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MPhil THESIS

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EFFECTS OF 1-MCP ON STORAGE OF "QUEEN COX" AND "BRAMLEY" APPLE FRUIT

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This thesis is submitted in fulfilment of the requirements for the degree of Master of Philosophy

ABSTRACT

Better maintenance of firmness and suppression of ethylene production in 'Queen Cox' and 'Bramley' apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] fruit was achieved by prestorage applications of 1-MCP. 1-MCP concentration, exposure time and exposure temperature ranges of 0.1 to 10.0 $\mu l\ l^{-1}$ 1-MCP, 6 to 48 h and 0 to 20°C, respectively, were effective on fruit subsequently stored for 2 ('Cox') and 3 ('Bramley') months in air at 3 to 4°C. However, 1-MCP had little effect on either firmness or ethylene production after 4 ('Cox') or 6 ('Bramley') months storage. Nonetheless, 1-MCP treated 'Bramley' fruit had reduced rot and superficial scald incidence compared with untreated control fruit.

1-MCP application was most effective when applied within 24 h of harvest compared to 14 d later. Earlier-harvested 'Cox' and 'Bramley' apple fruit showed better response to 1-MCP-treatment than those harvested towards the end of the picking season. 1-MCP-treatment was shown to improve apple storage alone and in combination with controlled atmosphere (CA) storage. Furthermore, 1-MCP-treatment maintained fruit quality during shelf-life better than CA storage alone. Chlorophyll fluorescence was not demonstrated to be an effective method to determine 'Cox' and 'Bramley' apple fruit quality.

There was no recorded correlation between the concentration of five antifungal compounds and 1-MCP-treatment after inoculation with *Penicillium expansum* or *Botrytis cinerea*.

1-MCP treatment for apple storage was developed for AgroFresh Inc., the holder of the 1-MCP patent. Part of this research was used for the UK efficacy trials for registration of 1-MCP as an apple storage treatment. On the 18th July 2002 the US Environmental Protection Agency (EPA) granted approval for 1-MCP to be applied to food crops. Approval was granted in the UK in time for the 2003 apple harvest, and for 2004 across Europe.

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ABREVIATIONS

Abbreviation	Full name
Ado	adenosine
ADP	adenosine diphosphate
ATP	adenosine triphosphate
AVG	L-2-amino-4-(2-aminoethyoxy)-*trans*-3-butenoic acid
AOA	(aminooxy)acetic acid
e.g.	(*exempli gratia*) for example
pH	(potential hydrogen) relative proton concentration in a solution
ACC	1-aminocyclopropane-1-carboxylic acid
1-MCP	1-methylcyclopropene
3,3-DMCP	3,3=dimethylcyclopropene
ANOVA	analysis of variance
ca	*circa* (approximately, thereabouts)
CRD	completely randomised design
CTR	constitutive triple response
CA	controlled atmosphere
CP	cyclopropene
d	day
°C	degrees Celsius
DAD	diode array detection
DPA	diphenylamine
DACP	diazocyclopentadiene
EPA	Environmental Protection Agency (US)
et al.	*et alia* (and others)
EFE	ethylene forming enzyme
EIN	Ethylene insensitive
ETR	ethylene receptor
ERF	ethylene response factor
ERS	ethylene response sensor
EREBP	ethylene-responsive element binding protein
FPP	farnesyl pyrophosphate
F_o	chlorophyll *a* constant fluorescence
F_m	chlorophyll *a* maximum fluorescence
F_v	chlorophyll *a* varialble fluorescence (F_m - F_o)
GC	gas chromatography
g	gram
HPLC	high performance liquid chromatography
h	hour
HR	hypersensitive response
IAA	indoleacetic acid
P_i	inorganic phosphate
IACR	Institute for Arable Crop Research

Abbreviation	Full name
K	kinase
kg	kilogram
l	litre
m	metre
µl	microlitre (10^{-6} l)
mg	milligram (10^{-3} g)
mg	milligram (10^{-3} g)
ml	millilitre (10^{-3} l)
MAP	mitogen-activated protein
MA	modified atmosphere
NDR	natural disease resistance
NR	never ripe
N	Newton
n/s	not significant
n	number of observations comprising a value
MBC	methyl benzimidazole carbamate
ms	millisecond
PQA	p-coumaryl-quinic acid
%	percent
PSD	Pesticide Safety Directorate (UK)
PCQ	p-coumaryl-quinic acid
PAL	phenylalanine ammonia lyase
PS I	photosystem I
PS II	photosystem II
P	probability
RH	relative humidity
RAN	responsive to antagonist
RPM	revolutions per minute
ROS	reactive oxygen species
AdoMet	S-adenosylmethionine
SAM	S-adenosylmethionine
s	second
STS	silver thiosulphate
sp. / spp.	species
s.e.m.	standard error of the mean
SAR	systemic acquired resistance
i.e.	that is
t	tonnes
TSS	total soluble solids
™	trademark

THESIS PLAN

1-Methylcyclopropene
(1-MCP)

Research funded by AgroFresh to determine the efficacy of 1-MCP in improving the storability of apples

Data compiled to register 1-MCP for commercial use in UK initially, then EU

5 Main Experiments

Experiment 1

Efficacy of 1-MCP concentration

Temperature of 1-MCP application

Duration of 1-MCP application

Experiment 2

Harvest maturity

Controlled atmosphere storage

The relationship between picking date and 1-MCP application

The comparison of 1-MCP treatment to fungicide application to prevent storage rot development

Experiment 3

1-MCP vs DPA to reduce scald in 'Bramley' apple fruit

Experiment 4

1-MCP-treatment on of phenolic compound concentrations in 'Queen Cox' apple fruit

Experiment 5

Commercial use of 1-MCP: Different 1-MCP-application strategies may need to be considered for different produce

Chapter 1 LITERATURE REVIEW

1.1 Introduction

1.1.1 Commercial importance of apples

Apples (*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf. are an important crop throughout the world. Global production in 2002 was 57,094,939 Mt, to which the UK supplied 176,700 Mt (FAO, 2003). Apples are a member of the pome group of fruits, together with pears (*Pyrus communis*), quince (*Cydonia oblonga*), oriental pear (*Pyrus serotina*), medlar (*Mespilus germanica*), and many other wild species of the Rosaceae.

In the UK, apples can be grown throughout most parts of England and Wales. However, the majority of orchards are in the southeast, particularly Kent, East Anglia and the West Midlands.

Apples have many uses, and specific apple cultivars are grown for particular purposes. In the UK, some cultivars, such as 'Queen Cox' are dessert apples. Others, primarily 'Bramley' are for culinary or processing fruit. However, low-grade dessert apples may also be processed into pies and sauces, frozen or canned.

1.1.2 Apple harvesting

Apples are generally harvested by hand in the weeks leading up to total ripeness. Apples are preferentially harvested earlier to minimise fruit loss due to abscission, senescence and pathogen attack, and increased fruit quality of early fruit over late fruit during storage. However, picking date ultimately depends on the market at that time. The market dictates how many fruit go for storage, straight to the shelves, to juicing or just left to fall. If there is a glut of apples, it may be cost effective to leave them rather than to pay picking, storage and transport costs (Giles Cannon, GER Fruit Ltd., pers. com.).

Fruit development and ripening is assessed weekly in the months leading up to harvest time. These checks of colour, firmness, sugar and starch content are recorded and used to let the buyers know exactly what stage the fruit is at in any particular orchard. This information is then used to inform growers what cultivars to pick, and when to pick them. Harvesting of all of the orchards under a buyer's control can be managed and the apples picked and stored progressively to maintain better prices by preventing the market being flooded with apples (Dr Martin Luton, Fruition, pers. com.). There may be other factors to consider with regard to picking date; 'Queen Cox' fruit, for example, are picked at 15% red colour due to buyer demands (Dr Martin Luton, Fruition, pers. com.).

1.2 Apple fruit physiology

1.2.1 Fruit growth, maturation and senescence

Following germination, there are three major physiological stages in the life of an apple: growth, maturation and senescence, although the distinction between these stages may be indistinct. During fruit development (the collective term for growth and maturation), the receptacle enlarges, enclosing and fusing with the ovary to form the edible portion of the fruit. There is little mitosis after early growth and the majority of fruit enlargement is by cell expansion. During growth, cells swell, separate. Mature apple fruit have *ca* 25% air space between the cells. The air spaces generally form radial canals through the cortex, and thus, fruit continually increase in volume during storage and ripening (Wills, 1987; Wills *et al.*, 1998).

Senescence is the phase where catabolic processes are greater than anabolic processes. Flesh firmness decreases during storage, and some of this may be attributed to decreasing cell-to-cell contact. However, it is difficult to distinguish this effect from ripening-related firmness losses. Cell separation generally stops when fruit become 'mealy'. Mealy fruit appear dry to taste; cells do not break on eating, and thus do not release juice.

1.2.2 Apple fruit ripening and the climacteric

Ripening may be considered the as the last stage of maturation and the onset of senescence. Apples are climacteric fruit. Apple fruit, like other climacteric fruit,

exhibit a climacteric rise in ethylene production and a subsequent rise in respiration rate. Kidd and West (1924), first described the rise in respiration rate, using apples as their model system.

As climacteric fruit exhibit these rises in ethylene and respiration, other changes occur throughout the fruit, such as the conversion of starch to sugars, loss of green colour, and reduced firmness (Wills *et al.*, 1998).

1.2.3 Ethylene

Ethylene (C_2H_4) is a naturally occurring, colourless and gaseous plant hormone, which diffuses readily within and from plant tissue.

Ethylene is a gaseous plant hormone whose signal is detectable at nanomolar concentrations (Rodríguez *et al.*, 1999). Exposure to biologically active levels of endogenous or exogenous ethylene will affect the biological activity of plant material (Saltveit, 1999). Ethylene particularly affects germination, flower and leaf senescence, leaf, flower and fruit abscission, root nodulation, programmed cell death and responsiveness to stress and pathogen attack, and ripening of climacteric fruit (Burg and Burg, 1965; Vendrell and McGlasson, 1971; Aharoni and Lieberman, 1979; Yu and Yang, 1980; Yang *et al.*, 1982; Stepanova and Ecker, 2000; Antunes and Sfakiotakis, 2002).

One of the most documented ethylene effects is the triple response, a phenotype exhibited by dicot seedlings grown in the dark under ethylene. Seedlings grown in the presence of ethylene develop a shorter, thicker root and hypocotyl and have enhanced curvature to the apical hook (Barry *et al.*, 2001). Arabidopsis (*Arabidopsis thaliana*) mutants expressing the triple response phenotype or not, in the absence or presence of ethylene, respectively, have been identified. These mutants have been cloned, and their genetic structure investigated. Arabidopsis and tomato (*Lycopersicon esculentum*) mutants with different ethylene responses have been used as tools to investigate ethylene binding, perception and signal transduction (Nakatsuka *et al.*, 1998; Chang and Shockey, 1999; Hall *et al.*, 1999; Barry *et al.*, 2000; Stepanova and Ecker, 2000; Hall *et al.*, 2000; Barry *et al.*, 2001).

1.2.4 Ethylene binding, perception and signal transduction

The method by which the ethylene signal is transferred from perception to effect is still under investigation. However, using evidence based on observations and similarities with other investigated pathways, a model of ethylene signal transduction has been proposed (Fig. 1.1).

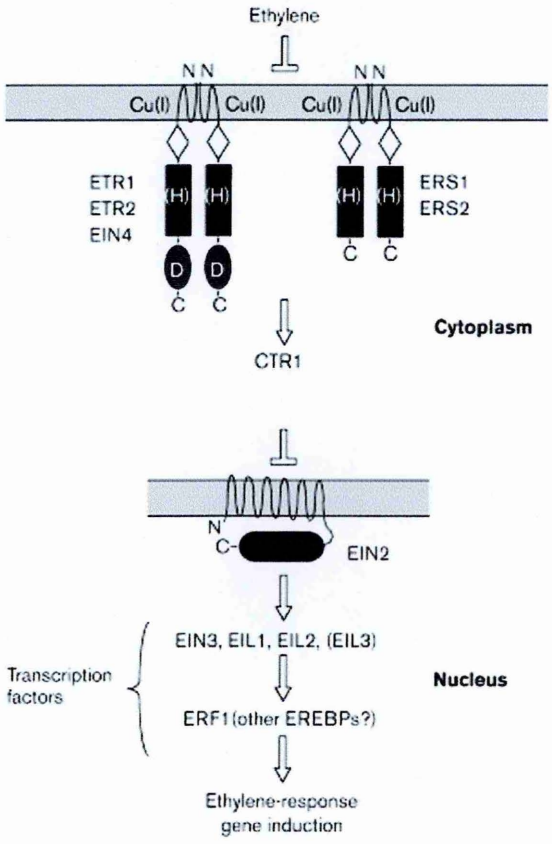

Figure 1.1 The ethylene signal transduction pathway. This is the current view, formulated using cloned Arabidopsis genes. Ethylene binds to membrane-localised Cu^{+}-containing ethylene receptors (Bengochea *et al.*, 1980; Rodríguez *et al.*, 1999). Ethylene is perceived by a family of ethylene receptor homodimers (ETR1, ERS1, ETR2, EIN4 and ERS2) (Schaller *et al.*, 1995; Hall *et al.*, 1999; Hall *et al.*, 2000; Stepanova and Ecker, 2000). In the absence of ethylene, the receptors repress ethylene responses of the downstream negative regulator, CTR1, possibly by direct action (Barry *et al.*, 2001). Ethylene binding on the receptor inhibits CTR1 from being activated by the receptor. Without the inhibitory effect of CTR1, The EIN2 integral membrane activates the carboxyl-terminal domain of EIN2, which in turn activates EIN3 (Chang and Shockey, 1999). EIN 3 is a positive regulator allowing expression of an EREBP transcription factor gene, ERF1 (Barry *et al.*, 2001). ERF1 causes the expression of the ethylene response by binding to the 'GCC box' promoter element of ethylene-regulated genes (Xu *et al.*, 1998). Figure after Chang and Shockey, (1999).

High affinity signal perception by plants usually involves the use of a transition metal co-factor to mediate the interaction between signal and a proteinaceous membrane-bound receptor (Rodríguez *et al.*, 1999). The efficacy of ethylene at low concentrations suggests the presence of high affinity receptors. It was hypothesised (Burg and Burg, 1965; Beyer, 1976) that ethylene binds to a Zn or Cu containing receptor. However, more recent studies have indicated that an ethylene-binding domain requires an associated copper ion for high-affinity binding activity (Rodríguez *et al.*, 1999; Stepanova and Ecker, 2000).

Five ethylene receptors have been identified in Arabidopsis. These have been divided into two sub-families of homodimers: ETR1 / ERS1 and ETR2 / EIN4 / ERS2, based on their gene and protein structures (Chang and Shockey, 1999). Ethylene receptors have been named after the phenotypes of the Arabidopsis mutants in which they were identified, ETR, ethylene receptor; EIN, ethylene insensitive; ERS, ethylene response sensor.

Ethylene binds to the amino termini of the hydrophobic pockets of the ethylene receptors, via a copper co-factor. The receptor molecules continually produce a positive regulatory signal, which maintains CTR1 protein activity. Ethylene binding inhibits this signal from being sent and, thus, deactivates the CTR1 protein (Schaller *et al.*, 1995; Hall *et al.*, 1999; Hall *et al.*, 2000; Stepanova and Ecker, 2000).

When activated, CTR1 protein activity promotes the positive regulator, EIN2. EIN2 is located in the nuclear membrane, but the precise location is unknown. When EIN2 fails to receive the CTR1 signal, it sends a signal to the EIN3 family of transcription factors (EIN3, EIL1, EIL2 and EIL3) (Stepanova and Ecker, 2000). The EIN3 family bind to the promoter of the ERF1 gene, activating it. ERF1 then interacts with ethylene response element binding proteins (EREBPs) (ethylene-response genes), thus inducing an ethylene-response (Chang and Shockey, 1999).

1.2.5 Ethylene biosynthetic pathway in planta

In addition to the perception of exogenous ethylene, plants biosynthesise endogenous ethylene. In higher plants, methionine is the sole precursor of ethylene (Yang, 1985; Fluhr and Mattoo, 1996), and is converted to ethylene in a series of reactions (Fig. 1.2). Methionine is converted to S-adenosylmethionine (SAM,

previously known as AdoMet) by SAM synthetase. SAM is then split into 1-aminocyclopropane-1-carboxylic acid (ACC) and 5'-methylthioadenosine by ACC synthase. 5'-methylthioadenosine is then recycled. ACC synthase has been characterised in many types of plant tissue (Kende and Boller, 1981). The conversion of ACC to ethylene is catalysed by ACC oxidase (previously known as ethylene forming enzyme, EFE). Both ACC synthase and ACC oxidase have been identified (Yang, 1987) and the genes that encode them have been cloned (Mehta *et al.*, 1997). ACC synthase and ACC oxidase gene expression exhibit well-documented increases during climacteric fruit ripening (Lelièvre *et al.*, 1997).

Ethylene production is regulated by ACC oxidase and ACC synthase gene expression. There are at least nine ACC synthase genes, and three ACC oxidase genes, identified from tomato fruit, each with differing levels of transcription activity. Conversely, gene expression of ACC oxidase may be ethylene regulated (Lelièvre *et al.*, 1997).

Other steps in the ethylene biosynthetic pathway may also regulate ethylene production. Not all of the ACC may be converted to ethylene. Yang and Hoffman (1984) suggest that ACC also forms NH_4, CO_2 and formic acid. However, it is more likely that ACC is converted to 1-malonyl ACC (MACC) (Hoffman *et al.*, 1982) by ACC *N*-malonyltransferase (Yang, 1985; Lelièvre *et al.*, 1997). This non-volatile compound cannot be converted to ethylene. *N*-malonyl ACC has an important role in the control of ethylene production, mainly by preventing ethylene overproduction (Amhrein *et al.*, 1982).

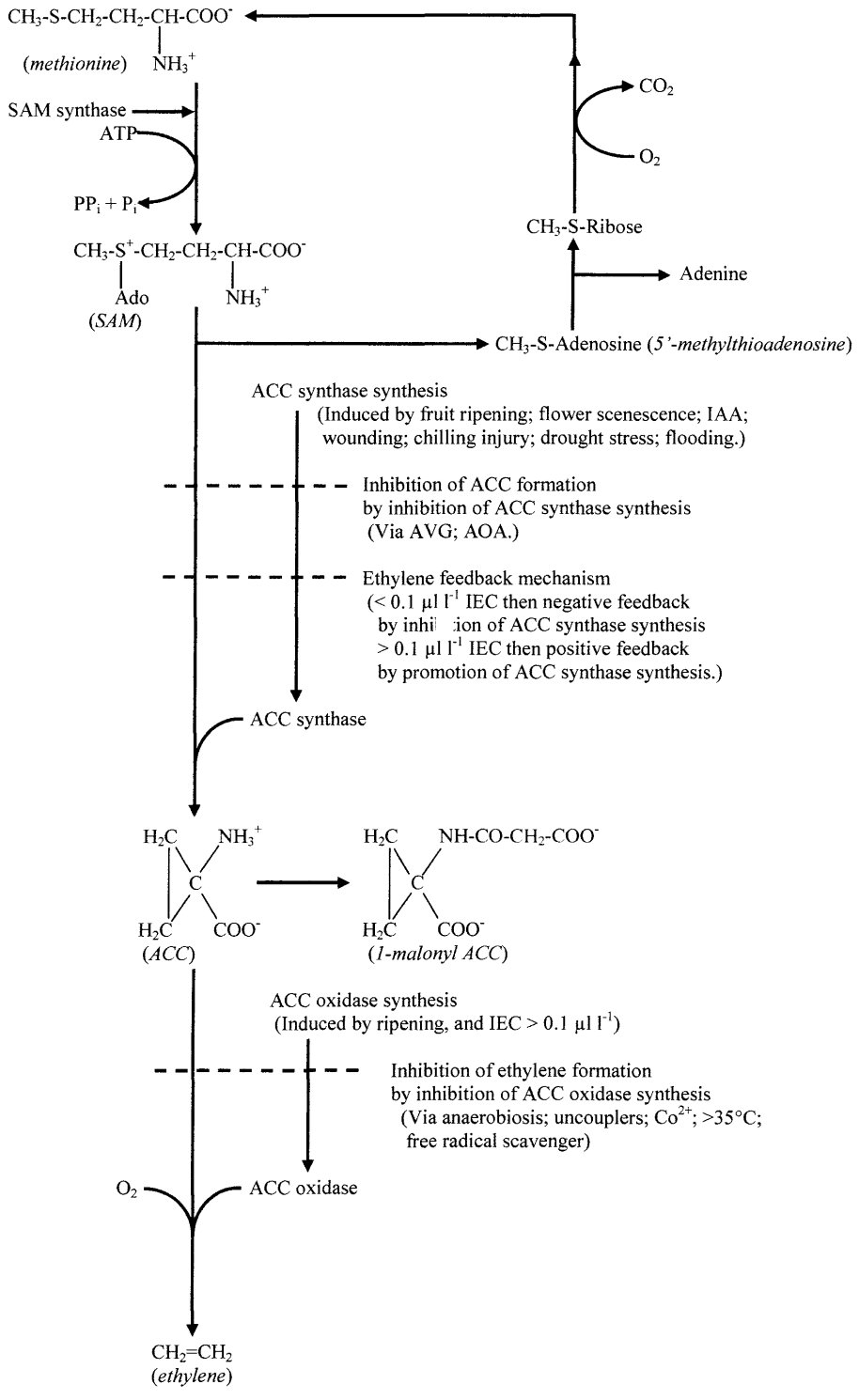

Figure 1.2 The biosynthesis and regulation of ethylene in plants. Methionine is found in low and constant concentrations in plant tissues. In ethylene-producing tissues, such as ripening climacteric fruit, a demand exists for methionine, which is supplied by recycling. The conversion of SAM to ACC is normally suppressed. During ripening, ethylene levels increase and promote the conversion of SAM to ACC and the conversion of ACC to ethylene in a positive feedback. ACC, 1-aminocyclopropane-1-carboxylic acid; SAM, S-adenosylmethionine; IEC, internal ethylene concentration; Ado, adenosine; P_i, inorganic phosphate. Modified from Yang (1985).

1.2.6 Ethylene effects on fruit ripening

For the sake of convenience, fruit can be classed as either climacteric or non-climacteric. Climacteric fruit, such as apples, bananas and some pears, exhibit a climacteric rise in respiration rate and ethylene production immediately prior and during ripening. Non-climacteric fruit, such as strawberries and oranges, do not exhibit these rises, but may be differently affected by ethylene.

ACC and ethylene levels in ripening fruit tissue increase during maturity, as does ethylene biosynthesis and ACC oxidase activity (Yang, 1987). A substantial increase in ethylene synthesis has been observed when 1-aminocylcopropane-1-carboxylic acid (ACC) is exogenously supplied to ethylene producing plant tissues, (Adams and Yang, 1979). It was also shown that application of ACC to unripe fruit only slightly enhances ethylene production, indicating that the activity of ACC oxidase is a critical step during ripening (Yang, 1987). Increased ethylene production as a stress response is also achieved by the conversion of SAM to ACC. Auxins can also promote ethylene synthesis by enhancing the conversion of SAM to ACC (Waring, 1982; Yang *et al.*, 1982).

Exposure of climacteric fruits to exogenous ethylene will trigger ripening and the auto-production of endogenous ethylene. Similarly, removal or inhibition of ethylene will inhibit ripening. The ability of ethylene perception to trigger the climacteric rise may be explained using the two-phase ethylene production hypothesis. First proposed for apple fruit by Knee (1985), system I and system II ethylene production has since been supported by studies on *Arabidopsis* mutants (Chang, 1996). System I operates in vegetative tissues, and both climacteric and non-climacteric fruit. Basal and wound-response ethylene production is believed to be produced by system I. System II is proposed to produce the ethylene for the climacteric rise (Lelièvre *et al.*, 1997). Positive feedback mechanisms exist in apples and other climacteric fruit where ethylene concentration affects ACC synthase and ACC oxidase activity (Bufler, 1984; Bufler, 1986; Lelièvre *et al.*, 1997; Nakatsuka *et al.*, 1998; Atta-Aly *et al.*, 2000).

The maintenance of low ethylene atmospheres (<0.05 $\mu l\ l^{-1}$) during apple storage prevents IEC from reaching the critical value of >0.1 $\mu l\ l^{-1}$ and thus initiating autocatalytic ethylene production (Knee and Tsantili, 1988; Stow *et al.*, 2000). During

this time system I is unaffected and basal ethylene production is observed. However, the perception of >0.1 $\mu l\ l^{-1}$ ethylene may exert a negative feedback on system I regulation, and enhance system I ethylene production. In addition, antagonists of ethylene action also enhance system I ethylene production in immature climacteric fruit (Lelièvre *et al.*, 1997). However, ethylene is auto-stimulatory in mature climacteric fruit. System II ethylene production and ripening is inhibited by the application of ethylene action inhibitors (Lelièvre *et al.*, 1997).

1.3 Inhibition of ethylene action

Inhibitors of ethylene biosynthesis or action, such as silver thiosulphate (STS) and 'Trion' have been shown to delay or prevent ripening (Taiz and Zeiger, 1991). Most recently, ethylene inhibitors based on, and including, cyclopropene (CP), such as 1-methylcyclopropene (1-MCP) and 3,3-dimethylcyclopropene (3,3-DMCP), have been investigated (Sisler and Serek, 1997; Golding *et al.*, 1998). These inhibitors are presumed to bind to a metal in the ethylene receptors, thus blocking ethylene molecules and their effects for prolonged periods. However, the actual mechanism by which either ethylene or the inhibitor binds to the receptor is still unknown (Sisler and Serek, 1997). It is presumed that the inhibitors remain bound to the receptors, and subsequent ripening effects are most likely due to the formation of new binding sites (Dauny and Joyce, 2002).

1.3.1 Chemistry of 1-methylcyclopropene (1-MCP)

1-MCP is a methylated propene ring (Fig. 1.3).

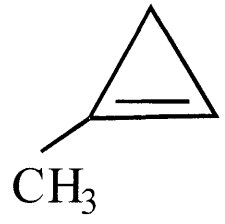

Figure 1.3 The chemical structure of 1-methylcyclopropene (1-MCP).

1-MCP is a vapour at room temperature and has no obvious odour (Sisler and Serek, 1997). 1-MCP is an ethylene reception inhibitor. It has been suggested (Sisler and Serek, 1997) that 1-MCP binds to the ethylene receptor in place of ethylene. However, there are differences between ethylene and 1-MCP in binding to the ethylene receptor. Whereas ethylene diffuses rapidly from the binding site, 1-MCP will remain attached for long periods (Sisler and Serek, 1997). The use of 1-MCP to maintain quality of ethylene-sensitive produce during storage is well established (Table 1.1), and is believed to work by inhibiting ethylene action, rather than having a direct chemical effect. Table 2.1 illustrates a range of 1-MCP treatment effects on different produce. A full and comprehensive list is published on the internet by Watkins and Miller (2004) and is updated regularly.

Table 1.1 Overview demonstrating 1-MCP effects on produce, with reference to 1-MCP treatment concentration, exposure time and exposure temperature.

1-MCP concentration ($\mu l\ l^{-1}$)	1-MCP exposure time (h)	1-MCP exposure temperature (°C)	Produce	Effects compared to non 1-MCP treated control	Reference
0.01	24	20	Banana	Increased shelf life	Jiang *et al.* (1999)
0.025(*)	6	dnp	Orange(n)	Inhibited C_2H_4-induced degreening	Porat *et al.* (1999)
0.05(*)	6	dnp	Orange(n)	Inhibited C_2H_4	Porat *et al.* (1999)
0.05	24	20	Banana	Increased shelf life	Jiang *et al.* (1999)
0.10(*)	6	dnp	Orange(n)	Inhibited C_2H_4, Increased disease incidence	Porat *et al.* (1999)
0.10	2	20	Strawberry(n)	Increased disease resistance, increased shelf life	Jiang *et al.* (2001)
0.10	2	20	Strawberry(n)	Decreased shelf life	Ku *et al.* (1999)
0.10	24	20	Banana	Increased shelf life	Jiang *et al.* (1999)
0.10	18	20	Apple	Reduced IEC, retention of firmness, reduced α-F	Rupasinghe *et al.* (2000)
0.20	2	20	Strawberry(n)	Decreased shelf life	Ku *et al.* (1999)
0.20	6	21	Waxflower	Inhibition of C_2H_4-induced bud & flower abscission	Serek *et al.* (1995)
0.25	2	20	Strawberry(n)	Increased disease resistance, increased postharvest life	Jiang *et al.* (2001)
0.25	14	20	Avocado	Delayed ripening, higher rot severity	Hofman *et al.* (2001)
0.25	14	20	Custard apple	Delayed ripening, higher rot severity	Hofman *et al.* (2001)
0.25	14	20	Mango	Delayed ripening, higher rot severity	Hofman *et al.* (2001)
0.25	14	20	Papaya	Delayed ripening, higher rot severity	Hofman *et al.* (2001)
0.50	2	20	Strawberry(n)	Decreased disease resistance, increased shelf life	Jiang *et al.* (2001)
0.50	2	20	Strawberry(n)	Decreased shelf life	Ku *et al.* (1999)
0.50(*)	7	20 – 25	Apple	Reduced IEC, retention of firmness, reduced α-F & CT	Watkins *et al.* (2000)
0.50	24	20	Banana	Increased shelf life	Jiang *et al.* (1999)
1.00	24	20	Banana	Increased shelf life	Jiang *et al.* (1999)
1.00(*)	7	20 – 25	Apple	Reduced IEC, retention of firmness, reduced α-F & CT	Watkins *et al.* (2000)
1.00	12	20	Apple	Reduced IEC, retention of firmness, reduced α-F & CT, higher TA	Fan *et al.* (1999b)
1.00	18	20	Apple	Reduced IEC, retention of firmness, reduced α-F	Rupasinghe *et al.* (2000)
1.00	2	20	Strawberry(n)	Decreased disease resistance, increased shelf life	Jiang *et al.* (2001)
2.00(*)	7	20 – 25	Apple	Reduced IEC, retention of firmness, reduced α-F & CT	Watkins *et al.* (2000)
10.00	18	20	Apple	Reduced IEC, retention of firmness, reduced α-F	Rupasinghe *et al.* (2000)
45.00	6	20	Banana	Delayed C_2H_4 and respiratory climacterics, increased green life	Golding *et al.* (1998)
100.00	18	20	Apple	Reduced IEC, retention of firmness, reduced α-F	Rupasinghe *et al.* (2000)
450.00	6	20	Banana	Delayed C_2H_4 and respiratory climacterics, increased green life	Golding *et al.* (1998)

(*) Non-quantified – based on 0.43% a.i. 1-MCP, dnp data not published, (n) non-climacteric fruit, IEC internal ethylene concentration, α-F α-farnesene, CT conjugated trienols, TA titratable acidity. Different cultivars or varieties of fruits may express a scale of response to 1-MCP that is not consistent with the majority of the species.

1.4 Apple storage

The techniques used to store apples depend on cultivar, maturity at harvest and storage duration. There are three main factors that can be altered to maintain optimal storage conditions. The first is temperature, then controlled atmosphere (CA) and the use of ethylene scrubbers. In addition, fruit may also be treated with chemicals after harvest to prevent disease and disorders.

'Cox' apple fruit are stored either in air or at 3 to 4°C under ultra-low oxygen CA conditions of 1.2% O_2, <1% CO_2 (Johnson and Colgan, 2003). 'Bramley' fruit may be stored at 3 to 4°C under CA conditions of 1% O_2, and 5% CO_2 (Colgan *et al.*, 1999) (Table 1.2).

Table 1.2 Optimal controlled atmosphere (CA) storage conditions for some apple fruit cultivars. Ideal storage times until termination of CA conditions are given. After (Colgan *et al.*, 1999; Johnson and Colgan, 2003).(1998)

Cultivar	CA conditions		Ideal termination date
	CO_2 (%)	O_2 (%)	
'Bramley'	5.0	1.0	June
'Cox'	< 1.0	1.2	March
'Gala'	8.0	2.0	January
'Jonagold'	8.0	1.2	July

However, 'Bramley' may also be stored under 12% O_2, 9% CO_2, particularly if there is a *Nectria* spp. problem (Ian Mitchell, Chairman of the Bramley Campaign, pers. Com, 2001). Unfortunately, 'Bramley' fruit are more susceptible to the storage disorder, superficial scald under 12:9 conditions. Therefore, 1% O_2, and 5% CO_2, is becoming the more common practice due to better storage under these conditions (Colgan *et al.*, 1999).

Retention of apple firmness during storage is greater when ethylene is scrubbed from the atmosphere, and IEC is maintained below 0.1 $\mu l\ l^{-1}$. This is due to inhibition of initiation of softening, rather than a reduction in the rate of softening (Stow *et al.*, 2000).

1.5 Storage disorders

Apple fruit quality decreases with length of storage; during which time fruit may develop different cultivar dependant disorders.

1.5.1 Softening

Maintenance of apple firmness is an aim of all apple storage management procedures, as softer fruit have reduced quality and thus less commercial value. Softening is influenced by the internal ethylene concentration (IEC) of fruit. In general, firmness of fruit stored in ethylene concentrations below 44 $\mu mol\ m^{-3}$ (1 $\mu l\ l^{-1}$), is greater than for those stored in higher ethylene concentrations (Stow *et al.*, 2000). However, for 'Cox' fruit, removal of atmospheric ethylene only delayed the accumulation to a critical IEC level of 0.1 $\mu l\ l^{-1}$ for 8 to 12 weeks. After this time, fruit softened; although less than for fruit stored in higher ethylene concentrations (Stow *et al.*, 2000).

1.5.2 Superficial scald

Superficial scald is a physiological disorder which affects many, but not all apple cultivars during cold storage (Fernández-Trujillo *et al.*, 2001). Superficial scald is visible as irregular brown patches of dead skin caused by progressive browning of hypodermal cells (Ingle and D'Souza, 1989) (Fig. 1.4).

Figure 1.4 Superficial scald on the skin of a 'Granny Smith' apple fruit (Photo: Allen Hilton).

In the most severe cases, superficial scald can be visible in cold storage. Superficial scald is not limited to the skin. As scald increases in severity, the browning may extend through five or six layers of the hypodermis. In the most severe cases, epidermal cells are affected and become brown, and there may be sunken patches where hypodermal cells have collapsed (Ingle and D'Souza, 1989).

Many factors influence scald development. Cultivar, maturity, seasonal environmental variation, cultural practices and postharvest conditions can affect both scald development and severity (Huelin and Coggiola, 1968; Ingle and D'Souza, 1989; Fan *et al.*, 1999b). Cultivars that are more scald-resistant include 'Bramley' and 'Granny Smith'. Scald-susceptible cultivars include 'Cox' and 'Crofton'.

Superficial scald develops 3 to 7 d from 're-warming' after *ca* 3 months cold storage. Superficial scald is not caused by the increase in temperature, but warming allows the symptoms to develop. It is believed that scald is a form of chilling injury (Watkins *et al.*, 1995).

Superficial scald is believed to be result from the auto oxidation of α-farnesene into conjugated trienes (CTs) and the associated formation of free radicals (Huelin and Coggiola, 1968; Anet and Coggiola, 1974; Du and Bramlage, 1994; Whitaker *et al.*, 2000). α-farnesene is an acrylic sesquiterpene hydrocarbon, one of the many volatiles and a component of apple surface wax (Rupasinghe *et al.*, 1998). During storage, CTs accumulate progressively on the surface of apples as α-farnesene oxidises. These oxidation products injure the cell membranes that result in cell death in the outermost layers. The concentration of CTs has a greater correlation with superficial scald severity than α-farnesene (Huelin and Coggiola, 1970; Rupasinghe *et al.*, 1998).

Biosynthesis of α-farnesene is via the isoprenoid pathway, and is converted to farnesyl pyrophosphate (FPP), catalysed by a single sesquiterpene synthase enzyme, α-farnesene synthase (Rupasinghe *et al.*, 1998). α-farnesene biosynthesis in apples is developmentally regulated, and increases rapidly during ripening and cold storage, parallel to increased internal ethylene concentration (Watkins *et al.*, 1993; Du and Bramlage, 1994; Ju and Curry, 2000). Cultivar is an important factor concerning the relationship between internal ethylene and α-farnesene concentrations. Cultivars that are more scald-resistant produce less α-farnesene and more ethylene than scald-

susceptible cultivars (Golding *et al.*, 2001). However, even scald-resistant cultivars show an increase in α-farnesene concentration with increased internal ethylene with storage (Golding *et al.*, 2001).

The relationship between internal ethylene and apple peel α-farnesene concentration is also dependent on storage temperature. 'Granny Smith' apples stored at 10°C exhibited a twenty-fold increase in ethylene production but only a doubling of peel α-farnesene concentration compared to fruit stored at 0°C (Golding *et al.*, 2001). In contrast, 'Crofton' fruit ethylene production increased nine-times, whereas peel α-farnesene concentration remained constant.

However, the exact mechanism by which ethylene interacts with α-farnesene biosynthesis is unclear. The evidence for this interaction is mostly circumstantial. Susceptibility to scald decreases and internal ethylene increases as fruit mature. Treatment with ethephon, an ethylene action analogue, advances fruit maturity and results in less scald development. Scald development and internal ethylene biosynthesis are reduced in controlled atmosphere (CA) storage. Diphenylamine (DPA), an antioxidant which also has been shown to suppress ethylene production, is commercially used to prevent scald (Fan *et al.*, 1999b). In addition, 'Granny Smith' apple fruit developed less scald after storage when treated with the ethylene action inhibitor diazocyclopentadiene (DACP) at harvest (Gong and Tian, 1998; Fan *et al.*, 1999b).

1.5.3 1-MCP as an apple storage technique

Currently, commercial systems utilise ethylene scrubbers and low-oxygen storage to reduce scald incidence (Colgan *et al.*, 1999), and maintain fruit quality. In addition, antioxidants, such as DPA are applied immediately after harvest. Certain countries do not permit the import of DPA-treated apples (Chervin. *et al.*, 2001), and the future use of DPA as a commercial scald treatment is unclear. However, experiments have shown 1-MCP-treated apples maintain their quality better compared to non-1-MCP-treated fruit, particularly in short-term storage (Rupasinghe *et al.*, 1998; Fan *et al.*, 1999a; Fan *et al.*, 1999b; Watkins *et al.*, 2000; Mir *et al.*, 2001; DeEll *et al.*, 2002; Dauny and Joyce, 2002).

1-MCP treatments for apple storage have been developed for AgroFresh Inc., a Rohm and Hass company that holds the patent for 1-MCP. Trials have been conducted across the UK, mainland Europe and the US. On the 18th July 2002 the US Environmental Protection Agency (EPA) granted approval for 1-MCP to be applied to food crops. Approval is expected in the UK in time for the 2003 apple harvest, and for 2004 across the rest of Europe (G. Regiroli, AgroFresh Inc., pers. comm., 2002). The use of 1-MCP to prolong the life of ornamentals has been permitted for some time.

1-MCP is supplied as a stable powder, known as SmartFresh™ for food crops, and Ethylbloc™ for ornamentals. The mode of application of 1-MCP will be via a supplied unit, which will be filled with water by the user and sealed in the storage room with the produce (G. Regiroli, AgroFresh Inc., pers. comm.).

1.6 Mechanisms of disease resistance *in-planta*, natural disease resistance (NDR)

Initially, the fungus and the host need to be compatible. Pathogen growth is prevented or retarded by incompatibility. Incompatibility is a resistance reaction that may be conditioned by a single interaction gene pair, a host resistance gene (*R*) and a pathogen avirulence gene (*Avr*) (Prusky, 1998). Flor postulated the gene-for-gene interaction in 1970. Flor showed that for each gene of resistance in the host there was a corresponding avirulence gene in the pathogen. In addition, Flor also reported that for each gene of virulence in the pathogen there was a corresponding susceptibility gene in the host (Agrios, 1997).

However, once a pathogen has cone into contact with a suitable host, there are many factors to be overcome before infection can begin and or develop. Assuming that the environmental factors (temperature, humidity, light conditions, etc.) are suitable for spore germination, spores which reach the plant's surface have to penetrate through the skin and overcome chemical defences before germination can occur (Grayer and Harborne, 1994; Grayer and Kokubun, 2001).

Plants have many levels of inherent defence mechanisms against fungal attack, referred to as natural disease resistance (NDR). NDR is defined here as the innate resistance to pathogen attack, and thus, infection. The level of NDR varies for different circumstances: environmental, crop handling, genotype and stage of

development. NDR decreases during development and after harvest, and thus, the susceptibility of produce to pathogen attack is increased (Prusky, 1996). The systems by which NDR may decline are the availability of nutrients for the pathogen; changes in preformed antifungal compounds with development and senescence; the ability of the host to produce antifungal compounds in response to attack (phytoalexins) (Prusky, 1996). Once these factors can be overcome by the pathogen, infection can develop.

1.6.1 The role of antifungal compounds in NDR

The role of preformed and elicited antifungal compounds, as part of an active defence response to pathogen attack is well documented (Nicholson and Hammerschmidt, 1992). Chemical defence by antifungal phenolic metabolites can be divided into two types, preformed and induced. Preformed antifungal compounds (e.g. prohibitins, phytoanticipins or pre-infectional metabolites) are permanently present in the tissues, normally in high enough concentrations to inhibit most fungi (Harborne, 1999), but may be accumulated in response to fungal attack (Grayer and Harborne, 1994).

Plants may also use other preformed defence compounds as a response to fungal attack. These are normally present as an inactive, bound form (e.g. a saponin), which are converted to an active antifungal compound (e.g. a sapogenin) in response to infection (Harborne, 1999). This is usually by a simple and quick chemical reaction, such as enzymic hydrolysis (Grayer and Harborne, 1994).

However, these are not to be confused with phytoalexins. Phytoalexins are low molecular weight antimicrobial compounds that are not normally present in healthy tissue. These induced compounds are the product of biosynthesis of non-immediate precursors as a specific defence response (Hammerschmidt, 1999). Phytoalexins are biosynthesised via the *de-novo* expression of the enzymes that are involved in their biosynthetic pathway in response to fungal attack, and may take up to three days to reach effective concentrations (Anderson, 1991; Grayer and Harborne, 1994). The first lines of defence are the outer layers (the cuticle, or peel), and antifungal compounds exuded to inhibit spore germination and germ tube elongation. Antifungal compounds are part of the range of constitutive (or preformed) antifungal compounds,

also known as preinfectional metabolites, prohibitins or phytoanticipins (Grayer and Kokubun, 2001).

If these defences fail to stop spore germination and the subsequent penetration of the epidermis by the hyphae, the plant may exhibit further responses to block or hinder fungal growth. A rapid increase in ethylene biosynthesis is one of the earliest detectable events of plant-pathogen interaction. Pathogen attack on a plant causes an increase in ACC synthase activity, and thus an increase in ethylene production (Ecker and Davis, 1987). In turn, increased ethylene production causes an accumulation of the plant defence response genes that code for defence mechanisms, particularly PAL, 4-coumarate CoA ligase (4-CL), chalcone synthase (CHS) and hydroxyproline-rich glycoproteins (HGRPs) (Ecker and Davis, 1987). As such, reactive oxygen species (ROS) may be generated as a warning signal to surrounding cells, which in turn may trigger defensive reactions. (Lamb and Dixon, 1997; Wojtaszek, 1997). These defensive reactions may include strengthening of the cell wall, production of polygalacturonase-inhibiting proteins (PGIPs), initiation of the hypersensitive response, induction of systemic acquired resistance (SAR), and the production and accumulation of antifungal compounds known as phytoaléxins.

It is important to note, however, that there is no absolute defence against pathogen attack. When considering the efficacy of a defence response, one must refer to the degree by which the disease is restricted, either as growth or damage (Mercier, 1996).

1.6.2 Preformed antifungals

For fungi to break into the plant cell, the plant polysaccharide-rich cell wall must be penetrated. Many fungi exude enzymes specifically to achieve this. One such enzyme family are the endopolyglacturonases (PGs), which cause cell wall degradation and macerate plant tissue. During this process, fungal PG action results in the plant cell wall releasing oligogalacuronide (OG) fragments by the action of by PGIPs. OG fragment release is favoured by PGIP action and PGIPs also inhibit and regulate PG action (de Lorenzo *et al.*, 2001). The PG-PGIP interaction is widespread in the plant kingdom. In apples, a PGIP gene has been cloned from 'Golden Delicious', Mdpgip1 and apple PGIP has been shown to inhibit *Nectria galligena*,

Phomopsis mali, Fusarium lateritium, Glomerella cingulata, Botrytis cinerea and *Venturia inaequalis*. However, apple PGIP was shown to inhibit neither *Penicillium expansum* nor *Phytophthora syringae* (de Lorenzo *et al.*, 2001).

In-vivo germination of *Gloesporium perennans* has been shown to be completely inhibited by cyanidin and *p*-coumaric acid (Hulme and Edney – Need Reference). Catechins and proanthocyanidins that are based on flavan-3-ols, which occur in a number of plant families. Leaves of *Rosaceae* species accumulate flavan-3-ols in a boundary layer of 2 mm around necrotic regions in response to fungal infection (Feucht *et al.*, 1992). Strawberry proanthocyanidins act as antifungal compounds. Strawberry proanthocyanidin extracts inhibit *B. cinerea* development *in-vitro*, and higher proanthocyanidin levels correlate with cultivar preservation differences (Hébert *et al.*, 2002). Avocado fruit exposed to elevated CO_2 show increased levels of epicatechin and an 'antifungal diene' [*sic.*] in their peel. These fruit with elevated levels of epicatechin and antifungal diene were observed to be more resistant to fungal attack by *Colletotrichum gloeosporioides* (Ardi *et al.*, 1998).

However, antifungal compounds may not work directly against the pathogen. Catechin undergoes both auto- and enzymic oxidation to H_2O_2, which may be linked to the synthesis of phytoalexins and the oxidation of induced and preformed phenolics, or act as a direct antifungal agent (hypersensitive response) (Jiang and Miles, 1993).

1.6.3 Induced antifungals

The majority of research into plant-pathogen interactions indicates that defence responses are expressed after infection to reduce pathogen development (Hammerschmidt, 1999). The post infection *de-novo* production of certain compounds that exhibit antifungal activity *in-vitro* has been associated with the defence response. Muller and Börger first proposed the hypothesis of elicited plant defence compounds in 1940. Using potato tubers infected with *Phytopthera infestans*, Muller and Bőrger identified compounds they called 'phytoalexins' from the Greek 'φυτον', plant; 'αλεξειν', to defend. However, Stoessl and Arditti (1984) state that observations of subsequent disease resistance after exposure to pathogen attack was recorded as far back as 1911 by the French botanist, Noel Bernard. Bernard discovered tubers of two orchid species, *Orchis morio* and *Loroglossum hircinum* (= *Himantoglossum*

hircinum), once infected with the fungus *Rhizoctonia repens* became resistant to subsequent fungal attack, and produced an unidentified inhibitor of fungal growth. Elicited antifungal compounds have since been found in most plant species.

The evidence to suggest that accumulation of phytoalexins is a defence response, rather than a response to infection, was shown by Keen (1981). Data was presented to quantify the localisation and timing of phytoalexin accumulation in infected tissue in relation to pathogen development'; 'a positive relationship between pathogen virulence and tolerance of phytoalexins'; and 'an increase of plant tissue resistance by stimulation of phytoalexin production prior to inoculation'.

The production of phytoalexins as a defence response is an economical use of plant resources. Carbon and energy sources are only used for phytoalexin production in the early stages of infection, and only at the sites of infection (Grayer and Kokubun, 2001).

1.6.4 Phenolic compounds in apple fruit

Large numbers of phenolic compounds have been found in apple fruit (Table 1.3). The phenolic composition of apple fruit peel and pulp has been quantified by HPLC-diode array detection (DAD) (Oleszek *et al.*, 1988; Burda *et al.*, 1990; Suárez *et al.*, 1996; Escarpa and González, 1998; Lattanzio *et al.*, 2001). Studies on a number of apple fruit cultivars have identified phenolic compounds in the peel and the pulp. The main phenolic compounds in 'Golden Delicious', 'Empire' and 'Rhode Island' apple fruit are epicatechin and procyanidin B2 (Burda *et al.*, 1990).

Apple phenolics are placed into four groups: simple phenols (such as chlorogeninc acid, and other phenolic acids), flavonoids (particularly quercetin and procyanidin), lignin and anthocyanin. Lignin is chemically stable, as are flavonoids and anthocyanin, unlike simple phenols which act as substrates for polyphenols oxidase (PPO), or auto-oxidise (Ju *et al.*, 1996).

There are a number of compounds associated with disease resistance reported for apple fruit. The two major compounds are the phytoalexin benzoic acid, and the phytoanticipin chlorogenic acid. In addition to these are flavonoid phytoanticipins.

Table 1.3 Phenolic compounds identified by HPLC-DAD analysis in the peel or pulp of 'Golden -' and 'Red Delicious', 'Granny Smith' and 'Green Reineta' apple fruit (Escarpa and González, 1998)[1], 'Rhode Island Greening' apple fruit (Oleszek *et al.*, 1988)[2], 'Elstar' and 'Jonagold' apple fruit, .(Awad *et al.*, 2000)[3], 'Golden Delicious' apple fruit (Lattanzio, *et al.*, 2001)[4].

Phenol	Location (Peel and / or pulp)
[1]Procyanidin B3	Peel & pulp
[1]Procyanidin B1	Peel & pulp
[1,3,4](+)-Catechin	Peel & pulp
[1]Procyanidin B2	Peel & pulp
[1,3,4]Chlorogenic acid	Peel & pulp
[1,4](-)-Epicatechin	Peel & pulp
[1]Caffeic acid	Peel & pulp
[1, 2,4] Phloretin derivative	Peel & pulp
[1, 2, 3]Phloridzin	Peel & pulp[1]
[1]Rutin	Peel
[1]Flavonol glycoside	Peel
[1]Flavonol glycoside	Peel
[1]Flavonol glycoside	Peel
[2]hyperin	Peel
[2]isoquercitrin	Peel
[2]3reynoutrin	Peel
[2]aviclarin	Peel
[2,4]quercitin	Peel

1.6.5 Benzoic acid

To date, benzoic acid has been identified as the only major phytoalexin in apples (Brown and Swinburne, 1971). Benzoic acid accumulates in the infected areas in response to certain pathogen attacks. The antifungal compound present in the necrotic tissue of immature 'Bramley' apple fruit infected with *Nectria galligena* was isolated, purified and identified as benzoic acid. Benzoic acid was found to be present in sufficiently large quantities to account for all the observed antifungal activity.

It is believed that during infection, *N. galligena* secretes proteases that elicits benzoic acid accumulation (Swinburne, 1975). Of the fungi tested, only *N. galligena*, *Pezicula malicorticis* and *Diaportha pernicosa* elicited benzoic acid *in-vivo*.

Penicillium expansum, *Botrytis cinerea*, *Phytopthera cactorum*, *Scerotinia fructigena*, *Aspergillus niger* and *Fusarium lateritium* were able to infect 'Bramley' fruit at any stage of maturity (Swinburne, 1975). However, only fungi that exhibit protease activity induce benzoic acid, and neither *B. cinerea* nor *P. expansum* have been shown to produce extracellular protease (Mercier, 1996).

Infection of 'Cox's Orange Pippin' and 'Bramley' fruit by *N. galligena* elicits more benzoic acid than infection by *P. malicorticis* (Swinburne, 1975; Noble and Drysdale, 1983). Benzoic acid is not usually found in healthy apple tissues, and is not induced by mechanical injury (Noble and Drysdale, 1983). However, attempts to replicate these experiments failed to isolate and identify benzoic acid from infected fruit of other cultivars (Harborne, 1999).

1.6.6 Chlorogenic acid

Chlorogenic acid is a preformed antifungal compound, not induced by pathogen infection. It is the major hyrdoxycinnamic acid derivative produced by apple fruit (Awad *et al.*, 2000). Chlorogenic acid is present, although differently distributed, throughout apple fruit tissue. Studies on 'Jonagold' and 'Elstar' cultivars showed that for both cultivars, chlorogenic acid concentration in the core (2.10 mg g dry weight (dw)) was higher than the surrounding tissue (0.48 mg g dw), which in turn was higher than in the peel (0.20 mg g dw) (Awad *et al.*, 2000).

Chlorogenic acid has been shown to reduce the germination of *P. expansum* conidia, *in-vitro* by *ca* 1.6-fold at 500 mg l^{-1} (Boonyakiat *et al.*, 1986). However, no effect was detected for reducing *P. expansum* growth after germination in the presence of ≤ 300 mg l^{-1} chlorogenic acid. Conversely, however, these authors also reported an increase in both germination of conidia and mycelial growth of *B. cinerea* in the presence of ≤ 200 mg l^{-1} chlorogenic acid. The fungal toxicity of phenolics may therefore be negligible or even stimulate fungal growth at particular concentrations (Boonyakiat *et al.*, 1986).

In addition to having direct antifungal activity against certain pathogens, chlorogenic acid also inhibits apple softening. Chlorogenic acid, catechins and quercetin glycosides have been identified as the principle constituents of ethyl acetate extracts of 'Spartan' apples that inhibit β–galactosidase (Dick *et al.*, 1985). There is

evidence to suggest that the enzyme, β-galactosidase, degrades cell-wall polysaccharides in apples (Knee, 1973; Bartley, 1977; Dick *et al.*, 1985). As such, β-galactosidase activity may be involved in the regulation of apple texture loss, as well as during infection (Dick *et al.*, 1984; Dick *et al.*, 1985). Apple extracts containing inhibitors of β-galactosidase (0.003% (w/v) quercetin, 0.1% (w/v) quercetin glycoside fraction, or 0.1% (w/v) chlorogenic acid in water) or crude apple extracts (200 unitsml^{-1}) applied as either a postharvest dip or by vacuum infiltration to harvested 'McIntosh' or 'Golden Delicious' apple fruit suppressed ripening (Dick *et al.*, 1985). Vacuum infiltration was more effective, and the infusion of 0.1% (w/v) chlorogenic acid alone suppressed fruit firmness loss at 20°C (Dick *et al.*, 1985).

1.6.7 Flavonoids

The major classes of flavonoids are flavonols, including quercetin 3-glycosides; monomeric and oligomeric flavan-3-ols, e.g. catechins, epicatechin and procyanidins; dihydrochalcones, e.g. phloridzin; and non antitifungal compounds, such as the anthocyanins, e.g. cyanidin 3-glycosides in red fruit. Unlike benzoic acid, flavonoids and chlorogenic acid are already present in the fruit, and flavonoid content has been correlated with disease resistance (Awad *et al.*, 2000). Flavonoids are present in all observed species and are present throughout the fruit structure, particularly the peel. Flavonoids have been observed at highest concentrations in immature fruit, and to decline during fruit maturation.

Flavonoids are phenolic plant metabolites, considered to express anti-oxidative, anti-microbial, anti-mutagenic and anti-carcinogenic properties (Awad *et al.*, 2000). These compounds are formed from precursors from the phenylpropanoid pathway which in turn are derived from the shikimate pathway. Flavonoids are often present as glycosides, particularly galactose in apple fruit. The role of flavonoids in conferring disease resistance is well established (Harborne and Williams, 2000).

'Red Delicious' apples harvested before July were shown to be resistant to *B. cinerea*. *B. cinerea* conidia germination and radial mycelial growth of *B. cinerea* were both inhibited by $\leq 50\ \mu g\ ml^{-1}$ and $\leq 100\ \mu g\ ml^{-1}$ extracted chlorogenic acid and p-coumaryl-quinic acids (PCQ), respectively. Furthermore, *P. expansum* mycelial

growth was inhibited by ≤ 100 μg ml^{-1} and ≤ 200 μg ml^{-1} extracted chlorogenic acid and PCQ, respectively (Ndubizu, 1976).

In this experiment, (Ndubizu, 1976) chlorogenic acid or PCQ was incorporated into Cruickshanks medium (Cruickshanks and Perrin, 1964) and seeded with 11 mm diameter mycelial plugs of *B. cinerea* or *P. expansum*, and stored at 22 to 24°C for 48 or 72 h. At these times, growth of the fungi was assessed as colony diameter, giving an index to the affect of the chlorogenic acid or PCQ on fungal growth (Ndubizu, 1976).

Chlorogenic acid and PCQ were sequentially extracted from frozen 'Red Delicious' apple tissue (unspecified). The extraction solvents were, in turn, 100% methanol (1:5 w/v), water, ethyl acetate (1:6 v/v) and the residue dissolved in 95% ethanol to give a final concentration of 3 g ml^{-1} (w/v). Chlorogenic acid PCQ and were both separated using 2-D thin layer chromatography in N-butyl alcohol-acetic acid-water (4:1:2.2) (Rfs not given). Concentrations were calculated by comparison with the absorbance (at 328 nm for chlorogenic acid; 310 nm for PCQ) to a known standard (Ndubizu, 1976).

Within the fruit themselves, the concentration of these phenolic compounds decreased during maturation from ca. 120 μg g^{-1} fw PCQ in mid July to ca. 18 μg g^{-1} fw by the end of September. Similarly, chlorogenic acid was shown to drop from ca. 350 μg g^{-1} fw to ca. 40 μg g^{-1} fw over the same period. This was matched with a decrease in resistance of mature fruit to attack after inoculation with these pathogens (details not stated) (Ndubizu, 1976).

Chlorogenic acid ($\leq 10^{-2}$ M) has been shown to have little effect on the *in-vitro* growth rate of *Pezicula malicorticis* (Noble and Drysdale, 1983).

Lattanzio and co-workers (2001) measured the content of chlorogenic acid, catechin, phloridzin and quercetin in fresh and stored 'Golden Delicious' apple fruit (Table 1.3). The compounds were tested for fungicidal activity against *Phlyctaena vagabunda*,. Chlorogenic acid was only shown to inhibit *P. vagabunda* germination and mycelial growth, but only *in-vitro*.

It is well established that apple fruit exhibit a rise in phenylalanine ammonia lyase (PAL) concurrent with the climacteric ethylene rise (Lattanzio *et al.*, 2001).

PAL is the major enzyme involved in the biosynthesis of phenolic compounds. It may be that system II ethylene production is the signal for the plant to produce defence against pathogen attack, particularly from quiescent infection, as it may also be the signal for the pathogen to terminate quiescence (Lattanzio *et al.*, 2001).

1.6.8 Changes in phenolic compounds during storage

Phenolic levels in apple fruit are generally agreed to decrease from ca 3 mgg^{-1} fresh weight (FW) to ca 0.5 mgg^{-1} during development, and maintain constant levels during maturation (Noble and Drysdale, 1983; Lattanzio *et al.*, 2001). However, changes in phenolic levels during cold storage is less clear. Burda *et al.* (1990) and Awad and de Jager (2000) both suggest that concentrations of individual phenolic compounds remain fairly constant during storage of 'Golden Delicious', 'Rhode Island Greening', 'Jonagold', 'Empire' and 'Elstar' apple fruit. Concentrations of preformed and induced phenolic substances on both 'Cox's Orange Pippin' and 'Bramley' fruit have also been reported to fall rapidly during development and reach constant levels from normal harvest time and through storage (Noble and Drysdale, 1983). However Lattanzio *et al.* (2001) reported a rise in individual phenolic concentrations during the first sixty days of storage, followed by their decrease.

There is a lower rate in the decrease of phenolic concentrations in cold storage than at room temperature (shelf life). This is due to enzyme metabolism being temperature dependent (Lattanzio *et al.*, 2001).

1.7 Storage diseases

Postharvest fungal attack of all produce results in losses in crop quantity and quality during storage, transit and retail (Mercier, 1996). Given the commercial value of apple fruit, the prevention, or at least the reduction of disease is of considerable importance. Apple fruit suffer from many diseases before and during storage. Two of the most important apple storage pathogens, both in terms of fruit damage and economic importance are *Penicillium expansum* and *Botrytis cinerea.*

1.7.1 Penicillium expansum (Link.) Thom.

Penicillium expansum is one of the most destructive pathogens of stored apples, worldwide. *P. expansum* can be isolated from most orchard soils. Although the disease is rare preharvest, it may occur on fallen fruit. Commonly known as blue mould, the disease is caused by *P. expansum*, that usually infects damaged or over-mature apples. The majority of infections occur when air- or waterborne conidia enter the fruit, usually via openings in the peel, lenticels, via *Mucor*, *Gloesporium* or *Phytopthera* infection sites, or bruises (Walker, 1969; Snowdon, 1990; Rosenberger, 1997a).

P. expansum spores are always present in the air of pome fruit packinghouses. Spores arise from decayed fruit, or from sporulation on bins and storage walls. However, the majority of *P. expansum* infections are from waterborne spores in postharvest drenches and flumes. Spores enter the water from decayed apples, orchard soil on dirty fruit and from contaminated bins (Rosenberger, 1997a).

A soft watery brown spot develops and rapidly enlarges, particularly at temperatures between 20 to 25°C (Snowdon, 1990). Blue green coremial fruiting structures later appear on the surface (Pitt and Hocking, 1997). *P. expansum* also produces between 2 to 100 μg per g of tissue of the carcinogenic mycotoxin, patulin, which may accumulate in fruit destined for processing, and result in off flavours (Janisiewicz, 1999; Barkai-Golan, 2001; Moodley *et al.*, 2002).

The best method of controlling *P. expansum* is by cultural control, e.g. by implementing best practices such as minimising bruising and wounding of fruit. In addition, infected bins should be cleaned and decayed fruit should be searched for and removed. The majority of *P. expansum* infections are by strains resistant to methyl benzimazole carbamate (MBC), the active ingredient of carbendazin, benomyl and thiophanate-methyl (Rosenberger, 1997a; FRAG-UK, 2002). However, it has been reported that DPA, applied as a drench, controls most MBC-resistant strains of *P. expansum*, and postharvest calcium treatments can increase fruit calcium levels and thus increase the resistance of the fruit to *P. expansum* decay (Rosenberger, 1997a).

1.7.2 Botrytis cinerea Pers.

Botrytis cinerea is the causal agent of grey mould. *B. cinerea* is a ubiquitous pathogen that causes disease on many harvested horticultural crops, worldwide. Grey mould results from *B. cinerea* infecting fruit via the cut stem, or more usually through wounds. Initially, dry dark lesion will appear which rapidly develop into a soft brown rot that engulfs the entire fruit, particularly at the cardinal temperature, 22°C. Under humid conditions the mould may produce grey-brown conidia. Black resting bodies (sclerotia) of a few mm in size may form eventually on windfall fruit, but not in storage. *B. cinerea* develops more rapidly during cold storage temperatures (3 - 5°C) than any other rot, with the exception of *Mucor* (Snowdon, 1990; Pitt and Hocking, 1997; Rosenberger, 1997b). There has been no reported mycotoxin production by *B. cinerea* (Pitt and Hocking, 1997).

B. cinerea infected fruit are rarely seen in the field, although common on the orchard floor. *B. cinerea* conidia may be airborne, although waterborne spores are more likely to be the cause of infection. Once *B. cinerea* has infected a fruit in storage, the disease can spread quickly to neighbouring healthy fruit, a phenomenon known as nesting (Rosenberger, 1997b).

B. cinerea infection may be controlled by MBCs and dicarboximides, but resistance to both of these controls has been reported and is becoming more common (FRAG-UK, 2002). *B. cinerea* is also sensitive to DPA (Rosenberger, 1997b). However, good handling practices may also reduce the risk of *B. cinerea* infection.

1.8 Fruit stress, and detection

Chlorophyll fluorescence has been used to detect stress in plant systems. Chlorophyll *a* florescence *in-vivo* is emitted during photosystem II (PS II). PS II is linked to the oxygen producing reactions and forms a complex that is particularly sensitive to cellular disturbances (Smillie and Hetherington, 1990). Chlorophyll *a* florescence emission may be separated into two components. The first or 'constant' fluorescence (F_o) is a fast rising response to applied visible light, occurring within 1 to 2 ms. The second component is maximum emission (F_m), which follows after 1 to 2 s. The rise in chlorophyll *a* fluorescence ($F_m - F_o$) is the variable florescence (F_v).

Changes in F_v should be regarded as direct indicators of the properties of excitation and energy conversion at PS II. However, PS II is intimately linked to other components of the photosynthetic apparatus. A wide range of environmental, chemical and biological stresses influence photosynthetic metabolism, and thus F_v may be used as an indicator of the entire photosynthetic process as a response to stress (Schreiber and Bilger, 1985; Smillie and Hetherington, 1990). Visible light affects photosynthesis, and thus measurements have to taken on dark-adapted samples. Dark-adaptation is the state where tissue has been kept away from light for long enough to give reproducible readings. The time taken for dark-adaptation may vary from minutes following exposure to low photo flux densities to several hours after exposure to prolonged sunlight (Smillie and Hetherington, 1990). However, modern equipment, particularly for field use, overcome this by detecting modulated fluorescence from chlorophyll excited by low intensity pulsed light.

Disturbances at the cellular level, such as injury or other stresses may be detected by associated changes in chlorophyll fluorescence (Smillie & Hetherington, 1990). For example, chlorophyll fluorescence will decline to zero when continually chilled, and thus chlorophyll fluorescence may be used to detect chilling injury.

Currently, most quality assessments of fruits and vegetables are destructive. Thus, a demand exists for rapid, cost-effective, non-destructive quality assessment (Watada, 1989). Chlorophyll florescence may not be able to quantify such quality parameters as sugar and acid content, firmness or ethylene production; however, it may be used to measure the underlying condition of the fruit as expressed in the ability of the produce to photosynthesise (Toivonen, 1992). Freshness, as defined as 'any deterioration or decline of tissue from a freshly harvested state', is an important component of quality and early changes in respiration, ethylene production, vitamin C, chlorophyll content and many other characteristics all contribute to loss of freshness and hence to further deterioration of the produce (Toivonen, 1992). To quantify these characteristics and relate them to freshness or loss of quality from harvest requires considerable time, expertise and equipment. F_v has been used to assess the quality of broccoli (Toivonen, 1992), banana, mango (Smillie *et al.*, 1987) and apple (Mir *et al.*, 2001).

Chapter 2 MATERIALS AND METHODS

2.1 Fruit Material

2.1.1 Harvest location

Apple fruit were harvested from a commercial orchard during September to October 2000, 2001 and 2002. Fruit were picked from the same trees each year from Broadfield Farm, Plaxtol, Kent, UK (51.2603 N, 0.2942 E). 'Queen Cox' (MM106 rootstock), were grown in 'Ragstone' orchard. 'Bramley' fruit (M26 rootstock), were grown in 'Pack house' orchard. All trees were less than five years old. There were no differences between pre-harvest treatments for any aspect of orchard husbandry between the apples used during any of the experiments and those grown for commercial purposes. All pre-harvest applications were equal for all subsequent treatments within each experiment.

2.1.2 Immediate handling

On arrival at the laboratory, fruit were randomised and labelled with an individual identification number using a black permanent Lumocolor marker (Staedtler, Neumarkt, Germany), and allocated to treatments. Fruit were weighed on the day of harvest, and placed inside cardboard fruit trays (59 x 37 x 14 cm; 30 fruit per tray).

2.1.3 Pre 1-MCP-treatment fungicide application

Unless otherwise stated, before 2001, 'Queen Cox' apples were dipped in 10 g l^{-1} Ridomil™ mbc 60WP (carbendazin [5 g l^{-1}] and metalaxyl [1 g l^{-1}]) (Syngenta, Bracknell, UK) to control *Phytophthora* spp. From 2001, 'Queen Cox' fruit were dipped in 0.6 g l^{-1} Derosal WDG (carbendazin [80% w/w]) to control *Phytophthora* spp. Fruit were air-dried and equilibrated to treatment temperature before 1-MCP-

treatment. 'Bramley' fruit were not dipped in fungicide, as per normal commercial practice.

2.1.4 Harvest maturity analysis

Initial fruit maturity for 'Queen Cox' (Table 2.1) and 'Bramley' (

Table 2.2) was determined by firmness and internal ethylene concentration (IEC), and was assessed within 24 h of harvest.

Table 2.1 Picking dates and harvest maturity of all 'Queen Cox' apple fruit picked during 2000, 2001 and 2002. Commercial maturity refers to either the beginning (early, E), middle (mid, M) or end (late, L) of the commercial harvest for that year. This was determined by the grower. Firmness and internal ethylene concentration (IEC) were determined for batches of 10 fruit ($^{z}n = 9$). Fruit firmness as an effect of harvest maturity is investigated specifically in Chapter 5, Experiment 3. Fruit were randomly selected from the total pool of available apples for each experiment (refer to section 2.9 for details).

Experiment	Harvest date	Commercial maturity	Firmness (N)	IEC ($\mu l\ l^{-1}$)
1a	13/9/2000	E	83.2 ± 2.7	0.5 ± 0.3
1b	20/9/2000	M	71.7 ± 1.9^{z}	0.4 ± 0.2^{z}
1c	27/9/2000	L	66.5 ± 2.0	25.3 ± 12.4
2a	11/9/2001	E	94.9 ± 2.5	0.0 ± 0.0
2b	17/9/2001	M	82.7 ± 1.6	0.0 ± 0.0
2c	23/9/2001	L	87.9 ± 2.5	0.0 ± 0.0
4	11/9/2002	E	76.4 ± 1.4^{z}	0.0 ± 0.0^{z}
4	18/9/2002	M	72.7 ± 4.6^{z}	0.9 ± 0.4^{z}
4	24/9/2002	L	76.3 ± 1.9^{z}	0.7 ± 0.4^{z}

Table 2.2 Picking dates and harvest maturity of all 'Bramley' apple fruit picked during 2000, 2001 and 2002. Commercial maturity refers to either the beginning (early, E), middle (mid, M) or end (late, L) of the commercial harvest for that year. This was determined by the grower. Firmness and internal ethylene concentration (IEC) were determined for batches of 10 fruit. Fruit firmness as an effect of harvest maturity is investigated specifically in Chapter 5, Experiment 3. Fruit were randomly selected from the total pool of available apples for each experiment (refer to section 2.9 for details).

Experiment	Harvest date	Commercial maturity	Firmness (N)	IEC ($\mu l\ l^{-1}$)
1a	6/9/2000	E	74.4 ± 1.8	0.0 ± 0.0
1b	20/9/2000	M	73.6 ± 2.0	0.0 ± 0.0
1c	4/10/2000	L	68.2 ± 1.8	0.2 ± 0.1
2a	5/9/2001	E	109.2 ± 3.2	0.0 ± 0.0
2b	11/9/2001	M	101.1 ± 3.4	0.0 ± 0.0
2c	17/9/2001	L	101.6 ± 2.8	0.0 ± 0.0
3	5/9/2002	E	91.0 ± 2.3	0.0 ± 0.0
3	11/9/2002	M	77.6 ± 1.2	0.0 ± 0.0
3	18/9/2002	L	82.1 ± 2.1	0.0 ± 0.0

2.1.5 Pre-1-MCP treatment handling of apple fruit

The randomised and allocated fruit were placed inside rigid polypropylene fumigation chambers (88 x 59 x 59 cm) (Fig. 2.1), and 1-MCP-treated 24 h after harvest. Each chamber was specific to a 1-MCP dose, and could accommodate four trays (a total of 120 fruit). Each chamber was stored at the 1-MCP treatment application temperature before 1-MCP application. Fruit temperature was measured on an extra sacrificial fruit of the same type (i.e. 'Queen Cox' or 'Bramley') before each experiment and it was found that the apples had temperature-equilibrated to the specific treatment temperature before 1-MCP treatment was due to start. Uniformity of pre-conditioning was assumed as a response of randomisation and the temperature measurements.

Figure 2.1 'Bramley' apple fruit in cardboard fruit trays inside a rigid polypropylene fumigation chamber. The tube and tap allowing samples of chamber atmosphere to be removed for 1-MCP quantification by gas chromatography (GC) can be seen on the front wall of the chamber. The trough around the top of the chamber was ¾ filled with water and the lid (not shown) fitted into this, submerged under the water, forming the seal. A small fan was attached to the underside of the lid to facilitate air circulation within the chamber Each chamber could accommodate 4 trays, each of which contained 30 fruit, giving a total treatment of 120 apples per chamber.

There was no ability to control relative humidity (RH). However, the 1-MCP treatment temperatures were constant and the systems the same for each treatment, so any variations in RH were assumed to have an equal effect for comparative treatments.

There was a potential for the packaging to hamper 1-MCP influx through the calyx. However, each fruit was placed calyx down on the tray to minimise any differences in 1-MCP influx. This was because placing fruit calyx-down on the tray was the only way to stabilise all apples in the same orientation and thus reduce errors due to a potential non-uniformity in calyx exposure.

2.2 1-MCP Manufacture

2.2.1 From base chemicals

1-MCP was prepared according to the method of (Macnish *et al.*, 1999). Briefly, 5 ml of lithium di-isopropylamide (Fluka, Buchs, Switzerland) was injected into a sealed test tube flushed with N_2. A 0.3 ml aliquot of 3-chloro-2-methylpropene (Fluka, Dorset, UK) was injected into the test tube over a period of 15 to 30 min to produce the lithium salt of 1-MCP. The liquid and precipitate mixture was held at room temperature for an additional 30 min with regular mixing using a vortex mixer. LiCl precipitated throughout this stage. The 1-MCP-evolving stock solution was stored at -20°C until required (Sisler and Serek, 1997). The probity of the stock solution was tested by GC before each 1-MCP application.

2.2.2 From SmartFresh™ (Ethylbloc™)

0.45 g SmartFresh (0.14 mg A.I. ml^{-1}) (AgroFresh Inc., Milan, Italy) was placed inside a 50 ml conical flask and sealed with a SubaSeal (Fisher, Leics, UK). A measured volume of distilled water at 40°C was injected into the flask through the seal. The flask was gently shaken until the SmartFresh had dissolved completely.

SmartFresh was still in the design-phase during Experiment 1, so 1-MCP had to be prepared from base chemicals. However, a similar substance, EthylBloc, was available, but with an incorrect formulation which did not correspond to the active ingredient calculation. Furthermore, there was not enough EthylBloc available in time

for the start of Experiment 1. SmartFresh was made available in a suitable quantity in time for the other experiments.

2.3 1-MCP application

A measured aliquot (Table 2.3) of the 1-MCP-evolving stock solution, or flask containing 1-MCP from SmartFresh was placed into a fumigation chamber, which was closed immediately. Air was circulated within the fumigation chamber, and gas samples were withdrawn for 1-MCP quantification.

Table 2.3 Expected maximum 1-MCP concentration inside a polypropylene fumigation chamber (88 x 59 x 59 cm) after sealing an exposed aliquot of 1-MCP-evolving stock solution inside.

Expected 1-MCP concentration ($\mu l\ l^{-1}$)	Aliquot volume (μl)
0.0	0
0.1	6
0.5	60
1.0	60
10.0	300

2.4 Quantification of 1-MCP and ethylene

1-MCP and ethylene concentrations were quantified using a Carlo Erba GC8340 GC fitted with an EL 980 FID and DP800 integrator (Thermoquest, Herts, UK). Oven and detector temperatures were set at 100°C. The 2 m long stainless steel column was packed with Chromosorb PAW mesh range 80 to 100, liquid phase OV1701 30% loading (Jones Chromatography, Mid Glamorgan, UK).

1-MCP was calibrated against 10.0 $\mu l\ l^{-1}$ n-butane (British Oxygen Company (BOC) Gases, Guildford, UK) (Sisler and Serek, 1997), although it is also possible to

calibrate against isobutylene (Fan *et al.*, 1999; Jiang *et al.*, 1999; Leverentz *et al.*, 2003). Ethylene was calibrated against 10.1 μl l^{-1} ethylene (BOC).

2.5 Post-1-MCP treatment handling of apple fruit

After 1-MCP treatment, unless otherwise stated, fruit were removed from the fumigation chambers, re-randomised and individually enclosed in perforated polypropylene bags (15 μm thickness, 400 μm diameter holes, 5 holes per cm^2 [Cryovac 250 Y]; Cryovac Sealed Air Ltd., Cambs, UK). Fruit were bagged to reduce the risk of 'nesting' of disease by isolating any fruit which developed disease during storage. The bags were from the same supply and production run, and folded over once to enclose the fruit. Fruit were then kept in cardboard fruit trays (30 fruit per tray) (Fig. 2.2).

Figure 2.2 'Bramley' apple fruit wrapped in perforated polypropylene bags, ready for storage. The wire leading into the box is a thermocouple wire for temperature measurement. Black markings visible on some fruit are identification numbers.

2.6 Post-treatment fruit storage

2.6.1 Cold air storage

Fruit were randomly stacked and stored in a constant temperature room at 3 to 4°C. Temperature was recorded throughout the storage periods using a Delta-T datalogger (Cambs, UK). Temperature was measured using calibrated type-T (copper-constantan) thermocouple probes placed inside random fruit boxes (Fig. 2.3).

Figure 2.3 Fruit boxes stacked randomly in a constant temperature room. The wires leading into the boxes are thermocouple wires for temperature measurement.

There was no ability to control RH or other gas control during cold air storage. However, the temperature was constant and the systems the same for each treatment, so any variations in RH or gas concentrations were assumed to have an equal effect for comparative treatments. As the storage temperature was set to 3 to 4°C, it was assumed that the RH was high enough to prevent dehydration. Furthermore, Natural ethylene produced by fruit undergoing the climacteric phase may have had some effect

on the experiment. However, the randomisation of the experiment was assumed to have minimised the effects of any unavoidable ethylene production.

2.6.2 Controlled atmosphere storage

Fruit were placed inside rigid polypropylene storage chambers (88 x 59 x 59 cm). 'Queen Cox' fruit were stored under controlled atmosphere (CA) conditions of 3 to 4°C and <1% CO_2, 1.2% O_2 or at atmospheric conditions in the chambers. 'Bramley' fruit were stored at 3 to 4°C under 5% CO_2, 1% O_2 or at atmospheric conditions in the chambers (Colgan *et al.*, 1999). Storage atmosphere was controlled using an Oxystat 2 CA system, attached to an Oxystat 2002 Controller, and Type 770 fruit store analyser (David Bishop Instruments, Sussex, UK). This system was self-calibrating every 24 h against 5% CO_2 in N_2 (BOC).

Space dictated that two constant temperature rooms were required for the CA experiments. Therefore, the 'Queen Cox' and the 'Bramley' CA storage were in separate constant temperature rooms. There was no ability for specific RH control, although the sealed units were flushed with air only supplied via the Oxystat system.

2.6.3 Shelf life

Upon removal from storage, fruit were taken out of their perforated bags and kept at 20°C and 50 to 60% relative humidity (RH) for 1, 7 d for all experiments, and 14 d for Experiments 1, 3 and 4. Fruit were also stored for 3 d for experiment 3.

Fruit were removed from their bags because they were checked regularly and mould apples could be identified before infecting other fruit. Furthermore, these apples were subject to checks such as weight, which would not be possible whilst wrapped. Fruit were placed under shelf-life conditions in the same cartons in which they were cold air / CA stored.

2.7 Experiment design

'Queen Cox' and 'Bramley' apple fruit (Tables 2.1; 2.2) harvested in 2000 were included into Experiment 1. Fruit harvested in 2001 were included into Experiment 2. Fruit harvested in 2002 were included into Experiments 3 and 4.

2.7.1 Experiment 1: 1-MCP concentration, exposure duration and temperature of application

Experiment 1 was designed to assess the efficacy of 1-MCP concentration, duration of 1-MCP exposure and temperature of 1-MCP application for improved storability of 'Queen Cox' and 'Bramley' apple fruit. 1-MCP was manufactured from base chemicals.

Experiment 1 was sub-divided into three experiments: a, b and c, for each cultivar. In Experiment 1a, fruit were exposed to 0.0, 0.1, 0.5, 1.0 or 10 $\mu l\ l^{-1}$ 1-MCP at either 0 or 20°C for 24 or 48 h. In Experiment 1b, fruit were exposed to either 0 or 1.0 $\mu l\ l^{-1}$ 1-MCP for 6, 12, 24 or 48 h at either 0 or 20°C. In Experiment 1c, fruit were exposed to either 0 or 1.0 $\mu l\ l^{-1}$ 1-MCP at 0, 5, 10, 15 or 20°C for either 24 or 48 h.

For each experiment, fruit were stored in cold air. 'Queen Cox' fruit were stored for 2, 4 or 6 months. 'Bramley' fruit were stored for 3, 6 or 9 months. Fruit condition was determined by quantification of internal ethylene concentration (IEC), fruit firmness, total soluble solids (TSS), peel colour and starch content (as described in section 2.8). 'Bramley' fruit were also assessed for scald by determining the percentage peel affected by scald per apple.

2.7.2 Experiment 2: Harvest maturity, CA storage, 1-MCP-treatment delay and fungicide application

Experiment 2 was designed to assess the efficacy of a single 0.65 $\mu l\ l^{-1}$ 1-MCP exposure at 3 to 4°C for improved storability of 'Queen Cox' and 'Bramley' apple fruit. 1-MCP efficacy was assessed by comparing harvest times, with or without CA

storage and with or without fungicide treatment. 1-MCP was produced from SmartFresh.

Experiment 2 was sub-divided into three experiments: a, b and c, for each cultivar. All fruit were temperature equilibrated to 3to 4°C during 24 h before treatment. In Experiment 2a, early-harvested fruit were exposed to either 0 or 0.6 $\mu l\ l^{-1}$ 1-MCP within 24 h of harvest for 24 h at 3 to 4°C. Fruit were then removed from treatment and stored at 3 to 4°C under CA conditions of <1% CO_2, 1.2% O_2 ('Queen Cox') or 5% CO_2, 1% O_2 ('Bramley'); or under atmospheric conditions within CA chambers; or in a constant temperature room.

For the 1-MCP-treatment delay experiment (2b), mid-harvest fruit were stored in air at 3 to 4°C and treated with either 0 or 0.6 $\mu l\ l^{-1}$ 1-MCP within 24 h of harvest, or at 7 or 14 d after harvest. During the delay, fruit were stored in air at 3 to 4°C. All fruit were subsequently stored in air at 3 to 4°C.

For the fungicide application experiment (2c), late-harvested fruit were either dipped in Derosal WDG or distilled water before exposure to either 0 or 0.65 $\mu l\ l^{-1}$ 1-MCP within 24 h of harvest. All fruit were subsequently stored in air at 3 to 4°C.

'Queen Cox' apples were stored for 2 and 4 months. 'Bramley' apple fruit were stored for 3 and 6 months. Upon removal from storage, fruit were taken out of their perforated bags and kept in a shelf-life room at 20°C and 50 to 60% RH for 0 or 7 d.

Fruit condition was determined by quantification of IEC, fruit firmness, TSS, titratable acidity (TA), chlorophyll fluorescence, and starch content (as described in section 3.8). 'Bramley' fruit were also assessed for scald by determining the percentage peel affected by scald per apple.

2.7.3 Experiment 3: 1-MCP vs diphenylamine (DPA) treatment for scald control for 'Bramley'

In Experiment 3, the efficacy of a single 0.65 $\mu l\ l^{-1}$ 1-MCP exposure at 3 to 4°C for scald prevention compared to current commercial practice was assessed.

'Bramley' apple fruit were either dipped in 2000 µl l^{-1} diphenylamine (DPA, Ian Mitchell, Foxbury Farm, Kent, UK) or distilled water for 2 min, then air dried. The fruit were then 1-MCP-treated, or put in storage immediately. Fruit were cold air stored for 0, 6 and 9 months. Fruit were assessed for scald by determining the percentage peel affected by scald per apple. Fruit were also assessed for disease incidence.

2.7.4 Experiment 4a: 1-MCP regulation of natural disease resistance in 'Queen Cox'

In Experiment 4, the efficacy of a single 0.65 µl l^{-1} 1-MCP exposure at 3 to 4°C for maintaining natural disease resistance (NDR) was assessed. On removal from storage, fruit were subsequently challenged by inoculation with pathogen and disease severity recorded. No fruit were treated with fungicide. 'Queen Cox' fruit were exposed to either 0 or 0.65 µl l^{-1} 1-MCP at 3 to 4°C, then cold air stored for 0, 2, 4, 8, 16 or 20 weeks until inoculation with single-spore isolates of either *Penicillium expansum* (IMI 319460; CABI Bioscience, Surrey, UK), or *Botrytis cinerea* (IMI 189121; CABI), respectively, the causal agents of blue and grey mould. Koch's postulate was performed every three months to maintain pathogenicity of each isolate.

Fruit condition was determined by quantification of IEC. Visible disease severity was determined by measurement of lesion diameter (section 2.7.4.5). Specific phenolic acids associated with NDR were also measured (section 2.7.5).

2.7.4.1 Pathogen storage

P. expansum and *B. cinerea* were grown in 9 cm plastic Petri dishes on ½ (19.5 g l^{-1}) potato dextrose agar (PDA; Oxoid, Unipath Ltd, Hants, UK) in an incubator at 25°C. Cultures were kept in the dark until growth was established and then under UV-A light to encourage spore production. Fungi were sub-cultured every two weeks, and all inoculations were from plates less than two weeks old.

2.7.4.2 Fruit preparation

'Queen Cox' fruit from Experiment 4 were removed from storage, allowed to equilibrate to room temperature (*ca* 6 h) then surface sterilised with 70% (v/v) ethanol for 2 min and air-dried.

2.7.4.3 Preparation of spore suspension

Spore suspensions were prepared immediately before use. All work was performed in a Bassaire 04HB laminar flow cabinet (Bassaire, Hampshire, UK). The Petri dish containing spores was flooded with sterile distilled water (SDW) containing 0.1% Tween 80™ (Sigma). Spores were scraped off the agar into the solution using a sterile no. 24 scalpel (Swann-Morton, Sheffield, UK). The spore-containing solution was filtered through cheesecloth into a sterile vessel to filter out mycelium. Spore concentration was determined and adjusted with SDW to 10^4 spores ml^{-1} (Ippolito *et al.*, 2000) using a haemocytometer (Weber scientific International Ltd., Middlesex, UK).

2.7.4.4 Fungal inoculation

Fruit were either inoculated with either *P. expansum* or *B. cinerea*. For those fruit inoculated with *P. expansum*, four holes (5 mm deep x 4 mm wide) were made at equidistant places around the lateral circumference of each fruit. These wounds were made using a using a sterile nail (Ippolito *et al.*, 2000; Fan and Tian, 2001; Vero *et al.*, 2002). A 20 µl spore suspension of *P. expansum* spores was pipetted over each of three holes.

For fruit inoculated with *B. cinerea*, three holes were made at equidistant places around the lateral circumference of each fruit. 20 µl conidial suspension of *B. cinerea* was pipetted over each of two wounds.

There were only three holes on the fruit inoculated with *B. cinerea* because the disease would spread too quickly that the over-lap between inoculation sites occurred before the experiment was completed (personal observations, based on a pre-experiment trial).

For all inoculated fruit, a further 20 µl drop of SDW was placed over the non-inoculated wound to act as a control. A further thirty-six non-inoculated fruit acted as controls.

Fruit were left in shelf-life conditions (as described in section 2.6.3) for 3, 7 or 14 d. The addition of day 3 on the shelf-life assessment was to determine the infections before 7 d. Other shelf-life measurements were not made at this time.

2.7.4.5 Determination of visible fungal infection

Nine random 'Queen Cox' fruit from each treatment were removed on each shelf life extraction time and the IEC of each fruit was quantified. It was acknowledged that wounding and infection may have had an effect on ethylene production, and the controls were used to assess the significance of any changes as a result of the treatment or the application of the treatment.

Lesion diameter was measured across the fruit circumference. Curvature was unavoidable, but lesions were measured by rolling a ruler across the curvature of the lesion, from one side to the other. The determination of fungal infection was purely visual. No internal fungal growth was determined.

The fruit were then cut into pieces and immediately snap-frozen in liquid N_2, and stored in labelled bags at -20°C awaiting analysis of phenolic compounds.

2.7.5 Analysis of antifungal compounds

2.7.5.1 Sample preparation

The extraction method was based on that of Escarpa and González (1998). Non-infected fruit were cut to give 2 g peel and 8 g pulp. Infected fruit were cut to give 2 g peel and 8 g of pulp, both from the area immediately surrounding the lesion (*ca* 2 mm from visible leading edge of the lesion) (Fig. 2.4).

Peel and pulp from the same sample were placed together into individual test-tubes and extracted at room temperature in the dark with HPLC grade methanol (Fisher) containing 1% 2,6-di-*tert*-butyl-4-methylphenol (BHT) (Sigma, Dorset, UK.)

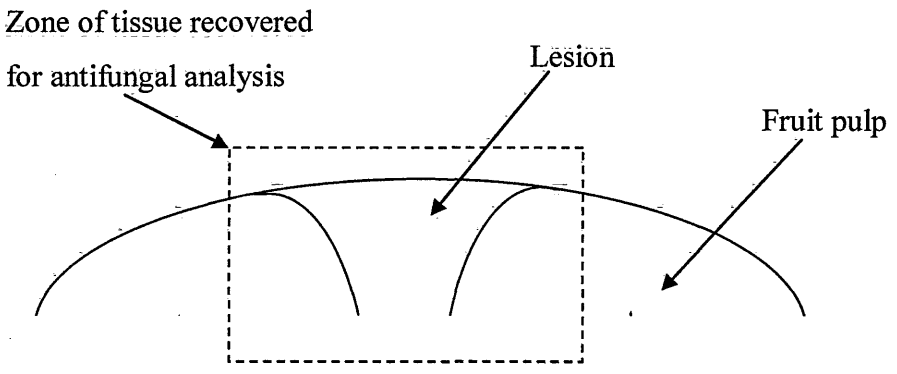

Figure 2.4 Schematic showing zone of tissues recovered for antifungal analysis.

using an ultrasonic bath (Sonicor SC-50-22TH, 50-60 Hz, Sonicor Instruments Corp., Copiague, N.Y. US). The sample was extracted with 10 ml of solvent for 1 h, 10 ml for 30 min, and then 5 ml for 30 min. The three extracts were combined to a final volume of 25 ml. Samples from this stock were withdrawn by syringe, and then filtered through a 25 mm diameter, 0.45 µm Optiflow PTFE syringe filter (Jaytee Biosciences Ltd., Kent, UK), into individual opaque HPLC vials (2=SV(A), Chromocol Ltd, Herts, UK), and sealed.

2.7.5.2 High Performance Liquid Chromatography of apple tissue samples

The HPLC analysis method was based on that of Escarpa and González (1998). Individual vials were loaded into a Kontron Instruments HPLC 360 autosampler, connected to a 335 dual UV detector, attached to a 325 pump (Sci-Tec Instruments, Beds, UK). The column used was a Nucleosil 120 C_{18} (25 cm x 0.46 cm I.D.) with 5 µm packing (AllTech Associates Ltd., Lancs, UK).

Detection was performed at 280 nm and the adsorption spectra of the compounds were recorded at 280 and 350 nm. Solvents were: aqueous 0.01 M phosphoric acid (Acros, Leics, UK), made up in HPLC grade water (Fisher), (solvent A) and 100% methanol (Fisher) (solvent B). Solvents A and B were both degassed using He for 20 minutes.

The samples were eluted according to the following gradient: 5% B as initial condition; 50% B for 10 min; 70% B for 5 min; 80% B for 5 in and finally 100% B for 5 min. The chromatic data on the peaks were integrated up to 25 min. The flow rate was 1 ml min^{-1} with a column head pressure of *ca* 110 kg cm^{-1}. The column was operated at room temperature. The sample injection was 20 µl.

Calibration of compounds was against 50 µg l^{-1} mixture of chlorogenic acid (Acros), benzoic acid (Sigma), (-)-epicatechin (Sigma), (+)-catechin hydrate (Sigma), p-hydroxycinamminic acid (Acros) in HPLC grade methanol (Escarpa and González (1998)).

2.7.6 Experiment 4b: In-vitro growth of pathogen with direct exposure to phenolic compounds

2.7.6.1 Thin layer chromatography

Individual stock solutions of 500 mg compound 10 ml^{-1} HPLC grade methanol was prepared for chlorogenic acid, benzoic acid, p-coumin, catechin and epicatechin. Using a micro-pipette, either 1 or 5 µl of each sample was spotted onto a glass-backed thin layer chromatography (TLC) plate (20 x 20 cm) coated with silica gel 60 F_{254} (Merck, Darmstat, Germany). In addition to these compounds, further spots of methanol, blanks and the protein synthesis inhibitor, cycloheximide (0.5 mg ml^{-1}) were used as controls (Terry, 2003).

TLC plates were developed in one dimension at *ca* 22°C in a TLC tank (20 x 20 x 10 cm) lined with filter paper (to saturate the atmosphere). Hexane: ethyl acetate: methanol (60:40:30 v/v/v) was used as the running solvent (Terry, 2003). TLC plates were left sealed (one per tank) until the solvent had reached 1 cm from the top of the plate. The TLC plate was removed, dried and used for bioassay (after Terry, 2002).

A spore suspension of *P. expansum* was prepared by flooding the spore-containing Petri dishes with Czapek Dox nutrient solution, and filtering through

cheese cloth. Developed TLC plates were sprayed with the spore suspension, and incubated at 20°C, 100% RH for up to 7 d.

Areas where fungal growth was absent (zones of inhibition) indicated antifungal activity (Klarman and Stanford, 1968; Homans and Fuchs, 1970).

2.8 Fruit quality analysis

2.8.1 Internal ethylene concentration (IEC)

Fruit IEC was determined as described by Saltveit (1982). A 1.1 mm diameter x 40 mm long stainless steel needle was inserted through the calyx and sealed in place with Blu-Tack™ (Bostic, Leics., UK). A 3 ml gas sample was withdrawn slowly from the air space of the apple core with a syringe. The syringe was sealed before removal from the apple and the sample tested immediately. The ethylene concentration in 1 ml of this sample was quantified by gas chromatography (as described in section 2.4).

2.8.2 Fruit firmness

Fruit firmness was measured on opposite sides of peeled fruit using a Digital Force Gauge (Mecmesin Ltd., West Sussex, UK) fitted with a 10.0 mm diameter flat end probe. The descent of the probe was controlled at 50 mm min^{-1} to simulate a steady, consistent pushing pressure into the fruit using the cross head of an Instron 1122 Universal Tester (Instron, Bucks, UK).

2.8.3 Measurement of total soluble solids

Fruit total soluble solids (TSS) were measured using a PR-1 digital refractometer (Atago, Tokyo, Japan). Fruit juice was extracted by crushing the flesh around the penetration holes with a wooden spatula. The refractometer was calibrated at zero using distilled water.

2.8.4 Peel colour

After each storage and shelf life time, fruit were weighed and the number of fruit affected with disease or superficial scald was recorded. Fruit were assessed for skin colour only in experiment 1. Peel colour was measured using a DP-100 colormeter (Minolta Co. Ltd., Osaka, Japan) with an 8 mm light path aperture. The instrument was calibrated with a Minolta standard white tile CR-200 (Y = 93.9, x = 0.3134, y = 0.3207). The mean of three readings (L* a* b*) taken from the green areas of the peel was recorded and the hue angle calculated (Sacks and Shaw, 1994) (**Equation 2.1**).

Equation 2.1 Hue angle equation, where a* = -60 to 0, and b* = 0 to +60.

$$(\mathrm{Tan}^{-1}(b^* / a^*)) + 180$$

2.8.5 Chlorophyll fluorescence

Chlorophyll fluorescence (F_o, F_m, F_v, F_v/F_m) was measured using an FMS2 fluorescence monitoring system (Hansatech Instruments Ltd., Norfolk, UK). Fruit were removed from cold storage and stored under shelf-life conditions (as described in 2.6.3). Fruit were placed in the dark overnight before analysis, to allow for dark adaptation. F_o is the constant (or minimal) fluorescence, and can only be seen prior to illumination. Therefore, this can be achieved by holding fruit in the absence of light (Krause and Weis, 1984). At 20°C, *ca* 95% of chlorophyll *a* fluorescence is emitted by PSII-associated chlorophyll molecules (Krause and Weis, 1984; 1991). F_m is the maximum total fluorescence, observed after exposure to light. F_v is the maximum variable fluorescence ($F_v = F_m - F_o$) (Krause and Weis, 1984). The F_v / F_m ratio was also calculated.

All measurements were taken in the dark. The tip of the fibre-optic leading from the light box was secured in a rigid black plastic shroud. The shroud was placed against the surface of a fruit and a measurement was taken. A 0.3 s, 14400 μmol m^{-2} s^{-1} saturating pulse was delivered. The shroud stopped light from the

pulse escaping and affecting other fruit in the dark room. The shroud also ensured that the light pulse was always administered at the same distance from the surface of each fruit.

2.8.6 Assessment of starch content

Starch content for all fruit was assessed by cutting the fruit in half along the equator and placing one exposed half into iodine solution and left to dry. Starch content was estimated by percentage staining.

2.8.7 Titratable acidity

Fruit were stored in a freezer at -20°C until analysis (less than 9 months). Fruit were removed from the freezer and defrosted using a microwave. Juice was squeezed from the apple. Titratable acidity (TA) of the apple juice was determined by titrating 5 ml of apple juice against 0.1 M NaOH. End point was determined using 1% phenolphthalein solution in propan-2-ol (BDH, Dorset UK).

2.9 Statistical design

Experiments 1a, b and c were 5 x 2 x 3 factorial designs. For Experiment 1a, n (number of fruit) = 20 for 2 months stored 'Queen Cox' and 3 months stored 'Bramley' fruit after 24 h exposure to 1-MCP and $n = 10$ for 4 and 6 months stored 'Queen Cox' and 6 months stored 'Bramley' after 48 h 1-MCP exposure. For Experiments 1b and c, $n = 10$ for each individual treatment. Total number of fruit = 4200.

Experiments 2a and b were 2 x 3 x 2 x 2 factorial designs. Experiment 2c was a 2 x 2 x 2 x 2 factorial design. For all experiments, $n = 20$ for each individual treatment. Batches of 10 fruit from each treatment were assessed 1 or 7 d after removal from storage. Total number of fruit = 1920.

Experiment 3 was a 3 x 2 x 2 x 3 x 3 factorial design, $n = 10$. Batches of 10 fruit from each treatment were assessed 1, 7 or 14 d after removal from storage. Total number of fruit = 1080.

Experiment 4a was a 3 x 2 x 6 x 4 factorial design. Batches of 9 fruit from each treatment were assessed 1, 3, 7 or 14 d after removal from storage. Therefore total number of fruit = 864. Experiment 4b was a 5 x 2 x 7 x 4 factorial design, $n = 5$.

All treatments for all experiments were arranged in a completely randomised design (CRD). Data were subjected to ANOVA using Genstat 5.0 (IACR Rothamstead, UK). LSDs were calculated for mean separation at the 5% level.

Percentage data was not subject to statistical analysis.

Chapter 3 RESULTS AND DISCUSSION

EXPERIMENT 1

EFFECT OF 1-MCP CONCENTRATION, EXPOSURE TIME AND APPLICATION TEMPERATURE ON APPLE FRUIT QUALITY

This chapter was published in part as: Dauny, P.T. and Joyce, D.C. (2002) 1-MCP improves storability of 'Queen Cox' and 'Bramley' apple fruit. *HortScience* **37**: 1082-1085.

3.1 Introduction

'Queen Cox' and 'Bramley' fruit [*Malus* x *sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] are important apple cultivars, particularly in the UK. 'Queen Cox' is a dessert apple that softens quickly under retail conditions. 'Bramley' is a cooking apple that is susceptible to softening and development of the storage disorder superficial scald.

Maintenance of apple firmness is an aim of all apple storage management procedures, as soft fruit have reduced quality and commercial value. Softening is influenced by the internal ethylene concentration (IEC) of fruit. Superficial scald is a form of chilling injury of apple and pear fruit manifest as brown or discoloured patches on the peel, with little or no physical damage to the pulp (Watkins *et al.*, 1995).

Superficial scald is caused by the action of conjugated trienes (CTs) released as oxidative-breakdown products of α-farnesene (Rupasinghe *et al.*, 1998). Production of α-farnesene in the skin tissue of apple fruit is influenced by ethylene levels in the fruit and storage atmosphere (Rupasinghe *et al.*, 1998). Susceptibility to

scald varies with cultivar, maturity, storage temperature and atmospheric ethylene levels (Huelin and Coggiola, 1968; 1970).

1-MCP binds to, and thus blocks, the ethylene receptor. As a result, ethylene is not able to bind to the receptor and exert an influence. 1-MCP treatment has been shown to reduce IEC (i.e. the suppression of autocatalytic ethylene production), maintain greater firmness, inhibit α-farnesene production and reduce superficial scald development in 'Delicious' 'Law Rome' and 'McIntosh' apples (Rupasinghe *et al.*, 1998; Watkins *et al.*, 2000). In addition, 1-MCP has also been shown to suppress other ripening-associated changes in apples. Reduced respiration rate and lower total soluble solids (TSS) have been found in 1-MCP treated apple cultivars, including 'Gala', 'Jonagold' and 'Delicious' (Fan and Mattheis, 1999; Watkins *et al.*, 2000).

The aim of this work was to determine the efficacy of 1-MCP applied at a range of concentrations, durations of exposure and exposure temperatures in reducing superficial scald development in 'Bramley' and softening of 'Bramley' and 'Queen Cox' fruit during storage at 3 to 4 °C in air.

3.2 Experiment 1a: The effect of 1-MCP concentration on the quality of 'Queen Cox' and 'Bramley' apple fruit storage

3.2.1 Fruit firmness and IEC

1-MCP applied at 0.1 to 10.0 $\mu l\ l^{-1}$ maintained 'Queen Cox' and 'Bramley' apple fruit firmness for 2 and 3 months, respectively. This was the first set of 1-MCP results to have been reported for either 'Queen Cox' or 'Bramley'.

Firmness at 24 h after harvest was *ca* 83 N for 'Queen Cox' and *ca* 74 N for 'Bramley' fruit (Tables 2.1 and 2.2, respectively). Overall, after 2 months storage, 1-MCP-treated 'Queen Cox' fruit exhibited a loss in firmness of *ca* 18 N, whereas non-1-MCP-treated fruit showed a *ca* 30 N loss in firmness. Similarly, after 3 months storage, 1-MCP treatment of 'Bramley' fruit resulted in a loss of *ca* 6 N, whereas non-1-MCP-treated fruit showed a *ca* 40 N loss in firmness.

On removal from cold storage and subsequent warming, IEC levels in 1-MCP-treated 'Queen Cox' (Fig.3.1a) and 'Bramley' (Fig.3.1b) were all <5 $\mu l\ l^{-1}$. IEC of non-1-MCP-treated 'Queen Cox' and 'Bramley' was *ca* 1.85 and 1.95-fold higher,

respectively. After 7 d shelf-life, 'Queen Cox' IEC increased regardless of treatment. IEC of non-1-MCP treated fruit was higher than 1-MCP-treated fruit. However, IEC was higher in fruit treated with higher concentrations (up to 10 $\mu l\ l^{-1}$) 1-MCP. This was repeated further in fruit stored for 14 d. Non-1-MCP-treated 'Bramley' fruit exhibited an IEC increase to 180 $\mu l\ l^{-1}$ at 7 d, then a decline to 150 $\mu l\ l^{-1}$ at 14 d. These 'Bramley' fruit may have been finishing the climacteric period of ethylene production. IEC of 1-MCP-treated 'Bramley' fruit continued to rise with shelf-life, but did not exceed 100 $\mu l\ l^{-1}$ throughout the shelf-life period. The ANOVA results are shown in ANOVA Table 3.1a ('Queen Cox') and 3.1b ('Bramley').

Fruit firmness decreased in non-1-MCP-treated fruit over the 14 d shelf-life period (Fig. 3.2). Fruit firmness was retained to a greater extent after 1-MCP treatment (in the range of 0.1 to 10 $\mu l\ l^{-1}$ 1-MCP), and after 14 d, firmness was >10 N higher than non-1-MCP-treated 'Queen Cox' fruit, and >30 N for 'Bramley' fruit. These results show a difference in the scale of 1-MCP treatment-effect between cultivars. Fruit firmness of 1-MCP-treated 'Queen Cox' apple fruit was >10 N higher than for non-treated fruit, and >30 N higher for 1-MCP-treated 'Bramley' fruit. The ANOVA results are shown in ANOVA Table 3.2a ('Queen Cox') and 3.2b ('Bramley').

Differences in firmness retention observed between 1-MCP-treated 'Queen Cox' and 'Bramley' fruit may lie in cultivar differences. As a comparison, Watkins *et* al. (2000) observed retained firmnesses of *ca* <5 N after 1-MCP treatment (0.5 to 2.0 $\mu l\ l^{-1}$) of US-grown 'Delicious', 5 N for both 'McIntosh' and 'Law Rome', and >10 N for 'Empire' apple fruit after 3 months air-storage.

However, the concentrations of 1-MCP used by Watkins *et al.* (2000) are questionable, as they were based on the application of a miscalculated dose of Ethylbloc™. Previously, 1-MCP active ingredient by weight was quoted by Rohm and Hass Inc. as 0.43%, when it should have been 0.14% (G. Regiroli (2001), pers. comm.).

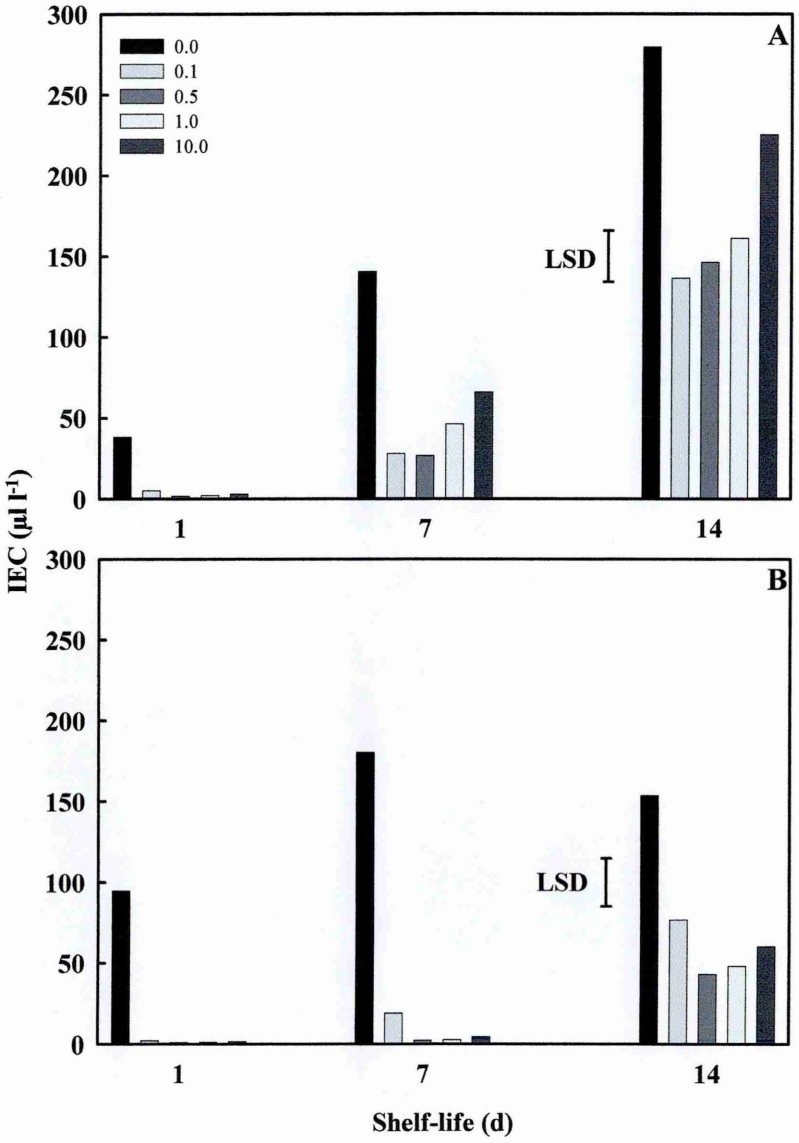

Figure 3.1 Fruit internal ethylene concentration (IEC µl l^{-1}) at 0, 7 and 14 d shelf life evaluations for Experiment 1a 'Queen Cox' (A) and 'Bramley' (B) apple fruit treated with 0, 0.1, 0.5, 1 or 10 µl l^{-1} 1-MCP for 24 h. Fruit were stored in air at 3 to 4°C for 2 months 'Queen Cox' or 3 months 'Bramley'. Data are the means for 20 individual replicate fruit (total number of fruit = 600). LSDs (P = 0.05).

ANOVA Table 3.1a ANOVA results for IEC of 'Queen Cox' fruit, Experiment 1a.

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Concentration (Conc.)	4	780549.	195137.	40.14	<.001
Temperature (Temp).	1	71697.	71697.	14.75	<.001
Day	2	3425593.	1712797.	352.32	<.001
Conc. x Temp.	4	173056.	43264.	8.90	<.001
Conc. x Day	8	207465.	25933.	5.33	<.001
Temperature x Day	2	32766.	16383.	3.37	0.048
Conc. x Temp. x Day	8	127418.	15927.	3.28	0.008

ANOVA Table 3.1b ANOVA results for IEC of 'Bramley' fruit, Experiment 1a.

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Concentration (Conc.)	4	598077.	149519.	10.77	<.001
Temperature (Temp).	1	1283.	1283.	0.09	0.761
Day	2	1341880.	670940.	48.31	<.001
Conc. x Temp.	4	167532.	41883.	3.02	0.019
Conc. x Day	8	146920.	18365.	1.32	0.232
Temperature x Day	2	25544.	12772.	0.92	0.400
Conc. x Temp. x Day	8	180928.	22616.	1.63	0.117

Similarly, Fan *et al.* (1999) showed greater fruit firmness in 'Delicious' apples treated with *ca* 0.2 to 1 $\mu l\ l^{-1}$ 1-MCP, with no further increase in firmness after treatments of 1, 2 or 3 $\mu l\ l^{-1}$ 1-MCP. Furthermore, these workers also reported treatment with *ca* 1 $\mu l\ l^{-1}$ 1-MCP had no effect on 'Gala' fruit firmness until 6 months air storage at 0°C. However, non-1-MCP-treated 'Gala' fruit maintained a firmness of >70 N after 6 months, compared to *ca* 100 N at harvest. Firmness of 'Queen Cox' treated with 10 $\mu l\ l^{-1}$ 1-MCP was slightly greater than that at the lower 1-MCP concentrations (Fig. 3.1a).

The higher concentration gradient at 10 $\mu l\ l^{-1}$ 1-MCP may have enhanced diffusion of 1-MCP into the fruit. Rupasinghe *et al.* (2000) treated 'McIntosh' and 'Delicious' apple fruit with 0, 0.01, 0.1, 1, 10, or 100 $\mu l\ l^{-1}$ 1-MCP at 20°C for 18 h. These workers found apple fruit treated with $\geq 1\ \mu l\ l^{-1}$ 1-MCP and stored for *ca* 2 months in cold air (0 to 1°C) showed increased firmness of *ca* 20 N over non-1-MCP-treated fruit, and reduced IEC of 1.96-fold upon removal from storage. Only one other published set of results shows data from apple fruit treated with >5 $\mu l\ l^{-1}$ 1-MCP.

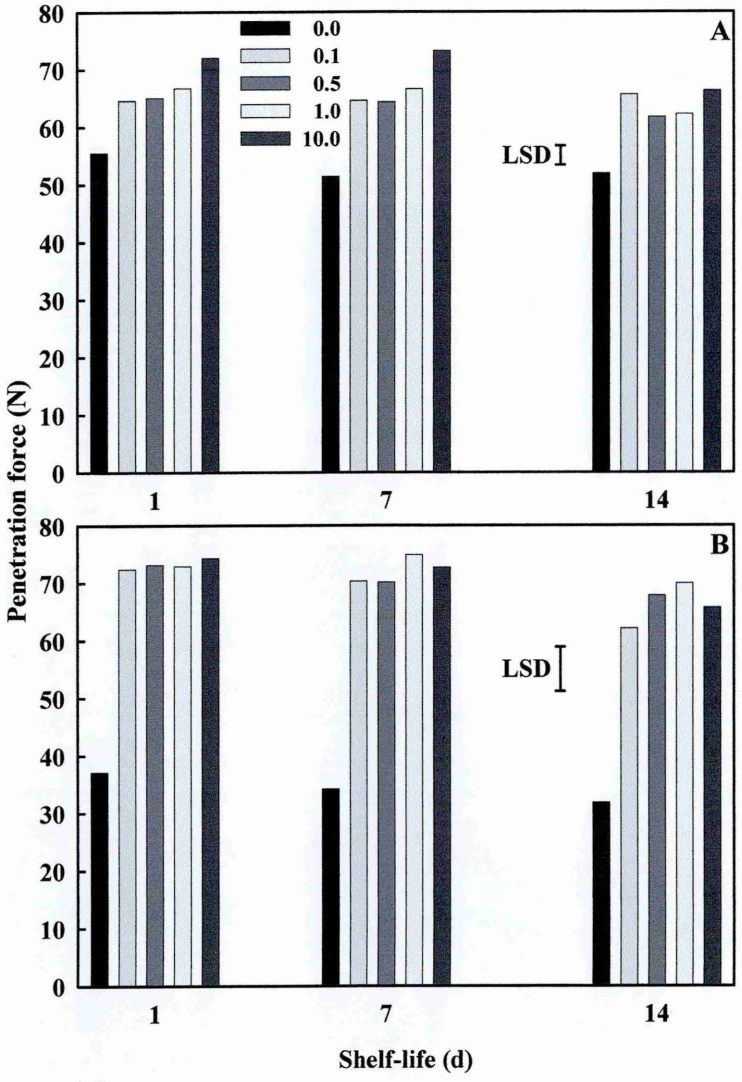

Figure 3.2 Fruit firmness (penetration force N) at 0, 7 and 14 d shelf life evaluations for Experiment 1a 'Queen Cox' (A) and 'Bramley' (B) apple fruit treated with 0, 0.1, 0.5, 1 or 10 $\mu l\ l^{-1}$ 1-MCP for 24 h. Fruit were stored in air at 3 to 4°C for 2 months 'Queen Cox' or 3 months 'Bramley'. Data are the means for 20 individual replicate fruit (total number of fruit = 600). LSDs (P = 0.05).

ANOVA Table 3.2a ANOVA results for firmness of 'Queen Cox' fruit, Experiment 1a.

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Concentration (Conc.)	4	20104.25	5026.06	93.16	<.001
Temperature (Temp).	1	6520.13	6520.13	120.85	<.001
Day	2	1129.20	564.60	10.46	<.001
Conc. x Temp.	4	3803.25	950.81	17.62	<.001
Conc. x Day	8	1127.57	140.95	2.61	0.027
Temperature x Day	2	55.46	27.73	0.51	0.603
Conc. x Temp. x Day	8	1372.72	171.59	3.18	0.010

ANOVA Table 3.2b ANOVA results for firmness of 'Bramley' fruit, Experiment 1a.

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Concentration (Conc.)	4	28351.4	7087.8	55.02	<.001
Temperature (Temp).	1	2863.1	2863.1	22.22	<.001
Day	2	4622.1	2311.0	17.94	<.001
Conc. x Temp.	4	887.8	221.9	2.72	0.045
Conc. x Day	8	845.4	105.7	2.82	0.041
Temperature x Day	2	516.6	258.3	2.00	0.137
Conc. x Temp. x Day	8	1920.7	240.1	1.86	0.066

Fan and Mattheis (1999) applied 10 $\mu l\ l^{-1}$ 1-MCP to climacteric 'Fuji' apple fruit (mean IEC of 32.5 $\mu l\ l^{-1}$), with no comparative 1-MCP concentration. No data for fruit firmness was presented; rather, the paper was primarily concerned with volatile production.

Work on other fresh produce shows little differences in 1-MCP efficacy at concentrations greater than *ca* 1 $\mu l\ l^{-1}$. 'Barlett' (Williams) pears (*Pyrus communis*) showed no difference in firmness for fruit treated with 0, 0.01 or 0.1 $\mu l\ l^{-1}$ 1-MCP, whereas treatment with 0.5 $\mu l\ l^{-1}$ 1-MCP showed *ca* 30 N greater firmness retention, and *ca* 50 N after treatment with 1 $\mu l\ l^{-1}$ 1-MCP. However, fruit treated with 1 $\mu l\ l^{-1}$ 1-MCP failed to soften, and were thus commercially unacceptable (Ekman *et al.*, 2004). Wills and Ku (2002) demonstrated an increase in time to ripening of green stage tomatoes (*Lycopersicon esculentum* Mill) of *ca* 1.7 to *ca* 2.10-fold after treatment with 5 to 100 $\mu l\ l^{-1}$ 1-MCP, respectively.

Jiang *et al.* (1999a) treated green 'Cavendish' banana fruit (*Musa sapentium*) with 0.01 to 1 $\mu l\ l^{-1}$ 1-MCP. It was concluded that treatment with higher concentrations of 1-MCP (up to 1 $\mu l\ l^{-1}$) resulted in more effective control over ripening. However, 1-MCP treatment of banana fruit resulted in an uneven development of peel colour during ripening. These workers suggested that there are positional differences in the rate of binding-site synthesis in the peel and the pulp.

It is possible that excess 1-MCP may be sorbed in some way by fruit tissue beyond saturation of ethylene-binding sites (Chapter 7). If so, 1-MCP may be slowly released during storage. 1-MCP would then be available to bind to newly synthesized or regenerated ethylene-binding sites (Sisler *et al.*, 1996; Golding *et al.*, 1998). 1-MCP is thought to bind irreversibly to ethylene-binding sites (Sisler and Blankenship, 1996). Therefore, any fruit that ripens, or any produce that regains ethylene-sensitivity, must do so as a result of ethylene binding to new ethylene receptors formed after 1-MCP application and in the absence of 1-MCP. Jiang *et al.* (2002) supported the synthesis hypothesis as the means to increasing ethylene-binding sites in banana fruit after treatment with 0.01, 0.1, 1 or 10 $\mu l\ l^{-1}$ 1-MCP. Furthermore, Jiang *et al.* (2002) suggest that binding-site synthesis may be enhanced with increased storage temperature. The greater firmness observed in 'Queen Cox' fruit treated with 10 $\mu l\ l^{-1}$ 1-MCP may result from 'free 1-MCP' blocking these newly-formed ethylene-binding sites.

IEC for both 'Queen Cox' and 'Bramley' exposed to 1-MCP was reduced relative to that of untreated fruit. Measurement of IEC is the most accurate method of ascertaining the climacteric state of climacteric fruit. Work on Kentish-grown 'Cox's Orange Pippin' apples (Stow *et al.*, 2000) showed that fruit with an IEC of *ca* 0.1 $\mu l\ l^{-1}$ 1-MCP are unable to initiate the climacteric. In addition, applications of 0.1 to 1 $\mu l\ l^{-1}$ ethylene have induced ripening in apple (Stow *et al*, 2000), banana, mango and plum (Burg and Burg, 1965). Therefore, for pre-ripe fruit, the lower the IEC, the less advanced the climacteric.

IEC for 'Queen Cox' treated with 10 $\mu l\ l^{-1}$ 1-MCP increased compared with other 1-MCP concentrations. The reason for this is unknown, but 1-MCP treatment may influence the ethylene-mediated negative-feedback mechanisms that control ethylene production *in-planta.* Two systems are involved in ethylene production: Systems I and II. System I ethylene generates low pre-climacteric ethylene, and inhibits System II ethylene-production until a critical ethylene concentration is reached (or made available, e.g. commercial ethylene-induced banana-ripening) and System II is triggered to produce climacteric ethylene (McMurchie *et al.*, 1972; Bufler, 1986).

There are examples of 1-MCP application increasing IEC of bananas, as compared to non-1-MCP treatment. Pelayo *et al.* (2003) reported an increase of *ca* 1 $\mu l\ kg^{-1}\ h^{-1}$ ethylene production after treatment with 1 $\mu l\ l^{-1}$ 1-MCP. This is in agreement with a similar finding by Golding *et al.* (1999).

1-MCP may enhance ACC synthase transcription (Golding *et al.*, 1999). Banana ACC synthase and ACC oxidase activities have been shown to increase in response to 1 $\mu l\ l^{-1}$ 1-MCP treatment (Pelayo *et al.*, 2003) and 60 $\mu l\ l^{-1}$ ethylene (Moya-León and John, 1994). Similarly, work by Nakano *et al.* (2002) on 'Tonewase' Japanese persimmon fruit (*Diospyros kaki* Thunb) showed increased ethylene production in response to water-stress (storage in low humidity: 40 to 60% RH). Water-stressed persimmons treated with 0.3 $\mu l\ l^{-1}$ 1-MCP at 0, 2, 4, 6 and 8 days after harvest showed a further increase in ethylene production rate of *ca* 2.5 $ml\ g^{-1}\ h^{-1}$ for the first two days at 20°C. ACC content of the calyx of the 1-MCP-treated persimmons was shown to be *ca* 0.4 to 0.8 $nmol\ g^{-1}$ higher until day 6. Subsequently, the rate of ethylene production in 1-MCP-treated fruit declined until effective suppression at day 5. This rise in ethylene production was thought to be in response to water-stress. A second ethylene production phase observed after day 6 storage for water-stressed non-1-MCP-treated persimmons was inhibited by 1-MCP treatment. Initially, non-1-MCP-treated fruit showed smaller increases in calyx ACC content than 1-MCP-treated fruit, but rose after day 6, and exhibited a relatively rapid rise (0 to *ca* 4 $nmol\ g^{-1}$) in peel ACC content from this time. ACC content of the pulp was unaffected by 1-MCP treatment. The second rise in ethylene production rate of non-1-MCP-treated persimmons was thought to be induced auto-catalytically under the action of the initial ethylene production, suggesting that 1-MCP interferes with the negative feedback regulation of ethylene production.

Firmness and IEC of 'Queen Cox' and 'Bramley' fruit were not affected by 1-MCP at 4 and 6 months and 6 months storage, respectively (Appendix 4.1). Loss of 1-MCP efficacy over time during storage was probably due to increasing availability of ethylene-binding sites in the fruit tissue (Sisler and Serek, 1999; Macnish *et al.*, 2000). A potentially rapid expression of ethylene-induced effects in previously 1-MCP-treated fruit may be due to a build-up of ACC, readily available for ethylene production. Once ethylene binding sites have been made available, and the fruit

become ethylene sensitive, the climacteric may be initiated. A ready supply of ACC may result in a faster expression of the climacteric than fruit without the ACC 'reserve'. This experiment did not quantify ACC levels due to time and resource restraints.

3.2.2 Total soluble solids

Treatment of 'Bramley' apple fruit with 1-MCP resulted in a small but significantly higher TSS of *ca* 0.6°Brix ($P = 0.05$) after 6 months storage (Table 3.1). There was no observed increase in TSS for 'Queen Cox', but conversely, there was a drop in TSS for fruit treated with the 10 $\mu l\ l^{-1}$ 1-MCP.

Fan *et al.* (1999) observed no difference in TSS of 1 $\mu l\ l^{-1}$ 1-MCP-treated 'Gala', 'Ginger Gold', or 'Jonagold' apple fruit up to 6 months air storage. Small increases of <0.5 and *ca* 1.5°Brix were observed in 1 $\mu l\ l^{-1}$ 1-MCP-treated 'Delicious' and 'Fuji' apples, respectively, but not until 6 months storage. Similarly, Rupasinghe *et al.* (2000) observed no differences in TSS of 'McIntosh' or 'Delicious' apple fruit treated with 1 to 100 $\mu l\ l^{-1}$ 1-MCP, after *ca* 2 months cold air storage. Furthermore, DeEll *et al.* (2002) showed no difference in TSS of 'Cortland' nor 'Empire' apple fruit treated with 0.6 $\mu l\ l^{-1}$ 1-MCP, and stored at 20°C for 7 d after 3 months cold air-storage (0 to 1°C). Similarly, 1-MCP did not affect TSS of 'Anna' (Pre-Aymard *et al.*, 2003), nor 'Granny Smith' apple fruits (Fan *et al.*, 1999; Zanella, 2003), banana fruit (Golding *et al.*, 1998), tomatoes (Wills and Ku, 2002), apricots and plums (Dong *et al.*, 2002).

Table 3.1 Total soluble solids (TSS °Brix) main factor means (i.e. across 0, 7 and 14 d shelf life evaluations) of Experiment 1a apple fruit treated with various 1-MCP concentrations for 24 h. Fruit were stored in air at 3 to 4°C for 2 months 'Queen Cox' or 3 months 'Bramley'. Data are the means for 60 individual replicate fruit (total number of fruit = 600). LSDs (P = 0.05).

Cultivar	1-MCP concentration (µl l^{-1})	TSS (°Brix)	*LSD*
'Bramley'	0.0	9.4	
	0.1	10.0	
	0.5	10.0	
	1.0	10.2	
	10.0	10.0	*0.27*
'Queen Cox'	0.0	14.0	
	0.1	14.0	
	0.5	13.8	
	1.0	13.6	
	10.0	13.4	*0.50*

Development of TSS during ripening and storage appeared to be independent of ethylene perception in climacteric fruits, including apples. Any differences in TSS for 'Queen Cox' or 'Bramley' fruit (Table 3.1) were small (0.1 and 0.3°Brix, respectively), and varied with cultivar and 1-MCP treatment concentration.

3.3 Experiment 1b: The effect of 1-MCP exposure time on the quality of 'Queen Cox' and 'Bramley' apple fruit storage

3.3.1 Fruit firmness and IEC

'Queen Cox' and 'Bramley' fruit were as firm after 2 and 3 months air storage, respectively, after 6 h exposure to 1.0 $\mu l\ l^{-1}$ 1-MCP as after the longest exposure time of 48 h (Fig. 3.3). 'Queen Cox' and 'Bramley' fruit maintained a consistent firmness of >50 N, *ca* 20 N higher than non-1-MCP-treated fruit. Firmness remained relatively consistent for both cultivars throughout the 14 d shelf-life period, regardless of 1-MCP-treatment. Furthermore, 1-MCP-treated fruit were consistently >10 N firmer than non-1-MCP treated fruit (Fig. 3.5).

IEC of 'Queen Cox' fruit was reduced in 1-MCP-treated fruit by *ca* 300 $\mu l\ l^{-1}$, to *ca* 75 $\mu l\ l^{-1}$, regardless of 1-MCP treatment duration (Fig. 3.3a). Similarly, 1-MCP-treated 'Bramley' fruit IEC was suppressed from *ca* 175 $\mu l\ l^{-1}$ for non-1-MCP-treated fruit to *ca* 25 $\mu l\ l^{-1}$, regardless of 1-MCP exposure duration (Fig. 3.3b). Unlike 1-MCP-treatment concentration (Experiment 1a) there was no cultivar difference in fruit firmness retention with 1-MCP exposure duration.

'Queen Cox' and 'Bramley' apple fruit appeared to reach the climacteric during shelf-life storage (Fig. 3.4a; b), although 'Bramley' fruit IEC was suppressed, as compared to Experiment 1a. This may be due to a maturity effect as fruit were harvested later than those in Experiment 1a (Tables 3.1; 3.2). Influence of picking date on apple quality is discussed further in Chapter 4.

IEC increased throughout shelf-life for both 1-MCP-treated 'Queen Cox' and 'Bramley', regardless of duration of 1-MCP treatment, to *ca* 170 $\mu l\ l^{-1}$ and 50 $\mu l\ l^{-1}$, respectively. However, IEC of non-1-MCP-treated fruits was 1.80-fold greater at 7 d shelf-life, and *ca* twice that of 1-MCP-treated fruit at 14 d.

The IEC ANOVA results are shown in ANOVA Tables 3.3a ('Queen Cox') and 3.3b ('Bramley'). The firmness ANOVA results are shown in ANOVA Tables 3.3a ('Queen Cox') and 3.3b ('Bramley').

'Cortland' apple fruit required a 9 h treatment with 0.6 $\mu l\ l^{-1}$ 1-MCP to achieve maximum retained firmness after 3 months cold-air storage (0 to 1°C) (DeEll *et al.*, 2002). 1-MCP treatment for 3 or 6 h resulted in less firmness of 'Cortland'

fruit than those treated for 9 h; there was no additional benefit for treating fruit for up to 24 h. DeEll *et al.* (2002) also reported that maximum firmness of 'Empire' apple fruit was achieved after 3 h 1-MCP-treatment, with no additional firmness after treatments between 6 to 24 h.

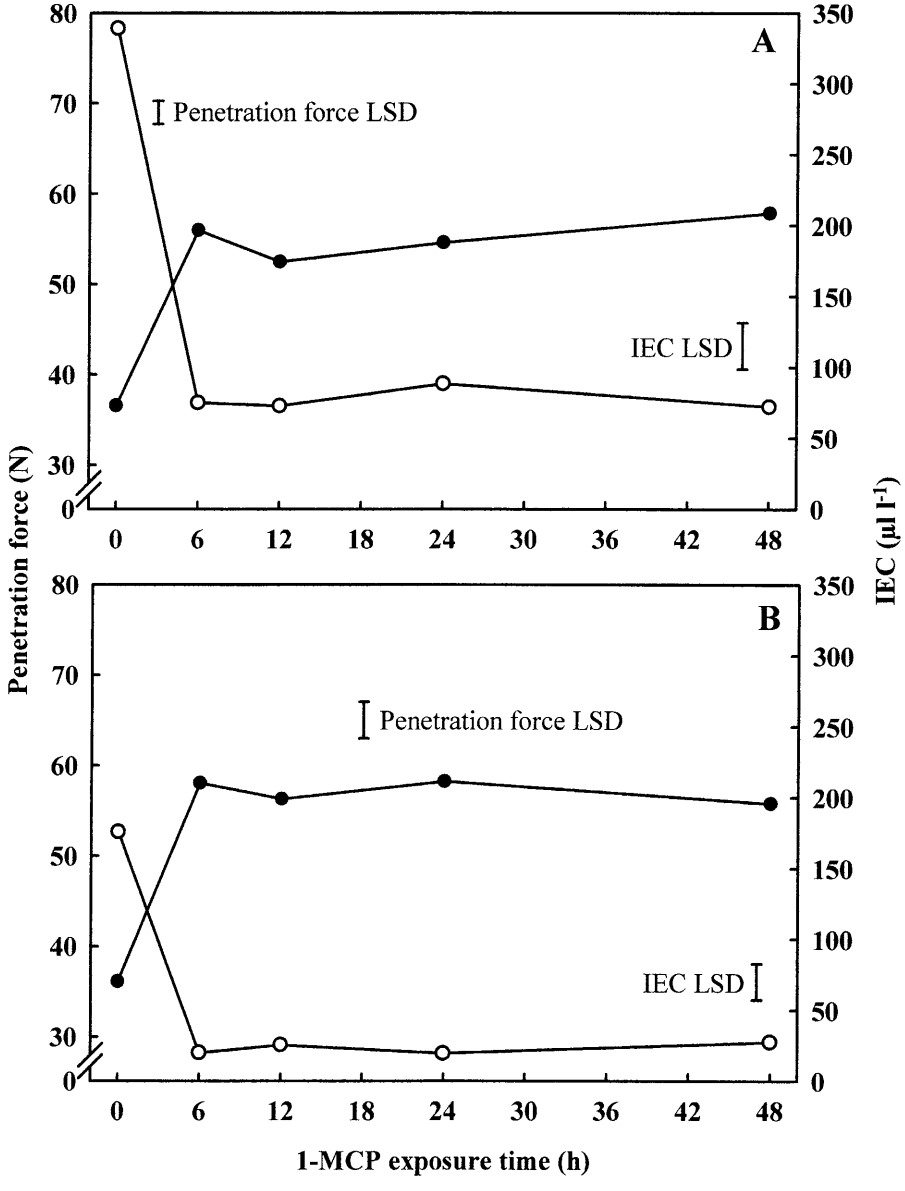

Figure 3.3 Fruit firmness (penetration force N, ●) and internal ethylene concentration (IEC µl l^{-1}, ○) main factor means (i.e. across 0, 7 and 14 d shelf life evaluations) for Experiment 1b 'Queen Cox' (A) and 'Bramley' (B) apple fruit treated with 1.0 µl l^{-1} 1-MCP for 0, 6, 12, 24 or 48 h. Fruit were stored in air at 3 to 4°C for 2 months 'Queen Cox' or 3 months 'Bramley'. Data are the means for 30 individual replicate fruit (total number of fruit = 300). LSDs (P = 0.05).

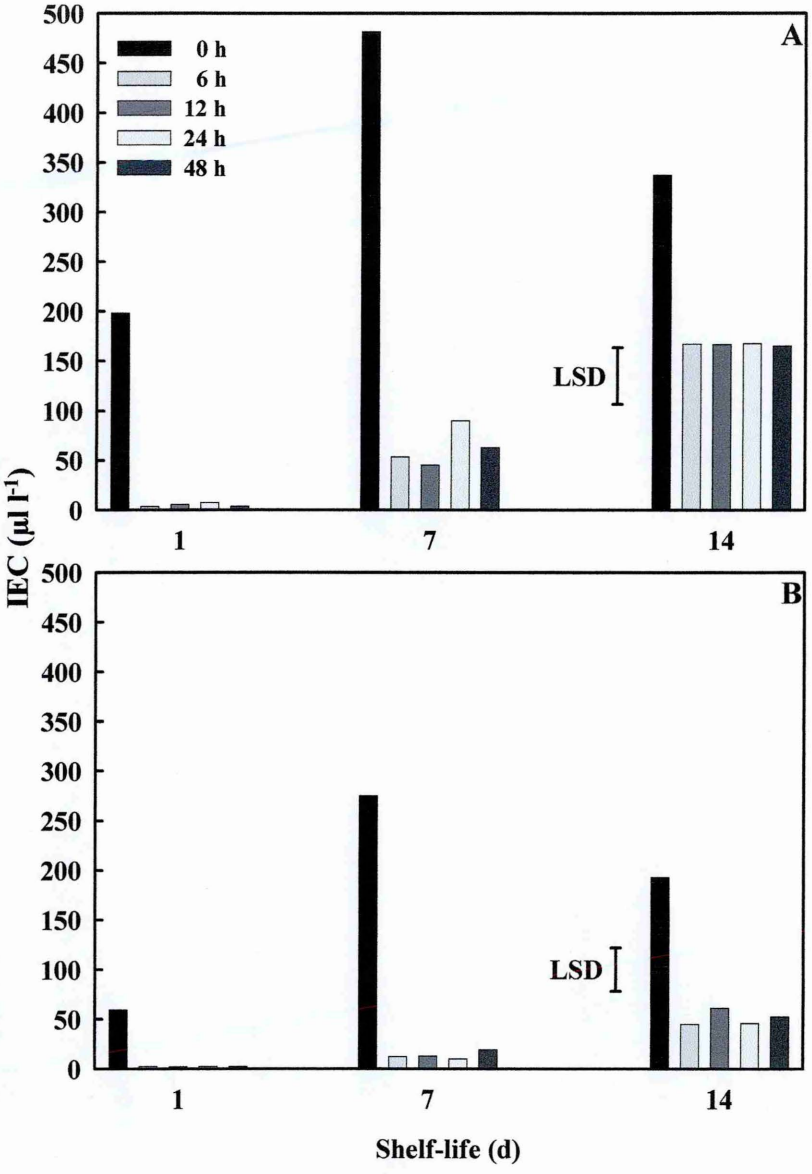

Figure 3.4 Fruit internal ethylene concentration (IEC µl l^{-1}) at 0, 7 and 14 d shelf life evaluations for Experiment 1b 'Queen Cox' (A) and 'Bramley' (B) apple fruit treated with 1.0 µl l^{-1} 1-MCP for 0, 6, 12, 24 or 48 h. Fruit were stored in air at 3 to 4°C for 2 months 'Queen Cox' or 3 months 'Bramley'. Data are the means for 10 individual replicate fruit (total number of fruit = 300). LSDs (P = 0.05).

Jiang and Joyce (2000) treated mango fruit (*Mangifera indica* L.) with either 50 or 100 µl l^{-1} 1-MCP at 20°C for 1, 3, 6, 12 or 24 h. The banana fruit were then dipped in 1000 µl l^{-1} ethephon for 3 min to initiate ripening. Fruit were then kept at 20°C for 7 d. The 100 µl l^{-1} 1-MCP treatment for 12 h appeared to minimise ethylene-induced softening (*ca* 0.6 mm less displaced fruit than non-1-MCP-treated fruit. In the case of mango fruit exposed to 50 µl l^{-1} 1-MCP, 12 h treatment was not

as effective as that for 12 h treatment at 100 µl l^{-1} 1-MCP, but was just as effective after 24 h exposure.

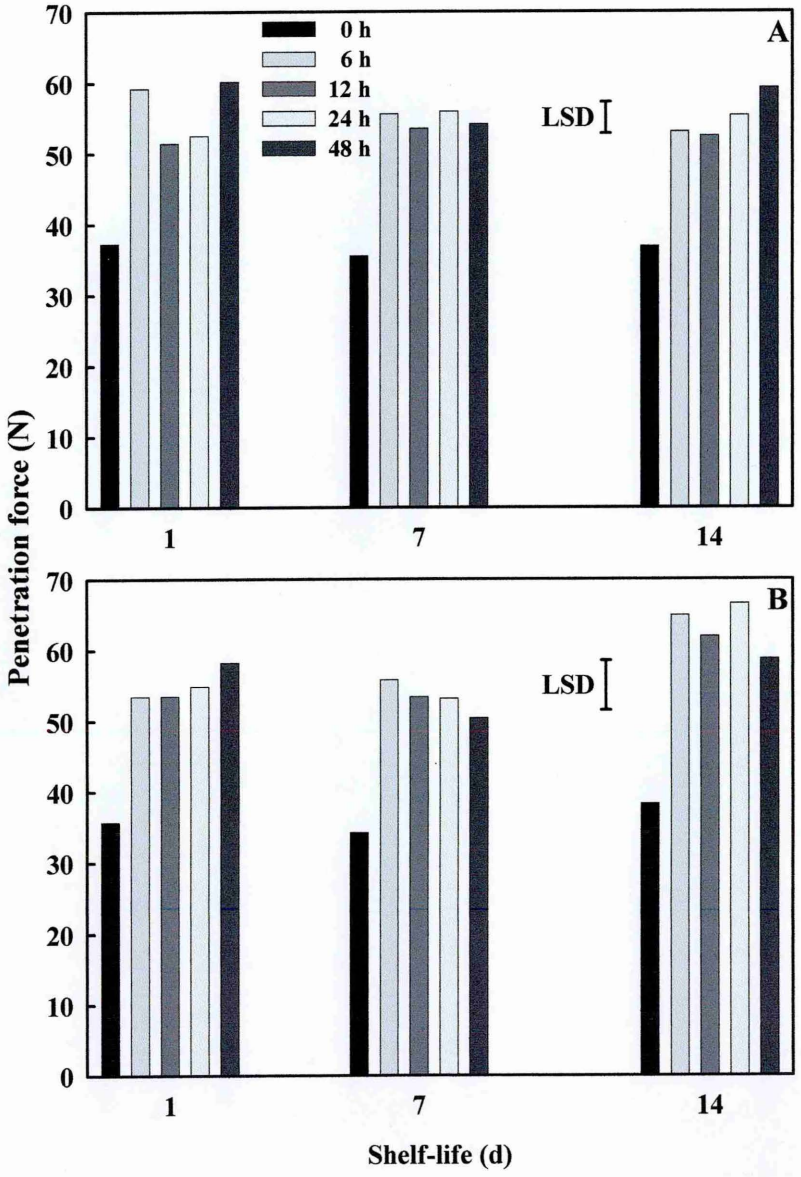

Figure 3.5 Fruit firmness (penetration force N) at 0, 7 and 14 d shelf life evaluations for Experiment 1b 'Queen Cox' (A) and 'Bramley' (B) apple fruit treated with 1.0 µl l^{-1} 1-MCP for 0, 6, 12, 24 or 48 h. Fruit were stored in air at 3 to 4°C for 2 months 'Queen Cox' or 3 months 'Bramley'. Data are the means for 10 individual replicate fruit (total number of fruit = 300). LSDs (P = 0.05).

ANOVA Table 3.3a ANOVA results for IEC of 'Queen Cox' fruit, Experiment 1b.

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Duration	4	3271448.	817862.	98.38	<.001
Temperature (Temp.)	1	78342.	78342.	9.42	0.002
Day	2	1274843.	637421.	76.68	<.001
Duration x Temp.	4	18666.	6222.	0.75	0.524
Duration x Day	8	615153.	76894.	9.25	<.001
Temp. x Day	2	25443.	12722.	1.53	0.219
Duration x Temp. x Day	8	34020.	5670.	0.68	0.664

ANOVA Table 3.3b ANOVA results for IEC of 'Bramley' fruit, Experiment 1b.

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Duration	4	1131119.	282780.	57.28	<.001
Temperature (Temp.)	1	13299.	13299.	2.69	0.102
Day	2	241621.	120811.	24.47	<.001
Duration x Temp.	4	6977.	1744.	0.35	0.842
Duration x Day	8	340675.	42584.	8.63	<.001
Temp. x Day	2	12177.	6089.	1.23	0.293
Duration x Temp. x Day	8	23681.	2960.	0.60	0.778

ANOVA Table 3.4a ANOVA results for firmness of 'Queen Cox' fruit, Experiment 1b.

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Duration	4	17645.28	4411.32	85.53	<.001
Temperature (Temp.)	1	499.08	499.08	9.68	0.002
Day	2	70.09	35.04	0.68	0.508
Duration x Temp.	4	788.23	262.74	5.09	0.002
Duration x Day	8	937.23	117.15	2.27	0.023
Temp. x Day	2	72.23	36.11	0.70	0.497
Duration x Temp. x Day	8	84.25	14.04	0.27	0.950

ANOVA Table 3.4b ANOVA results for firmness of 'Bramley' fruit, Experiment 1b.

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Duration	4	21407.9	5352.0	42.05	<.001
Temperature (Temp.)	1	709.7	709.7	5.58	0.019
Day	2	4231.5	2115.8	16.62	<.001
Duration x Temp.	4	400.3	100.1	0.79	0.535
Duration x Day	8	1361.1	170.1	1.34	0.225
Temp. x Day	2	275.0	137.5	1.08	0.341
Duration x Temp. x Day	8	958.5	119.8	0.94	0.483

Jiang and Joyce (2000) also suggested that, for mango, treatment periods greater than 24 h would be commercial unacceptable. However, with apples, 1-MCP treatment may be applied during storage. Work on tomato by Wills and Ku (2002) showed a 1.68-fold extension of time to ripen after 1 h of 5 $\mu l\ l^{-1}$ 1-MCP treatment at 20°C, as compared to non-1-MCP-treated fruit. Time to ripen was significantly ($P = 0.05$) increased to 105% after 5 h 1-MCP-treatment. However, these workers did

not demonstrate the 1-MCP exposure duration required to maximise time to ripen tomato fruit.

Jiang *et al.* (1999b) treated mature green 'Cavendish' bananas with 0.1 or 1 $\mu l\ l^{-1}$ 1-MCP at 20°C then immediately with 100 $\mu l\ l^{-1}$ ethylene for 24 h at 20°C, and stored at 20°C for 1, 3 or 5 d. Fruit treated with 0.1 $\mu l\ l^{-1}$ 1-MCP for 0.5 and 1 h showed <1.1-fold more firmness than non-1-MCP-treated fruit. As 1-MCP exposure duration increased to 3 or 6 h, firmness was *ca* 1.3 and 1.1-fold higher for fruit held at 1 and 5 d, respectively. After 12 h 1-MCP treatment, fruit firmness of bananas held at 1 and 5 d was 50 to 30% higher, respectively. Banana fruit treated with 1 $\mu l\ l^{-1}$ 1-MCP had a less varied response. Fruit treated for 0.5 h and held for 1, 3 or 5 d showed greater firmnesses of *ca* 1.4, 1.3 and 1.2-fold, respectively. However, after 1-MCP treatment for 1 or 3 h, fruit were *ca* 50% firmer than non-1-MCP-treated fruit. Fruit exposed to 1$\mu l\ l^{-1}$ 1-MCP for 6 or 12 h showed no further firmness retention when held at 1 or 3 d, and increased to *ca* 1.65-fold after holding for 5 d.

Other work on bananas treated with 1 $\mu l\ l^{-1}$ 1-MCP for either 12 or 24 h exhibited no difference in either firmness or IEC up to 6 d air-storage at 20°C (Pelayo *et al.*, 2003). However, as with Jiang *et al.* (1999b) there was no long-term storage for these fruit. Also, Pelayo *et al.* (2003) applied 1-MCP after the climacteric had begun (see section 3.2.1).

An absence of a differential exposure time (Fig. 3.3) effect suggests that 1-MCP rapidly permeates throughout apple fruit. Mature apple fruit have *ca* 25% air space between the cells (Wills, 1987).

As in Experiment 1a, differences in firmness and IEC for untreated and 1-MCP treated fruit were not significant ($P > 0.05$) for longer storage periods of 4 and 6 months for 'Queen Cox' and 6 months for 'Bramley'.

3.3.2 Total soluble solids

Similarly to Experiment 1a, treatment of 'Bramley' apple fruit with 1-MCP resulted in a small (<1°Brix) but significantly higher TSS ($P = 0.05$) after 3 months storage (Table 3.2). Contrary to Experiment 1a, there was also a small (<0.5°Brix) but significantly higher ($P = 0.05$) observed increase in TSS after 2 months storage for 'Queen Cox' for all exposure times.

Table 3.2 Total soluble solids (TSS °Brix) main factor means (i.e. across 0, 7 and 14 d shelf life evaluations) of Experiment 1b apple fruit treated with 1.0 $\mu l\ l^{-1}$ 1-MCP for different exposure times. Fruit were stored in air at 3 to 4°C for 2 months 'Queen Cox' or 3 months 'Bramley'. Data are the means for 30 individual replicate fruit (total number of fruit = 300). LSDs (P = 0.05).

Cultivar	1-MCP exposure time (h)	TSS (°Brix)	*LSD*
'Bramley'	0	10.4	
	6	11.4	
	12	11.3	
	24	11.3	
	48	11.3	*0.22*
'Queen Cox'	0	14.0	
	6	14.2	
	12	14.4	
	24	14.5	
	48	14.4	*0.19*

3.4 Experiment 1c: The effect of 1-MCP exposure temperature on the quality of 'Queen Cox' and 'Bramley' apple fruit storage

3.4.1 Fruit firmness and IEC

Application temperature did not affect efficacy of 1-MCP of 'Queen Cox' and 'Bramley' fruit after 2 and 3 months storage in air, respectively (Fig. 3.6). 'Queen Cox' apple fruit treated with 1 $\mu l\ l^{-1}$ 1-MCP for 24 h maintained a firmness of *ca* 48 N when 1-MCP was applied at 0, 5, 10 or 15°C. When 1-MCP was applied at 20°C, fruit firmness was *ca* 5 N less. 'Queen Cox' fruit treated with 1 $\mu l\ l^{-1}$ 1-MCP for 48 h showed a firmness of 45 to 50 N regardless of application temperature, except for fruit treated at 10°C which had a firmness of *ca* 54 N. Non-1-MCP-treated fruit firmness was *ca* 35 N, regardless of the pre-storage holding temperature (0, 5, 10, 15 or 20°C). Similarly, IEC of 'Queen Cox' fruit was *ca* 200 $\mu l\ l^{-1}$ less than for non-1-MCP-treated fruit, regardless of 1-MCP application temperature for either 24 or 48 h (Fig. 3.6a).

'Bramley' fruit treated with 1-MCP for 24 or 48 h at 0, 10, 15 or 20°C had a firmness of *ca* 45 N. 'Bramley' fruit treated at 5°C showed a firmness of 48 or 53 N after 24 or 48 h 1-MCP-treatement, respectively. Non-1-MCP-treated 'Bramley' fruit maintained a firmness of <35 N, as with 'Queen Cox', regardless of pre-storage holding temperature. Similarly, IEC of 'Bramley' fruit was *ca* 150 $\mu l\ l^{-1}$ less than for non-1-MCP-treated fruit, regardless of temperature of 1-MCP application for either 24 or 48 h (Fig. 3.6b). As for Experiment 1b (Fig. 3.3), there was no cultivar difference in response to 1-MCP treatment temperature for fruit firmness of these fruit, unlike 1-MCP-treatment concentration (Experiment 1a). Again, fruit appear to achieve climacteric ethylene production during shelf-life storage ('Queen Cox', Fig. 3.7), or increase towards the peak ('Bramley', Fig. 3.8). Ethylene production increased over 14 d for all 1-MCP-treated fruit, although IEC was less than non-1-MCP-treated fruit. However, for 'Queen Cox' fruit, after 14 d shelf-life, fruit treated at the higher temperatures (15 and 20°C) show higher ethylene production than fruit treated at lower temperatures (Fig. 3.7). Although the fruit treated with 1-MCP at higher temperatures had similar IECs to non-1-MCP-treated fruit, ethylene production was increasing for 1-MCP-treated fruit and decreasing for-1-MCP-treated fruit. It would

appear that 1-MCP treatment of 'Queen Cox' is most effective when applied at lower temperatures. No such distinction was shown for 'Bramley' fruit.

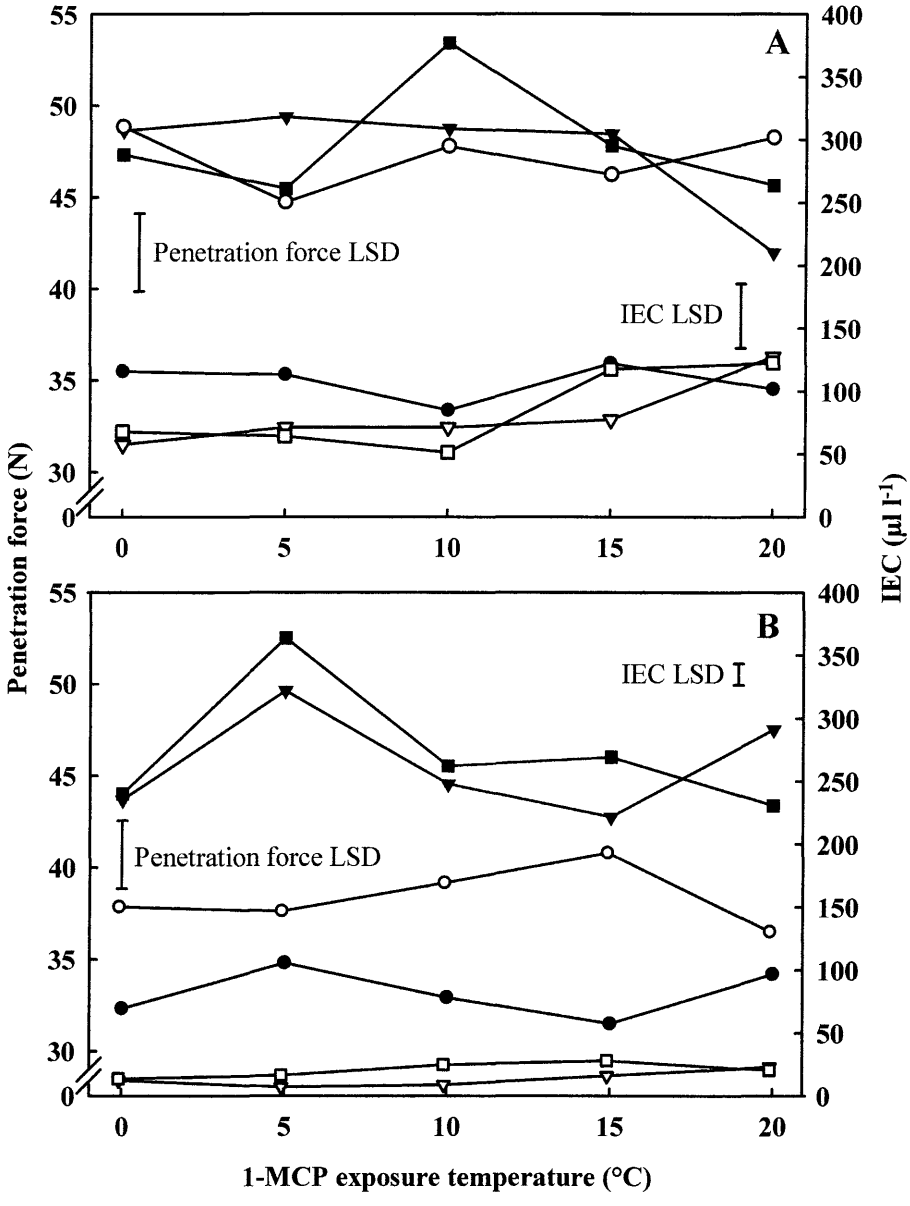

Figure 3.6 Fruit firmness (penetration force N, closed symbols) and internal ethylene concentration (IEC µl l^{-1}, open symbols) main factor means (i.e. across 0, 7 and 14 d shelf life evaluations) for Experiment 1c 'Queen Cox' (A) and 'Bramley' (B) apple fruit treated with 1.0 µl l^{-1} 1-MCP at different temperatures for 0 (●, ○), 24 (▼, ▽) or 48 h (■, □). Fruit were stored in air at 3 to 4°C for 2 months 'Queen Cox' or 3 months 'Bramley'. Data are the means for 30 individual fruit (total number of fruit = 900). LSDs (P = 0.05).

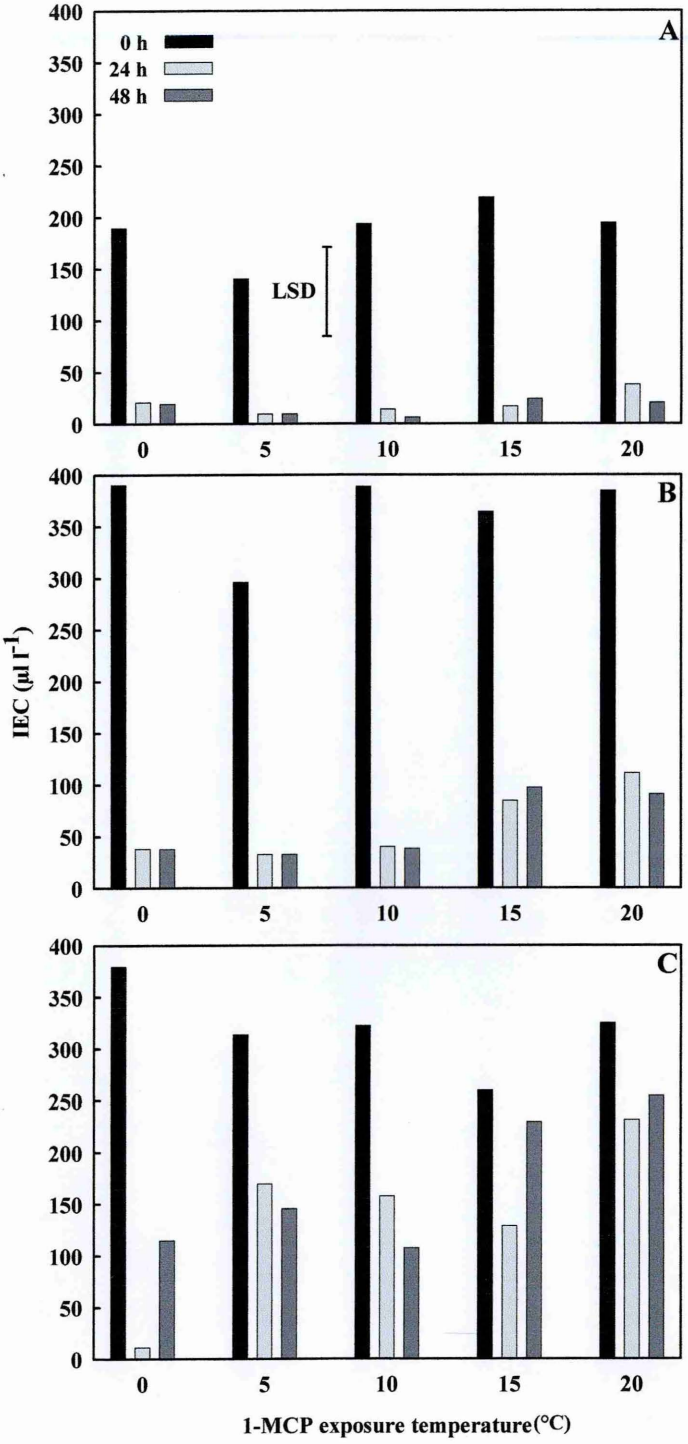

Figure 3.7 'Queen Cox' fruit internal ethylene concentration (IEC µl l^{-1}) at 0 (A), 7 (B) and 14 d (C) shelf life evaluations for Experiment 1c. Fruit were treated with 1.0 µl l^{-1} 1-MCP for 0, 24 or 48 h, then stored in air at 3 to 4°C for 2 months. Data are the means for 10 individual fruit (total number of fruit = 450). LSD inclusive for A, B and C (P = 0.05).

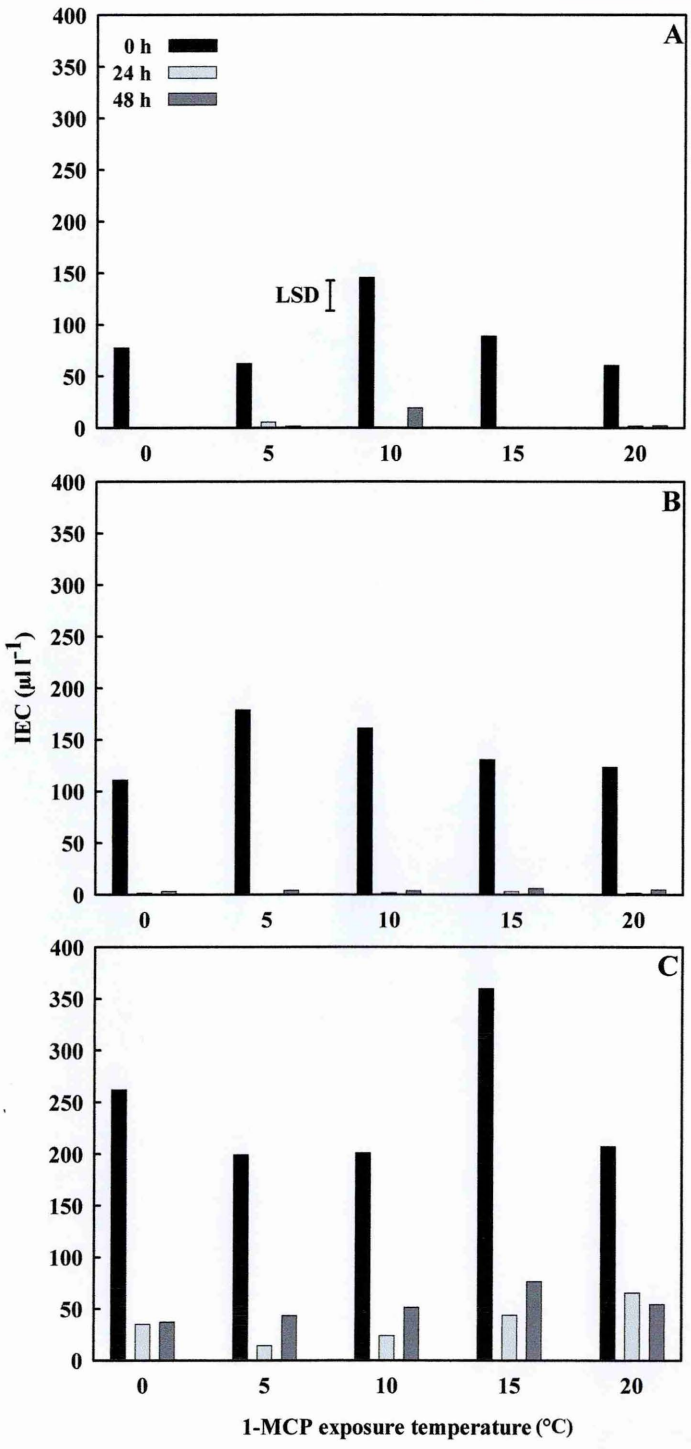

Figure 3.8 'Bramley' fruit IEC at 0 (A), 7 (B) and 14 d (C) shelf life evaluations for Experiment 1c. Fruit were treated with 1.0 µl l^{-1} 1-MCP for 0, 24 or 48 h, then stored in air at 3 to 4°C for 3 months. Data are the means for 10 individual fruit (total number of fruit = 450). LSD inclusive for A, B and C (P = 0.05).

The IEC ANOVA results are shown in ANOVA Tables 3.5a ('Queen Cox') and 3.5b ('Bramley'). The firmness ANOVA results are shown in ANOVA Tables 3.6a ('Queen Cox') and 3.6b ('Bramley').

ANOVA Table 3.5a ANOVA results for IEC of 'Queen Cox' fruit, Experiment 1c.

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Duration	2	4130638.	2065319.	203.13	<.001
Temperature (Temp.)	4	157279.	39320.	3.87	0.004
Day	2	1574796.	787398.	77.44	<.001
Duration x Temp.	8	128058.	16007.	1.57	0.131
Duration x Day	4	379207.	94802.	9.32	<.001
Temp. x Day	8	61290.	7661.	0.75	0.644
Duration x Temp. x Day	16	137197.	8575.	0.84	0.636

ANOVA Table 3.5b ANOVA results for IEC of 'Bramley' fruit, Experiment 1c.

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Duration	2	1991735.	995867.	230.60	<.001
Temperature (Temp.)	4	31787.	7947.	1.84	0.120
Day	2	534085.	267043.	61.83	<.001
Duration x Temp.	8	46072.	5759.	1.33	0.225
Duration x Day	4	233974.	58493.	13.54	<.001
Temp. x Day	8	103725.	12966.	3.00	0.003
Duration x Temp. x Day	16	114912.	7182.	1.66	0.051

ANOVA Table 3.6a ANOVA results for firmness of 'Queen Cox' fruit, Experiment 1c.

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Duration	2	16270.77	8135.38	116.07	<.001
Temperature (Temp.)	4	982.04	245.51	3.50	0.008
Day	2	442.43	221.22	3.16	0.044
Duration x Temp.	8	1522.87	190.36	2.72	0.006
Duration x Day	4	348.78	87.19	1.24	0.292
Temp. x Day	8	329.91	41.24	0.59	0.788
Duration x Temp. x Day	16	1032.05	64.50	0.92	0.546

ANOVA Table 3.6b ANOVA results for firmness of 'Bramley' fruit, Experiment 1c.

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Duration	2	16477.28	8238.64	154.50	<.001
Temperature (Temp.)	4	1944.01	486.00	9.11	<.001
Day	2	205.82	102.91	1.93	0.147
Duration x Temp.	8	853.51	106.69	2.00	0.045
Duration x Day	4	640.60	160.15	3.00	0.018
Temp. x Day	8	465.12	58.14	1.09	0.369
Duration x Temp. x Day	16	996.23	62.26	1.17	0.291

1-MCP efficacy at different temperatures is not widely reported. Few published papers compare 1-MCP application at different temperatures; most results are from 1-MCP treatments at 1 or 20°C (Table 2.1). Where 1-MCP has been applied at different temperatures, little research has fully determined the effective range of temperatures conducive to successful 1-MCP application. In addition, even fewer report the effect of 1-MCP treatment temperature on produce stored for more than 1 or 2 weeks.

'Cavendish' banana fruit failed to exhibit a differential 1-MCP treatment-temperature effect (Macnish *et al.*, 2000). Treatment of banana fruit with 15 $\mu l\ l^{-1}$ 1-MCP at 2.4, 5, 7.5, 10, 12.5, 15 or 20°C, followed by an immediate exposure to either 0 or 100 $\mu l\ l^{-1}$ ethylene at 20°C were shown to be equally effective at delaying fruit softening, skin degreening and extending shelf-life by *ca* 1 month. Macnish *et al.* (2000) also treated grevillea 'Sylvia' (*Grevillea banksii* R. Brown x *G. whiteana* D.J. McGillivray) inflorescences and Geraldton waxflower (*Chamelaucium uncinatum* Schauer) flowers with 0.01 $\mu l\ l^{-1}$ 1-MCP for 12 h at 2 or 20°C, followed by a daily 12 h exposure to 10 $\mu l\ l^{-1}$ ethylene. 1-MCP treatment of grevillea at 2°C was ineffective at inhibiting ethylene action. However, 1-MCP treatment at 20°C did inhibit ethylene action for 2 days. Waxflowers treated with 1-MCP at 2°C were protected against ethylene action for *ca* 2 d, and for *ca* 4 d after treatment at 20°C.

DeEll *et al.* (2002) treated 'Cortland' and 'Empire' apples with 0.6 $\mu l\ l^{-1}$ 1-MCP applied at 3, 13 or 23°C, then stored in cold air (0 to 1°C) for 3 months. After this storage period, 'Cortland' apple fruit treated with 1-MCP for 9 to 24 h had a firmness of *ca* 65 N, regardless of 1-MCP treatment temperature. Similarly, all 1-MCP-treated 'Empire' apples had a firmness of >70 N after 3 to 24 h treatment. However, treatment temperature was not constant. Apples treated at 3 or 13°C were cooling from 20°C throughout treatment. Thus, it may be better to say that these 'Cortland' and 'Delicious' fruit were 1-MCP-treated over a temperature gradient. Furthermore, fruit treated for longer were cooled more (to 3°C after 24 h) than those only treated for 3 h (to 17°C).

Treatment of 'Anna' apple fruit with 1 $\mu l\ l^{-1}$ 1-MCP showed no difference in 1-MCP-response after exposure for 4, 10 or 24 h (Pre-Aymard *et al.*, 2003).

However, these fruit were treated then monitored over 12 d at 20°C; there was no storage involved.

Experiments on banana fruit (Pelayo *et al.*, 2003) showed that 1-MCP treatment at either 14°C (commercial ripening temperature of banana fruit) or 20°C gave inconsistent results. However, these workers applied a treatment of 1 $\mu l\ l^{-1}$ 1-MCP *ca* 36 to 48 h after commercial ethylene treatment. Although this was the purpose of their study, most workers agree that 1-MCP is most effective when applied before the climacteric has been initiated, and 1-MCP efficacy decreases with increasing IEC.

Differences in firmness and IEC between control and 1-MCP treatments were not significant ($P > 0.05$) (Appendix 4.3) for longer storage periods of 4 and 6 months for 'Queen Cox' and 6 months for 'Bramley'.

3.4.2 Total soluble solids

Treatment of 'Bramley' apple fruit with 1-MCP at 0, 5, 10, 15 or 20°C resulted in small increase (≤1°Brix) in TSS after 3 months storage (Table 3.3). At the extremes of application temperature: 0, 15 and 20°C, 1-MCP-treated 'Queen Cox' fruit had a small increase (≤0.7°Brix) in TSS than non-1-MCP-treated fruit. However, 1-MCP treatment at 5 and 10°C had no effect on 'Queen Cox' apple fruit TSS. Unlike Experiments 1a and 1b, there was no clear relationship between 1-MCP application and TSS.

Table 3.3 Total soluble solids (TSS °Brix) main factor means (i.e. across 0, 7 and 14 d shelf life evaluations) of Experiment 1c apple fruit treated with 1.0 µl l^{-1} 1-MCP for 24 h at different temperatures. Fruit were stored in air at 3 to 4°C for 2 months 'Queen Cox' or 3 months 'Bramley'. Data are the means for 30 individual fruit (total number of fruit = 900). LSDs (P = 0.05).

Cultivar	Treatment temperature (°C)	1-MCP concentration (µl l^{-1})		*LSD*
		0.0	**1.0**	
'Bramley'	0	10.9	11.7	
	5	10.3	11.3	
	10	10.4	11.5	
	15	10.7	11.3	
	20	10.8	11.6	*0.30*
'Queen Cox'	0	13.7	14.3	
	5	14.0	14.0	
	10	14.2	14.5	
	15	13.9	14.3	
	20	13.9	14.3	*0.38*

3.5 The effect of 1-MCP treatment on other storage factors

3.5.1 Peel colour measurement

Peel colour of 'Queen Cox' and 'Bramley' fruit was unaffected by 1-MCP treatment, regardless of 1-MCP concentration, exposure temperature or time (Appendix 4.4.3). This result is in agreement with work on 'Granny Smith' fruit treated with 1 µl l^{-1} 1-MCP, then stored in air for 0, 5 or 10°C for 3 months showed no significant differences in hue angle, L* or chroma (Eqn. 3.1) after 1-MCP treatment, regardless of storage temperature, or duration. After 6 months storage, 1-MCP-treated fruit had retained *ca* 5% L*, Hue angle and chroma compared to non-1-MCP-treated fruit, indicating that 1-MCP-treated fruit were greener (Fan *et al.*, 1999).

Equation 3.1 Chroma calculation for colour measurement.

$$chroma = \sqrt{(a^*)^2 + (b^*)^2}$$

Pre-Aymard *et al.* (2003) demonstrated a change from green towards yellow (112° to 107° hue angle) for 'Anna' apples ripened at 20°C, but showed no clear data defining a 1-MCP treatment effect. However, it was reported that 1-MCP treatment (0.01 to 1 $\mu l\ l^{-1}$) of these apples for 4 to 24 h reduced de-greening, regardless of duration, and fruit treated with 1 $\mu l\ l^{-1}$ 1-MCP remained more green than fruit from other treatments.

'Gala' apple fruit treated with 0.5 $\mu l\ l^{-1}$ 1-MCP for 12 h, then stored in air at 20°C for 3 weeks showed a reduced chroma of 42.7, as compared to 48.7 in non-1-MCP-treated fruit, and no difference in hue angle was observed, although these results were not discussed (Fan and Mattheis, 2001). However, if chroma is reduced with no change in hue angle, both a* and b* must be reduced, resulting in loss of both green and yellowness.

Some climacteric fruits that exhibit gross changes in colour during ripening (e.g. bananas from green to yellow; tomatoes from green to red) show differences in hue angle and colour as a result of 1-MCP treatment. However, this is due to 1-MCP action blocking ethylene reception, and the subsequent delay in ripening. Colour change (i.e. loss of chlorophyll and, as in banana, the subsequent unmasking of carotenoids) in fruit is mediated by ethylene-induced ripening. Consequently, differences in hue angle reported between 1-MCP-treated and non-treated fruit (at the same time after treatment) may be accounted for in this way. However, a 1-MCP-effect on colour may be observed should 1-MCP-treated fruit not attain the same colour as non-1-MCP-treated fruit at the end of the ripening period. 'Cavendish' banana fruit exhibit such a difference.

As discussed in Section 3.3.1, Jiang *et al.* (1999a) treated green 'Cavendish' banana fruit with 0.01 to 1 $\mu l\ l^{-1}$ 1-MCP, resulting in an uneven development of peel colour during ripening. These workers suggest that there are positional differences in the rate of binding-site synthesis in the peel and the pulp. Banana peel and pulp differ in both ethylene production and response to ethylene application (McGlasson, 1985; Oetiker and Yang, 1995). It may be that ethylene-feedback regulation is in someway disrupted by 1-MCP-action such that insufficient ethylene is produced to bind to all the available ethylene-binding sites, thus ripening may be localized. Moreover, it may be that 1-MCP is still available to bind to some ethylene binding sites. An alternative

suggestion is that 1-MCP-treated bananas ripen from pulp-evolved ethylene, rather than from the peel-evolved ethylene. As such, there may be insufficient ethylene available to the ethylene-receptors in the peel to cause the full colour change to yellow; but enough for the pulp to ripen, and thus for ripe 1-MCP-treated banana fruit to be no different in any other respect to non-1-MCP-treated fruit other than peel colour. However, it may be the case that loss of chlorophyll may be unaffected. Banana peel colour pigments may be broken down and/or the transition of chloroplasts to chromaplasts may become disrupted.

3.5.2 Superficial scald incidence on 'Bramley' apple fruit

1-MCP treatment reduced superficial scald development in 'Bramley' fruit after 6 months storage by *ca* 1.97 and *ca* 1.99-fold in Experiments 1a and 1b, respectively (Table 3.4). There was no development of superficial scald on 'Bramley' fruit stored for 3 months, or 'Queen Cox' fruit at any time. Superficial scald develops only in certain cultivars, and is expressed as a chilling injury during prolonged low-temperature storage, primarily on early-harvested and less-mature fruit (Watkins *et al.*, 1995).

Table 3.4 Percentage superficial scald observed after 6 months air storage at 3 to 4°C for 'Bramley' apple fruit harvested in 2000. $^{w}n = 180$; $^{x}n = 720$; $^{y}n = 150$; $^{z}n = 300$.

Experiment	Non 1-MCP-treated	1-MCP-treated
1a (1-MCP concentration)	17.2^{w}	0.6^{x}
1b (1-MCP exposure time)	31.6^{w}	0.1^{x}
1c (1-MCP exposure temperature)	0.7^{y}	1.0^{z}

Superficial scald is believed to result from the auto-oxidation of α-farnesene into conjugated trienes (CTs) and the associated formation of free radicals (Huelin and Coggiola, 1968; Anet and Coggiola, 1974; Du and Bramlage, 1994; Whitaker *et al.*, 2000).

Inhibition of scald-development by 1-MCP treatment has been shown for some North American-grown cultivars. Superficial scald development in 'Cortland' apple fruit has been shown to be reduced by 1-MCP-treatment duration, but not temperature (DeEll *et al.*, 2002); whereas 100% of non-1-MCP-treated 'Cortland' fruit developed scald, fruit treated with 0.6 $\mu l\ l^{-1}$ 1-MCP for 3 h reduced scald-development to under 1.3-fold, and to less than 1.5-fold after 6 h treatment.

'McIntosh' and 'Delicious' apple fruit treated with 1 $\mu l\ l^{-1}$ 1-MCP and stored for *ca* 4 months showed less scald with increased 1-MCP concentration (0.01 to 100 $\mu l\ l^{-1}$), with a concurrent decrease of α–farnesene levels (229 and 766 to 49 and 10 $\mu g\ g\ FW^{-1}$, respectively). Similarly, CT levels were shown to decrease with 1-MCP concentration (Rupasinghe *et al.*, 2000).

Fan *et al.* (1999) treated 'Granny Smith', 'Red Chief Delicious' and 'Fuji' apple fruit with 1 $\mu l\ l^{-1}$ 1-MCP for 12 h at 20°C, then stored in air at 0°C. 'Granny Smith' fruit 1-MCP-treated at 0, 5 or 10°C and stored for 3, 4, 6 or 8 months exhibited no scald, whereas non-1-MCP-treated fruit showed scald coverage ranging from <33 to 66% of the surface area. α-Farnesene levels in 'Granny Smith' fruit stored for 4 or 8 months was unaffected by 1-MCP treatment at either 1 or 7 d shelf-life at 20°C. However, levels of MHO (6-methyl-5-hepten-2-one; the major oxidation product of α-farnesene) and CTs were found to be lower in 1-MCP-treated fruit at 1 or 7 d shelf life after 4 or 8 months storage. No scald developed in 1-MCP-treated 'Red Chief Delicious' fruit after 3 or 6 months storage and 1 or 7 d shelf-life; non-1-MCP-treated fruit developed scald after 6 months storage. MHO levels were also lower in 1-MCP-treated 'Red Chief Delicious' fruit. However, 'Fuji' fruit exhibited no scald, but produced MHO. This indicated that MHO production alone does not cause scald development. Rather, CT production was suggested as the major correlation with scald development, in keeping with the suggestion that α-farnesene oxidation products have a role in scald development (Huelin and Coggiola, 1968).

During storage, CTs accumulate progressively on the surface of apples as α-farnesene oxidises, resulting in cell death in the outermost layers. The concentration of CTs has a greater correlation with superficial scald severity than α-farnesene (Huelin and Coggiola, 1970; Rupasinghe *et al.*, 1998). Diphenylamine (DPA), an antioxidant that reduces the oxidation of α–farnesene to CTs, and been

shown to suppress ethylene production, and is used commercially to prevent scald (Fan *et al.*, 1999).

The role of ethylene in scald development is unclear. However, if ethylene levels remain low, α-farnesene levels remain low (Du and Bramlage, 1994). Ethylene appears to mediate α-farnesene production. α–Farnesene levels were undetectable in 'Granny Smith' and 'Delicious' apples with an IEC of <1 $\mu l\ l^{-1}$. Application of 200 $mg\ l^{-1}$ aminoethoxyvinylglycine (AVG; an inhibitor of ACC synthase, and therefore of ethylene production) inhibited both IEC and α–farnesene production. Similarly, application of 200 $mg\ l^{-1}$ ethephon increased IEC and α–farnesene production (Ju and Curry, 2000). Furthermore, 'Granny Smith' apple fruit developed less scald after storage when treated with the ethylene action inhibitor diazocyclopentadiene (DACP) at harvest (Gong and Tian, 1998; Fan *et al.*, 1999).

1-MCP-treatment of 'Bramley' fruit suppresses IEC (Figs. 3.3b, 3.6b). As such, α–farnesene production may be reduced in earlier harvested fruit (Experiments 1a, b; Table 3.2), and therefore less CTs and associated free radicals would be formed. Consequently, less scald develops in 1-MCP-treated fruit (Table 3.4).

Scald incidence was *ca* 1% in Experiment 1c, regardless of 1-MCP treatment. This overall reduction in observed scald in Experiment 1c, as compared with Experiments 1a and 1b, may have been due to the later harvest of these fruit (Table 3.2). A similar fruit maturity effect on scald development has been reported for 'Cortland', 'Delicious', 'Granny Smith' and 'Crofton' (Huelin and Coggiola, 1968; Watkins *et al.*, 1993).

3.5.3 Observed disease incidence on 'Queen Cox' and 'Bramley' apple fruit

Diseases observed during storage were *Botrytis cinerea, Monilinia fructigena, Nectria galligena, Penicillium expansum and Phytopthera* spp. Observed disease incidence (ODI) was low in both 'Queen Cox' and 'Bramley' fruit following storage for 2 and 3 months, respectively, regardless of 1-MCP treatment (Appendix 4.4.2). However, after 6 months storage, disease incidence in 'Bramley' was reduced by 1-MCP application regardless of 1-MCP concentration, exposure time or exposure

temperature (Table 3.5). The difference in 1-MCP treatment on ODI was less for Experiment 1a (*ca* 2%) than for Experiments 1b (*ca* 9%) and 1c (*ca* 28%).

Table 3.5 Percentage observed disease incidence after 6 months air storage at 3 to 4°C for 'Bramley' apple fruit harvested in 2000. $^{w}n = 180$; $^{x}n = 720$; $^{y}n = 150$; $^{z}n = 300$.

Experiment	Non 1-MCP-treated	1-MCP-treated
1a (1-MCP concentration)	3.9^{w}	1.7^{z}
1b (1-MCP exposure time)	10.0^{w}	0.8^{x}
1c (1-MCP exposure temperature)	32.7^{y}	4.3^{z}

Similarly, after 4 months storage, overall ODI in 'Queen Cox' was reduced after 1-MCP treatment (Table 3.6).

Table 3.6 Percentage observed disease incidence after 4 months air storage at 3 to 4°C for 'Queen Cox' apple fruit harvested in 2000. $^{u}n = 240$, $^{v}n = 960$, $^{w}n = 120$; $^{x}n = 480$; $^{y}n = 180$; $^{z}n = 720$.

Experiment	Non 1-MCP-treated	1-MCP-treated
1a (1-MCP concentration)	2.9^{u}	4.2^{v}
1b (1-MCP exposure time)	6.7^{w}	0.8^{x}
1c (1-MCP exposure temperature)	13.3^{y}	7.2^{z}

As with 'Bramley' fruit, later-harvested non-1-MCP treated 'Queen Cox' fruit had greater ODI than earlier harvested fruit. However, the efficacy of 1-MCP treatment to reduce ODI was less for 'Queen Cox' than for 'Bramley'; ODI was reduced by 6% after 1-MCP-treatment (Experiments 1b, c). There was no decrease in ODI in Experiment 1a; conversely, these earlier-picked fruit showed more ODI after 1-MCP treatment than without. However, the difference between ODI was *ca* 1%, compared to *ca* 2% difference for 'Bramley'.

In both 'Bramley' and 'Queen Cox' fruit, proportionally greater ODI in non-1-MCP treated fruit was observed with lateness of harvest date. This tendency for more observed disease in later-picked fruit may have been a feature of decreasing

inherent disease resistance in late-harvested fruit. Immature apple fruit are relatively resistant to diseases, and subsequently lose this resistance during maturation and ripening (Ndubizu, 1976).

Reduced decay in association with 1-MCP treatment of 'Bramley' apple fruit contrasts some other 1-MCP studies. For avocado, custard apple, mango, orange, papaya and strawberry fruit, certain 1-MCP treatments enhanced disease compared to untreated controls (Ku *et al.*, 1999; Porat *et al.*, 1999; Hofman *et al.*, 2001; Jiang *et al.*, 2001).

3.6 Conclusion

In summary, 1-MCP enhanced storage quality of 'Queen Cox' and 'Bramley' apple fruit. Fruit firmness was better retained, storage disease incidence was reduced, and, for 'Bramley' fruit, superficial scald was inhibited. 1-MCP treatments ranging between 0.1 to 10 $\mu l\ l^{-1}$ applied for 6 to 48 h at 0 to 20°C were equally effective, although treatment of 'Queen Cox' fruit at lower temperatures may give additional benefit (lower IEC, greater firmness and higher TSS) than treatment at higher temperatures.

Chapter 4 RESULTS AND DISCUSSION

EXPERIMENT 2

EFFECT OF HARVEST MATURITY, CA STORAGE, TREATMENT DELAY AND FUNGICIDE APPLICATION ON 1-MCP TREATMENT

4.1 Introduction

Data acquired from both Experiment 1 and other sources such as further storage tests (one in the UK and the rest in mainland Europe), toxicity trials and residue analysis allowed AgroFresh to determine that 1-MCP was a viable apple storage management tool, and that the application concentration should be 0.6 $\mu l\ l^{-1}$ 1-MCP (G.Regiroli, AgroFresh Inc, 2001).

Results obtained from Experiment 1 also highlighted areas of further investigation. Initially, the improved storability of 'Queen Cox' and 'Bramley' apple fruit after a range of 1-MCP-treatments were comparative within an air-only storage system. Commercial apple storage systems use controlled atmosphere to maintain apple quality, lowering O_2 and raising CO_2 to best suit particular cultivars. An experiment was designed to assess the benefits of a 1-MCP-treatment of 0.6 $\mu l\ l^{-1}$ prior to CA storage.

In addition, there were observed differences in response to 1-MCP-treatment from those fruit harvested at the beginning of the trial to those harvested at the end of the trial. As such, Experiment 2 considered the effect of picking date on the efficacy of 1-MCP-treatment.

Furthermore, little or no effect was observed after 1-MCP-treatment on apple peel colour as determined by hue angle. As such, it was decided that this parameter was not to be investigated during Experiment 2. Instead, the observed increase in storability of 1-MCP-treated apple fruit raised questions about the differences in stress

levels between fruit of low storage-quality and the higher quality 1-MCP-treated fruit. Chlorophyll fluorescence has been used to detect stresses in plant systems. Chlorophyll *a* florescence *in-vivo* is emitted during photosystem II (PS II). Chlorophyll *a* florescence emission may be separated into two components. The first or 'constant' fluorescence (F_o) is a fast rising response to applied visible light, occurring within 1 to 2 ms. The second component is maximum emission (F_m), which follows after 1 to 2 s. The temporary rise in chlorophyll *a* fluorescence ($F_m - F_o$) is the variable florescence (F_v). Changes in F_v should be regarded as direct indicators of the properties of excitation and energy conversion at PS II. However, PS II is intimately linked to other components of the photosynthetic apparatus. A wide range of environmental, chemical and biological stresses influence photosynthetic metabolism, and thus F_v may be used as an indicator of the entire photosynthetic process as a response to stress (Schreiber and Bilger, 1985; Smillie and Hetherington, 1990). Visible light affects photosynthesis. Chlorophyll florescence occurs all the time and samples being measured are exposed to saturating levels of illumination after a period of dark-adaptation to accurately monitor the time of fluorescence induction of a sample. Dark-adaptation is the state where tissue has been kept away from light. After a dark period, the electron transport pathway of PSII is open since Q_A, the primary electron acceptor of PSII, is fully oxidised. In dark-adapted plant tissues, fluorescence is minimal (F_0). If continuous illumination is started, chlorophyll fluorescence temporarily achieves a maximum (F_m) due to Q_A reduction (Krause and Weis, 1991). The time taken for dark-adaptation may vary from minutes following exposure to low photo flux densities to several hours after exposure to prolonged sunlight (Smillie and Hetherington, 1990). However, modern equipment, particularly for field use, overcomes this by detecting modulated fluorescence from chlorophyll excited by low intensity pulsed light.

Disturbances at the cellular level, such as mechanical injury or other stresses may be detected by associated changes in chlorophyll fluorescence (Smillie & Hetherington, 1990). Currently, most quality assessments of fruits and vegetables are destructive. Thus, a demand exists for rapid, cost-effective, non-destructive quality assessment (Watada, 1989). Chlorophyll florescence may not be able to quantify such quality parameters as sugar and acid content, firmness or ethylene production;

however, it may be used to measure the underlying condition of the fruit as expressed as the measured level of available photosynthetic activity (Toivonen, 1992). Freshness, as defined as 'any deterioration or decline of tissue from a freshly harvested state', is an important component of quality and early changes in respiration, ethylene production, vitamin C, chlorophyll content and many other characteristics all contribute to loss of freshness and hence to further deterioration of the produce (Toivonen, 1992). To correlate Fv/Fm decline to decline in quality after harvest with freshness or loss of quality from harvest requires considerable time, expertise and equipment. F_v/F_m has been used to assess the quality of broccoli (Toivonen, 1992), banana, mango (Smillie *et al.*, 1987), apple (Mir *et al.*, 2001) and kangaroo paw flowers (Miranda *et al.*, 2000).

Delay before 1-MCP application was also investigated. From a practical and commercial point of view, it may take a while to fill an apple store before treatment can commence. As such, the first fruit placed inside the store may have been harvested for more than 24 h before treatment can occur. Experiment 2 investigated three delay times of 1, 7 or 14 d between harvest and 1-MCP-treatment.

The final area of investigation based on the results from Experiment 1 was the reduced incidence of storage rot development in 1-MCP-treated fruit, particularly in those harvested on the last picking dates. The effect of 1-MCP-treatment against commercial fungicide-treatment to reduce storage rot incidence was investigated in late-harvested fruit.

4.2 Experiment 2a: Early harvest - CA storage

4.2.1 Fruit firmness and IEC

Treatment of 'Queen Cox' with 0.6 $\mu l\ l^{-1}$ 1-MCP applied within 24 h of harvest at 3 to 4°C maintained fruit firmness (Fig. 4.1) and suppressed IEC (Fig. 4.2) for 2 months storage under CA or cold air storage. However, after 7 d shelf-life, the IEC observed in non-1-MCP-treated CA stored fruit increased to >350 $\mu l\ l^{-1}$ (Fig. 4.2 B), whereas 1-MCP-treated fruit IEC remained suppressed. Furthermore, other non-1-MCP-treated fruit were observed to have increased IEC by *ca* 200 $\mu l\ l^{-1}$. After 4 months storage, 1-MCP-treated fruit showed suppressed IEC (<50 $\mu l\ l^{-1}$) 1 d after

removal from storage (Fig. 4.2 A). After 7 d shelf-life 1-MCP-treated fruit showed increased IEC to between 100 to 200 μl l^{-1} (Fig. 4.2 B). Furthermore, the IEC of all non-1-MCP-treated 'Queen Cox' fruit doubled to >300 N.

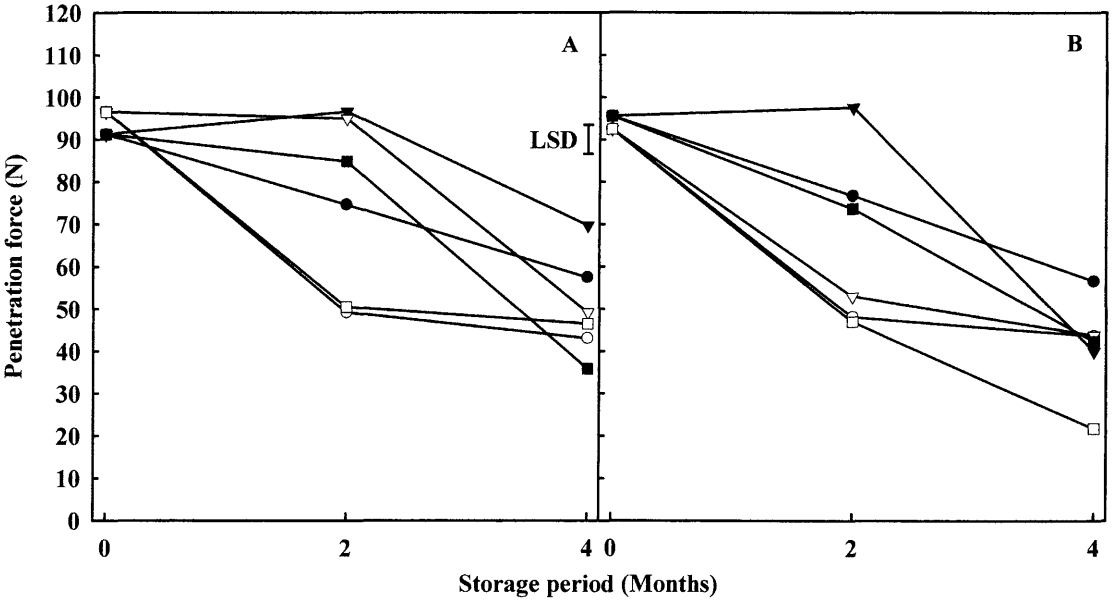

Figure 4.1 Fruit firmness (penetration force, N) for Experiment 2a 'Queen Cox' apple fruit treated with 1.0 μl l^{-1} 1-MCP (closed symbols) and stored in air (●,○), controlled atmosphere (CA, <1% CO_2, 1.2% O_2) (▼,▽) or atmospheric conditions, controlled by the CA system (■, □). Fruit were stored for 2 or 4 months before air storage at 20°C for 1 (A) and 7 d (B). Data are the means for 10 individual fruit (total number of fruit (n) = 540). LSD (P = 0.05).

'Queen Cox' fruit removed after storage for 2 months showed unchanged levels of firmness after CA storage, regardless of 1-MCP-treatment (Fig. 4.1 A). Firmness was also retained by non-CA stored 1-MCP-treated fruit. Non-1-MCP-treated fruit either stored in air or under atmospheric air concentrations inside CA chambers showed significant (P = 0.05) loss in firmness from *ca* 90 N to *ca* 50 N. After 7 d shelf-life (Fig. 4.1 B), firmness was similar to that for 1 d, except for the non-1-MCP-treated, CA stored fruit; in this case, firmness was observed to drop *ca* 50 N, from *ca* 95 to 55 N.

After 4 months storage, the storage-treatment effects become less clear, although there was still a delay in the onset of the climacteric. However, on removal

from storage (Fig. 4.1 A), the combined effects of 1-MCP-treatment and CA storage resulted in the greatest retention of firmness, followed by 1-MCP-treatment with subsequent air-storage. 1-MCP-treatment combined with storage under atmospheric conditions in a CA chamber resulted in a loss of initial harvest firmness of *ca* 65 N. After 7 d shelf-life (Fig. 4.1B), 1-MCP-treated fruit stored in air was shown to maintain a firmness of *ca* 60 N, similar to 1 d. Fruit exposed to 1-MCP then stored under CA conditions showed a drop in firmness from *ca* 70 to 40 N between removal from storage and 7 d shelf life. Fruit stored under atmospheric conditions in CA chambers without a 1-MCP-treatment were observed to have lost the most firmness, from *ca* 95 N at harvest to *ca* 25 N after 4 months storage, and 7 d shelf-life.

IEC was suppressed in 'Queen Cox' apple fruit treated with 1-MCP, and / or stored under CA conditions for 2 months, observed 1 d after removal from storage (Fig. 4.2 A).

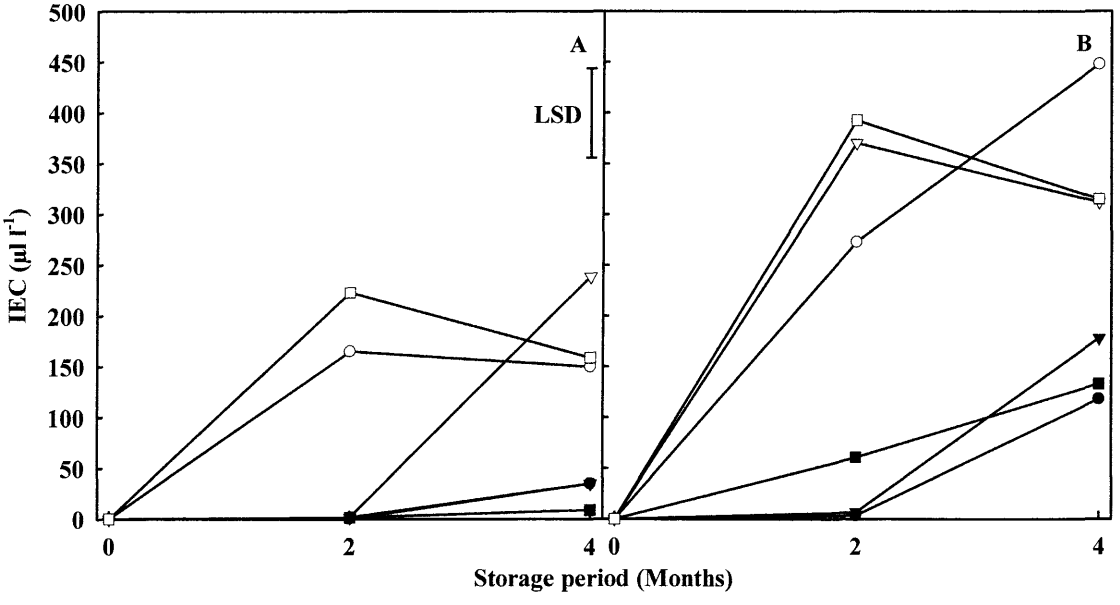

Figure 4.2 Fruit internal ethylene concentration (IEC, µl l^{-1}) for Experiment 2a 'Queen Cox' apple fruit treated with 1.0 µl l^{-1} 1-MCP (closed symbols) and stored in air (●,○), controlled atmosphere (CA, <1% CO_2, 1.2% O_2) (▼,▽) or atmospheric conditions, controlled by the CA system (■, □). Fruit were stored for 2 or 4 months before air storage at 20°C for 1 (A) and 7 d (B). Data are the means for 10 individual fruit (total number of fruit (n) = 540). LSD (P = 0.05).

However, after 7 d shelf-life, the IEC observed in non-1-MCP-treated CA stored fruit increased to >350 $\mu l\ l^{-1}$ (Fig. 4.2 B), whereas 1-MCP-treated fruit IEC remained suppressed. Furthermore, other non-1-MCP-treated fruit were observed to have increased IEC by *ca* 200 $\mu l\ l^{-1}$. After 4 months storage, 1-MCP-treated fruit showed suppressed IEC (<50 $\mu l\ l^{-1}$) 1 d after removal from storage (Fig. 4.2 A). After 7 d shelf-life 1-MCP-treated fruit showed increased IEC to between 100 to 200 $\mu l\ l^{-1}$ (Fig. 4.2 B). Furthermore, the IEC of all non-1-MCP-treated 'Queen Cox' fruit doubled to >300 N. The 'Queen Cox' IEC and firmness ANOVA results are shown in ANOVA Tables 4.1 and 4.2, respectively.

Similarly, treatment of 'Bramley' with 0.6 $\mu l\ l^{-1}$ 1-MCP applied within 24 h of harvest at 3 to 4°C maintained fruit firmness (Fig 4.3) and suppressed IEC (Fig 4.4) for 2 months storage under CA or cold air storage.

There were no additional benefits, in terms of maintenance of fruit firmness or IEC suppression, of applying 1-MCP to 'Bramley' fruit subsequently stored under CA conditions. However, 1-MCP-treatment alone gave similar results to CA storage alone for 3 months storage. After 6 months storage, 1-MCP-treatment effect lessened.

As observed in the 'Queen Cox' experiment, storage of fruit under atmospheric conditions inside CA chambers showed unexplained variances in both firmness and IEC, regardless of 1-MCP-treatment.

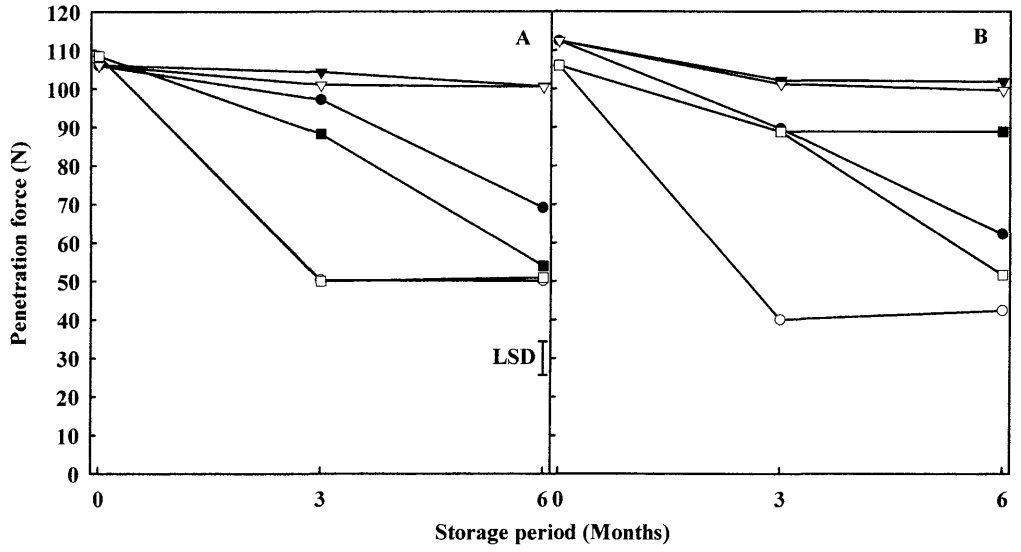

Figure 4.3 Fruit firmness (penetration force, N) for Experiment 2a 'Bramley' apple fruit treated with 1.0 µl l^{-1} 1-MCP (closed symbols) and stored in air (●,○), controlled atmosphere (CA, 5% CO_2, 1% O_2) (▼,▽) or atmospheric conditions, controlled by the CA system (■, □). Fruit were stored for 2 or 4 months before air storage at 20°C for 1 (A) and 7 d (B). Data are the means for 10 individual fruit (total number of fruit (n) = 540). LSD (P = 0.05).

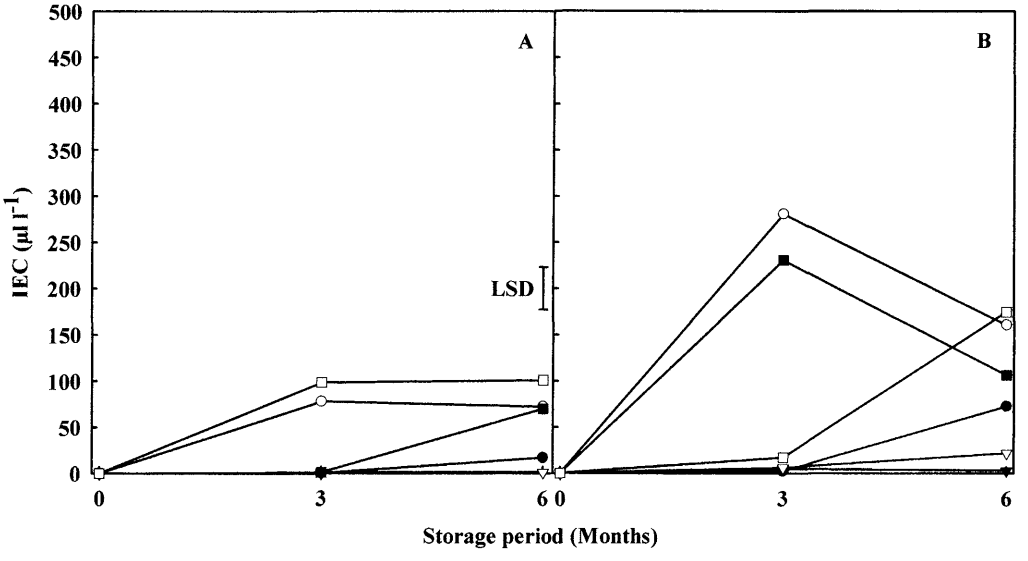

Figure 4.4 Fruit internal ethylene concentration (IEC, µl l^{-1}) for Experiment 2a 'Bramley' apple fruit treated with 1.0 µl l^{-1} 1-MCP (closed symbols) and stored in air (●,○), controlled atmosphere (CA, 5% CO_2, 1% O_2) (▼,▽) or atmospheric conditions, controlled by the CA system (■, □). Fruit were stored for 2 or 4 months before air storage at 20°C for 1 (A) and 7 d (B). Data are the means for 10 individual fruit (total number of fruit (n) = 540). LSD (P = 0.05).

ANOVA Table 4.1a ANOVA results for IEC of 'Queen Cox' fruit, Experiment 2a.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1618934.	323787.	32.21	<.001
Shelf life	1	727853.	727853.	72.41	<.001
Storage	2	2149149.	1074574.	106.90	<.001
Treatment x Shelf life	5	139409.	27882.	2.77	0.018
Treatment x Storage	10	1021010.	102101.	10.16	<.001
Shelf life x Storage	2	387171.	193586.	19.26	<.001
Treatment x Shelf life x Storage	10	448816.	44882.	4.46	<.001

ANOVA Table 4.2a ANOVA results for firmness of 'Queen Cox' fruit, Experiment 2a.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	17714.78	3542.96	58.06	<.001
Shelf life	1	3241.80	3241.80	53.12	<.001
Storage	2	138966.31	69483.16	1138.62	<.001
Treatment x Shelf life	5	3241.22	648.24	10.62	<.001
Treatment x Storage	10	27921.22	2792.12	45.75	<.001
Shelf life x Storage	2	1645.80	822.90	13.48	<.001
Treatment x Shelf life x Storage	10	7574.57	757.46	12.41	<.001

'Queen Cox' fruit treated with 1-MCP were shown to have maintained photosynthetic activity (Table 4.1). The initial relative fluorescence ratio (F_v/F_m) was measured as 0.85 in early harvested fruit, similar to the published figure of *ca* 0.83 given as the typical ratio for healthy tissue (Krause and Weis, 1991; Miranda *et al.*, 2000). After 2 months storage, There was no significant difference (P = 0.05) between F_m, the maximum chlorophyll florescence, between 1-MCP-treated fruit stored under CA conditions or cold-air storage. Furthermore, there was no difference between CA only storage and 1-MCP-treatment only. F_o was also highest in 1-MCP-treated fruit. F_v/F_m was shown to drop from 0.85 to within 0.78 to 0.81 with no significant difference (P = 0.05) between 1-MCP-treatment and / or CA storage.

After 4 months storage, the variation between the individual components, F_m, F_o and F_v was higher, and FM was observed to be highest in non-1-MCP-treated, CA-stored fruit. There were no observed differences between F_m in 1-MCP treatment regardless of CA storage, or non-1-MCP-treated cold-air stored fruit. F_v/F_m values were shown to be highest in 1-MCP-treated, CA-stored fruit, followed by CA storage alone. 1-MCP was not shown to maintain F_v/F_m in cold-air storage.

This would suggest that 1-MCP applied to 'Queen Cox' fruit prior to CA storage has a beneficial effect of reducing stress levels during storage. Miranda *et al.* (2000) demonstrated a loss in F_v/F_m as a stress indicator during chilling injury and

senescence of Kangaroo paw flowers; similar findings have been demonstrated in broccoli (Toivonen, 1992).

Results obtained for early harvested 'Bramley' fruit were less clear (Table 4.2), with little correlation between F_m with 1-MCP-treatment and CA storage at 3 months, although the highest F_v/F_m were observed for CA-stored fruit. After 6 months, however, F_m and F_v/F_m were highest after CA-storage than air-storage, with no observed 1-MCP effect.

Table 4.1 Chlorophyll fluorescence values for Early harvest 'Queen Cox' apple fruit treated with 0.65 µl l^{-1} 1-MCP for 24 h at 3 to 4°C, within 24 h of harvest. Fruit were stored at 3 to 4°C in air, or under <1% CO_2, 1.2% O_2, or as atmospheric conditions in the chambers for 0, 2 or 4 months. n/s, non-significant at P = 0.05. Number of replicates (n) = 20.

Treatment and storage	**Chlorophyll fluorescence value after storage period (months)**											
	F_m			**F_o**			**F_v**			**F_v / F_m**		
	0	**2**	**4**	**0**	**2**	**4**	**0**	**2**	**4**	**0**	**2**	**4**
Control, air storage	1367	964	978	204.5	211.9	329.5	1163	752	648	0.850	0.770	0.650
1-MCP, air storage	1432	1171	860	213.4	252.6	316.3	1218	917	544	0.850	0.780	0.616
Control, CA storage	-	1090	1180	-	202.5	331.8	-	887	856	-	0.809	0.690
1-MCP, CA storage	-	1238	890	-	233.4	254.5	-	1005	635	-	0.811	0.709
Control, CA atmospheric conditions storage	-	806	644	-	199.9	330.3	-	607	313	-	0.748	0.480
1-MCP, CA atmospheric conditions storage	-	963	386	-	223.7	197.1	-	723	189	-	0.757	0.433
LSD (P = 0.05) for treatment per storage	*n/s*	*116*	*140.5*	*n/s*	*19.5*	*44.3*	*n/s*	*106*	*115*	*n/s*	*0.024*	*0.005*
LSD (P = 0.05) for all storage		*118*			*28.8*			*103*			*0.034*	

Table 4.2 Chlorophyll fluorescence values for Early harvest 'Bramley' apple fruit treated with 0.65 µl l^{-1} 1-MCP for 24 h at 3 to 4°C, within 24 h of harvest. Fruit were stored at 3 to 4°C in air, or under 5% CO_2, 1% O_2, or as atmospheric conditions in the chambers for 0, 3 or 6 months. n/s, non-significant at P = 0.05. Number of replicates (n) = 20.

Treatment and storage	**Chlorophyll fluorescence value after storage period (months)**											
	F_m			**F_o**			**F_v**			**F_v / F_m**		
	0	**3**	**6**	**0**	**3**	**6**	**0**	**3**	**6**	**0**	**3**	**6**
Control, air storage	1512	1914	1586	240.9	386.5	580.2	1272	1510	1004	0.840	0.787	0.596
1-MCP, air storage	1517	1683	1235	237.1	408.4	612.2	1280	1275	622	0.843	0.751	0.476
Control, CA storage	-	1728	1890	-	321.5	412.7	-	1406	1477	-	0.810	0.782
1-MCP, CA storage	-	1763	1790	-	345.5	402.8	-	1417	1387	-	0.801	0.753
Control, CA atmospheric conditions storage	-	1756	1537	-	398.9	695.4	-	1352	842	-	0.765	0.518
1-MCP, CA atmospheric conditions storage	-	1520	1019	-	407.3	634.9	-	1113	383	-	0.722	0.346
LSD (P = 0.05) for treatment per storage	*n/s*	*189*	*263*	*n/s*	*44.3*	*96.1*	*n/s*	*179.0*	*239.7*	*n/s*	*0.030*	*0.087*
LSD (P = 0.05) for all storage		*195*			*61.1*			*180*			*0.053*	

4.3 Experiment 2b: Mid harvest - delay of 1-MCP application

1-MCP-treated 'Queen Cox' (Fig. 4.5 A) apple fruit showed retention of fruit firmness >15 N after 2 months cold-air storage and up to 7 d subsequent shelf-life at 20°C when treated within 24 of harvest.

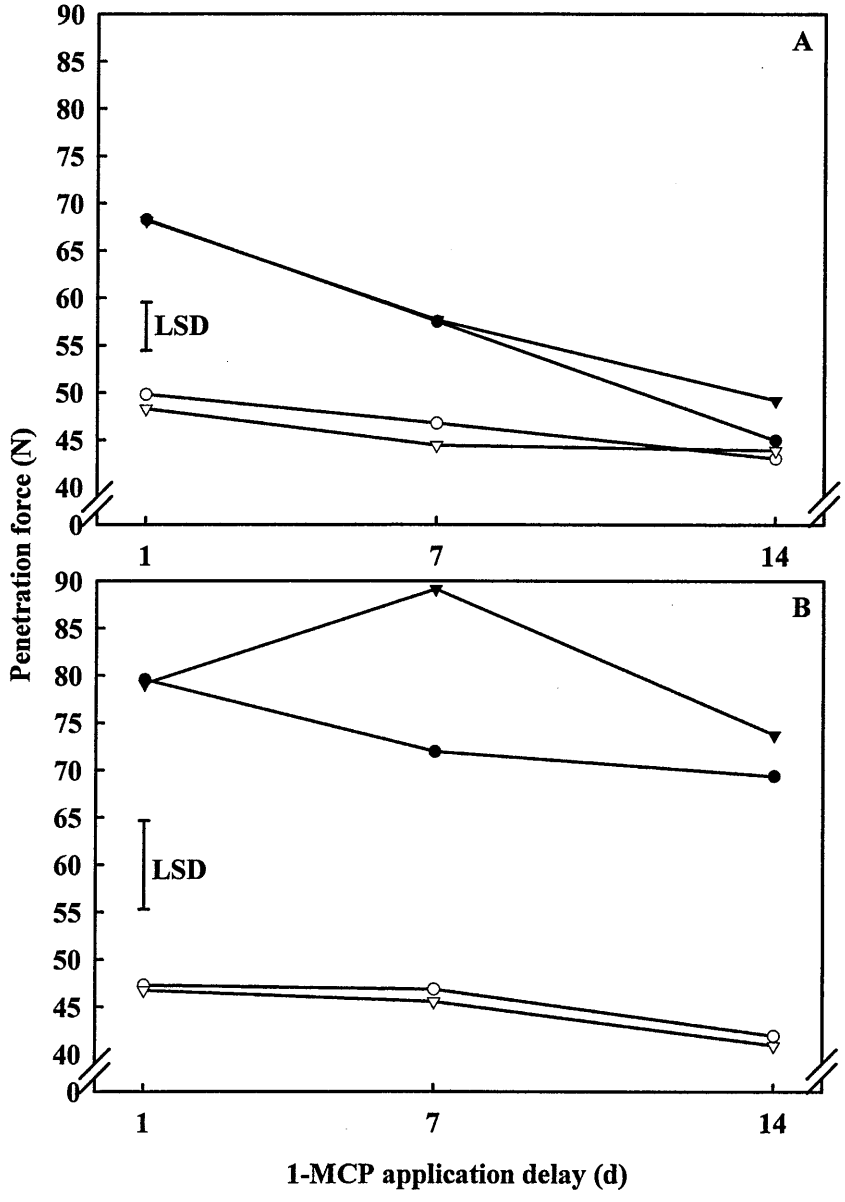

Figure 4.5 Fruit firmness (penetration force, N) of 'Queen Cox' (A) and 'Bramley' (B) apple fruit treated with 0.6 µl l^{-1} 1-MCP (closed symbols) after 2 months cold air storage (3 to 4°C) and stored in air at 20°C for 1 (●,○) or 7 (▼,▽) d after removal from storage. Data are the grand means for 10 individual fruit (total number of fruit (n) = 240). LSD (P = 0.05).

Delaying 1-MCP-treatment of mid-harvest 'Queen Cox' by 7 d from picking date was also resulted in greater firmness retention compared to non-treatment, but the firmness was *ca* 10 N less than those fruit exposed to 1-MCP within 24 h of harvest. However, leaving 14 d between harvest and 1-MCP-treatment resulted in no firmness retention.

'Bramley' fruit showed a greater response to 1-MCP-treatment (Fig. 4.5 B). 'Bramley' fruit treated with 1-MCP were shown to have no significant loss of firmness after 3 months cold-air storage, as a result of delaying 1-MCP application by 1, 7 or 14 d after harvest, remaining >20 N firmer than non-1-MCP-treated 'Bramley' fruit.

Measurement of IEC mirrored the firmness result for 'Queen Cox' (Fig. 4.6 A) and 'Bramley' fruit (Fig. 4.6 B). 'Queen Cox' fruit treated with 1-MCP with 24 h of harvest showed no increase in IEC. There was less suppression of IEC after 7 d delay between harvest and 1-MCP-treatment, and less again after 14 d delay. 'However, after 7 d shelf-life, there was no suppression of IEC for the 14 d delayed fruit.

'Bramley' fruit showed suppressed IEC when exposed to 1-MCP 1, 7 or 14 d after harvest, or 1 or 7 d shelf life.

The IEC ANOVA results are shown in ANOVA Tables 4.3a ('Queen Cox') and 4.3b ('Bramley'). The firmness ANOVA results are shown in ANOVA Tables 4.3a ('Queen Cox') and 4.3b ('Bramley').

There was no significant difference ($P = 0.05$) in chlorophyll florescence regardless of 1-MCP-treatment or delay of application for mid-harvested 'Queen Cox' (Table 4.3) or 'Bramley' (Table 4.4) apple fruit. However, data suggests that 1-MCP-treated 'Queen Cox' and 'Bramley' fruit generally had higher chlorophyll fluorescence than non-treated fruit. Furthermore, storage of the fruit before 1-MCP-treatment showed reduced chlorophyll fluorescence at the time of application, as compared to harvest.

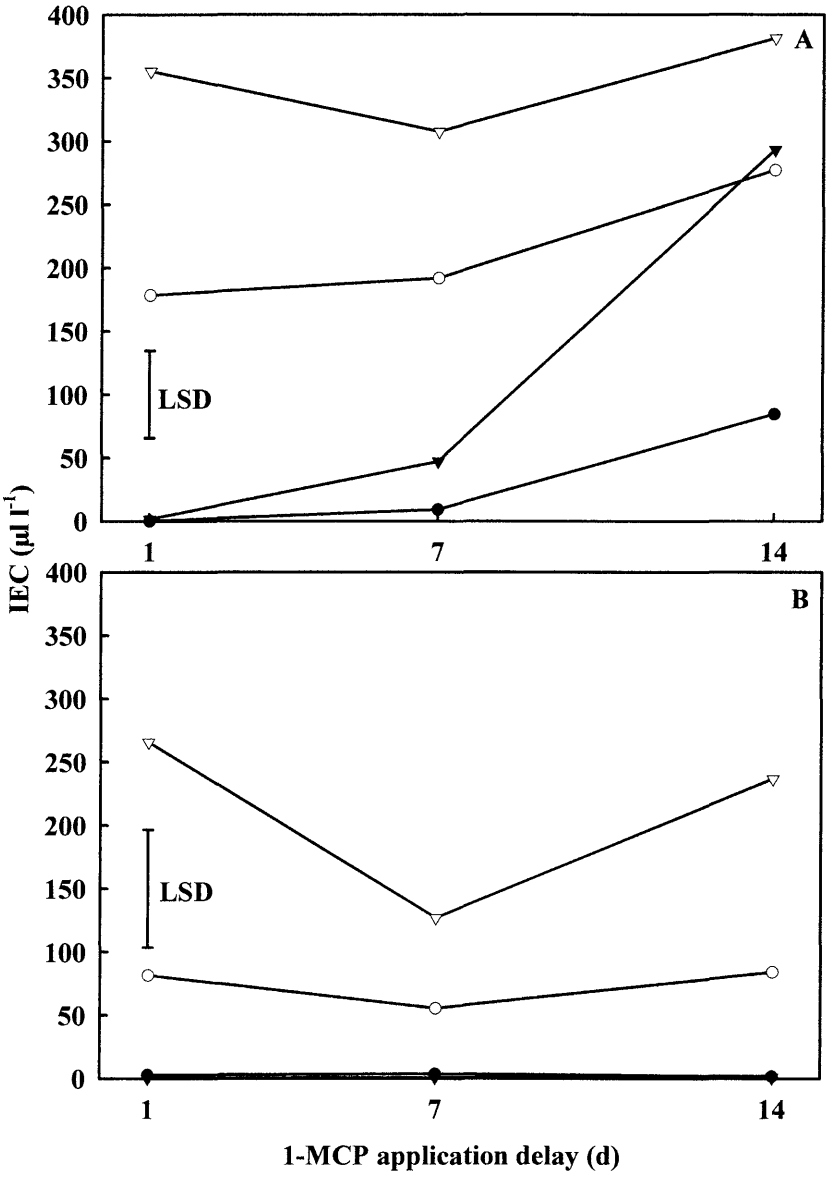

Figure 4.6 Fruit internal ethylene concentration (IEC, µl l^{-1}) of 'Queen Cox' (A) and 'Bramley' (B) apple fruit treated with 0.6 µl l^{-1} 1-MCP (closed symbols) after 2 months cold air storage (3 to 4°C) and stored in air at 20°C for 1 (●,○) or 7 (▼,▽) d after removal from storage. Data are the grand means for 10 individual fruit (total number of fruit (n) = 240). LSD (P = 0.05).

ANOVA Table 4.3a ANOVA results for IEC of 'Queen Cox' fruit, Experiment 2b.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	1	1692988.	1692988.	147.77	<.001
Delay	2	795498.	397749.	34.72	<.001
Storage time	2	2410790.	1205395.	105.21	<.001
Shelf life	1	1574112.	1574112.	137.39	<.001
Treatment x Delay	2	91904.	45952.	4.01	0.019
Treatment x Storage	2	235480.	117740.	10.28	<.001
Delay x Storage	4	176297.	44074.	3.85	0.005
Treatment x Shelf life	1	67818.	67818.	5.92	0.016
Delay x Shelf life	2	92630.	46315.	4.04	0.018
Storage x Shelf life	2	205557.	102778.	8.97	<.001
Treatment x Delay x Storage	4	330006.	82501.	7.20	<.001
Treatment x Delay x Shelf life	2	42784.	21392.	1.87	0.156
Treatment x Storage x Shelf life	2	147925.	73962.	6.46	0.002
Delay x Storage x Shelf life	4	66499.	16625.	1.45	0.217
Treatment x Delay x Storage x Shelf life	4	211556.	52889.	4.62	0.001

ANOVA Table 4.3b ANOVA results for IEC of 'Bramley' fruit, Experiment 2b.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	1	727059.	727059.	83.91	<.001
Delay	2	28319.	14160.	1.63	0.197
Shelf life	1	361602.	361602.	41.73	<.001
Storage time	2	953990.	476995.	55.05	<.001
Treatment x Delay	2	16833.	8416.	0.97	0.380
Treatment x Shelf life	1	129905.	129905.	14.99	<.001
Delay x Shelf life	2	4379.	2189.	0.25	0.777
Treatment x Storage	2	331667.	165833.	19.14	<.001
Delay x Storage	4	65049.	16262.	1.88	0.114
Storage x Shelf life	2	196079.	98039.	11.31	<.001
Treatment x Delay x Shelf life	2	11020.	5510.	0.64	0.530
Treatment x Delay x Storage	4	35873.	8968.	1.04	0.389
Treatment x Storage x Shelf life	2	62709.	31355.	3.62	0.028
Delay x Storage x Shelf life	4	20494.	5124.	0.59	0.669
Treatment x Delay x Storage x Shelf life	4	10142.	2535.	0.29	0.883

ANOVA Table 4.4a ANOVA results for firmness of 'Queen Cox' fruit, Experiment 2b.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	1	5531.12	5531.12	105.52	<.001
Delay	2	13611.48	6805.74	129.83	<.001
Storage time	2	69073.42	34536.71	658.85	<.001
Shelf life	1	2548.81	2548.81	48.62	<.001
Treatment x Delay	2	1574.37	787.18	15.02	<.001
Treatment x Storage	2	823.28	411.64	7.85	<.001
Delay x Storage	4	4099.37	1024.84	19.55	<.001
Treatment x Shelf life	1	568.77	568.77	10.85	0.001
Delay x Shelf life	2	698.78	349.39	6.67	0.001
Storage x Shelf life	2	3942.37	1971.18	37.60	<.001
Treatment x Delay x Storage	4	1420.37	355.09	6.77	<.001
Treatment x Delay x Shelf life	2	320.80	160.40	3.06	0.048
Treatment x Storage x Shelf life	2	1779.05	889.53	16.97	<.001
Delay x Storage x Shelf life	4	1859.46	464.87	8.87	<.001
Treatment x Delay x Storage x Shelf life	4	325.30	81.33	1.55	0.187

ANOVA Table 4.4b ANOVA results for firmness of 'Bramley' fruit, Experiment 2b.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	1	165033.	165033.	2.72	0.100
Delay	2	101599.	50800.	0.84	0.434
Shelf life	1	56363.	56363.	0.93	0.336
Storage time	2	564072.	282036.	4.64	0.010
Treatment x Delay	2	117362.	58681.	0.97	0.382
Treatment x Shelf life	1	69486.	69486.	1.14	0.286
Delay x Shelf life	2	128006.	64003.	1.05	0.350
Treatment x Storage	2	46213.	23106.	0.38	0.684
Delay x Storage	4	240475.	60119.	0.99	0.413
Storage x Shelf life	2	109155.	54578.	0.90	0.408
Treatment x Delay x Shelf life	2	117099.	58550.	0.96	0.383
Treatment x Delay x Storage	4	244342.	61085.	1.01	0.405
Treatment x Storage x Shelf life	2	112948.	56474.	0.93	0.396
Delay x Storage x Shelf life	4	238635.	59659.	0.98	0.418
Treatment x Delay x Storage x Shelf life	4	248208.	62052.	1.02	0.396

Table 4.3 Chlorophyll fluorescence values for mid harvest 'Queen Cox' apple fruit treated with 0.65 µl l^{-1} 1-MCP for 24 h at 3 to 4°C, at 1, 7 or 14 d after harvest. Fruit were stored at 3 to 4°C in air, or under <1% CO_2, 1.2% O_2, or as atmospheric conditions in the chambers for 0, 2 or 4 months. n/s, non-significant at P = 0.05. Number of replicates (n) = 60.

Treatment and storage	**Chlorophyll fluorescence value after storage period (months)**											
	F_m			**F_o**			**F_v**			**F_v / F_m**		
	0	**2**	**4**	**0**	**2**	**4**	**0**	**2**	**4**	**0**	**2**	**4**
0 d delay control	1266	994	941	209.7	210.4	317.3	1056	784	673	0.829	0.783	0.649
0 d delay 1-MCP	1337	1126	880	228.2	231.8	308.2	1109	894	572	0.824	0.789	0.638
7 d delay control	1209	1066	989	192.7	230.4	356.0	1016	831	582	0.839	0.782	0.621
7 d delay 1-MCP	1298	1183	940	214.6	259.7	372.6	1083	925	565	0.834	1.120	0.606
14 d delay control	1127	1011	1049	185.9	260.4	420.1	941	749	629	0.832	0.730	0.572
14 d delay 1-MCP	1209	1219	903	197.0	277.8	376.6	970	941	526	0.835	0.774	0.544
LSD (P = 0.05) for treatment per storage	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*
LSD (P = 0.05) for all storage		*n/s*			*n/s*			*n/s*			*n/s*	

Table 4.4 Chlorophyll fluorescence values for mid harvest 'Bramley' apple fruit treated with 0.65 µl l^{-1} 1-MCP for 24 h at 3 to 4°C, at 1, 7 or 14 d after harvest. Fruit were stored at 3 to 4°C in air, or under 5% CO_2, 1% O_2, or as atmospheric conditions in the chambers for 0, 2 or 4 months. n/s, non-significant at P = 0.05. Number of replicates (n) = 60.

Treatment and storage	**Chlorophyll fluorescence value after storage period (months)**											
	F_m			**F_o**			**F_v**			**F_v / F_m**		
	0	**3**	**6**	**0**	**3**	**6**	**0**	**3**	**6**	**0**	**3**	**6**
0 d delay control,	1500	1820	1329	233.4	407.0	714.0	1267	1413	615	0.843	0.772	0.372
0 d delay 1-1-MCP	1540	1773	1203	246.7	441.2	657.0	1293	1339	546	0.836	0.744	0.420
7 d delay control,	1585	1683	1149	243.6	453.9	516.0	1341	1229	633	0.845	0.710	0.525
7 d delay 1-1-MCP	1551	1736	966	241.4	483.1	593.0	1310	1253	373	0.843	0.716	0.368
14 d delay control,	1444	1707	1350	241.1	436.5	558.0	1203	1265	793	0.831	0.737	0.571
14 d delay 1-1-MCP	1539	1671	868	255.3	462.1	524.0	1284	1209	344	0.833	0.710	0.365
LSD (P = 0.05) for treatment per storage	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	0.100
LSD (P = 0.05) for all storage		n/s			n/s			n/s			0.064	

4.4 Experiment 2c: Late harvest - use of fungicide

There was no significant (P = 0.05) difference in firmness, IEC or chlorophyll fluorescence due to dipping either 'Queen Cox' or 'Bramley' fruit in Derosal WDG. Firmness and IEC data for dipped and non-dipped (combined) 1-MCP-treated 'Queen Cox' fruit are shown in Table 4.6 and 4.7, respectively. Firmness and IEC data for dipped and non-dipped (combined) 1-MCP-treated 'Bramley' fruit are shown in and Table 4.8 and 4.9, respectively.

Furthermore, no storage rots developed in 'Queen Cox' or 'Bramley' fruit, regardless of treatment or storage time. Furthermore, no storage rots developed in fruit harvested in the early or mid season.

However, there was significant ($P < 0.05$) greater maintenance of firmness and reduction in IEC for 'Queen Cox' and 'Bramley' in response to 1-MCP treatment. Furthermore, this response was greater for early-harvested fruit than for mid-harvested fruit, and greater for mid-harvested fruit than late-harvested fruit (Tables 4.6 – 4.9).

Table 4.5 Chlorophyll fluorescence values for late harvest 'Queen Cox' and 'Bramley' apple fruit treated with 0.65 µl l^{-1} 1-MCP for 24 h at 3 to 4°C, within 24 h of harvest. Fruit were stored at 3 to 4°C in air for 0, 2 or 4 months ('Queen Cox') or 0,3 or 6 months ('Bramley'). n/s, non-significant at P = 0.05. Number of replicates (n) = 40.

Cultivar	**Treatment and storage**	**Chlorophyll fluorescence value after storage period (months)**											
		Fm			**Fo**			**Fv**			**Fv/Fm**		
'Queen Cox'		**0**	**2**	**4**	**0**	**2**	**4**	**0**	**2**	**4**	**0**	**2**	**4**
	Control, air storage	1078	857	761	183.5	188.0	290.1	895	669	496	0.824	0.767	0.594
	1-MCP, air storage	1203	945	696	194.6	208.0	260.8	1009	737	433	0.836	0.769	0.606
	LSD (P = 0.05) for treatment per storage	*91*	*n/s*	*n/s*	*n/s*	*14*	*n/s*	*86.5*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*
	LSD (P = 0.05) for all storage		*99.5*			*21.8*			*88*			*n/s*	
'Bramley'		**0**	**3**	**6**	**0**	**3**	**6**	**0**	**3**	**6**	**0**	**3**	**6**
	Control, air storage	1398	1812	975	246.8	447.3	547.1	1151	1364	623	0.825	0.744	0.482
	1-MCP, air storage	1463	1736	1013	248.1	435.7	455.3	1218	1300	363	0.823	0.743	0.409
	LSD (P = 0.05) for treatment per storage	*n/s*	*n/s*	*146*	*n/s*	*n/s*	*70.4*	*n/s*	*n/s*	*138*	*n/s*	*n/s*	*n/s*
	LSD (P = 0.05) for all storage		*n/s*			*46.3*			*137*			*n/s*	

Table 4.6 Fruit firmness (penetration force, N) of 'Queen Cox' fruit harvested Early, mid and late season, treated within 24 h of harvest with 0.6 μl l^{-1} 1-MCP for 24 h then stored in cold air at 3 to 4°C for 2 or 4 months, then removed for shelf life storage for 1 or 7 d at 20°C. Main factor means, number of replicates (n) = 20.

Storage (months)	Harvest and treatment					
	Early		Mid		Late	
	Control	1-MCP	Control	1-MCP	Control	1-MCP
0	94.5	93.4	87.8	91.3	79.0	91.1
2	48.6	75.7	49.1	68.3	46.0	64.3
4	43.3	57.0	44.7	53.3	44.4	50.2
LSD (P = 0.05)			*4.67*			

Table 4.7 Fruit internal ethylene concentration (IEC, μl l^{-1}) of 'Queen Cox' fruit harvested Early, mid and late season, treated within 24 h of harvest with 0.6 μl l^{-1} 1-MCP for 24 h then stored in cold air at 3 to 4°C for 2 or 4 months, then removed for shelf life storage for 1 or 7 d at 20°C. Main factor means, number of replicates (n) = 20.

Storage (months)	Harvest and treatment					
	Early		Mid		Late	
	Control	1-MCP	Control	1-MCP	Control	1-MCP
0	0.0	0.0	3.0	0.4	147.0	0.3
2	218.8	1.8	267.0	0.8	278.5	3.1
4	299.0	111.6	272.3	97.9	285.4	142.5
LSD (P = 0.05)			*57.12*			

Table 4.8 Fruit firmness (penetration force, N) of 'Bramley' fruit harvested Early, mid and late season, treated within 24 h of harvest with 0.6 μl l^{-1} 1-MCP for 24 h then stored in cold air at 3 to 4°C for 2 or 4 months, then removed for shelf life storage for 1 or 7 d at 20°C. Main factor means, number of replicates (n) = 20.

Storage (months)	Harvest and treatment					
	Early		Mid		Late	
	Control	1-MCP	Control	1-MCP	Control	1-MCP
0	107.2	109.3	107.3	99.0	102.8	105.7
3	45.2	93.4	47.0	79.4	42.9	76.7
6	46.3	65.6	42.3	65.8	44.2	68.0
LSD (P = 0.05)			*7.32*			

Table 4.9 Fruit internal ethylene concentration (IEC, $\mu l\ l^{-1}$) of 'Bramley' fruit harvested Early, mid and late season, treated within 24 h of harvest with 0.6 $\mu l\ l^{-1}$ 1-MCP for 24 h then stored in cold air at 3 to 4°C for 2 or 4 months, then removed for shelf life storage for 1 or 7 d at 20°C. Main factor means, number of replicates (n) = 20.

Storage (months)	Harvest and treatment					
	Early		Mid		Late	
	Control	1-MCP	Control	1-MCP	Control	1-MCP
0	0.0	0.0	0.0	0.0	0.1	0.0
3	179.2	1.2	173.8	1.6	101.3	0.3
6	116.3	44.6	150.1	50.9	167.1	85.9
LSD (P = 0.05)			*47.07*			

4.5 TA and TSS

There were little, if any significant (P = 0.05) differences in TA or TSS in response to either 1-MCP-treament or storage method or harvest maturity Tables 4.10 – 4.14). It was thought by the author that fruit stored under CA conditions would have shown higher TA and TSS than those stored under air, in keeping with commercial practices. It is possible that the CA storage mechanism was at fault, but there was no indication that any of the gas flow controls were incorrect or that the chambers were not sealed correctly.

Results from the previous experiments did not suggest a significant (P = 0.05) difference in TA or TSS would be observed in response to 1-MCP treatment. The TA and TSS results from Experiment 2 are in-keeping with those from Experiment 1.

Table 4.10 Fruit titratable acidity (TS) and total soluble solids (TSS) values for Early harvest 'Queen Cox' apple fruit treated with 0.65 µl l^{-1} 1-MCP for 24 h at 3 to 4°C, within 24 h of harvest. Fruit were stored at 3 to 4°C in air, or under <1% CO_2, 1.2% O_2, or as atmospheric conditions in the chambers for 0, 2 or 4 months. n/s, non-significant at P = 0.05. Number of replicates (n) = 20.

Treatment and storage	TSS (°Brix)			TA (ml NaOH)		
	0	2	4	0	2	4
Control, air storage	11.3	13.3	12.4	5.7	3.8	2.6
1-MCP, air storage	12.6	13.5	12.8	5.8	5.3	3.1
Control, CA storage	11.3	12.7	12.5	5.7	4.1	2.9
1-MCP, CA storage	12.6	12.8	13.2	5.8	4.0	2.7
Control, CA atmospheric conditions storage	11.3	12.3	11.8	5.7	3.5	2.3
1-MCP, CA atmospheric conditions storage	12.6	12.3	11.9	5.8	3.4	1.8
LSD (P = 0.05) for treatment per storage	*0.8*	*0.6*	*0.6*	*n/s*	*0.5*	*0.4*
LSD (P = 0.05) for all storage		*0.7*			*0.5*	

Table 4.11 Fruit titratable acidity (TS) and total soluble solids (TSS) values for early harvest 'Bramley' apple fruit treated with 0.65 µl l^{-1} 1-MCP for 24 h at 3 to 4°C, within 24 h of harvest. Fruit were stored at 3 to 4°C in air, or under <1% CO_2, 1.2% O_2, or as atmospheric conditions in the chambers for 0, 2 or 4 months. n/s, non-significant at P = 0.05. Number of replicates (n) = 20.

Treatment and storage	TSS (°Brix)			TA (ml NaOH)		
	0	2	4	0	2	4
Control, air storage	11.5	11.9	10.9	11.0	8.1	6.7
1-MCP, air storage	11.9	13.4	11.9	10.9	10.2	8.5
Control, CA storage	-	13.1	12.6	-	9.8	9.1
1-MCP, CA storage	-	13.2	11.9	-	9.5	8.7
Control, CA atmospheric conditions storage	-	11.4	10.4	-	7.8	6.3
1-MCP, CA atmospheric conditions storage	-	12.7	10.8	-	9.6	7.4
LSD (P = 0.05) for treatment per storage	*n/s*	*0.8*	*0.7*	*n/s*	*0.8*	*0.8*
LSD (P = 0.05) for all storage		*0.7*			*0.8*	

Table 4.12 Fruit titratable acidity (TS) and total soluble solids (TSS) values for mid harvest 'Queen Cox' apple fruit treated with 0.65 µl l^{-1} 1-MCP for 24 h at 3 to 4°C, at 1, 7 or 14 d after harvest. Fruit were stored at 3 to 4°C in air, or under <1% CO_2, 1.2% O_2, or as atmospheric conditions in the chambers for 0, 2 or 4 months. n/s, non-significant at P = 0.05. Number of replicates (n) = 60.

Treatment and storage	TSS (°Brix)			TA (ml NaOH)		
	0	2	4	0	2	4
0 d delay control	11.8	12.8	11.8	4.8	3.4	2.3
0 d delay 1-MCP	11.5	13.3	12.2	4.8	0.9	3.3
7 d delay control	12.3	13.4	12.0	4.5	3.2	1.9
7 d delay 1-MCP	12.4	13.7	12.0	4.5	4.1	2.8
14 d delay control	13.5	12.7	12.0	4.2	3.0	2.0
14 d delay 1-MCP	13.3	12.7	12.1	4.9	6.7	2.2
LSD (P = 0.05) for treatment per storage	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*0.3*
LSD (P = 0.05) for all storage		*n/s*			*0.5*	

Table 4.13 Fruit titratable acidity (TS) and total soluble solids (TSS) values for mid harvest 'Bramley' apple fruit treated with 0.65 µl l^{-1} 1-MCP for 24 h at 3 to 4°C, at 1, 7 or 14 d after harvest. Fruit were stored at 3 to 4°C in air, or under <1% CO_2, 1.2% O_2, or as atmospheric conditions in the chambers for 0, 2 or 4 months. n/s, non-significant at P = 0.05. Number of replicates (n) = 60.

Treatment and storage	TSS (°Brix)			TA (ml NaOH)		
	0	2	4	0	2	4
0 d delay control	11.8	11.2	11.6	10.3	7.3	6.3
0 d delay 1-MCP	12.1	12.5	11.8	10.2	8.5	7.8
7 d delay control	13.8	11.4	11.1	14.5	7.9	5.6
7 d delay 1-MCP	13.8	12.8	11.4	13.7	9.8	7.0
14 d delay control	14.2	11.4	11.3	13.1	6.9	5.6
14 d delay 1-MCP	13.9	13.4	12.2	13.2	9.2	7.1
LSD (P = 0.05) for treatment per storage	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*
LSD (P = 0.05) for all storage		*n/s*			*n/s*	

Table 4.14 Fruit titratable acidity (TS) and total soluble solids (TSS) values for Late harvest 'Queen Cox' and 'Bramley' apple fruit treated with 0.65 µl l^{-1} 1-MCP for 24 h at 3 to 4°C, within 24 h of harvest. Fruit were stored at 3 to 4°C in air for 0, 2 or 4 months ('Queen Cox') or 0,3 or 6 months ('Bramley'). n/s, non-significant at P = 0.05. Number of replicates (n) = 40.

Cultivar	Treatment and storage	TSS (°Brix)			TA (ml NaOH)		
'Queen Cox'		**0**	**2**	**4**	**0**	**2**	**4**
	Control, air storage	14.8	13.3	12.1	6.6	3.6	2.2
	1-MCP, air storage	15.4	13.9	12.4	7.0	4.4	2.7
	LSD (P = 0.05) for treatment per storage	*0.6*	*0.6*	*0.3*	*n/s*	*0.5*	*0.2*
	LSD (P = 0.05) for all storage		*n/s*			*n/s*	
'Bramley'		**0**	**3**	**6**	**0**	**3**	**6**
	Control, air storage	12.9	11.8	11.0	12.6	7.4	5.6
	1-MCP, air storage	12.4	12.9	12.1	12.3	8.4	7.3
	LSD (P = 0.05) for treatment per storage	*0.5*	*0.4*	*0.4*	*n/s*	*0.5*	*0.4*
	LSD (P = 0.05) for all storage		*0.4*			*0.6*	

4.6 Conclusion

Early-harvested fruit responded better to 1-MCP-treatment than later-harvested fruit, this is probably due to more ethylene receptors being available in early fruit. The use of CA storage is effective at storing apples. However, upon removal from CA store, 'Queen Cox' and 'Bramley' apple fruit had reduced quality after 7 d shelf-life than 1-MCP-treated fruit. This may also be due to the accumulation of ACC in the fruit during storage (please refer to the Discussion in Experiment 1).

1-MCP-treatment is effective at storing apple fruit in air at low temperatures. Evidence is presented here that for short-term storage it may be possible to treat 'Queen Cox' and 'Bramley' fruit with 1-MCP, and store at low temperature rather than under CA conditions. However, it is also shown that for longer storage 1-MCP becomes less effective, thus, a combination of 1-MCP-treatment and CA storage may result in the best storage management technique.

TA and TSS were demonstrated to be unaffected by either 1-MCP-treatment or storage technique.

Chlorophyll florescence may have a limited use as a tool to determine the quality of 'Queen Cox' and 'Bramley' fruit. Results are mixed, but some benefit may be gained for use on early-harvested fruit.

Chapter 5 RESULTS AND DISCUSSION

EXPERIMENT 3

THE USE OF 1-MCP AS AN ALTERNATIVE TO DPA TO PREVENT SCALD IN 'BRAMLEY' APPLE FRUIT

5.1 Introduction

Superficial scald is a physiological disorder which affects many, but not all apple cultivars during cold storage (Fernández-Trujillo et al., 2001). Superficial scald is visible as irregular brown patches of dead skin caused by progressive browning of hypodermal cells (Ingle and D'Souza, 1989)

In the most severe cases, superficial scald can be visible in cold storage. Superficial scald is not limited to the skin. As scald increases in severity, the browning may extend through five or six layers of the hypodermis. In the most severe cases, epidermal cells are affected and become brown, and there may be sunken patches where hypodermal cells have collapsed (Ingle and D'Souza, 1989).

Many factors influence scald development. Cultivar, maturity, seasonal environmental variation, cultural practices and postharvest conditions can affect both scald development and severity (Huelin and Coggiola, 1968; Ingle and D'Souza, 1989; Fan *et al.*, 1999b). Cultivars that are more scald-resistant include 'Bramley' and 'Granny Smith'. Scald-susceptible cultivars include 'Cox' and 'Crofton'.

Superficial scald develops 3 to 7 d from 're-warming' after *ca* 3 months cold storage. Superficial scald is not caused by the increase in temperature, but warming allows the symptoms to develop. It is believed that scald is a form of chilling injury (Watkins *et al.*, 1995).

Superficial scald is believed to be result from the auto oxidation of α-farnesene

into conjugated trienes (CTs) and the associated formation of free radicals (Huelin and Coggiola, 1968; Anet and Coggiola, 1974; Du and Bramlage, 1994; Whitaker *et al.*, 2000). α -farnesene is an acrylic sesquiterpene hydrocarbon, one of the many volatiles and a component of apple surface wax (Rupasinghe *et al.*, 1998). During storage, CTs accumulate progressively on the surface of apples as α-farnesene oxidises. These oxidation products injure the cell membranes that result in cell death in the outermost layers. The concentration of CTs has a greater correlation with superficial scald severity than α-farnesene (Huelin and Coggiola, 1970; Rupasinghe *et al.*, 1998).

Biosynthesis of α-farnesene is via the isoprenoid pathway, and is converted to farnesyl pyrophosphate (FPP), catalysed by a single sesquiterpene synthase enzyme, α-farnesene synthase (Rupasinghe *et al.*, 1998). α -farnesene biosynthesis in apples is developmentally regulated, and increases rapidly during ripening and cold storage, parallel to increased internal ethylene concentration (Watkins *et al.*, 1993; Du and Bramlage, 1994; Ju and Curry, 2000). Cultivar is an important factor concerning the relationship between internal ethylene and α-farnesene concentrations. Cultivars that are more scald-resistant produce less α-farnesene and more ethylene than scald-susceptible cultivars (Golding *et al.*, 2001). However, even scald-resistant cultivars show an increase in α-farnesene concentration with increased internal ethylene with storage (Golding *et al.*, 2001).

The relationship between internal ethylene and apple peel α-farnesene concentration is also dependent on storage temperature. 'Granny Smith' apples stored at 10°C exhibited a twenty-fold increase in ethylene production but only a doubling of peel α-farnesene concentration compared to fruit stored at 0°C (Golding *et al.*, 2001). In contrast, 'Crofton' fruit ethylene production increased nine-times, whereas peel α-farnesene concentration remained constant.

However, the exact mechanism by which ethylene interacts with α-farnesene biosynthesis is unclear. The evidence for this interaction is mostly circumstantial. Susceptibility to scald decreases and internal ethylene increases as fruit mature. Treatment with ethephon, an ethylene action analogue, advances fruit maturity and results in less scald development. Scald development and internal ethylene biosynthesis are

reduced in controlled atmosphere (CA) storage. Diphenylamine (DPA), an antioxidant which also has been shown to suppress ethylene production, is commercially used to prevent scald (Fan *et al.*, 1999b). In addition, 'Granny Smith' apple fruit developed less scald after storage when treated with the ethylene action inhibitor diazocyclopentadiene (DACP) at harvest (Gong and Tian, 1998; Fan *et al.*, 1999b).

Currently, commercial systems utilise ethylene scrubbers and low-oxygen storage to reduce scald incidence (Colgan *et al.*, 1999), and maintain fruit quality. In addition, antioxidants, such as DPA are applied immediately after harvest. Certain countries do not permit the import of DPA-treated apples (Chervin. *et al.*, 2001), and the future use of DPA as a commercial scald treatment is unclear. However, experiments have shown 1-MCP-treated apples maintain their quality better compared to non-1-MCP-treated fruit, particularly in short-term storage (Rupasinghe *et al.*, 1998; Fan *et al.*, 1999a; Fan *et al.*, 1999b; Watkins *et al.*, 2000; Mir *et al.*, 2001; DeEll *et al.*, 2002; Dauny and Joyce, 2002).

In this experiment the previous method of scald measurement was replaced. Previously, the total percentage coverage of scald per apple was determined. This was due give a more precise quantification of scald development. However, in this instance, it was decided to determine scald by the Laurie Scale (Table 5.1); a system used extensively within the apple industry. The reason for this was to show greater realism with the commercial world.

Table 5.2 The Laurie Scale. A commercially-used measurement of scald severity.

Laurie Score	Percentage Scald Coverage on Fruit Surface
0	0
1	1 – 25
2	26 – 50
3	> 50

5.2 Results and Discussion

The use of 1-MCP reduces scald development in 'Bramley' apple fruit by *ca* half compared to current commercial DPA treatment. Furthermore, DPA treatment combined with 1-MCP-treatment showed no significant difference (P = 0.05) in scald development (Table 5.2), meaning that there was no additional benefit observed to using both systems in conjunction with each other.

Table 5.2 Combined mean data for Experiment 4. 1-MCP and DPA-treatment to reduce scald development (mean Laurie Scale: 0 = no scald; 1 = 1-25% scald coverage; 2 = 26-50% scald coverage; 3 = >50% scald coverage) during storage. Number of replicates (n) = 45, total number of fruit = 360.

Treatment	Mean Scald development (Laurie scale)
+ 1-MCP, +DPA	0.5
+1-MCP, - DPA	0.6
-1-MCP, + DPA	1.2
-1-MCP, -DPA	1.5

There was greater scald development in early-harvested 'Bramley' apple fruit than late-harvested fruit, and for fruit stored for 9 months compared to 6 months, and for 14 d shelf-life compared to 1 d (Table 5.3).

The use of 1-MCP-treatment to prevent scald development in apples was demonstrated in Experiment 1. However, current commercial practice is to drench apple fruit in DPA before storage. The future of this practice is uncertain. Furthermore, it has been demonstrated here that a 1-MCP-treatment to maintain storage quality as a commercial practice would reduce the requirement for DPA-treatment.

The method change in scald development was to better represent the analysis performed in a commercial situation. The Laurie Scale was chosen to better reflect the

commercial world. This experiment was heavily based on 'real-world' practicality, and the author considered that this should be reflected with 'real-world' measurements. As such, the experiment was designed as a factorial to equalise the treatments and replication. The Laurie Score, being non-parametric data, could not be analysed using ANOVA. The data was considered to be relative.

Table 5.3 Combined mean data for Experiment 4. 1-MCP and DPA treatment of 'Bramley' fruit to reduce scald development (mean Laurie Scale: 0 = no scald; 1 = 1-25% scald coverage; 2 = 26-50% scald coverage; 3 = >50% scald coverage) during storage and shelf life. Total number of fruit per table = 360. (Continued on next page.)

1-MCP-treatment (µl l^{-1})	Mean Scald development (Laurie scale)
0.0	1.3
0.6	0.5

DPA-treatment (µl l^{-1})	Mean Scald development (Laurie scale)
0	1.0
2000	0.8

Harvest	Mean Scald development (Laurie scale)
Early	1.2
Mid	0.7
Late	0.9

Storage (months)	Mean Scald development (Laurie scale)
6	0.6
9	1.3

Table 5.3 ***Continued*** 1-MCP and DPA treatment of 'Bramley' fruit to reduce scald development (mean Laurie Scale: 0 = no scald; 1 = 1-25% scald coverage; 2 = 26-50% scald coverage; 3 = >50% scald coverage) during storage and shelf life. Total number of fruit per table = 360. (Continued from previous page.)

Shelf-life (d)	Mean Scald development (Laurie scale)
1	0.5
7	1.1
14	1.3

Chapter 6 RESULTS AND DISCUSSION

EXPERIMENT 4

THE EFFECT OF 1-MCP ON NATURAL DISEASE RESISTANCE

6.1 Introduction

It was observed throughout Experiments 1 and 2 that 1-MCP-treated apple fruit developed less storage disease than non-1-MCP-treated fruit, with or without an antifungal treatment. Plants have many levels of inherent defence mechanisms against fungal attack, referred to as natural disease resistance (NDR). NDR decreases during development and after harvest, and thus, the susceptibility of produce to pathogen attack is increased (Prusky, 1996). The systems by which NDR may decline are the availability of nutrients for the pathogen; changes in preformed antifungal compounds with development and senescence; the ability of the host to produce antifungal compounds in response to attack (phytoalexins) (Prusky, 1996). Once these factors can be overcome by the pathogen, infection can develop. The use of 1-MCP-treatment to inhibit ethylene responses and subsequently slow ripening may have a direct influence on the rate of decline of NDR; the retained quality observed in stored 1-MCP-treated apple fruit may include retention of NDR.

There are a number of compounds associated with disease resistance reported for apple fruit, including certain phenolic compounds (Table 1.3). The phenolic composition of apple fruit peel and pulp has been quantified by HPLC-diode array detection (DAD) (Oleszek *et al.*, 1988; Burda *et al.*, 1990; Suárez *et al.*, 1996; Escarpa and González, 1998; Lattanzio *et al.*, 2001). The main phenolic compounds in 'Golden Delicious', 'Empire' and 'Rhode Island' apple fruit are epicatechin and procyanidin B2 (Burda *et al.*, 1990). Further to these are the phytoalexin benzoic acid, and the phytoanticipin chlorogenic acid, and flavonoid phytoanticipins.

To date, benzoic acid has been identified as the only major phytoalexin in apples (Brown and Swinburne, 1971). Benzoic acid accumulates in the infected areas in response to certain pathogen attacks. The antifungal compound present in the necrotic tissue of immature 'Bramley' apple fruit infected with *Nectria galligena* was isolated, purified and identified as benzoic acid. Benzoic acid was found to be present in sufficiently large quantities to account for all the observed antifungal activity. Benzoic acid is not usually found in healthy apple tissues, and is not induced by mechanical injury (Noble and Drysdale, 1983). However, attempts to replicate these experiments failed to isolate and identify benzoic acid from infected fruit of other cultivars (Harborne, 1999).

Chlorogenic acid is a preformed antifungal compound, not induced by pathogen infection. It is the major hyrdoxycinnamic acid derivative produced by apple fruit (Awad *et al.*, 2000). Chlorogenic acid is present, although differently distributed, throughout apple fruit tissue. Studies on 'Jonagold' and 'Elstar' cultivars showed that for both cultivars, chlorogenic acid concentration in the core (2.10 mg g^{-1} dry weight (dw)) was higher than the surrounding tissue (0.48 mg g^{-1} dw), which in turn was higher than in the peel (0.20 mg g^{-1} dw) (Awad *et al.*, 2000).

Chlorogenic acid has been shown to reduce the germination of *P. expansum* conidia, *in-vitro* by *ca* 1.6-fold at 500 mg l^{-1} (Boonyakiat *et al.*, 1986). However, no effect was detected for reducing *P. expansum* growth after germination in the presence of = 300 mg l^{-1} chlorogenic acid. Conversely, however, these authors also reported an increase in both germination of conidia and mycelial growth of *B. cinerea* in the presence of = 200 mg l^{-1} chlorogenic acid. The fungal toxicity of phenolics may therefore be negligible or even stimulate fungal growth at particular concentrations (Boonyakiat *et al.*, 1986).

The major classes of flavonoids are flavonols, including quercetin 3-glycosides; monomeric and oligomeric flavan-3-ols, e.g. catechins, epicatechin and procyanidins; dihydrochalcones, e.g. phloridzin; and non antitifungal compounds, such as the anthocyanins, e.g. cyanidin 3-glycosides in red fruit. Unlike benzoic acid, flavonoids and chlorogenic acid are already present in the fruit, and flavonoid content has been correlated with disease resistance (Awad *et al.*, 2000). Flavonoids are present in all

observed species and are present throughout the fruit structure, particularly the peel. Flavonoids have been observed at highest concentrations in immature fruit, and to decline during fruit maturation.

Flavonoids are phenolic plant metabolites, formed from precursors from the phenylpropanoid pathway which in turn are derived from the shikimate pathway. The role of flavonoids in conferring disease resistance is well established (Harborne and Williams, 2000). 'Red Delicious' apples harvested before July were shown to be resistant to *B. cinerea*. *B. cinerea* conidia germination and radial mycelial growth of *B. cinerea* were both inhibited by = 50 $\mu g\ ml^{-1}$ and = 100 $\mu g\ ml^{-1}$ extracted chlorogenic acid and p-coumaryl-quinic acids (PCQ), respectively. Furthermore, *P. expansum* mycelial growth was inhibited by = 100 $\mu g\ ml^{-1}$ and = 200 $\mu g\ ml^{-1}$ extracted chlorogenic acid and PCQ, respectively (Ndubizu, 1976). Ndubizu incorporated chlorogenic acid or PCQ into Cruickshanks medium (Cruickshanks and Perrin, 1964) and seeded with 11 mm diameter mycelial plugs of *B. cinerea* or *P. expansum*, and stored at 22 to 24°C for 48 or 72 h. At these times, growth of the fungi was assessed as colony diameter, giving an index to the affect of the chlorogenic acid or PCQ on fungal growth (Ndubizu, 1976).

Chlorogenic acid and PCQ were sequentially extracted from frozen 'Red Delicious' apple tissue (unspecified). Within the fruit themselves, the concentration of these phenolic compounds decreased during maturation from *ca* 120 $\mu g\ g^{-1}$ fw PCQ in mid July to *ca* 18 $\mu g\ g^{-1}$ fw by the end of September. Similarly, chlorogenic acid was shown to drop from *ca* 350 $\mu g\ g^{-1}$ fw to *ca* 40 $\mu g\ g^{-1}$ fw over the same period. This was matched with a decrease in resistance of mature fruit to attack after inoculation with these pathogens (details not stated) (Ndubizu, 1976).

Chlorogenic acid (= 10^{-2} M) has been shown to have little effect on the *in-vitro* growth rate of *Pezicula malicorticis* (Noble and Drysdale, 1983). Lattanzio and co-workers (2001) measured the content of chlorogenic acid, catechin, phloridzin and quercetin in fresh and stored 'Golden Delicious' apple fruit. The compounds were tested for fungicidal activity against *Phlyctaena vagabunda*,. Chlorogenic acid was only shown to inhibit *P. vagabunda* germination and mycelial growth, but only *in-vitro*.

It is well established that apple fruit exhibit a rise in phenylalanine ammonia lyase

(PAL) concurrent with the climacteric ethylene rise (Lattanzio *et al.*, 2001). PAL is the major enzyme involved in the biosynthesis of phenolic compounds. It may be that system II ethylene production is the signal for the plant to produce defence against pathogen attack, particularly from quiescent infection, as it may also be the signal for the pathogen to terminate quiescence (Lattanzio *et al.*, 2001).

Phenolic levels in apple fruit are generally agreed to decrease from *ca* 3 mg g^{-1} fresh weight (FW) to *ca* 0.5 mg g^{-1} during development, and maintain constant levels during maturation (Noble and Drysdale, 1983; Lattanzio *et al.*, 2001). However, changes in phenolic levels during cold storage are less clear. Burda *et al.* (1990) and Awad and de Jager (2000) both suggest that concentrations of individual phenolic compounds remain fairly constant during storage of 'Golden Delicious', 'Rhode Island Greening', 'Jonagold', 'Empire' and 'Elstar' apple fruit. Concentrations of preformed and induced phenolic substances on both 'Cox's Orange Pippin' and 'Bramley' fruit have also been reported to fall rapidly during development and reach constant levels from normal harvest time and through storage (Noble and Drysdale, 1983). However Lattanzio *et al.* (2001) reported a rise in individual phenolic concentrations during the first sixty days of storage, followed by their decrease. There is a lower rate in the decrease of phenolic concentrations in cold storage than at room temperature (shelf life). This is due to enzyme metabolism being temperature dependent (Lattanzio *et al.*, 2001).

Postharvest fungal attack of all produce results in losses in crop quantity and quality during storage, transit and retail (Mercier, 1996). Given the commercial value of apple fruit, the prevention, or at least the reduction of disease is of considerable importance. Coupled with pressure to reduce chemical treatments of produce, a storage treatment such as 1-MCP which has a non-primary antifungal action may become an even more important postharvest tool than it may with only one of these properties. Apple fruit suffer from many diseases before and during storage. Two of the most important apple storage pathogens, both in terms of fruit damage and economic importance are *Penicillium expansum* and *Botrytis cinerea*. *Penicillium expansum* is one of the most destructive pathogens of stored apples, worldwide. *P. expansum* can be isolated from most orchard soils. Although the disease is rare preharvest, it may occur on fallen fruit.

Commonly known as blue mould, the disease is caused by *P. expansum*, that usually infects damaged or over-mature apples. The majority of infections occur when air- or waterborne conidia enter the fruit, usually via openings in the peel, lenticels, via *Mucor*, *Gloesporium* or *Phytopthera* infection sites, or bruises (Walker, 1969; Snowdon, 1990; Rosenberger, 1997a).

P. expansum spores are always present in the air of pome fruit packinghouses. Spores arise from decayed fruit, or from sporulation on bins and storage walls. However, the majority of *P. expansum* infections are from waterborne spores in postharvest drenches and flumes. Spores enter the water from decayed apples, orchard soil on dirty fruit and from contaminated bins (Rosenberger, 1997a). A soft watery brown spot develops and rapidly enlarges, particularly at temperatures between 20 to 25°C (Snowdon, 1990). Blue green coremial fruiting structures later appear on the surface (Pitt and Hocking, 1997). *P. expansum* also produces between 2 to 100 μg per g of tissue of the carcinogenic mycotoxin, patulin, which may accumulate in fruit destined for processing, and result in off flavours (Janisiewicz, 1999; Barkai-Golan, 2001; Moodley *et al.*, 2002). The majority of *P. expansum* infections are by strains resistant to methyl benzimazole carbamate (MBC), the active ingredient of carbendazin, benomyl and thiophanate-methyl (Rosenberger, 1997a; FRAG-UK, 2002).

Botrytis cinerea is the causal agent of grey mould. *B. cinerea* is a ubiquitous pathogen that causes disease on many harvested horticultural crops, worldwide. Grey mould results from *B. cinerea* infecting fruit via the cut stem, or more usually through wounds. Initially, dry dark lesion will appear which rapidly develop into a soft brown rot that engulfs the entire fruit, particularly at the cardinal temperature, 22°C. Under humid conditions the mould may produce grey-brown conidia. Black resting bodies (sclerotia) of a few mm in size may form eventually on windfall fruit, but not in storage. *B. cinerea* develops more rapidly during cold storage temperatures (3 - 5°C) than any other rot, with the exception of *Mucor* (Snowdon, 1990; Pitt and Hocking, 1997; Rosenberger, 1997b). There has been no reported mycotoxin production by *B. cinerea* (Pitt and Hocking, 1997).

B. cinerea infected fruit are rarely seen in the field, although common on the

orchard floor. *B. cinerea* conidia may be airborne, although waterborne spores are more likely to be the cause of infection. Once *B. cinerea* has infected a fruit in storage, the disease can spread quickly to neighbouring healthy fruit, a phenomenon known as nesting (Rosenberger, 1997b). *B. cinerea* infection may be controlled by MBCs and dicarboximides, but resistance to both of these controls has been reported and is becoming more common (FRAG-UK, 2002).

Due to time constraints, only one strain of *P. expansum* could be used in this experiment. The use of a single spore isolate meant that any 1-MCP / pathogen interactions would be confined to this isolate. As such, a bioassay was performed (Experiment 4b) to visualise the effect of the antifungal compounds on the specific isolate used in Experiment 4.

6.2 Results and Discussion

6.2.1 Experiment 4a

6.2.1.1 The effect of inoculation and 1-MCP treatment on IEC in 'Queen Cox' apple fruit.

Inoculating 'Queen Cox' fruit with *P. expansum* resulted in a rise in IEC observed at 3 d after inoculation (Fig. 6.1), for early and late-harvested fruit, stored for *ca* 2 months. 1-MCP-treatment effectively suppressed IEC of inoculated and non-inoculated fruit. This is in-keeping with results from the previous experiments.

6.2.1.2 The effect of inoculation and 1-MCP treatment on lesion development in 'Queen Cox' apple fruit.

1-MCP-treatment showed no significant difference ($P = 0.05$) in reducing lesion development after inoculation with 10^4 spores / conidia ml^{-1} (Fig. 6.2).

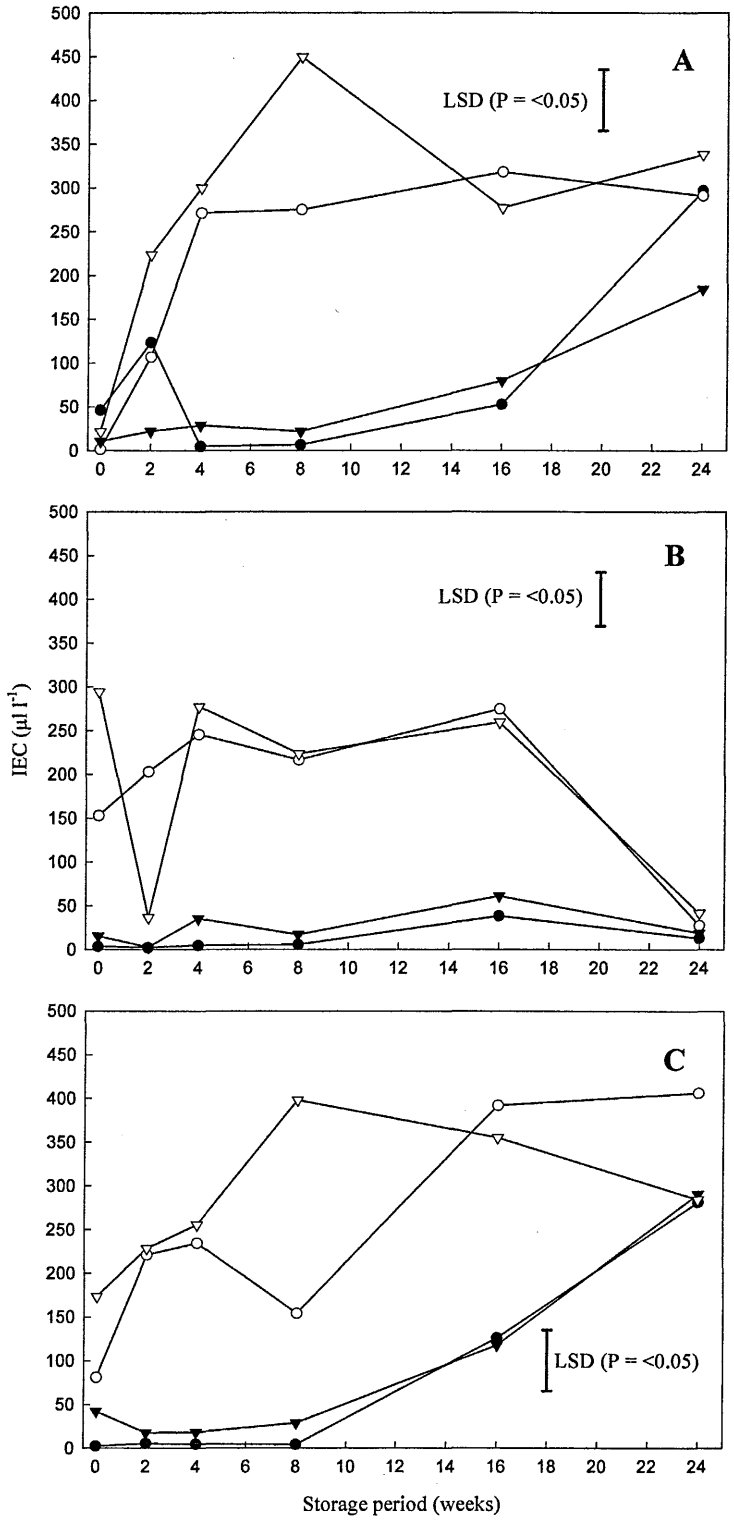

Figure 6.1 IEC (µl l^{-1}) 3 d after inoculation with *Penicillium expansum* for 0.6 µl l^{-1} 1-MCP treated (▼) or non 1-MCP treated (▽); and non inoculated 0.6 µl l^{-1} 1-MCP treated (●) or non 1-MCP treated (○) early (A), mid (B) and late (C) harvested 'Queen Cox' apple fruit (2002-2003 season). After 1-MCP treatment, fruit were stored in air at 3 to 4°C until removal from storage and subsequent inoculation. Data are the means for 9 individual replicate fruit.

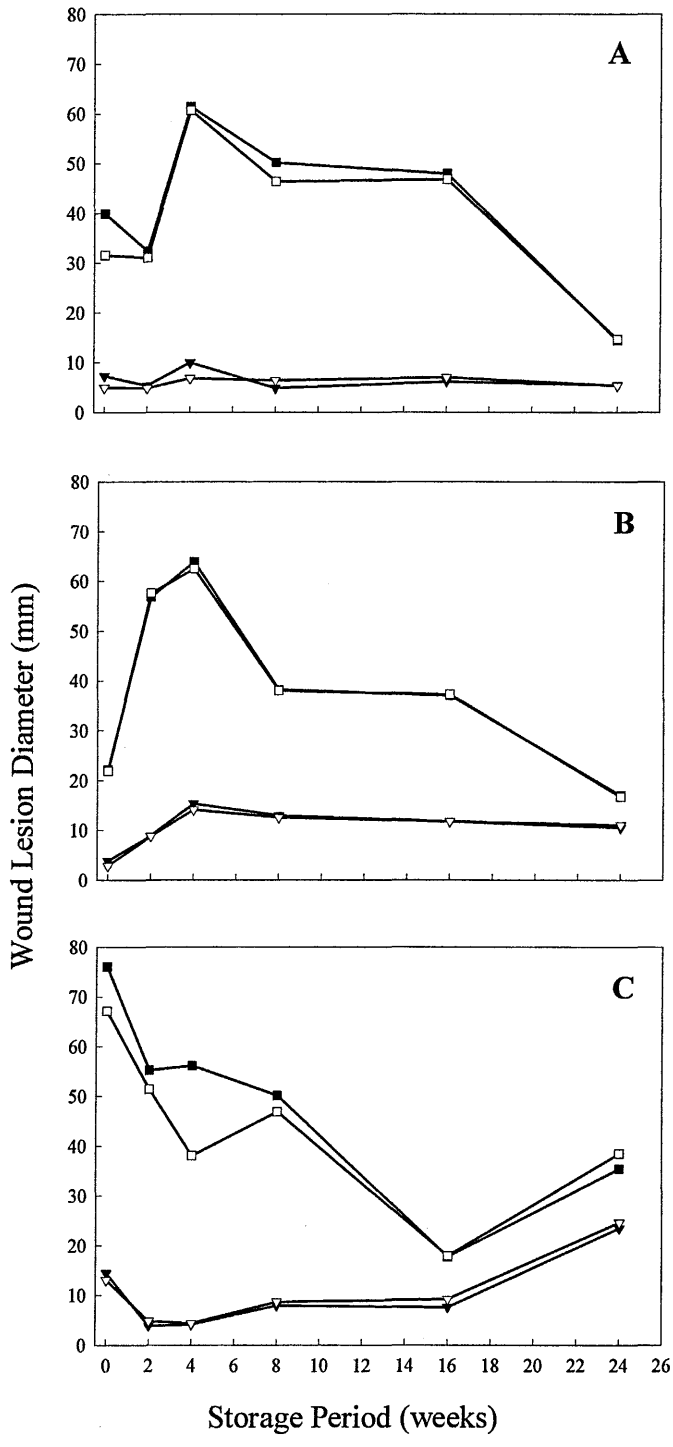

Figure 6.2 Wound lesion diameter (mm) for Early (A), mid (B) and Late (C) harvested 'Queen Cox' apple fruit inoculated with 20 µl 10^4 spores ml^{-1} *Penicillium expansum* (▼,▽) or *Botrytis* cinerea (■,□). Fruit were pre-treated with 0.65 µl l^{-1} 1-MCP (closed symbols) or air (open symbols) within 24 h of harvest and stored at 3 to 4°C in air. There was no significant difference (P = 0.05) between wound lesion diameter in 1-MCP and non 1-MCP-treated fruit.

ANOVA Table 6.1 ANOVA results for early, mid or late-harvested 'Queen Cox' apple fruit exposed to +/- 1-MCP, +/- infection with *P. expansum*, and 3 d after storage for 0, 2, 4, 8, 16 or 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Maturity (Early, Mid, Late)	2	562577.	281289.	54.30	<.001
1-MCP (0 or 0.6 µl l^{-1})	1	4925853.	4925853.	950.92	<.001
Pathogen (+ / -)	1	38487.	38487.	7.43	0.007
Storage (0, 2, 4, 8, 16, 24 w)	5	1514334.	302867.	58.47	<.001
Maturity x 1-MCP	2	13327.	6663.	1.29	0.277
Maturity x Pathogen	2	7810.	3905.	0.75	0.471
1-MCP x Pathogen	1	41248.	41248.	7.96	0.005
Maturity x Storage	10	1736310.	173631.	33.52	<.001
1-MCP x Storage	5	1089117.	217823.	42.05	<.001
Pathogen x Storage	5	237189.	47438.	9.16	<.001
Maturity x 1-MCP x Pathogen	2	70669.	35335.	6.82	0.001
Maturity x 1-MCP x Storage	10	417067.	41707.	8.05	<.001
Maturity x Pathogen x Storage	10	178778.	17878.	3.45	<.001
1-MCP x Pathogen Storage	5	122584.	24517.	4.73	<.001
Maturity x 1-MCP x Pathogen x Storage	10	263144.	26314.	5.08	<.001

This is contrary to observed storage disease development reported in Experiment 1. However, it may be that an inoculation of this magnitude out-competed any NDR inherent in the fruit and potentially masked maintained NDR in stored 1-MCP-treated fruit. It is possible that NDR is limited to inoculation concentration of the pathogen. It is possible that the reduction of storage rots in 1-MCP-treated fruit observed in Experiment 1 was due to a lower fungal load, one within the effective ability of the fruit to defend against.

6.2.1.3 Quantification of antifungal compounds after storage in non-inoculated 'Queen Cox' apple fruit

Concentrations of benzoic acid, catechin, chlorogenic acid, epicatechin and p-coumin in 'Queen Cox' at 24 h after removal from different durations of cold-air storage were measured (Figs 6.3, 6.4, 6.5, 6.6 and 6.7, respectively).

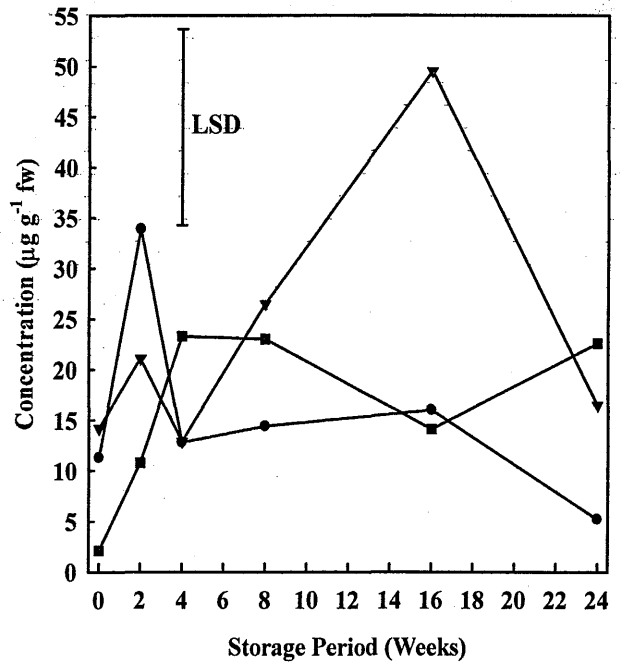

Figure 6.3 Benzoic acid concentration (µg g^{-1} fw) of Early (●), Mid (▼) and Late (■) harvested 'Queen Cox' apple fruit at 24 h after removal from storage at 3 to 4°C in air. Data are the means of 18 individual replicate fruit (total number of fruit = 54). LSD (P = 0.05).

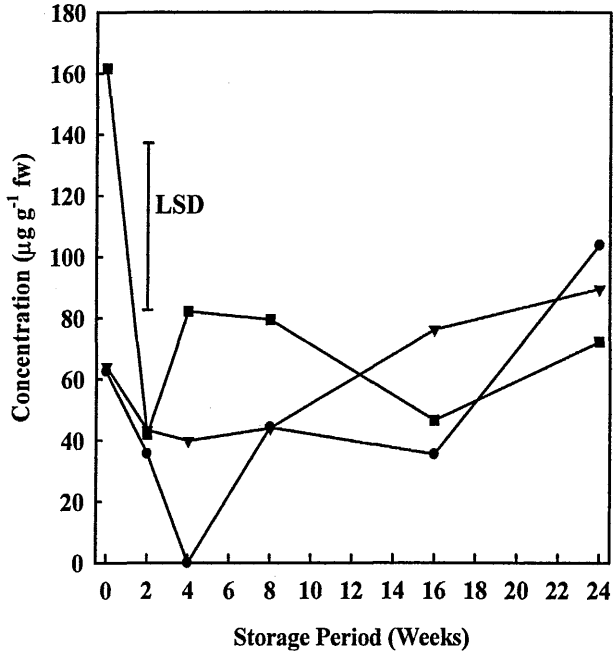

Figure 6.4 Catechin concentration (µg g^{-1} fw) of Early (●), Mid (▼) and Late (■) harvested 'Queen Cox' apple fruit at 24 h after removal from storage at 3 to 4°C in air. Data are the means of 18 individual replicate fruit (total number of fruit = 54). LSD (P = 0.05).

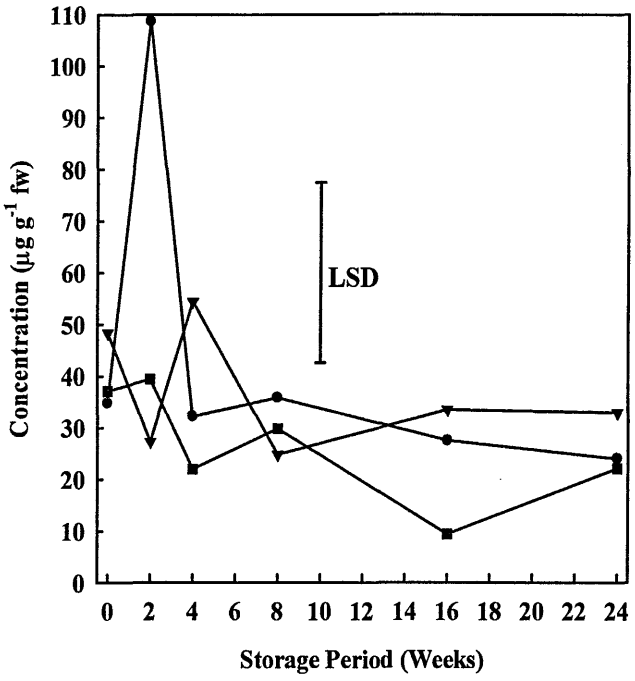

Figure 6.5 Chlorogenic acid concentration (µg g^{-1} fw) of Early (●), Mid (▼) and Late (■) harvested 'Queen Cox' apple fruit at 24 h after removal from storage at 3 to 4°C in air. Data are the means of 18 individual replicate fruit (total number of fruit = 54). LSD (P = 0.05).

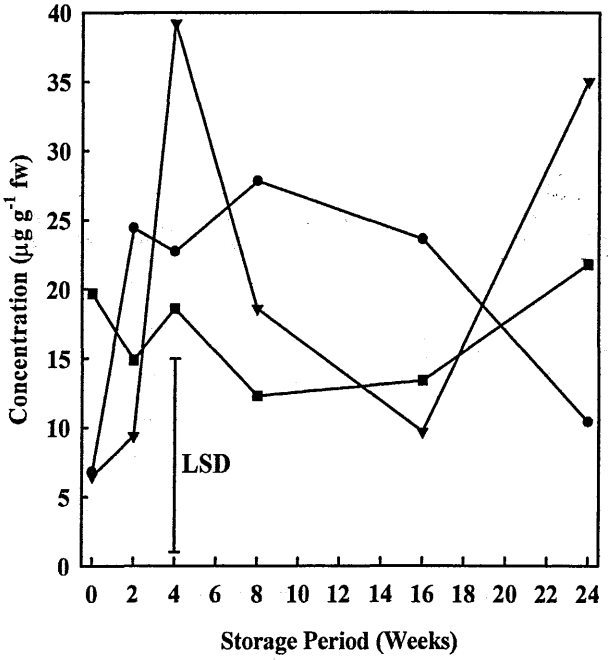

Figure 6.6 Epicatechin acid concentration (µg g^{-1} fw) of Early (●), Mid (▼) and Late (■) harvested 'Queen Cox' apple fruit at 24 h after removal from storage at 3 to 4°C in air. Data are the means of 18 individual replicate fruit (total number of fruit = 54). LSD (P = 0.05).

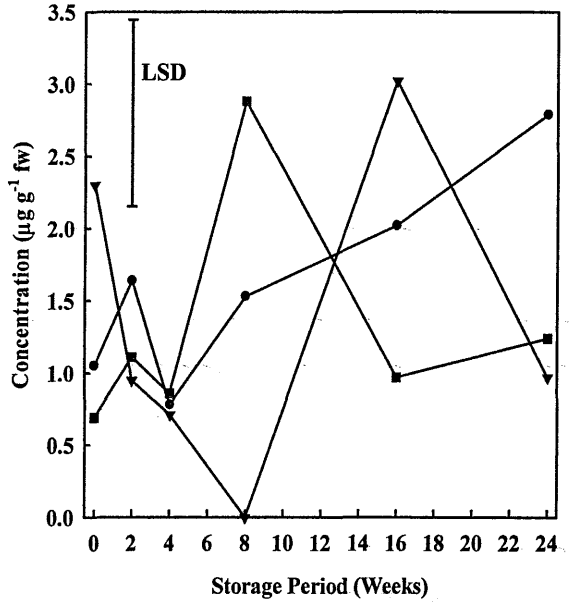

Figure 6.6 p-Coumin concentration (µg g^{-1} fw) of Early (●), Mid (▼) and Late (■) harvested 'Queen Cox' apple fruit at 24 h after removal from storage at 3 to 4°C in air. Data are the means of 18 individual replicate fruit (total number of fruit = 54). LSD (P = 0.05).

Benzoic acid levels in 'Queen Cox' fruit inoculated with *P. expansum* showed no significant difference (P = 0.05) regardless of 1-MCP-treatment. Neither was there any difference in chlorogenic acid or p-coumin levels. However, Catechin showed higher levels in 1-MCP-treated fruit (5.76 µg l^{-1} g^{-1} fw) compared to non-1-MCP-treated fruit (4.35 µg l^{-1} g^{-1} fw; lsd = 0.921). 1-MCP-treatment also resulted in higher observed levels of epicatechin after infection (2.70 µg l^{-1} g^{-1} fw) than non-1-MCP-treated fruit (2.1 µg l^{-1} g^{-1} fw; lsd = 0.549). Conversely, 'Queen Cox' fruit inoculated with *B. cinerea* showed higher benzoic acid levels in 1-MCP-treated fruit (2.54 µg l^{-1} g^{-1} fw) than non-1-MCP-treated fruit (1.12 µg l^{-1} g^{-1} fw; lsd = 0.88). There was no significant difference in chlorogenic acid, p-coumin, catechin or epicatechin ANOVA tables are shown in Appendix.

6.2.1.4 Disease development after 1-MCP treatment of 'Queen Cox' apple fruit.

Observed disease development in stored 'Queen Cox' may be higher in non-1-MCP-treated fruit due to increased availability of catechin and epicatechin in response

to *P. expansum* infection and by increased availability of benzoic acid in response to *B. cinerea*. Further research is required to quantify phenolic levels in response to applied infection.

The use of a single spore isolate meant that any 1-MCP / pathogen interactions would be confined to this isolate. However, there was not enough time to repeat this experiment or to make the experiment any larger, to incorporate any additional *P. expansum* strains.

Fruit inoculated with the *B. cinerea* isolate developed infection so rapidly that the experiment was unable to be run effectively. It is not known if this particular isolate is especially virulent. It is neither known if the apples from this particular harvest were especially susceptible to this disease. Therefore, apart from a few isolated data points, there is no further mention of *B. cinerea* / 1-MCP interactions.

There were no clear trends regarding 1-MCP / pathogen interaction. A full series of ANOVAs were performed and displayed in the appendix. However, as no clear trends exist, it is difficult to ascertain any 1-MCP effects on NDR over time. Table 6.1 isolates various significant interactions. The presence of these significant interactions may suggest that 1-MCP does influence either *P. expansum* growth directly or via decreasing ethylene sensitivity, thus influencing the availability of antifungal compounds, either by their manufacture, or position to relation to the infection site.

Furthermore, by maintaining fruit firmness and therefore fruit structural integrity, it may also be possible that as 1-MCP-treatment maintains a firmer fruit, there may be a greater physical barrier against infection. Firmness is maintained in 1-MCP-treated 'Queen Cox' and 'Bramley' apple fruit, and it may be this greater structural integrity that reduces the ability of pathogens to attack the fruit in store.

Table 6.1 A selection of Significant interactions after ANOVA analysis (Please refer to Appendix for ANOVA Tables) per treatment. 1-MCP refers to 1-MCP v. control (0.6 µl l^{-1} or 0 µl l^{-1}); Harvest refers to harvest maturity (early, mid or late); Infection refers to infection with *P. expansum* or control (no infection); Storage refers to the storage interval (0, 2, 4, 8 16 or 34 weeks; Shelf refers to the shelf life period (0, 3, 7 or 14 d).

Antifungal compound	**Significant Interactions**		
	Non-infected	**Non-Infected x *P. expansum***	***P. expansum x Wound***
Benzoic acid	[1-MCP x Harvest x Storage]	[1-MCP x Infection x Harvest x Storage]	[1-MCP x Infection x Harvest]
	<0.001	<0.001	0.032
	[1-MCP x Harvest x Storage x Shelf]	[1-MCP x Harvest x Storage x Shelf]	[1-MCP x Harvest x Storage x Shelf]
	0.016	0.003	0.002
Catechin	[1-MCP x Harvest x Storage]	[1-MCP x Infection x Harvest x Storage]	[1-MCP x Infection x Harvest x Storage]
	<0.001	<0.001	0.009
	[1-MCP x Harvest x Shelf]	[Infection x Harvest x Storage x Shelf]	[1-MCP x Harvest x Storage x Shelf]
	0.002	<0.001	0.032
Chlorogenic acid	[1-MCP x Harvest x Storage]	[Infection x Harvest x Storage]	[1-MCP x Harvest x Storage]
	0.017	<0.007	0.004
			[1-MCP x Harvest x Storage x Shelf]
			0.036
Epicatechin	[Harvest x Storage]	[Infection x Harvest x Storage]	[Infection x Harvest x Storage]
	<0.001	<0.001	<0.001
	[1-MCP x Storage x Shelf]	1-MCP x Infection x Harvest x Storage]	[Harvest x Storage x Shelf]
	<0.001	0.039	0.018
p-Coumin	[1-MCP x Harvest x Storage]	[1-MCP x Infection x Storage]	[1-MCP x Infection x Harvest]
	0.016	0.016	0.003
		[Infection x Harvest x Shelf]	[1-MCP x Harvest x Storage x Shelf]
		0.020	0.020

6.2.1 Experiment 4b

TLC bioassay demonstrated the antifungal capacity of 1 µl 0.5 g 10 ml^{-1} catechin, benzoic acid, epicatechin and p-coumin to inhibit growth of *P. expansum* (Fig. 6.7). The antifungal effect of these compounds is seen more clearly when the volume of the compound applied to the plate is increased to 5 µl (Fig. 6.8).

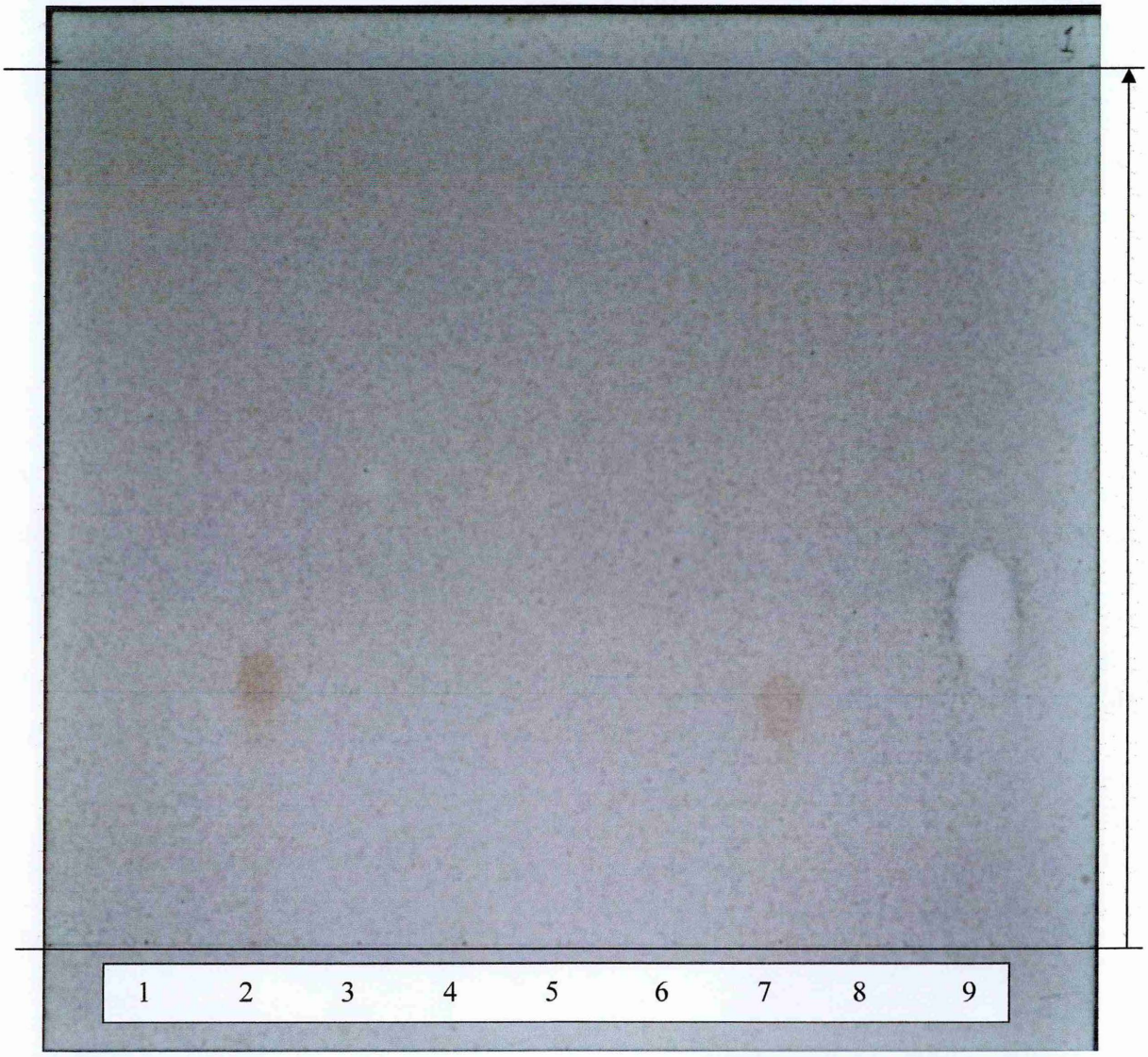

Figure 6.7 Thin-layer Chromatogram (TLC) spotted with 1 µl: 1, HPLC grade methanol; 0.5 g 10 ml^{-1}: 2, catechin; 3, cycolhexamide; 4, chlorogenic acid; 5, blank; 6, benzoic acid; 7, epicatechin; 8, blank; 9, p-coumin. Running solvent: 60:40:30 hexane : ethylacetate : methanol. Arrow indicates direction of solvent. Horizontal bars indicate base line (bottom) and solvent front (top). Clear areas indicate inhibition of *P. expansum* growth.

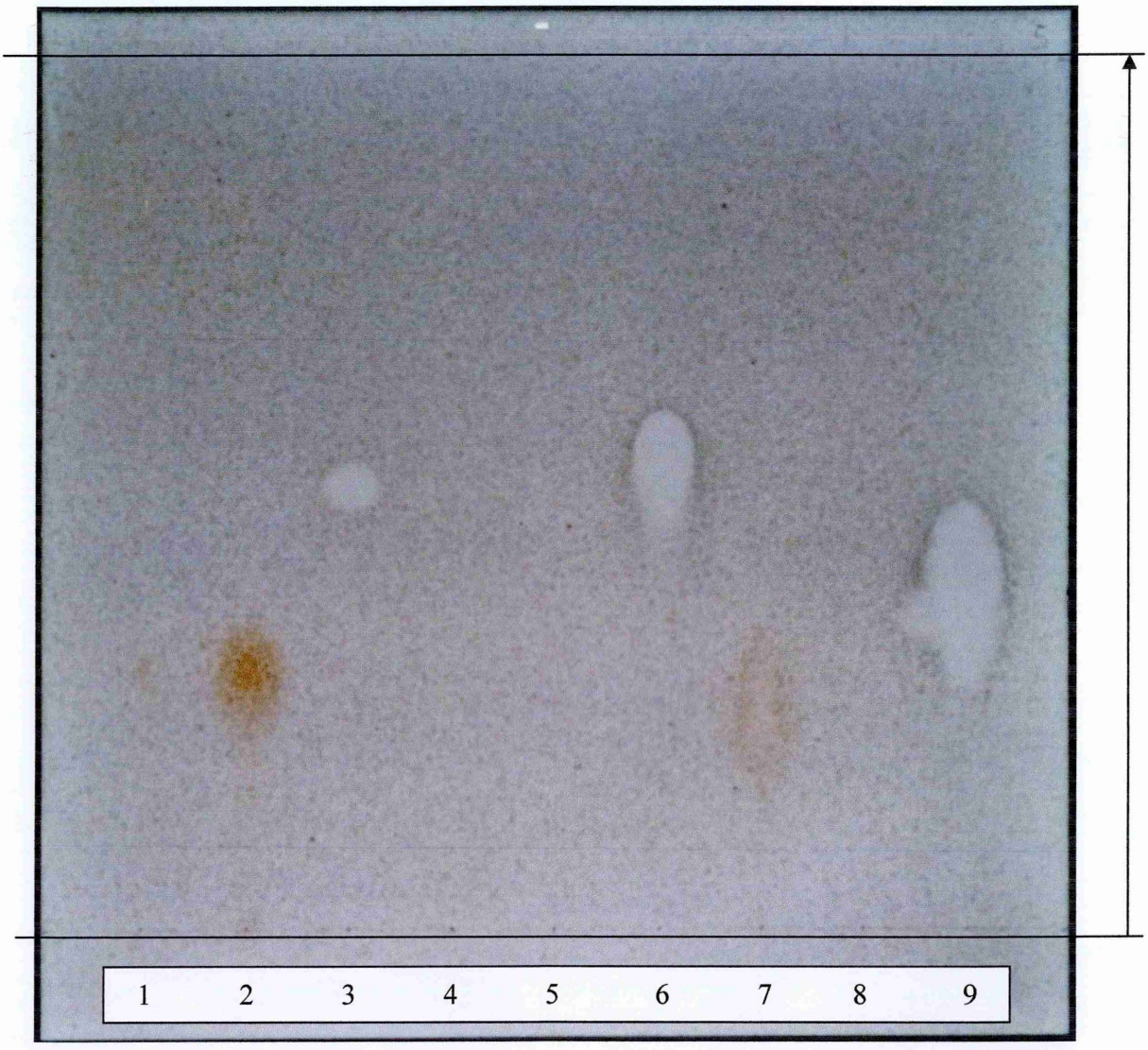

Figure 6.8 Thin-layer Chromatogram (TLC) spotted with 5 µl: 1, HPLC grade methanol; 0.5 g 10 ml^{-1}: 2, catechin; 3, cycolhexamide; 4, chlorogenic acid; 5, blank; 6, benzoic acid; 7, epicatechin; 8, blank; 9, p-coumin. Running solvent: 60:40:30 hexane : ethylacetate : methanol. Arrow indicates direction of solvent. Horizontal bars indicate base line (bottom) and solvent front (top). Clear areas indicate inhibition of *P. expansum* growth.

There were significant alteration in chlorogenic acid levels *in-vivo* between chlorogenic acid and *P. expansum* in response to infection (Table 6.1). However, figures

6.7 and 6.8 do not show any ability of chlorogenic acid, at these amounts, to inhibit growth of this isolate of *P. expansum*.

For both Experiments 4a and 4b, only limited information could be gained regarding 1-MCP / pathogen interactions. The *P. expansum* spores were cultured from a single spore isolate. As such, to fully explore any 1-MCP / pathogen interactions, a separate experiment needs to be performed which repeats the basis of this work, but replicates for other strains of *P. expansum*. There was a limited amount of pre-published work regarding 1-MCP / *P. expansum* interactions, and therefore it is difficult to compare these results to other findings.

6.3 Conclusion

1-MCP-treatment did show some level of influence on antifungal compounds and disease development as a result of inoculation. However, there were no clear trends to indicate any specific response in antifungal compound levels as a response to either 1-MCP treatment or inoculation.

There may be a different antifungal responses dependant on attacking pathogen species, which may be increased by 1-MCP-treatment before storage. Observed resistance to storage pathogens exhibited by 1-MCP-treated 'Queen Cox' fruit (Experiment 1) may be linked to maintenance of firmness and suppression of ethylene production.

Chapter 7 1-METHYLCYCLOPROPENE INFLUX AND EFFLUX FOR 'COX' APPLE AND 'HASS' AVOCADO FRUIT

This chapter was published as: Dauny, P.T., Joyce, D.C. and Gamby, C. (2003) 1-Methylcyclopropene influx and efflux in 'Cox' apple and 'Hass' avocado fruit. *Postharvest Biology and Technology* **29**: 101-105.

7.1 Introduction

The use of the ethylene binding site blocker 1-methylcyclopropene (1-MCP) to extend the postharvest longevity of ethylene-sensitive produce has been reported widely. Most findings have been based on a single pre-storage application of 1-MCP. The efficacy of 1-MCP varies with the crop. Cut flowers have an immediate response to 1-MCP but typically show re-sensitivity to ethylene within a few days of 1-MCP treatment (Sisler *et al.*, 1996; Macnish *et al.*, 1999; Çelikel *et al.*, 2002). Application of >0.1 $\mu l\ l^{-1}$ 1-MCP has been shown to extend avocado fruit shelf life by 40% (Hofman *et al.*, 2001; Pesis *et al.*, 2002). Apples treated with >0.1 $\mu l\ l^{-1}$ 1-MCP have been shown to maintain quality during air storage for as long as 9 months (Watkins *et al.*, 2000; Dauny and Joyce, 2002).

Firmness of 'Cox' fruit treated with 10.0 $\mu l\ l^{-1}$ 1-MCP has been shown to be slightly greater than that for 1-MCP concentrations of <1.0 $\mu l\ l^{-1}$ (Dauny and Joyce, 2002). It was suggested that the higher concentration gradient at 10 $\mu l\ l^{-1}$ 1-MCP might have enhanced diffusion of 1-MCP into the fruit. It was also thought possible that excess 1-MCP may be sorbed in some way by fruit tissue beyond saturation of ethylene-binding sites. If so, 1-MCP may be slowly de-sorbed during storage to become available to bind to newly synthesized or regenerated ethylene-binding sites (Golding *et al.*, 1998).

The ability of produce to retain 1-MCP may be directly related to plant tissue composition. 1-MCP should be preferentially sorbed into lipid versus aqueous compartments. Organic chemicals are typically more hydrophobic than hydrophilic. 1-MCP has no carboxylic or amino groups and thus would partition into oil/lipids and not water (K. Karim, 2003, Cranfield University, pers. comm.). Low polarity alkenes

are much more soluble in non-polar than in polar solvents (Solomons, 1978). Apples (0.1 g fat 100 g^{-1} edible portion; Wills, 1987) may have less 1-MCP sorbing capacity than oil-containing avocados (23.0 g 100^{-1}). In order to test this hypothesis, apples and avocados were sealed in an atmosphere containing 1-MCP. The 1-MCP concentration of the atmosphere was measured repeatedly over a 48 h period to determine if 1-MCP was being taken up into the produce. To further test the proposition, oil was extracted from avocado and 1-MCP uptake was compared to a water control. The practical applications of commercial 1-MCP application are considered.

7.2 Materials and methods

7.2.1 Whole fruit

'Cox' apple and 'Hass' avocado fruit were obtained from a local wholesaler (Wilkinsons Ltd., Bedfordshire, UK). Three of each type of fruit were labelled. Each fruit was placed into individual sealable 1.5 l jars. These jars also held a 200 ml Pyrex beaker containing a weighed amount of SmartFresh (Ethylbloc)™ (0.14% a.i. ml^{-1}) (AgroFresh Inc., Gessate, Italy). Water (20 ml) at 50°C was added to the beaker to liberate 1-MCP gas and the jar was sealed immediately. This process achieved initial concentrations of *ca* 120 µl l^{-1} 1-MCP within the jars. The jars were kept sealed for 48 h at 20°C. Every hour after 1 h, 1-MCP concentration in a 1 ml sample of air extracted from each jar was quantified by GC (see section 3.4). Air removed from the jar was replaced with 1 ml of nitrogen gas.

In follow-up experimentation, a further three apple and avocado fruit were treated with *ca* 120 µl l^{-1} 1-MCP. 1-MCP was quantified after 1, 6, 12, 24 and 48 h. These fruit were then removed from the 1.5 l jars, and individually placed into 500 ml jars. These jars were connected to a flow-through gas-system, and the air supply regulated to give one air change every 8 h. Individual 1 ml samples of exhaust gas from each jar were removed after 1 h. 1-MCP concentrations in these samples were quantified by GC. 1-MCP was subsequently measured hourly for the first 48 h, then three times a day until a fruit showed signs of decay.

7.2.2 Avocado oil

'Hass' avocado fruit were peeled and the flesh (200 g fresh weight (FW) per avocado) was cut into small pieces. Tissue was snap-frozen in liquid nitrogen and freeze-dried. Each freeze-dried sample was ground and *ca* 3 ml g^{-1} FW of 99% (v/v) hexane at 20°C was added. The mixture was homogenised at 20,500 rpm using an Ultra-Turrax T25 homogeniser (Janick and Kunkel, Stafen, Germany) for 5 min at 20°C. The homogenate was filtered under vacuum through Whatman No. 3 filter paper using a 5.5 cm diameter Buchner funnel. The solvent was removed using a rotary evaporator (Buchi Rotovapor, Büchi Labortechnik AG, Flawil, Switzerland) under vacuum (0.6 kPa) at 35°C.

Avocado oil (15 ml) was placed into each of three 250 ml volumetric flasks. Distilled water (15 ml) was placed into each of three other 250 ml volumetric flasks. Three more 250 ml volumetric flasks were left empty. All the flasks were sealed with Suba seals (Fisher Scientific, Leicestershire, UK). 1-MCP gas was prepared as a stock. Equal volumes of stock gas were injected into each of the flasks through the seal to give a concentration of *ca* 10 µl l^{-1} 1-MCP. Every hour after 1 h, 1-MCP concentrations in a 2 ml sample of air extracted from each flask were quantified by GC. Air removed from the flasks was replaced with 2 ml of nitrogen gas. After 24 h, the seals were removed and the flasks flushed with nitrogen for 15 min and resealed. From that time and every 3 h after, 1-MCP concentrations in 2 ml samples of air extracted from each flask were quantified by GC. Air removed from the flasks was replaced with 2 ml of nitrogen gas.

7.3 Results and Discussion

1-MCP concentrations in a sealed atmosphere decreased both faster and to a greater extent for avocado fruit than for apple fruit (Fig. 7.1 A). Thus the avocado fruit sorbed more 1-MCP than the apple fruit. When 1-MCP-treated fruit were placed into a flow-through system, 1-MCP concentrations in the outflow air stream showed that avocado fruit exposed to 1-MCP released more 1-MCP than apple fruit (Fig. 7.1 B). Avocado fruit contain oil (Wills, 1987), which apparently acts as a sink for the cycloalkene 1-MCP.

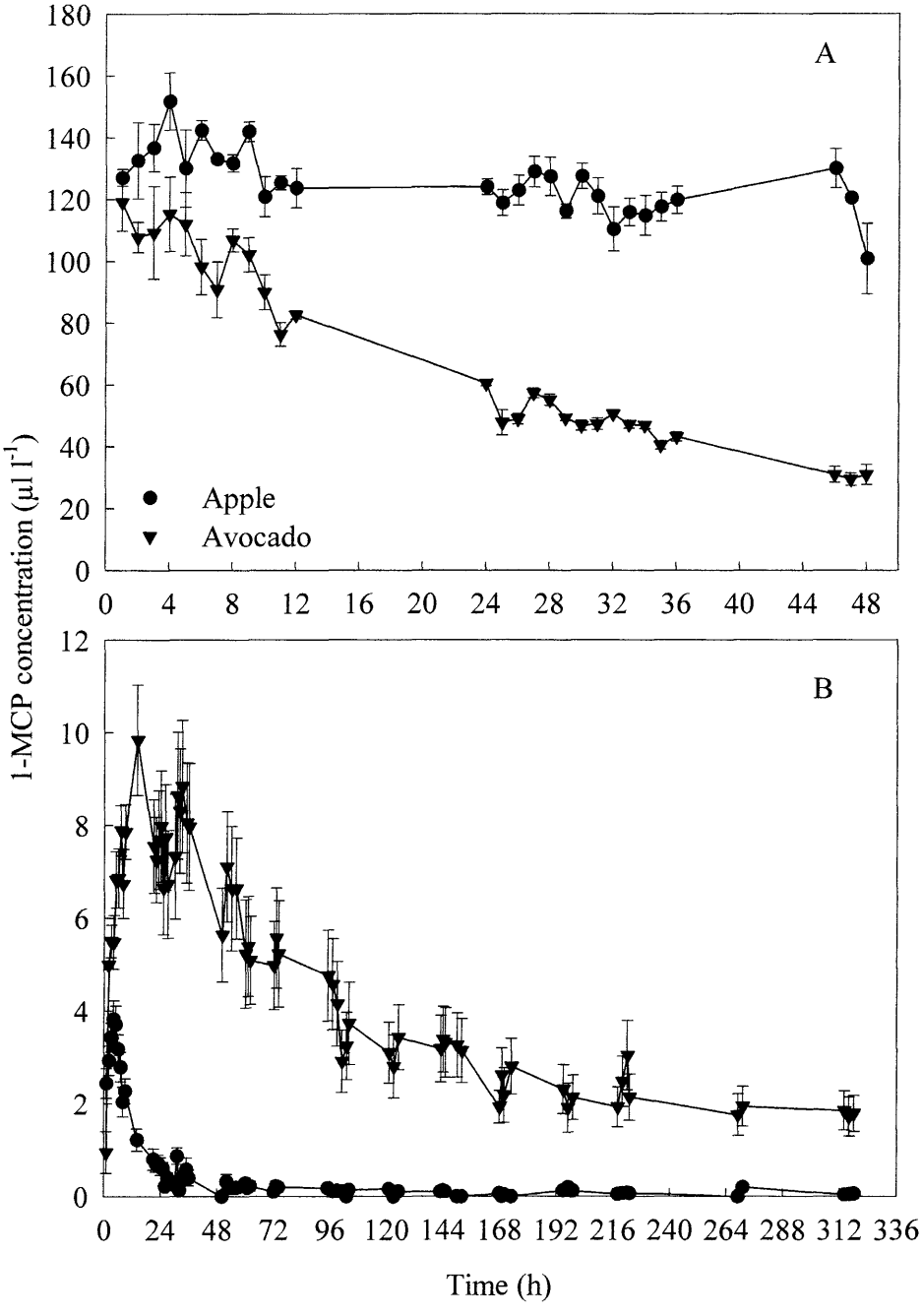

Figure 7.1 **A** 1-MCP concentrations (µl l^{-1}) over 48 h in the headspace of sealed 1.5 l jars containing either an individual 'Cox' apple or 'Hass' avocado fruit (influx). 1-MCP (120 µl l^{-1}) was added as SmartFresh™ at 0 h. **B** 1-MCP concentrations (µl l^{-1}) over 312 h in outflow air over either 'Cox' apple and 'Hass' avocado fruit stored in individual ventilated 0.5 l jars with an air flow through rate of 4 ml min^{-1} (efflux). Fruit were previously exposed to 120 µl l^{-1} 1-MCP for 48 h, then transferred to ventilated jars within 1 h. Fruit were placed into ventilated jars at 0 h. Vertical bars show the standard errors of the means (n = 3). Where no vertical bars are visible the standard errors were smaller than the size of the symbols.

1-MCP concentrations in sealed containers decreased both faster and to a greater extent over oil extracted from avocado fruit than over distilled water (Fig. 7.2 A).

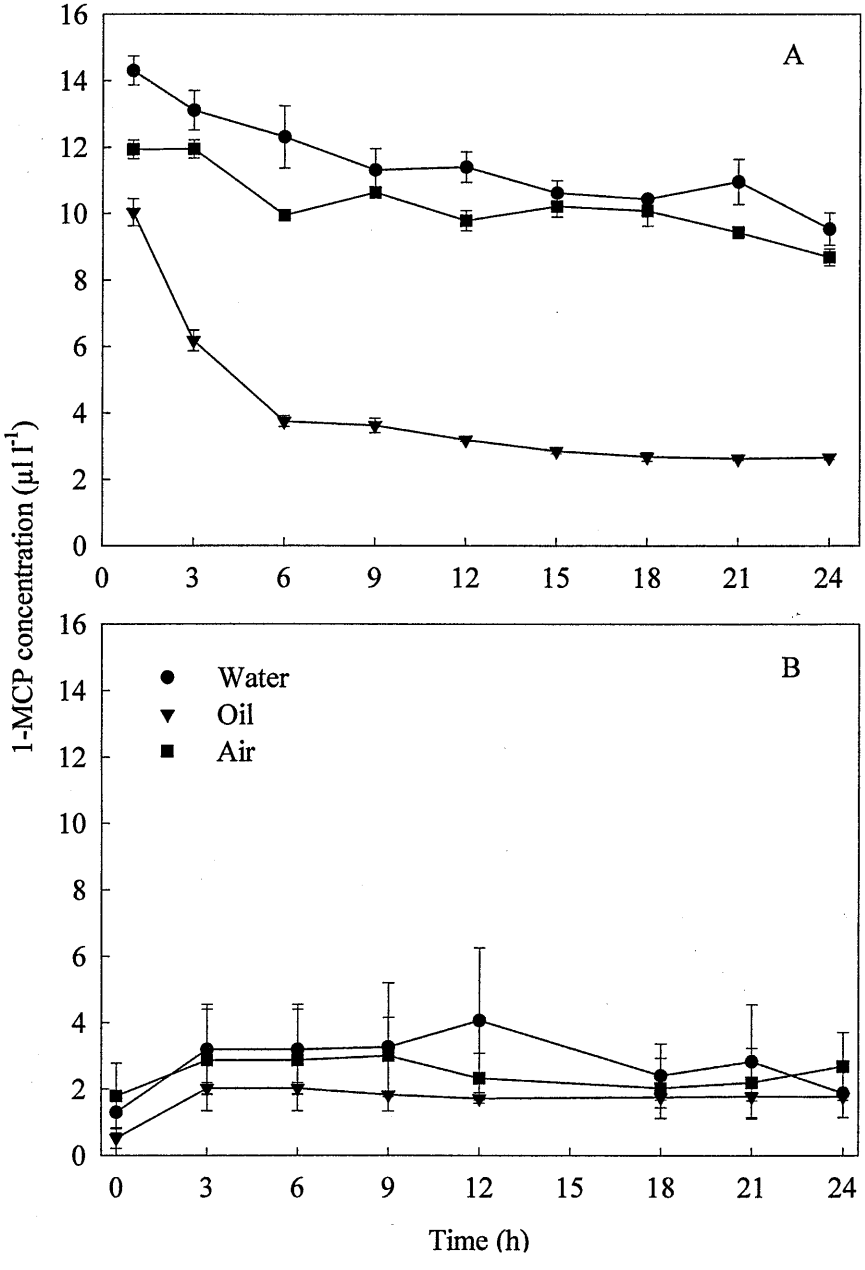

Figure 7.2 **A** 1-MCP concentrations (µl l^{-1}) over 24 h in the headspace of sealed 250 ml volumetric flasks containing 15 ml of avocado oil or distilled water plus an air control (influx). 1-MCP (10 µl l^{-1}) was injected into the flasks at 0 h. **B** 1-MCP concentrations (µl l^{-1}) over 24 h in the headspace of sealed individual 250 ml volumetric flasks containing 15 ml of either avocado oil or distilled water plus an air control (efflux). The flasks were previously injected with 10 µl l^{-1} 1-MCP and the contents left for 48 h followed by flushing with nitrogen gas for 15 min. Vertical bars show the standard errors of the means (n = 3). Where no vertical bars are visible the standard errors were smaller than the size of the symbols.

When the headspace over oil or water exposed to 1-MCP was then flushed with nitrogen gas, 1-MCP concentrations in the headspace were similar. Although apparently inconsistent with the whole fruit experiment, this result suggests that 1-MCP preferentially partitioned into the oil versus the air in the headspace over the oil (Fig. 7.2 B). The apparent inconsistency may be due to the different set-up for the two experiments. The whole fruit experiment was continually ventilated. Thus, there was no opportunity for the 1-MCP in the oil and the headspace to equilibrate according to the partition coefficient.

To facilitate measurements of 1-MCP influx and efflux (in a flow-through system) into and from fruit, respectively, an initial treatment of >100 $\mu l\ l^{-1}$ 1-MCP was used. The relative differences between avocado and apple were clear in terms of greater 1-MCP sorption by the high-oil avocado fruit. Similar differences were evident when extracted avocado oil and water were compared using a 1-MCP treatment concentration of 10 $\mu l\ l^{-1}$, which is closer to the recommended commercial treatment concentration of <1 $\mu l\ l^{-1}$ (G. Regiroli, 2002, AgroFresh Inc., pers. comm.). Preferential partitioning into oil is to be expected at all 1-MCP concentrations (see 7.1).

These results pose questions about the commercial application of 1-MCP for different fruit types. For example, the high oil content of avocados may allow avocados to be treated with a lower 1-MCP concentration than apples due to their greater ability to store 1-MCP. In this case the oil may release 1-MCP to bind to ethylene binding sites when 1-MCP is no longer present in the surrounding atmosphere.

Differences among products in 1-MCP treatment responses in terms of degree of effect (e.g. on ripening retardation or abscission prevention) and the loss of 1-MCP efficacy over time may be due primarily to the synthesis of new ethylene binding sites in plant tissues (Sisler and Serek, 1999; Macnish *et al.*, 2000). Catabolism of 1-MCP remains a possibility. In addition, product physiological (e.g. magnitude and duration of the ethylene climacteric) and physicochemical characteristics (e.g. cuticular and tissue resistance to gas diffusion) could have effects. However, non-specific sorption in lipids could also modulate efficacy, for example, if there were subsequent de-sorption of 1-MCP to bind to newly-formed ethylene binding sites. In an applied

context, non-specific sorption helps to explain the disappearance of 1-MCP over time in commercial fumigation rooms (G. Regiroli, 2002, pers. comm.) and also in laboratory treatment systems at greater rates than expected due to either leakage or specific binding. Unless trickle delivery systems are developed, commercial treatment recommendations may need to be made on the basis of the concentration of the initial dose.

Chapter 8 CONCLUSIONS

1-MCP–treatment maintains 'Queen Cox' and 'Bramley' apple fruit quality during storage. For both cultivars, 1-MCP-treatment suppressed IEC which in-turn controlled ripening. Firmness was also maintained. However, 1-MCP efficacy was shown to be dependent on maturity of 'Queen Cox' and 'Bramley' at harvest. Early harvested 'Queen Cox' and 'Bramley' maintain storage quality more than late-harvested fruit. The time between picking and 1-MCP-treatment was critical for 1-MCP-efficacy. Fruit treated within 24 h of harvest was found to maintain greater quality during storage than those treated 14 d after harvest.

Application of 1-MCP reduced scald development in 'Bramley' apple fruit. 1-MCP-treatment was demonstrated to reduce scald development compared to the current commercial practice of DPA-treatment. 1-MCP-treatment may offer an alternative scald-prevention technique to DPA-treatment.

1-MCP-treatment may be used in conjunction with CA storage to best maintain fruit quality. Short-term CA and 1-MCP-treatment are similar in the ability to store fruit but CA-stored fruit have decreased quality as shelf-life is extended. 1-MCP-treatment maintains quality through a similar shelf-life period.

Fruit type needs to be considered when planning 1-MCP-treatment strategies. Fruits with high fat contents, such as avocado may require different application concentrations or exposure durations than those with less fat, such as apples.

Chlorophyll fluorescence has limited use to quantify 'Queen Cox' and 'Bramley' apple fruit quality.

1-MCP-treatment resulted in reduced incidence of storage rots in 'Queen Cox' and 'Bramley' apple fruit. However, the method of action is unclear. Quantification of antifungal compounds in stored 'Queen Cox' apple fruit by HPLC showed little differences due to 1-MCP-treatment. However, 1-MCP may reduce storage rots due to the maintenance of firmness and the co-incident maintenance of fruit mechanical structure.

Furthermore, it may be possible that 'Queen Cox' apple fruit produces different biochemical responses to different pathogen attack. 1-MCP-treatment may increase the amount of this response.

REFERENCES

Adams, D.O. and Yang, S.F. (1979) Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. *Proceedings of the National Academy of Science, USA* **76**, 170-174.

Agrios, G.N. (1997) Plant Pathology. 4 edn, 635pp California: Academic Press.

Aharoni, N. and Lieberman, M. (1979) Ethylene as a regulator of senescence in tobacco leaf discs. *Plant Physiology* **64**, 801-804.

Amhrein, N., Breuing, F., Eberle, J., Skorupka, H. and Tophof, S. (1982) The metabolism of 1-aminocyclopropane-1-carboxylic acid. In: Wareing P.F. (Ed.) *Plant Growth Substances*, pp. 249-258. London: Academic Press

Anet, E.F.L.J. and Coggiola, I.M. (1974) Superficial scald, a functional disorder of stored apples. X. Control of α-farnesene autooxidation. *Journal of the Science of Food and Agriculture* **25**, 293-298.

Antunes, M.D.C. and Sfakiotakis, E.M. (2002) Ethylene biosynthesis and ripening behaviour of 'Hayward' kiwifruit subjected to some controlled atmospheres. *Postharvest Biology and Technology* **26**, 167-179.

Ardi, R., Kobiler, I., Jacoby, B., Keen, N.T. and Prusky, D. (1998) Involvement of epicatechin biosynthesis in the activation of the mechanism of resistance of avocado fruits to *Colletotrichum gloeosporioides*. *Physiological and Molecular Plant Pathology* **53** (5-6):269-285.

Atta-Aly, M.A., Brecht, J.K. and Huber, D.J. (2000) Ethylene feedback mechanisms in tomato and strawberry fruit tissues in relation to fruit ripening and climacteric patterns. *Postharvest Biology and Technology* 151-162.

Awad, M.A. and de Jager, A. (2000) Flavonoid and chlorogenic acid concentrations in skin of 'Jonagold' and 'Elstar' apples during and after regular and ultra low oxygen storage. *Postharvest Biology and Technology* **20**, 15-24.

Awad, M.A., de Jager, A. and van Westing, L.M. (2000) Flavonoid and chlorogeninc acid levels in apple fruit: characterisation of variation. *Scientia Horticulturae* **83**, 249-263.

Barry, C.S., Fox, E.A., Yen, H., Lee, S., Ying, T., Grierson, D. and Giovannoni, J.J. (2001) Analysis of the ethylene response in the *epinastic* mutant of tomato. *Plant Physiology* **127**, 58-66.

Barry, C.S., Llop-Tous, I. and Grierson, D. (2000) The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. *Plant Physiology* **123**, 979-986.

Bartley, I.M. (1977) A further study of β-galactosidase activity in apples ripening in store. *Journal of Experimental Botany* **28**, 943-948.

Bengochea, T., Dodds, J.H., Evans, D.E., Jerie, P.H., Niepel, B., Shaari, A.R. and Hall, M.A. (1980) Studies on ethylene binding by cell-free preparations from cotyledons of *Phaseolus vulgaris* L. *Plant Physiology* **58**, 268-271.

Beyer, E.M. (1976) A potent inhibitor of ethylene action in plants. *Plant Physiology* **58**, 268-271.

Boonyakiat, D., Spotts, R.A. and Richardson, D.G. (1986) Effects of chlorogenic acid and arbutin on growth and spore germination of decay fungi. *HortScience* **21**, 309-310.

Brown, A.E. and Swinburne, T.R. (1971) Benzoic acid: an antifungal compound formed in Bramley's Seedling apple fruits following infection by *Nectria galligena* Bres. *Physiological Plant Pathology* **1**, 469-475.

Bufler, G. (1984) Ethylene-enhanced 1-aminocyclopropene-1-carboxylic acid synthase activity in ripening apples. *Plant Physiology* **75**, 192-195.

Bufler, G. (1986) Ethylene-promoted conversion of 1-aminocyclopropene-1-carboxylic acid to ethylene in peel of apple at various stages of fruit development. *Plant Physiology* **80**, 539-543.

Burda, S., Oleszek, W. and Lee, C.Y. (1990) Phenolic compounds and their changes in apples during maturation and cold storage. *Journal of Agriculture, Food and Chemistry* **38**, 945-948.

Burg, S.P. and Burg, E.A. (1965) Ethylene action and the ripening of fruits. *Science* **148**, 1190-1196.

Chang, C. (1996) The ethylene signal transduction pathway in *Arabidopsis*: an emerging paradigm. *Trends in Biochemical Sciences* **21**, 129-133.

Chang, C. and Shockey, J.A. (1999) The ethylene-response pathway: signal perception to gene regulation. *Current Opinion in Plant Biology* **2**, 352-358.

Chervin., C., Raynal, J., André, N., Bonneau, A. and Westercamp, P. (2001) Combining controlled atmosphere storage and ethanol vapors to control superficial scald of apple. *HortScience* **36**, 951-952.

Colgan, R.J., Dover, C.J., Johnson, D.S. and Pearson, K. (1999) Delayed CA and oxygen at 1 kPa or less control superficial scald without CO_2 injury on Bramley's Seedling apples. *Postharvest Biology and Technology* **16**, 223-231.

Dauny, P.T. and Joyce, D.C. (2002) 1-MCP improves storability of 'Queen Cox' and 'Bramley' apple fruit. *HortScience* **37**, 1082-1085.

de Lorenzo, G., d'Ovidio, R. and Cervone, F. (2001) The role of polygalacturonase-inhibiting proteins (PGIPs) in defense against pathogenic fungi. *Annual Review of Phytopathology* **39**, 313-335.

DeEll, J.R., Murr, D.P., Porteous, M.D. and Rupasinghe, H.P.V. (2002) Influence of temperature and duration of 1-methylcyclopropene (1-MCP) treatment on apple quality. *Postharvest Biology and Technology* **24**, 349-353.

Dick, A.J., Laskey, G. and Lidster, P.D. (1984) Inhibition of β-galactosidase isolated from 'McIntosh' apples. *HortScience* **19**, 552-553.

Dick, A.J., Williams, R., Bearne, S.L. and Lidster, P.D. (1985) Quercetin glycosides and chlorogeninc acid: inhibitors of apple β-galactosidase and of apple softening. *Journal of Agriculture, Food and Chemistry* **33**, 298-800.

Dong, L., Lurie, S. and Zhou, H. (2002) Effect of 1-methylcyclopropene on ripening of 'Canino' apricots and 'Royal Zee' plums. *Postharvest Biology and Technology* **24**, 135-145.

Du, Z. and Bramlage, W.J. (1994) Roles of ethylene in the development of superficial scald in 'Cortland' apples. *Journal of the American Society for Horticultural Science* **119**, 516-523.

Ecker, J.R. and Davis, R.W. (1987) Plant defense genes are regulated by ethylene. *Proceedings of the National Academy of Science, USA* **84**, 5202-5206.

Ekman, J.H., Clayton, M., Biasi, W.V. and Mitcham, E.J. (2004) Interactions between 1-MCP concentration, treatment interval and storage time for 'Bartlett' pears. *Postharvest Biology and Technology* **31**, 127-136.

Escarpa, A. and González, M.C. (1998) High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. *Journal of Chromatography A* **823**, 331-337.

Fan, Q. and Tian, S. (2001) Postharvest biological control of grey mold and blue mold on apple by *Cryptococcus albidus* (Saito) Skinner. *Postharvest Biology and Technology* **21**, 341-350.

Fan, X., Blankenship, S. and Mattheis, J.P. (1999a) 1-methylcyclopropene inhibits apple ripening. *Journal of the American Society for Horticultural Science* **124**, 690-695.

Fan, X., Mattheis, J.P. and Blankenship, S. (1999b) Development of apple superficial scald, soft scald, core flush, and greasiness is reduced by MCP. *Journal of Agriculture, Food and Chemistry* **47**, 3063-3068.

Fernández-Trujillo, J.P., Nock, J.F. and Watkins, C.B. (2001) Superficial scald, carbon dioxide injury, and changes of fermentation products and organic acids in 'Cortland' and 'Law Rome' apples after high carbon dioxide stress treatment. *Journal of the American Society for Horticultural Science* **126**, 235-241.

Feucht, W., Truetter, D. and Christ, E. (1992) The precise localization of catechins and proanthocyanidins in protective layers around fungal infections. *Journal of Plant Diseases and Protection* **99**, 404-413.

Fluhr, R. and Mattoo, A.K. (1996) Ethylene biosynthesis and perception. *Critical Reviews in Plant Sciences* **15**, 479-523.

Golding, J.B., McGlasson, W.B. and Wyllie, S.G. (2001) Relationship between production of ethylene and α-farnesene in apples, and how it is influenced by the timing of diphenylamine treatment. *Postharvest Biology and Technology* **21**, 225-233.

Golding, J.B., Shearer, D., Wyllie, S.G. and McGlasson, W.B. (1998) Application of 1-MCP and propylene to identify ethylene-dependent ripening processes in mature banana fruit. *Postharvest Biology and Technology* **14**, 87-98.

Gong, Y. and Tian, M.S. (1998) Inhibitory effect of diazocyclopentadiene on the development of superficial scald in Granny Smith apple. *Plant Growth Regulation* **26**, 117-121.

Grayer, R.J. and Harborne, J.B. (1994) A survey of antifungal compounds from higher plants, 1982-1993. *Phytochemistry* **37**, 19-42.

Grayer, R.J. and Kokubun, T. (2001) Plant-fungal interactions: the search for phytoalexins and other antifungal compounds from higher plants. *Phytochemistry* **56**, 253-263.

Hall, A.E., Chen, Q.C., Findell, J.L., Schaller, G.E. and Bleecker, A.B. (1999) The relationship between ethylene binding and dominant insensitivity conferred by mutant forms of the ETR1 ethylene receptor. *Plant Physiology* **121**, 291-300.

Hall, A.E., Findell, J.L., Schaler, G.E., Sisler, E.C. and Bleecker, A.B. (2000) Ethylene perception by the ERS1 protein in Arabidopsis. *Plant Physiology* **123**, 1449-1457.

Hammerschmidt, R. (1999) Phytoalexins: what have we learned after 60 years? *Annual Review of Phytopathology* **37**, 285-306.

Harborne, J.B. (1999) The comparative biochemistry of phytoalexin induction in plants. *Biochemical Systematics and Ecology* **27**, 335-367.

Harborne, J.B. and Williams, C.A. (2000) Advances in flavonoid research since 1992. *Phytochemistry* **55**, 481-504.

Hébert, C., Charles, M.T., Gauthier, L., Willemot, C., Khanizadeh, S. and Cousineau, J. (2002) Strawberry proanthocyanidins: biochemical markers for *Botrytis cinerea* resistance and shelf-life predictability. *Acta Horticulturae* **567**, 659-661.

Hoffman, N.E., Yang, S.F. and McKeon, T. (1982) Identification of 1-(malonylamino) cyclopropane-1-carboxylic acid as a major conjugate of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor in higher plants. *Biochemical and Biophysical Research Communication* **104**, 765-770.

Hofman, P.J., Jobin-Decor, M., Meiburg, G.F., Macnish, A.J. and Joyce, D.C. (2001) Ripening and quality responses of avocado, custard apple, mango and papaya fruit to 1-methylcyclopropene. *Australian Journal of Experimental Agriculture* **41**, 567-572.

Huelin, F.E. and Coggiola, I.M. (1968) Superficial scald, a functional disorder of stored apples. IV. Effect of variety, maturity, oiled wraps and diphenylamine on the concentration of α-farnesene in the fruit. *Journal of the Science of Food and Agriculture* **19**, 297-301.

Huelin, F.E. and Coggiola, I.M. (1970) Superficial scald, a functional disorder of stored apples. VII. Effect of applied α-farnesene, temperature and diphenylamine on scald and the concentration and oxidation of α-farnesene in the fruit. *Journal of the Science of Food and Agriculture* **21**, 584-589.

Ingle, M. and D'Souza, M.C. (1989) Physiology and control of superficial scald of apples: a review. *HortScience* **24**, 28-31.

Ippolito, A., Ghaouth, A.E., Wilson, C.L. and Wisniewski, M. (2000) Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. *Postharvest Biology and Technology* **19**, 265-272.

Jiang, Y. and Joyce, D.C. (2000) Effects of 1-methylcyclopropene alone and in combination with polyethylene bags on the postharvest life of mango fruit. *Annals of Applied Biology* **137**, 321-327.

Jiang, Y., Joyce, D.C. and Macnish, A.J. (1999) Extension of the shelf life of banana fruit by 1-methylcyclopropene in combination with polyethylene bags. *Postharvest Biology and Technology* **16**, 187-193.

Jiang, Y., Joyce, D.C. and Macnish, A.J. (1999b) Responses of banana fruit to treatment with 1-methylcyclopropene. *Plant Growth Regulation* **28**, 77-82.

Jiang, Y., Joyce, D.C. and Macnish, A.J. (2002) Softening response of banana fruit treated with 1-methylcyclopropene to high temperature exposure. *Plant Growth Regulation* **36**, 7-11.

Jiang, Y., Joyce, D.C. and Terry, L.A. (2001) 1-MCP treatment affects strawberry fruit decay. *Postharvest Biology and Technology* **23**, 227-232.

Jiang, Y. and Miles, P.W. (1993) Generation of H_2O_2 during enzymic oxidation of catechin. *Phytochemistry* **33**, 29-34.

Johnson, D.S. and Colgan, R.J. (2003) Low ethylene controlled atmosphere induces adverse effects on the quality of 'Cox's Orange Pipin' apples treated with aminoethoxyvinylglycine during fruit development. *Postharvest Biology and Technology* **27**, 59-68.

Ju, Z. and Curry, E.A. (2000) Evidence that α -farnesene biosynthesis during fruit ripening is mediated by ethylene regulated gene expression in apples. *Postharvest Biology and Technology* **19**, 9-16.

Ju, Z., Yuan, Y., Liu, C., Zhan, S. and Wang, M. (1996) Relationships among simple phenol, flavonoid and anthocyanin in apple fruit peel at harvest and scald susceptibility. *Postharvest Biology and Technology* **8** 83-93.

Kende, H. and Boller, T. (1981) Wound ethylene and 1-aminocyclopropane-1-carboxylate synthase in ripening tomato fruit. *Planta* **151**, 476-481.

Kidd, F. and West, C. (1924) The course of respiratory activity throughout the life of an apple. In: Anonymous *Annual Report of the Food Investigations Board*, pp. 27-32. London:

Knee, M. (1973) Polysaccharide changes in cell walls of ripening apples. *Phytochemistry* **12**, 1543-1549.

Knee, M. and Tsantili, E. (1988) Storage of 'Gloster 69' apples in the preclimacteric state for up to 200 days after harvest. *Physiologia Plantarum* **74**, 499-503.

Krause, G.H. and Weis, E. (1984) Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. *Photosynthesis Research* **5**, 139-157.

Krause, G.H. and Weis, E. (1991) Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 313-349.

Ku, V.V.V., Wills, R.B.H. and Ben-Yehoshua, S. (1999) 1-methylcyclopropene can differentially affect the postharvest life of strawberries exposed to ethylene. *HortScience* **34**, 119-120.

Lattanzio, V., di Venere, D., Linsalata, V., Bertolini, P., Ippolito, A. and Salerno, M. (2001) Low temperature metabolism of apple phenolics and quiescence of *Phlyctaena vagabunda*. *Journal of Agricultural and Food Chemistry* **49**, 5817-5821.

Lelièvre, J., Latché, A., Jones, B., Bouzayen, M. and Pech, J. (1997) Ethylene and fruit ripening. *Physiologia Plantarum* **101**, 729-739.

Leverentz, B., Conway, W.S., Janisiewicz, W.J., Saftner, R.A. and Camp, M.J. (2003) Effect of combining MCP treatment, heat treatment, and biocontrol on the reduction of postharvest decay of 'Golden Delicious' apples. *Postharvest Biology and Technology* **27**, 221-233.

Macnish, A.J., Joyce, D.C., Hofman, P.J. and Simons, D.H. (1999) Involvement of ethylene in postharvest senescence of *Boronia heterophylla* flowers. *Australian Journal of Experimental Agriculture* **39**, 911-913.

McGlasson, W.B. (1985) Ethylene and fruit ripening. *HortScience* **20**, 51-54.

McMurchie, E.J., McGlasson, W.B. and Eaks, I.L. (1972) Treatment of fruit with propylene gives information about the biogenesis of ethylene. *Nature* **237**, 235-236.

Mir, N.A., Curell, E., Khan, N., Whitaker, M. and Beaudry, R.M. (2001) Harvest maturity, storage temperature, and 1-MCP application frequency alter firmness retention and chlorophyll fluorescence of 'Redchief Delicious' apples. *Journal of the American Society for Horticultural Science* **126**, 618-624.

Moodley, R.S., Govinden, R. and Odhav, B. (2002) The effect of modified atmospheres and packaging on patulin production in apples. *Journal of Food Protection* **65**, 867-871.

Moya-León, M.A. and John, P. (1994) Activity of 1-aminocyclopropane-1-carboxylate (ACC) oxidase (ethylene-forming enzyme) in the pulp and the peel of ripening bananas. *Journal of Horticultural Science* **69**, 243-250.

Nakano, R., Inoue, S., Kubo, Y. and Inaba, A. (2002) Water stress-induced ethylene in the calyx triggers autocatalytic ethylene production and fruit softening in 'Tonewase' persimmon grown in a heated plastic-house. *Postharvest Biology and Technology* **25**, 293-300.

Nakatsuka, A., Murachi, S., Okunishi, H., Shiomi, S., Nakano, R., Kubo, Y. and Inaba, A. (1998) Differential expression and internal feedback regulation of 1-aminocyclopropane-1-carboxylate synthase, 1-aminocyclopropane-1-carboxylate oxidase, and ethylene receptor genes in tomato fruit during development and ripening. *Plant Physiology* **118**, 1295-1305.

Ndubizu, T.O.C. (1976) Relations of phenolic inhibitors to resistance of immature apple fruits to rot. *Journal of Horticultural Science* **51**, 311-319.

Nicholson, R.L. and Hammerschmidt, R. (1992) Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology* **30**, 369-389.

Noble, J.P. and Drysdale, R.B. (1983) The role of benzoic acid and phenolic compounds in latency in fruits of two apple cultivars infected with *Pezicula malicorticis* or *Nectria galligena*. *Physiological Plant Pathology* **23**, 207-216.

Oetiker, J.H. and Yang, S.F. (1995) The role of ethylene in fruit ripening. *Acta Horticulturae* **398**, 167-178.

Oleszek, W., Lee, C.Y., Jaworski, A.W. and Price, K.R. (1988) Identification of some phenolic compounds in apples. *Journal of Agriculture, Food and Chemistry* **36**, 430-432.

Pelayo, C., Vilas-Boas, E.V.d.B., Benichou, M. and Kader, A.A. (2003) Variability in responses of partially ripe bananas to 1-methylcyclopropene. *Postharvest Biology and Technology* **28**, 75-85.

Pitt, J.I. and Hocking, A.D. (1997) Fungi and Food Spoilage. 2 edn, London: Blackie Academic & Professional (Chapman & Hall).

Porat, R., Weiss, B., Cohen, L., Daus, A., Goren, R. and Droby, S. (1999) Effects of ethylene and 1-methylcyclopropene on the postharvest qualities of 'Shamouti' oranges. *Postharvest Biology and Technology* **15**, 155-163.

Prusky, D. (1996) Pathogen quiescence in postharvest diseases. *Annual Review of Phytopathology* **34**, 413-434.

Rodríguez, F.I., Esch, J.J., Hall, A.E., Binder, B.M., Schaller, G.E. and Bleecker, A.B. (1999) A copper cofactor for the ethylene receptor ETR1 from Arabidopsis. *Science* **283**, 996-998.

Rupasinghe, H.P.V., Murr, D.P., Paliyath, G. and Skog, L. (2000) Inhibitory effect of 1-MCP on ripening and superficial scald in 'McIntosh' and 'Delicious' apples. *Journal of Horticultural Science and Biotechnology* **75**, 271-276.

Rupasinghe, H.P.V., Paliyath, G. and Murr, D.P. (1998) Biosynthesis of α-farnesene and its relation to superficial scald development in 'Delicious' apples. *Journal of the American Society for Horticultural Science* **123**, 882-886.

Sacks, E.J. and Shaw, D.J. (1994) Optimum allocation of objective color measurements for evaluating fresh strawberries. *Journal of the American Society for Horticultural Science* **119**, 330-334.

Saltveit, M.E. (1982) Procedures for extracting and analyzing internal gas samples from plant tissues by gas chromatography. *HortScience* **17**, 878-881.

Saltveit, M.E. (1999) Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biology and Technology* **15**, 279-292.

Schaller, G.E., Ladd, A.N., Lanahan, M.B., Spanbauer, J.M. and Bleecker, A.B. (1995) The ethylene response mediator ETR1 from Arabidopsis forms a disulfide-linked dimer. *Journal of Biological Chemistry* **270**, 12526-12530.

Sisler, E.C. and Blankenship, S.M. (1996) USA Patent No. 5518988.

Sisler, E.C., Dupille, E. and Serek, M. (1996) Effect of 1-methylcyclopropene and methylenecyclopropene on ethylene binding and ethylene action on cut carnations. *Plant Growth Regulation* **18**, 79-86.

Sisler, E.C. and Serek, M. (1997) Inhibitors of ethylene responses in plants at the receptor level: Recent developments. *Physiologia Plantarum* **100**, 577-582.

Sisler, E.C. and Serek, M. (1999) Compounds controlling the ethylene receptor. *Botanical Bulletin of Academia Sinica* **40**, 1-7.

Smillie, R.M., Hetherington, S.E., Nott, R., Chaplin, G.R. and Wade, N.L. (1987) Applications of chlorophyll fluorescence to the postharvest physiology and storage of mango and banana fruit and the chilling tolerance of mango cultivars. *Asean Food Journal* **3**, 55-59.

Stepanova, A.N. and Ecker, J.R. (2000) Ethylene signaling: from mutants to molecules. *Current Opinion in Plant Biology* **3** (5):353-360.

Stow, J.R., Dover, C.J. and Genge, P.M. (2000) Control of ethylene biosynthesis and softening in 'Cox's Orange Pippin' apples during low-ethylene, low-oxygen storage. *Postharvest Biology and Technology* **18**, 215-225.

Suárez, B., Picinelli, A. and Mangas, J.J. (1996) Solid-phase extraction and high performance liquid chromatographic determination of polyphenols in apple musts and ciders. *Journal of Chromatography A* **727**, 203-209.

Swinburne, T.R. (1975) Microbial proteases as elicitors of benzoic acid accumulation in apples. *Phytopathologische Zeitschrift* **82**, 152-162.

Taiz, L. and Zeiger, E. (1991) Plant Physiology. California: The Benjamin/Cummings Publishing Company, Inc.

Toivonen, P.M.A. (1992) Chlorophyll fluorescence as a nondestructive indicator of freshness in harvested broccoli. *HortScience* **27**, 1014-1015.

Vendrell, M. and McGlasson, W.B. (1971) Inhibition of ethylene production in banana fruit tissue by ethylene treatment. *Australian Journal of Biological Science* **24**, 885-895.

Vero, S., Mondino, P., Burgueño, J., Soubes, M. and Winiewski, M. (2002) Characterization of biocontrol activity of two yeast strains from Uruguay against blue mold of apples. *Postharvest Biology and Technology* **26**, 91-98.

Walker, J.R.L. (1969) Inhibition of the apple phenolase system through infection by *Penicillium expansum*. *Phytochemistry* **8**, 561-566.

Waring, P.F. (1982) Plant Growth Substances. In: Imaskei, H., Yoshii, Y. and Todaka, I., (Eds.) *Regulation of auxin-induced ethylene biosynthesis in plants*, pp. 259-268. London: Academic Press]

Watada, A.E. (1989) Nondestructive methods of evaluating quality of fresh fruits and vegetables. *Acta Horticulturae* **258**, 321-329.

Watkins, C.B., Barden, C.L. and Bramlage, W.J. (1993) Relationships between alpha-farnesene, ethylene production and superficial scald development in apples. *Acta Horticulturae* **343**, 155-160.

Watkins, C.B., Bramlage, W.J. and Cregoe, B.A. (1995) Superficial scald of 'Granny Smith' apples is expressed as a typical chilling injury. *Journal of the American Society for Horticultural Science* **120**, 88-94.

Watkins, C.B., Nock, J.F. and Whitaker, B.D. (2000) Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biology and Technology* **19**, 17-32.

Whitaker, B.D., Nock, J.F. and Watkins, C.B. (2000) Peel tissue α-farnesene and conjugated trienol concentrations during storage of 'White Angel' x 'Rome Beauty' hybrid apple selections susceptible and resistant to superficial scald. *Postharvest Biology and Technology* **20**, 231-241.

Wills, R., McGlasson, B., Graham, D. and Joyce, D. (1998) Postharvest. An Introduction to the Physiology and Handling of Fruit, Vegetables and Ornamentals. 4th edn, Oxon, UK: CAB International.

Wills, R.B.H. (1987) Composition of Australian fresh fruit and vegetables. *Food Technology in Australia* **39**, 523-526.

Wills, R.B.H. and Ku, V.V.V. (2002) Use of 1-MCP to extend the time to ripen of green tomatoes and postharvest life of ripe tomatoes. *Postharvest Biology and Technology* **26**, 85-90.

Xu, P., Narasimhan, M.L., Samson, T., Coca, M.A., Huh, G.H., Zhou, J., Martin, G.B., Hasegawa, P.M. and Bressan, R.A. (1998) A nitrilase-like protein interacts with GCC Box DNA-binding proteins involved in ethylene and defense responses. *Plant Physiology* **118**, 867-874.

Yang, S.F. (1985) Biosynthesis and action of ethylene. *HortScience* **20**, 41-45.

Yang, S.F. (1987) The role of ethylene and ethylene synthesis in fruit ripening. In: Thompson W.W., Nothnagel E.A. and Huffaker R.C., (Eds.) *Plant Senescence: Its Biochemistry and Physiology*, pp. 156-166. Rockville, Md: American Society of Plant Physiology

Yang, S.F., Hoffman, N.E., McKeon, T., Riov, J., Kao, C.H. and Yung, K.H. (1982) Mechanism and regulation of ethylene biosynthesis. In: Wareing P.F., (Ed.) *Plant Growth Substances*, pp. 239-248. London: Academic Press]

Yu, Y.B. and Yang, S.F. (1980) Biosynthesis of wound ethylene. *Plant Physiology* **66**, 281-285.

Zanella, A. (2003) Control of apple superficial scald and ripening - a comparison between 1-methylcyclopropene and diphenylamine postharvest treatments, initial low oxygen stress and ultra low oxygen storage. *Postharvest Biology and Technology* **27**, 69-78.

APPENDIX

QUANTIFICATION OF ANTIFUNGAL COMPOUNDS BY HPLC AND STATISTICAL ANALYSIS OF DATA FOR 'QUEEN COX' APPLE FRUIT HARVESTED IN 2002.

Genstat 5 Release 3.2 (PC/Windows NT) 31 December 2004 22:31:05
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Genstat 5 Second Edition (for Windows)
Genstat 5 Procedure Library Release 3[3] (PL9)

Non Infected Only

Analysis of variance

Benzoic acid

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	698.	698.	0.60	0.439
Harvest	2	5630.	2815.	2.42	0.091
Storage	5	13670.	2734.	2.35	0.041
Shelf	3	7345.	2448.	2.11	0.100
MCP.Harvest	2	5234.	2617.	2.25	0.107
MCP.Storage	5	4527.	905.	0.78	0.565
Harvest.Storage	10	29125.	2912.	2.51	0.007
MCP.Shelf	3	31392.	10464.	9.01	<.001
Harvest.Shelf	6	10433.	1739.	1.50	0.180
Storage.Shelf	15	27023.	1802.	1.55	0.088
MCP.Harvest.Storage	10	63667.	6367.	5.48	<.001
MCP.Harvest.Shelf	6	21991.	3665.	3.16	0.005
MCP.Storage.Shelf	15	29730.	1982.	1.71	0.050
Harvest.Storage.Shelf	25(5)	63410.	2536.	2.18	0.001
MCP.Harvest.Storage.Shelf	16(14)	36666.	2292.	1.97	0.016
Residual	240(48)	278679.	1161.		
Total	364(67)	531987.			

Tables of means

Grand mean 18.3

MCP	Control	MCP
	17.1	19.6

Harvest	Early	Late	Mid
	15.6	16.0	23.4

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	9.2	21.9	16.4	21.3	26.5	14.8

Shelf	0.00	3.00	7.00	14.00
	20.8	23.0	17.6	12.0

MCP	Harvest	Early	Late	Mid
Control		18.0	15.7	17.5
MCP		13.2	16.3	29.4

MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		10.1	24.0	12.7	21.6	19.2	14.9
MCP		8.3	19.9	20.1	21.0	33.9	14.6

Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		11.3	33.9	12.8	14.4	16.0	5.2
Late		2.1	10.8	23.3	23.0	14.1	22.6
Mid		14.2	21.1	12.9	26.5	49.5	16.5

MCP	Shelf	0.00	3.00	7.00	14.00
Control		9.3	16.8	19.1	23.1
MCP		32.3	29.1	16.2	0.9

Harvest	Shelf	0.00	3.00	7.00	14.00
Early		21.0	15.4	9.9	16.1
Late		12.1	22.5	16.7	12.7
Mid		29.3	31.0	26.3	7.2

Storage	Shelf	0.00	3.00	7.00	14.00
0.00		14.5	13.4	10.1	-1.4
2.00		13.3	40.7	18.9	14.7
4.00		18.3	26.6	6.5	14.0
8.00		29.6	8.3	37.1	10.4
16.00		38.6	25.3	15.1	27.1
24.00		10.4	23.3	18.2	7.1

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		11.0	64.6	10.5	-3.0	18.8
	Late		5.4	7.8	11.0	27.7	19.8
	Mid		13.8	-0.5	16.5	40.1	19.0
MCP	Early		11.6	3.1	15.2	31.8	13.1
	Late		-1.3	13.8	35.7	18.3	8.4
	Mid		14.5	42.7	9.3	13.0	80.1

MCP	Harvest	Storage	24.00
Control	Early		6.1
	Late		22.3
	Mid		16.3
MCP	Early		4.3
	Late		22.9
	Mid		16.6

MCP	Harvest	Shelf	0.00	3.00	7.00	14.00
Control	Early		11.1	24.5	14.6	21.8
	Late		10.8	14.4	13.7	23.9
	Mid		5.9	11.4	29.0	23.7
MCP	Early		30.8	6.2	5.2	10.5
	Late		13.5	30.6	19.7	1.4
	Mid		52.6	50.6	23.6	-9.3

MCP	Storage	Shelf	0.00	3.00	7.00	14.00
Control	0.00		7.0	7.5	5.9	19.9
	2.00		-0.5	38.6	36.4	21.4
	4.00		11.3	15.2	7.7	16.5
	8.00		24.4	12.4	18.0	31.5
	16.00		7.8	7.7	25.1	36.2
	24.00		5.5	19.2	21.6	13.3
MCP	0.00		22.1	19.4	14.3	-22.6
	2.00		27.1	42.8	1.4	8.1
	4.00		25.3	38.1	5.3	11.6
	8.00		34.7	4.1	56.1	-10.8
	16.00		69.5	43.0	5.1	17.9
	24.00		15.2	27.4	14.8	0.9

Harvest	Storage	Shelf	0.00	3.00	7.00	14.00
Early	0.00		13.1	5.1	9.6	17.3
	2.00		16.5	59.8	26.2	32.9
	4.00		27.8	3.5	6.0	14.2
	8.00		49.5	7.5	-11.2	11.7
	16.00		13.6	8.6	25.4	16.3
	24.00		5.3	7.6	3.4	4.5
Late	0.00		8.8	11.7	4.6	-16.8
	2.00		7.6	13.9	16.1	5.7
	4.00		10.2	57.9	6.2	19.0
	8.00		26.3	8.5	36.0	21.3
	16.00		5.4	10.4	11.1	29.5
	24.00		14.5	32.7	26.2	17.1
Mid	0.00		21.7	23.6	16.0	-4.7
	2.00		15.9	48.4	14.4	5.6
	4.00		16.9	18.6	7.3	8.9
	8.00		12.9	8.7	86.4	-1.9
	16.00		96.9	57.0	8.8	35.5
	24.00		11.3	29.7	25.0	-0.2

MCP	Harvest	Storage	Shelf	0.00	3.00	7.00	14.00
Control	Early	0.00		1.2	2.3	8.7	31.8
		2.00		17.8	111.4	71.6	57.8
		4.00		19.9	6.2	6.0	10.0
		8.00		10.6	9.4	-45.2	13.3
		16.00		8.2	8.4	44.2	14.5
		24.00		9.1	9.5	2.4	3.5
	Late	0.00		2.2	18.9	-3.2	3.7
		2.00		9.0	1.6	19.5	1.3
		4.00		2.8	5.1	11.2	24.9
		8.00		47.7	11.7	10.7	40.8
		16.00		-3.3	10.7	20.6	51.2
		24.00		6.1	38.3	23.7	21.2
	Mid	0.00		17.4	1.4	12.1	24.2
		2.00		-28.2	2.7	18.3	5.0
		4.00		11.1	34.5	5.9	14.4
		8.00		14.9	16.3	88.6	40.5
		16.00		18.5	3.8	10.5	43.0
		24.00		1.4	9.9	38.7	15.2
MCP	Early	0.00		25.0	7.9	10.5	2.9
		2.00		15.2	8.2	-19.1	8.0
		4.00		35.6	0.7	5.9	18.3
		8.00		88.5	5.7	22.9	10.1
		16.00		18.9	8.9	6.5	18.1
		24.00		1.5	5.7	4.4	5.4
	Late	0.00		15.3	4.5	12.4	-37.3
		2.00		6.1	26.2	12.8	10.1
		4.00		17.6	110.7	1.2	13.2
		8.00		4.8	5.3	61.3	1.9
		16.00		14.1	10.1	1.6	7.8
		24.00		22.9	27.1	28.7	12.9
	Mid	0.00		26.0	45.8	20.0	-33.6
		2.00		59.9	94.2	10.5	6.2
		4.00		22.6	2.7	8.7	3.4
		8.00		10.9	1.2	84.2	-44.4
		16.00		175.3	110.1	7.1	27.9
		24.00		21.1	49.5	11.3	-15.5

Standard errors of differences of means

Table	MCP	Harvest	Storage	Shelf
rep.	216	144	72	108
d.f.	240	240	240	240
s.e.d.	3.28	4.02	5.68	4.64

Table	MCP Harvest	MCP Storage	Harvest Storage	MCP Shelf
rep.	72	36	24	54
d.f.	240	240	240	240
s.e.d.	5.68	8.03	9.84	6.56

Table	Harvest Shelf	Storage Shelf	MCP Harvest Storage	MCP Harvest Shelf
rep.	36	18	12	18
d.f.	240	240	240	240
s.e.d.	8.03	11.36	13.91	11.36

Table	MCP Storage Shelf	Harvest Storage Shelf	MCP Harvest Storage Shelf
rep.	9	6	3
d.f.	240	240	240
s.e.d.	16.06	19.67	27.82

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Harvest	Storage	Shelf
rep.	216	144	72	108
d.f.	240	240	240	240
l.s.d.	6.46	7.91	11.19	9.13

Table	MCP Harvest	MCP Storage	Harvest Storage	MCP Shelf
rep.	72	36	24	54
d.f.	240	240	240	240
l.s.d.	11.19	15.82	19.38	12.92

Table	Harvest Shelf	Storage Shelf	MCP Harvest Storage	MCP Harvest Shelf
rep.	36	18	12	18
d.f.	240	240	240	240
l.s.d.	15.82	22.38	27.40	22.38

Table	MCP Storage Shelf	Harvest Storage Shelf	MCP Harvest Storage Shelf
rep.	9	6	3
d.f.	240	240	240
l.s.d.	31.64	38.76	54.81

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
240	34.08	185.7

Catechin

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	75123.	75123.	8.18	0.005
Harvest	2	84604.	42302.	4.61	0.011
Storage	5	211632.	42326.	4.61	<.001
Shelf	3	62108.	20703.	2.25	0.083
MCP.Harvest	2	70023.	35011.	3.81	0.023
MCP.Storage	5	99364.	19873.	2.16	0.059
Harvest.Storage	10	207992.	20799.	2.27	0.015
MCP.Shelf	3	39329.	13110.	1.43	0.235
Harvest.Shelf	6	81657.	13609.	1.48	0.185
Storage.Shelf	15	427182.	28479.	3.10	<.001
MCP.Harvest.Storage	10	475413.	47541.	5.18	<.001
MCP.Harvest.Shelf	6	196912.	32819.	3.57	0.002
MCP.Storage.Shelf	15	164862.	10991.	1.20	0.275
Harvest.Storage.Shelf	25(5)	186563.	7463.	0.81	0.724
MCP.Harvest.Storage.Shelf	16(14)	244968.	15310.	1.67	0.054
Residual	240(48)	2203761.	9182.		
Total	364(67)	3737480.			

Tables of means

Grand mean 62.3

MCP	Control	MCP
	49.1	75.5

Harvest	Early	Late	Mid
	46.7	80.7	59.5

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	96.1	40.3	40.2	55.9	52.7	88.6

Shelf	0.00	3.00	7.00	14.00
	59.6	48.9	59.0	81.7

MCP	Harvest	Early	Late	Mid
Control		43.6	49.5	54.2
MCP		49.8	111.8	64.8

MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		50.0	33.0	26.9	48.5	50.1	86.2
MCP		142.2	47.6	53.5	63.3	55.3	91.0

Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		62.5	35.7	-1.5	44.3	35.4	103.9
Late		161.6	41.9	82.2	79.5	46.5	72.3
Mid		64.2	43.4	39.9	44.0	76.2	89.6

MCP	Shelf	0.00	3.00	7.00	14.00
Control		58.9	41.6	35.3	60.6
MCP		60.2	56.3	82.6	102.9

Harvest	Shelf	0.00	3.00	7.00	14.00
Early		40.4	39.4	36.1	70.9
Late		93.6	46.0	99.0	84.1
Mid		44.7	61.5	41.7	90.2

Storage	Shelf	0.00	3.00	7.00	14.00
0.00		169.8	45.2	78.3	91.1
2.00		53.0	43.1	20.6	44.5
4.00		-4.2	46.5	79.7	38.8
8.00		39.2	51.6	84.8	48.1
16.00		44.0	33.7	46.2	86.8
24.00		55.6	73.6	44.2	181.1

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		72.0	5.7	26.5	34.0	41.7
	Late		5.9	48.5	38.9	70.0	38.6
	Mid		72.1	44.8	15.2	41.6	70.0
MCP	Early		53.1	65.7	-29.5	54.5	29.2
	Late		317.2	35.2	125.6	89.0	54.3
	Mid		56.3	42.0	64.5	46.4	82.3

MCP	Harvest	Storage	24.00
Control	Early		81.9
	Late		95.1
	Mid		81.7
MCP	Early		126.0
	Late		49.6
	Mid		97.4

MCP	Harvest	Shelf	0.00	3.00	7.00	14.00
Control	Early		80.3	27.3	24.0	42.8
	Late		41.6	52.5	34.7	69.2
	Mid		54.8	45.1	47.2	69.8
MCP	Early		0.6	51.5	48.3	99.0
	Late		145.6	39.4	163.3	98.9
	Mid		34.5	77.9	36.3	110.7

MCP	Storage	Shelf	0.00	3.00	7.00	14.00
Control	0.00		96.5	44.4	18.3	40.7
	2.00		59.8	44.3	1.2	26.8
	4.00		36.9	9.4	24.4	36.8
	8.00		30.0	55.2	75.2	33.7
	16.00		46.7	38.5	39.4	75.8
	24.00		83.5	58.1	53.3	149.9
MCP	0.00		243.0	46.0	138.2	141.6
	2.00		46.3	41.9	40.1	62.2
	4.00		-45.3	83.6	135.0	40.8
	8.00		48.4	48.0	94.3	62.6
	16.00		41.4	29.0	53.0	97.8
	24.00		27.6	89.1	35.1	212.3

Harvest	Storage	Shelf	0.00	3.00	7.00	14.00
Early	0.00		123.7	56.9	31.6	37.9
	2.00		39.3	56.5	8.9	38.0
	4.00		-83.2	0.8	43.7	32.7
	8.00		34.1	23.9	62.3	56.7
	16.00		47.7	17.1	25.1	51.8
	24.00		81.0	81.1	45.2	208.4
Late	0.00		294.9	20.3	164.6	166.4
	2.00		76.3	29.5	29.7	31.9
	4.00		35.7	57.0	184.9	51.4
	8.00		61.6	76.6	128.4	51.6
	16.00		48.0	32.4	44.1	61.4
	24.00		44.9	60.0	42.5	142.0
Mid	0.00		90.7	58.4	38.6	69.2
	2.00		43.4	43.3	23.3	63.7
	4.00		34.8	81.8	10.5	32.3
	8.00		22.0	54.2	63.7	36.2
	16.00		36.4	51.7	69.4	147.2
	24.00		40.8	79.7	44.8	193.0

MCP	Harvest	Storage	Shelf	0.00	3.00	7.00	14.00
Control	Early	0.00		191.9	66.9	8.4	20.8
		2.00		15.6	57.0	-31.4	-18.5
		4.00		27.7	1.0	28.1	49.4
		8.00		36.6	11.4	50.2	37.6
		16.00		67.9	13.4	32.4	53.0
		24.00		142.1	14.0	56.5	114.8
	Late	0.00		2.6	13.1	-26.7	34.7
		2.00		101.9	38.2	29.6	24.5
		4.00		45.2	18.4	35.0	56.8
		8.00		29.8	108.7	97.0	44.6
		16.00		11.9	48.4	25.0	69.2
		24.00		58.2	88.3	48.2	185.7
	Mid	0.00		95.1	53.3	73.1	66.7
		2.00		61.9	37.5	5.4	74.5
		4.00		37.7	8.9	10.0	4.1
		8.00		23.7	45.3	78.4	18.9
		16.00		60.3	53.5	61.0	105.2
		24.00		50.3	72.0	55.2	149.3
MCP	Early	0.00		55.4	47.0	54.9	55.0
		2.00		63.1	55.9	49.3	94.4
		4.00		-194.1	0.6	59.4	16.0
		8.00		31.5	36.5	74.3	75.8
		16.00		27.6	20.8	17.9	50.6
		24.00		20.0	148.2	33.8	302.0
	Late	0.00		587.3	27.5	355.8	298.1
		2.00		50.8	20.9	29.8	39.3
		4.00		26.2	95.6	334.7	45.9
		8.00		93.4	44.5	159.7	58.5
		16.00		84.1	16.3	63.2	53.6
		24.00		31.6	31.6	36.9	98.2
	Mid	0.00		86.2	63.4	4.0	71.7
		2.00		25.0	49.0	41.2	52.8
		4.00		31.9	154.6	11.1	60.5
		8.00		20.2	63.0	49.0	53.4
		16.00		12.5	49.8	77.8	189.2
		24.00		31.3	87.4	34.5	236.6

Standard errors of differences of means

Table	MCP	Harvest	Storage	Shelf
rep.	216	144	72	108
d.f.	240	240	240	240
s.e.d.	9.22	11.29	15.97	13.04

Table	MCP Harvest	MCP Storage	Harvest Storage	MCP Shelf
rep.	72	36	24	54
d.f.	240	240	240	240
s.e.d.	15.97	22.59	27.66	18.44

Table	Harvest Shelf	Storage Shelf	MCP Harvest Storage	MCP Harvest Shelf
rep.	36	18	12	18
d.f.	240	240	240	240
s.e.d.	22.59	31.94	39.12	31.94

Table	MCP Storage Shelf	Harvest Storage Shelf	MCP Harvest Storage Shelf
rep.	9	6	3
d.f.	240	240	240
s.e.d.	45.17	55.32	78.24

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Harvest	Storage	Shelf
rep.	216	144	72	108
d.f.	240	240	240	240
l.s.d.	18.16	22.25	31.46	25.69

Table	MCP Harvest	MCP Storage	Harvest Storage	MCP Shelf
rep.	72	36	24	54
d.f.	240	240	240	240
l.s.d.	31.46	44.49	54.49	36.33

Table	Harvest Shelf	Storage Shelf	MCP Harvest Storage	MCP Harvest Shelf
rep.	36	18	12	18
d.f.	240	240	240	240
l.s.d.	44.49	62.92	77.06	62.92

Table	MCP Storage Shelf	Harvest Storage Shelf	MCP Harvest Storage Shelf
rep.	9	6	3
d.f.	240	240	240
l.s.d.	88.98	108.98	154.13

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
240	95.82	153.8

Chlorogenic acid

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	2515.	2515.	0.67	0.415
Harvest	2	21604.	10802.	2.87	0.059
Storage	5	58119.	11624.	3.09	0.010
Shelf	3	3090.	1030.	0.27	0.844
MCP.Harvest	2	4503.	2252.	0.60	0.551
MCP.Storage	5	13035.	2607.	0.69	0.630
Harvest.Storage	10	97321.	9732.	2.59	0.005
MCP.Shelf	3	5003.	1668.	0.44	0.722
Harvest.Shelf	6	16879.	2813.	0.75	0.612
Storage.Shelf	15	80704.	5380.	1.43	0.134
MCP.Harvest.Storage	10	83942.	8394.	2.23	0.017
MCP.Harvest.Shelf	6	27075.	4513.	1.20	0.308
MCP.Storage.Shelf	15	63023.	4202.	1.12	0.342
Harvest.Storage.Shelf	25(5)	93485.	3739.	0.99	0.477
MCP.Harvest.Storage.Shelf	16(14)	53758.	3360.	0.89	0.578
Residual	240(48)	903460.	3764.		
Total	364(67)	1345815.			

Tables of means

Grand mean 35.8

MCP	Control	MCP
	33.4	38.2

Harvest	Early	Late	Mid
	43.9	26.6	36.9

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	40.1	58.5	36.2	30.2	23.5	26.3

Shelf	0.00	3.00	7.00	14.00
	34.8	39.9	32.5	36.1

MCP	Harvest	Early	Late	Mid
Control		44.8	25.2	30.1
MCP		42.9	28.0	43.7

MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		32.1	64.3	27.3	30.6	25.5	20.5
MCP		48.1	52.6	45.1	29.7	21.4	32.2

Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		34.8	108.7	32.2	35.8	27.5	24.0
Late		37.1	39.4	22.0	29.8	9.4	22.1
Mid		48.4	27.3	54.4	24.8	33.5	32.9

MCP	Shelf	0.00	3.00	7.00	14.00
Control		29.7	43.1	29.8	31.0
MCP		39.9	36.6	35.1	41.2

Harvest	Shelf	0.00	3.00	7.00	14.00
Early		46.8	46.4	44.2	38.0
Late		34.9	28.4	20.0	23.3
Mid		22.6	44.8	33.3	46.9

Storage	Shelf	0.00	3.00	7.00	14.00
0.00		50.8	34.8	48.0	26.8
2.00		12.8	86.2	69.0	65.9
4.00		50.7	27.1	31.1	35.9
8.00		35.6	23.1	19.7	42.2
16.00		23.9	30.2	11.8	27.9
24.00		34.8	37.6	15.2	17.8

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		23.1	154.7	17.2	36.6	23.8
	Late		22.8	43.2	28.3	29.8	9.5
	Mid		50.3	-4.8	36.5	25.3	43.0
MCP	Early		46.5	62.8	47.3	35.1	31.1
	Late		51.3	35.6	15.7	29.9	9.3
	Mid		46.5	59.5	72.3	24.3	23.9

MCP	Harvest	Storage	24.00
Control	Early		13.3
	Late		17.8
	Mid		30.4
MCP	Early		34.8
	Late		26.4
	Mid		35.5

MCP	Harvest	Shelf	0.00	3.00	7.00	14.00
Control	Early		27.0	61.6	51.0	39.6
	Late		42.1	23.3	18.2	17.4
	Mid		19.9	44.3	20.3	35.9
MCP	Early		66.7	31.2	37.4	36.4
	Late		27.6	33.5	21.7	29.3
	Mid		25.4	45.2	46.2	57.8

MCP	Storage	Shelf	0.00	3.00	7.00	14.00
Control	0.00		11.4	47.6	41.2	28.2
	2.00		14.5	116.8	59.2	66.9
	4.00		54.3	16.3	21.5	17.1
	8.00		41.8	12.6	27.2	40.7
	16.00		32.6	45.8	11.7	11.8
	24.00		23.3	19.3	18.2	21.1
MCP	0.00		90.2	22.0	54.8	25.4
	2.00		11.1	55.7	78.9	64.9
	4.00		47.2	37.9	40.6	54.7
	8.00		29.5	33.6	12.2	43.6
	16.00		15.2	14.6	12.0	44.0
	24.00		46.2	55.9	12.2	14.4

Harvest	Storage	Shelf	0.00	3.00	7.00	14.00
Early	0.00		74.4	21.1	36.5	7.2
	2.00		26.4	181.8	121.7	105.0
	4.00		63.6	15.8	35.2	14.3
	8.00		50.6	17.4	39.9	35.4
	16.00		19.9	20.7	14.1	55.3
	24.00		46.1	21.6	17.8	10.6
Late	0.00		58.4	19.4	41.8	28.7
	2.00		42.7	31.7	26.0	57.3
	4.00		24.4	26.1	10.2	27.3
	8.00		30.9	36.9	16.8	34.8
	16.00		27.9	16.2	10.3	-16.8
	24.00		25.0	39.9	14.6	8.8
Mid	0.00		19.6	63.9	65.8	44.4
	2.00		-30.7	45.1	59.4	35.4
	4.00		64.2	39.5	47.8	66.1
	8.00		25.4	15.0	2.4	56.3
	16.00		24.0	53.7	11.1	45.2
	24.00		33.2	51.4	13.2	33.9

MCP	Harvest	Storage	Shelf	0.00	3.00	7.00	14.00
Control	Early	0.00		1.2	29.4	54.7	7.3
		2.00		25.7	290.1	156.9	146.0
		4.00		44.6	16.2	7.0	0.9
		8.00		38.6	11.6	52.6	43.7
		16.00		35.0	17.3	16.3	26.8
		24.00		16.8	5.2	18.5	12.7
	Late	0.00		20.8	18.9	28.8	22.8
		2.00		77.4	36.9	12.0	46.4
		4.00		41.2	19.7	18.3	33.9
		8.00		47.3	21.1	25.7	25.0
		16.00		45.8	25.4	2.3	-35.3
		24.00		20.2	17.7	21.7	11.6
	Mid	0.00		12.2	94.5	40.2	54.4
		2.00		-59.7	23.3	8.8	8.2
		4.00		77.3	13.2	39.3	16.4
		8.00		39.5	5.0	3.2	53.5
		16.00		17.0	94.8	16.4	43.9
		24.00		33.0	35.1	14.3	39.1
MCP	Early	0.00		147.7	12.9	18.4	7.1
		2.00		27.1	73.5	86.5	64.0
		4.00		82.7	15.5	63.4	27.7
		8.00		62.6	23.2	27.3	27.2
		16.00		4.7	24.1	11.8	83.8
		24.00		75.4	38.1	17.1	8.5
	Late	0.00		95.9	19.9	54.7	34.6
		2.00		7.9	26.5	40.0	68.1
		4.00		7.6	32.5	2.2	20.7
		8.00		14.4	52.6	7.9	44.7
		16.00		10.0	7.1	18.3	1.7
		24.00		29.8	62.1	7.4	6.1
	Mid	0.00		27.0	33.3	91.4	34.5
		2.00		-1.6	67.0	110.1	62.6
		4.00		51.2	65.9	56.4	115.7
		8.00		11.4	25.0	1.6	59.1
		16.00		30.9	12.6	5.8	46.4
		24.00		33.4	67.6	12.2	28.7

Standard errors of differences of means

Table	MCP	Harvest	Storage	Shelf
rep.	216	144	72	108
d.f.	240	240	240	240
s.e.d.	5.90	7.23	10.23	8.35

Table	MCP Harvest	MCP Storage	Harvest Storage	MCP Shelf
rep.	72	36	24	54
d.f.	240	240	240	240
s.e.d.	10.23	14.46	17.71	11.81

Table	Harvest Shelf	Storage Shelf	MCP Harvest Storage	MCP Harvest Shelf
rep.	36	18	12	18
d.f.	240	240	240	240
s.e.d.	14.46	20.45	25.05	20.45

Table	MCP Storage Shelf	Harvest Storage Shelf	MCP Harvest Storage Shelf
rep.	9	6	3
d.f.	240	240	240
s.e.d.	28.92	35.42	50.10

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Harvest	Storage	Shelf
rep.	216	144	72	108
d.f.	240	240	240	240
l.s.d.	11.63	14.24	20.14	16.45

Table	MCP Harvest	MCP Storage	Harvest Storage	MCP Shelf
rep.	72	36	24	54
d.f.	240	240	240	240
l.s.d.	20.14	28.49	34.89	23.26

Table	Harvest Shelf	Storage Shelf	MCP Harvest Storage	MCP Harvest Shelf
rep.	36	18	12	18
d.f.	240	240	240	240
l.s.d.	28.49	40.29	49.34	40.29

Table	MCP Storage Shelf	Harvest Storage Shelf	MCP Harvest Storage Shelf
rep.	9	6	3
d.f.	240	240	240
l.s.d.	56.98	69.78	98.68

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
240	61.35	171.4

Epicatechin

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	2650.4	2650.4	4.41	0.037
Harvest	2	722.7	361.4	0.60	0.549
Storage	5	11197.6	2239.5	3.72	0.003
Shelf	3	1955.0	651.7	1.08	0.357
MCP.Harvest	2	548.3	274.2	0.46	0.635
MCP.Storage	5	2547.3	509.5	0.85	0.518
Harvest.Storage	10	23137.5	2313.8	3.85	<.001
MCP.Shelf	3	552.1	184.0	0.31	0.821
Harvest.Shelf	6	9813.0	1635.5	2.72	0.014
Storage.Shelf	15	9104.2	606.9	1.01	0.446
MCP.Harvest.Storage	10	9162.3	916.2	1.52	0.132
MCP.Harvest.Shelf	6	8510.0	1418.3	2.36	0.031
MCP.Storage.Shelf	15	24978.9	1665.3	2.77	<.001
Harvest.Storage.Shelf	25(5)	17585.8	703.4	1.17	0.269
MCP.Harvest.Storage.Shelf	16(14)	10661.7	666.4	1.11	0.348
Residual	240(48)	144383.3	601.6		
Total	364(67)	253441.6			

Tables of means

Grand mean 18.6

MCP	Control	MCP
	16.1	21.1

Harvest	Early	Late	Mid
	19.3	16.8	19.7

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	11.0	16.3	26.8	19.6	15.5	22.4

Shelf	0.00	3.00	7.00	14.00
	15.5	20.6	20.5	17.8

MCP	Harvest	Early	Late	Mid
Control		15.7	15.9	16.8
MCP		22.9	17.7	22.6

MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		8.8	14.9	19.5	16.1	14.9	22.5
MCP		13.2	17.7	34.1	23.0	16.2	22.3

Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		6.8	24.4	22.7	27.8	23.6	10.4
Late		19.7	14.9	18.6	12.3	13.4	21.8
Mid		6.5	9.4	39.2	18.6	9.7	35.0

MCP	Shelf	0.00	3.00	7.00	14.00
Control		11.3	19.6	18.4	15.1
MCP		19.6	21.6	22.5	20.6

Harvest	Shelf	0.00	3.00	7.00	14.00
Early		14.6	18.7	28.0	15.7
Late		20.9	21.2	14.2	10.9
Mid		10.8	22.0	19.2	27.0

Storage	Shelf	0.00	3.00	7.00	14.00
0.00		8.1	13.0	15.7	7.3
2.00		11.8	18.8	17.6	16.8
4.00		29.8	24.5	26.5	26.6
8.00		18.0	20.3	21.8	18.2
16.00		13.9	11.6	12.5	24.1
24.00		11.1	35.6	28.8	14.0

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		4.7	20.1	13.7	23.0	19.5
	Late		14.1	10.0	12.8	20.7	12.9
	Mid		7.6	14.5	32.2	4.7	12.3
MCP	Early		9.0	28.8	31.7	32.5	27.6
	Late		25.3	19.8	24.4	3.9	13.9
	Mid		5.5	4.4	46.3	32.5	7.1

MCP	Harvest	Storage	24.00
Control	Early		13.1
	Late		24.6
	Mid		29.9
MCP	Early		7.6
	Late		19.1
	Mid		40.1

MCP	Harvest	Shelf	0.00	3.00	7.00	14.00
Control	Early		6.8	23.7	18.3	13.8
	Late		22.9	20.7	12.5	7.2
	Mid		4.3	14.4	24.5	24.2
MCP	Early		22.4	13.6	37.8	17.6
	Late		18.9	21.6	15.9	14.5
	Mid		17.3	29.6	13.9	29.7

MCP	Storage	Shelf	0.00	3.00	7.00	14.00
Control	0.00		12.4	11.9	5.1	5.7
	2.00		-0.2	23.8	10.3	25.6
	4.00		3.2	21.9	35.3	17.8
	8.00		29.5	10.6	13.5	10.9
	16.00		11.3	13.5	15.8	19.0
	24.00		11.8	36.0	30.6	11.6
MCP	0.00		3.8	14.2	26.2	8.8
	2.00		23.9	13.7	24.9	8.1
	4.00		56.4	27.0	17.7	35.4
	8.00		6.5	29.9	30.1	25.5
	16.00		16.5	9.8	9.2	29.3
	24.00		10.4	35.2	27.1	16.4

Harvest	Storage	Shelf	0.00	3.00	7.00	14.00
Early	0.00		0.9	5.2	18.1	3.1
	2.00		22.8	30.9	32.5	11.6
	4.00		30.7	7.3	35.0	17.7
	8.00		15.2	21.4	50.9	23.6
	16.00		16.7	23.1	18.5	35.9
	24.00		1.5	24.2	13.3	2.4
Late	0.00		20.5	30.0	20.0	8.2
	2.00		13.2	15.3	15.0	16.1
	4.00		33.1	22.6	13.1	5.6
	8.00		32.1	8.3	3.4	5.5
	16.00		19.3	5.2	4.6	24.5
	24.00		7.3	45.6	29.3	5.2
Mid	0.00		2.9	3.8	9.0	10.5
	2.00		-0.5	10.1	5.3	22.8
	4.00		25.6	43.5	31.3	56.5
	8.00		6.7	31.0	11.3	25.5
	16.00		5.6	6.6	14.4	12.1
	24.00		24.6	37.0	43.9	34.4

MCP	Harvest	Storage	Shelf	0.00	3.00	7.00	14.00
Control	Early	0.00		1.5	9.5	4.6	3.1
		2.00		5.0	40.5	15.6	19.2
		4.00		0.6	11.4	32.7	10.2
		8.00		23.3	13.4	34.2	21.1
		16.00		8.8	22.4	21.2	25.6
		24.00		1.8	45.2	1.7	3.6
	Late	0.00		31.0	19.2	5.0	1.2
		2.00		2.7	11.9	8.6	16.8
		4.00		8.0	18.0	22.6	2.4
		8.00		62.9	12.4	5.5	2.0
		16.00		21.4	6.3	7.1	16.9
		24.00		11.5	56.6	26.5	3.9
	Mid	0.00		4.7	6.9	5.8	12.9
		2.00		-8.3	18.9	6.7	40.7
		4.00		1.0	36.4	50.6	40.7
		8.00		2.3	6.0	1.0	9.5
		16.00		3.7	11.7	19.2	14.4
		24.00		22.3	6.3	63.5	27.4
MCP	Early	0.00		0.3	1.0	31.6	3.0
		2.00		40.6	21.3	49.4	4.0
		4.00		60.8	3.3	37.4	25.2
		8.00		7.0	29.4	67.6	26.0
		16.00		24.6	23.8	15.8	46.2
		24.00		1.2	3.2	24.9	1.2
	Late	0.00		10.0	40.8	34.9	15.2
		2.00		23.7	18.7	21.4	15.4
		4.00		58.2	27.1	3.7	8.8
		8.00		1.3	4.2	1.2	9.0
		16.00		17.2	4.0	2.2	32.0
		24.00		3.1	34.5	32.1	6.5
	Mid	0.00		1.1	0.7	12.1	8.1
		2.00		7.2	1.3	4.0	5.0
		4.00		50.2	50.6	11.9	72.4
		8.00		11.0	56.0	21.6	41.5
		16.00		7.5	1.5	9.6	9.7
		24.00		27.0	67.7	24.2	41.5

Standard errors of differences of means

Table	MCP	Harvest	Storage	Shelf
rep.	216	144	72	108
d.f.	240	240	240	240
s.e.d.	2.36	2.89	4.09	3.34

Table	MCP Harvest	MCP Storage	Harvest Storage	MCP Shelf
rep.	72	36	24	54
d.f.	240	240	240	240
s.e.d.	4.09	5.78	7.08	4.72

Table	Harvest Shelf	Storage Shelf	MCP Harvest Storage	MCP Harvest Shelf
rep.	36	18	12	18
d.f.	240	240	240	240
s.e.d.	5.78	8.18	10.01	8.18

Table	MCP Storage Shelf	Harvest Storage Shelf	MCP Harvest Storage Shelf
rep.	9	6	3
d.f.	240	240	240
s.e.d.	11.56	14.16	20.03

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Harvest	Storage	Shelf
rep.	216	144	72	108
d.f.	240	240	240	240
l.s.d.	4.65	5.69	8.05	6.58

Table	MCP Harvest	MCP Storage	Harvest Storage	MCP Shelf
rep.	72	36	24	54
d.f.	240	240	240	240
l.s.d.	8.05	11.39	13.95	9.30

Table	Harvest Shelf	Storage Shelf	MCP Harvest Storage	MCP Harvest Shelf
rep.	36	18	12	18
d.f.	240	240	240	240
l.s.d.	11.39	16.11	19.73	16.11

Table	MCP Storage Shelf	Harvest Storage Shelf	MCP Harvest Storage Shelf
rep.	9	6	3
d.f.	240	240	240
l.s.d.	22.78	27.90	39.45

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
240	24.53	131.9

p-coumin

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	0.067	0.067	0.01	0.910
Harvest	2	10.297	5.148	1.00	0.370
Storage	5	61.049	12.210	2.37	0.040
Shelf	3	10.303	3.434	0.67	0.574
MCP.Harvest	2	11.305	5.652	1.09	0.336
MCP.Storage	5	56.422	11.284	2.19	0.056
Harvest.Storage	10	227.915	22.792	4.42	<.001
MCP.Shelf	3	23.977	7.992	1.55	0.203
Harvest.Shelf	6	36.530	6.088	1.18	0.318
Storage.Shelf	15	131.311	8.754	1.70	0.052
MCP.Harvest.Storage	10	116.330	11.633	2.25	0.016
MCP.Harvest.Shelf	6	36.972	6.162	1.19	0.310
MCP.Storage.Shelf	15	69.901	4.660	0.90	0.562
Harvest.Storage.Shelf	25(5)	114.337	4.573	0.89	0.625
MCP.Harvest.Storage.Shelf	16(14)	98.213	6.138	1.19	0.277
Residual	240(48)	1238.876	5.162		
Total	364(67)	1984.661			

Tables of means

Grand mean 1.42

MCP	Control	MCP
	1.43	1.40

Harvest	Early	Late	Mid
	1.63	1.29	1.32

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	1.34	1.23	0.78	1.46	2.00	1.66

Shelf	0.00	3.00	7.00	14.00
	1.58	1.52	1.17	1.39

MCP	Harvest	Early	Late	Mid
Control		1.77	1.08	1.44
MCP		1.50	1.51	1.20

MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		1.55	1.46	0.78	1.29	1.34	2.13
MCP		1.13	1.00	0.79	1.64	2.66	1.20

Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		1.05	1.64	0.78	1.53	2.02	2.79
Late		0.69	1.11	0.86	2.88	0.97	1.24
Mid		2.30	0.95	0.71	-0.02	3.02	0.97

MCP	Shelf	0.00	3.00	7.00	14.00
Control		1.24	1.68	1.11	1.68
MCP		1.91	1.35	1.24	1.11

Harvest	Shelf	0.00	3.00	7.00	14.00
Early		1.87	1.95	0.78	1.93
Late		1.52	1.34	1.07	1.22
Mid		1.34	1.25	1.67	1.03

Storage	Shelf	0.00	3.00	7.00	14.00
0.00		1.44	1.04	1.78	1.11
2.00		0.36	1.79	0.76	2.01
4.00		0.85	0.84	0.71	0.74
8.00		3.21	0.91	0.53	1.21
16.00		2.22	2.03	2.16	1.60
24.00		1.39	2.49	1.09	1.70

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		1.14	2.24	0.74	0.64	1.81
	Late		0.44	0.85	0.95	2.12	0.61
	Mid		3.08	1.30	0.65	1.12	1.61
MCP	Early		0.95	1.03	0.81	2.42	2.24
	Late		0.94	1.37	0.78	3.64	1.33
	Mid		1.51	0.60	0.78	-1.15	4.43

MCP	Harvest	Storage	24.00
Control	Early		4.02
	Late		1.48
	Mid		0.89
MCP	Early		1.55
	Late		0.99
	Mid		1.04

MCP	Harvest	Shelf	0.00	3.00	7.00	14.00
Control	Early		1.81	2.58	0.69	1.99
	Late		0.70	1.48	1.00	1.12
	Mid		1.23	0.99	1.63	1.92
MCP	Early		1.93	1.33	0.86	1.88
	Late		2.35	1.20	1.15	1.32
	Mid		1.45	1.52	1.71	0.13

MCP	Storage	Shelf	0.00	3.00	7.00	14.00
Control	0.00		1.60	0.86	1.77	1.99
	2.00		0.42	2.04	1.10	2.30
	4.00		0.95	0.88	0.73	0.55
	8.00		1.43	1.14	0.71	1.89
	16.00		1.26	1.64	1.24	1.23
	24.00		1.82	3.53	1.08	2.10
MCP	0.00		1.28	1.22	1.80	0.23
	2.00		0.31	1.55	0.42	1.72
	4.00		0.75	0.80	0.69	0.92
	8.00		4.99	0.68	0.36	0.52
	16.00		3.19	2.41	3.08	1.98
	24.00		0.95	1.44	1.09	1.30

Harvest	Storage	Shelf	0.00	3.00	7.00	14.00
Early	0.00		0.96	0.59	1.32	1.32
	2.00		0.80	3.15	0.58	2.02
	4.00		0.81	0.84	0.89	0.56
	8.00		3.27	1.36	-0.40	1.89
	16.00		2.97	1.28	1.48	2.37
	24.00		2.41	4.51	0.79	3.43
Late	0.00		0.60	0.84	1.16	0.17
	2.00		0.39	1.43	1.28	1.33
	4.00		0.65	1.54	0.30	0.96
	8.00		5.77	1.36	1.35	3.05
	16.00		0.67	1.12	1.15	0.93
	24.00		1.06	1.77	1.22	0.90
Mid	0.00		2.75	1.70	2.88	1.85
	2.00		-0.11	0.81	0.42	2.66
	4.00		1.07	0.14	0.95	0.69
	8.00		0.59	0.01	0.65	-1.32
	16.00		3.03	3.67	3.85	1.51
	24.00		0.69	1.17	1.26	0.76

MCP	Harvest	Storage	Shelf	0.00	3.00	7.00	14.00
Control	Early	0.00		0.57	0.43	1.68	1.88
		2.00		0.47	4.97	1.17	2.37
		4.00		0.97	0.41	0.88	0.71
		8.00		0.97	0.74	-1.01	1.85
		16.00		3.40	1.42	0.90	1.51
		24.00		4.47	7.50	0.53	3.59
	Late	0.00		0.04	0.51	0.88	0.34
		2.00		0.57	0.94	1.65	0.25
		4.00		0.47	2.13	0.37	0.82
		8.00		2.45	1.87	1.22	2.93
		16.00		-0.22	1.12	0.76	0.77
		24.00		0.88	2.33	1.11	1.61
	Mid	0.00		4.18	1.64	2.75	3.75
		2.00		0.21	0.22	0.49	4.27
		4.00		1.40	0.12	0.94	0.13
		8.00		0.87	0.80	1.91	0.89
		16.00		0.59	2.38	2.07	1.41
		24.00		0.11	0.76	1.62	1.08
MCP	Early	0.00		1.35	0.75	0.96	0.75
		2.00		1.12	1.32	0.00	1.68
		4.00		0.66	1.27	0.89	0.41
		8.00		5.56	1.97	0.21	1.94
		16.00		2.53	1.15	2.05	3.22
		24.00		0.35	1.52	1.05	3.27
	Late	0.00		1.16	1.16	1.43	-0.01
		2.00		0.22	1.92	0.91	2.42
		4.00		0.83	0.96	0.22	1.09
		8.00		9.09	0.84	1.48	3.16
		16.00		1.56	1.12	1.54	1.09
		24.00		1.24	1.22	1.32	0.19
	Mid	0.00		1.32	1.76	3.01	-0.04
		2.00		-0.43	1.40	0.35	1.06
		4.00		0.75	0.17	0.96	1.25
		8.00		0.31	-0.77	-0.61	-3.54
		16.00		5.47	4.97	5.64	1.62
		24.00		1.27	1.58	0.89	0.43

Standard errors of differences of means

Table	MCP	Harvest	Storage	Shelf
rep.	216	144	72	108
d.f.	240	240	240	240
s.e.d.	0.219	0.268	0.379	0.309

Table	MCP Harvest	MCP Storage	Harvest Storage	MCP Shelf
rep.	72	36	24	54
d.f.	240	240	240	240
s.e.d.	0.379	0.536	0.656	0.437

Table	Harvest Shelf	Storage Shelf	MCP Harvest Storage	MCP Harvest Shelf
rep.	36	18	12	18
d.f.	240	240	240	240
s.e.d.	0.536	0.757	0.928	0.757

Table	MCP Storage Shelf	Harvest Storage Shelf	MCP Harvest Storage Shelf
rep.	9	6	3
d.f.	240	240	240
s.e.d.	1.071	1.312	1.855

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Harvest	Storage	Shelf
rep.	216	144	72	108
d.f.	240	240	240	240
l.s.d.	0.431	0.527	0.746	0.609

Table	MCP Harvest	MCP Storage	Harvest Storage	MCP Shelf
rep.	72	36	24	54
d.f.	240	240	240	240
l.s.d.	0.746	1.055	1.292	0.861

Table	Harvest Shelf	Storage Shelf	MCP Harvest Storage	MCP Harvest Shelf
rep.	36	18	12	18
d.f.	240	240	240	240
l.s.d.	1.055	1.492	1.827	1.492

Table	MCP Storage Shelf	Harvest Storage Shelf	MCP Harvest Storage Shelf
rep.	9	6	3
d.f.	240	240	240
l.s.d.	2.110	2.584	3.654

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
240	2.272	160.6

HPLC Data
Non Infected and *Penicillium expansum*

Analysis of variance

Benzoic acid

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	1439.2	1439.2	1.47	0.226
Infectio	1	4512.5	4512.5	4.62	0.033
Harvest	2	8773.0	4386.5	4.49	0.012
Storage	5	13147.5	2629.5	2.69	0.022
Shelf	1	189.1	189.1	0.19	0.660
MCP.Infectio	1	38.4	38.4	0.04	0.843
MCP.Harvest	2	10413.1	5206.6	5.33	0.005
Infectio.Harvest	2	4210.1	2105.0	2.16	0.118
MCP.Storage	5	8948.3	1789.7	1.83	0.107
Infectio.Storage	5	3705.8	741.2	0.76	0.580
Harvest.Storage	10	40945.5	4094.5	4.19	<.001
MCP.Shelf	1	1945.4	1945.4	1.99	0.159
Infectio.Shelf	1	524.5	524.5	0.54	0.464
Harvest.Shelf	2	1024.0	512.0	0.52	0.593
Storage.Shelf	5	11924.9	2385.0	2.44	0.035
MCP.Infectio.Harvest	2	8265.5	4132.7	4.23	0.016
MCP.Infectio.Storage	5	4571.9	914.4	0.94	0.458
MCP.Harvest.Storage	10	22429.3	2242.9	2.30	0.014
Infectio.Harvest.Storage	10	17400.7	1740.1	1.78	0.065
MCP.Infectio.Shelf	1	4866.3	4866.3	4.98	0.027
MCP.Harvest.Shelf	2	2145.9	1072.9	1.10	0.335
Infectio.Harvest.Shelf	2	496.0	248.0	0.25	0.776
MCP.Storage.Shelf	5	8263.5	1652.7	1.69	0.137
Infectio.Storage.Shelf	5	6011.6	1202.3	1.23	0.295
Harvest.Storage.Shelf	8(2)	21434.2	2679.3	2.74	0.006
MCP.Infectio.Harvest.Storage	9(1)	28798.1	3199.8	3.28	<.001
MCP.Infectio.Harvest.Shelf	2	2918.8	1459.4	1.49	0.226
MCP.Infectio.Storage.Shelf	5	5913.3	1182.7	1.21	0.305
MCP.Harvest.Storage.Shelf	7(3)	21499.9	3071.4	3.15	0.003
Infectio.Harvest.Storage.Shelf	6(4)	9269.8	1545.0	1.58	0.153
MCP.Infectio.Harvest.Storage.Shelf	-3(13)	1903.2			
Residual	238(50)	232395.9	976.5		
Total	358(73)	409791.6			

Tables of means

Grand mean 19.4

MCP	Control	MCP
	17.6	21.2

Infectio	NI	PE
	22.6	16.2

Harvest	Early	Late	Mid
	16.4	16.0	25.8

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	11.7	24.6	15.6	28.2	18.7	17.5

Shelf	3.00	7.00
	20.0	18.7

MCP	Infectio	NI	PE
Control		20.5	14.6
MCP		24.7	17.7

MCP	Harvest	Early	Late	Mid
Control		21.4	11.8	19.5
MCP		11.3	20.3	32.0

Infectio	Harvest	Early	Late	Mid
NI		15.5	19.9	32.4
PE		17.2	12.1	19.1

MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		8.0	31.1	12.3	19.6	17.9	16.4
MCP		15.4	18.0	19.0	36.9	19.4	18.5

Infectio	Storage	0.00	2.00	4.00	8.00	16.00	24.00
NI		12.4	33.9	16.6	31.8	20.2	20.8
PE		11.0	15.3	14.7	24.6	17.1	14.1

Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		13.1	45.6	2.4	13.8	18.0	5.3
Late		8.0	11.9	24.4	23.5	7.2	21.2
Mid		14.0	16.3	20.1	47.3	30.9	25.8

MCP	Shelf	3.00	7.00
Control		16.1	19.0
MCP		24.0	18.4

Infectio	Shelf	3.00	7.00
NI		24.4	20.9
PE		15.7	16.6

Harvest	Shelf	3.00	7.00
Early		15.3	17.4
Late		18.7	13.4
Mid		26.1	25.4

Storage	Shelf	3.00	7.00
0.00		14.5	8.9
2.00		27.5	21.7
4.00		18.3	12.9
8.00		17.3	39.2
16.00		23.3	14.0
24.00		19.4	15.5

	Infectio	NI			PE		
MCP	Harvest	Early	Late	Mid	Early	Late	Mid
Control		25.2	16.1	20.2	17.6	7.4	18.8
MCP		5.8	23.8	44.6	16.9	16.8	19.3

MCP	Infectio	Storage	0.00	2.00	4.00	8.00	16.00
Control	NI		10.7	45.4	11.5	18.5	16.4
	PE		5.3	16.8	13.1	20.7	19.5
MCP	NI		14.1	22.3	21.7	45.2	24.1
	PE		16.7	13.7	16.4	28.6	14.8

MCP	Infectio	Storage	24.00
Control	NI		20.4
	PE		12.4
MCP	NI		21.1
	PE		15.9

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		6.7	75.8	7.8	11.2	22.6
	Late		8.1	11.1	9.2	10.0	12.0
	Mid		9.3	6.5	19.8	37.5	19.2
MCP	Early		19.5	15.4	-2.9	16.5	13.3
	Late		7.9	12.7	39.6	37.0	2.4
	Mid		18.7	26.1	20.4	57.2	42.6

MCP	Harvest	Storage	24.00
Control	Early		4.2
	Late		20.1
	Mid		24.8
MCP	Early		6.3
	Late		22.3
	Mid		26.9

Infectio	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
NI	Early		7.4	55.2	4.7	3.1	17.0
	Late		10.1	15.0	32.0	22.3	10.8
	Mid		19.8	31.4	13.0	70.2	32.9
PE	Early		18.8	35.9	0.2	24.6	18.9
	Late		5.9	8.8	16.7	24.8	3.6
	Mid		8.3	1.2	27.3	24.5	28.9

Infectio	Harvest	Storage	24.00
NI	Early		5.5
	Late		29.4
	Mid		27.4
PE	Early		5.0
	Late		13.0
	Mid		24.3

	Infectio	NI		PE			
MCP	Shelf	3.00	7.00	3.00	7.00		
Control		16.8	24.2	15.4	13.8		
MCP		32.0	17.5	16.0	19.4		

	Harvest	Early		Late		Mid	
MCP	Shelf	3.00	7.00	3.00	7.00	3.00	7.00
Control		21.1	21.6	11.9	11.6	15.3	23.8
MCP		9.5	13.2	25.5	15.1	36.9	27.0

	Harvest	Early		Late		Mid	
Infectio	Shelf	3.00	7.00	3.00	7.00	3.00	7.00
NI		15.4	15.6	22.5	17.4	35.3	29.6
PE		15.3	19.2	14.9	9.3	16.9	21.2

MCP	Storage	Shelf	3.00	7.00
Control	0.00		8.8	7.2
	2.00		26.4	35.9
	4.00		15.0	9.6
	8.00		14.4	24.8
	16.00		16.2	19.7
	24.00		15.9	16.9
MCP	0.00		20.2	10.6
	2.00		28.6	7.5
	4.00		21.7	16.3
	8.00		20.2	53.5
	16.00		30.5	8.4
	24.00		22.9	14.2

Infectio	Storage	Shelf	3.00	7.00
NI	0.00		13.4	11.4
	2.00		40.7	27.1
	4.00		26.6	6.5
	8.00		16.8	46.9
	16.00		25.3	15.1
	24.00		23.3	18.2
PE	0.00		15.6	6.4
	2.00		14.2	16.4
	4.00		10.0	19.4
	8.00		17.8	31.5
	16.00		21.3	13.0
	24.00		15.4	12.9

Harvest	Storage	Shelf	3.00	7.00
Early	0.00		11.7	14.4
	2.00		46.8	44.3
	4.00		-4.3	9.2
	8.00		12.4	15.3
	16.00		17.6	18.3
	24.00		7.7	2.8
Late	0.00		12.5	3.4
	2.00		11.4	12.4
	4.00		41.0	7.8
	8.00		19.8	27.2
	16.00		7.7	6.7
	24.00		19.9	22.6
Mid	0.00		19.2	8.8
	2.00		24.1	8.5
	4.00		18.3	21.9
	8.00		19.7	75.0
	16.00		44.7	17.1
	24.00		30.5	21.1

MCP	Infectio	Harvest	Storage	0.00	2.00	4.00	8.00
Control	NI	Early		5.5	115.3	6.1	-8.1
		Late		20.0	10.5	8.1	11.2
		Mid		6.8	10.5	20.2	52.4
	PE	Early		7.8	36.3	9.5	30.6
		Late		-3.8	11.7	10.3	8.9
		Mid		11.9	2.6	19.4	22.6
MCP	NI	Early		9.2	-4.8	3.3	14.3
		Late		0.2	19.5	55.9	33.3
		Mid		32.9	52.3	5.7	87.9
	PE	Early		29.8	35.5	-9.2	18.6
		Late		15.5	5.9	23.2	40.6
		Mid		4.6	-0.2	35.1	26.5

MCP	Infectio	Harvest	Storage	16.00	24.00
Control	NI	Early		26.3	5.9
		Late		15.7	31.0
		Mid		7.2	24.3
	PE	Early		18.9	2.5
		Late		8.3	9.3
		Mid		31.2	25.3
MCP	NI	Early		7.7	5.1
		Late		5.9	27.9
		Mid		58.6	30.4
	PE	Early		18.9	7.6
		Late		-1.0	16.8
		Mid		26.6	23.3

MCP	Infectio	Harvest	Shelf	3.00	7.00
Control	NI	Early		24.5	25.8
		Late		14.4	17.8
		Mid		11.4	29.0
	PE	Early		17.7	17.5
		Late		9.4	5.4
		Mid		19.1	18.6
MCP	NI	Early		6.2	5.4
		Late		30.6	16.9
		Mid		59.1	30.2
	PE	Early		12.8	21.0
		Late		20.5	13.2
		Mid		14.8	23.9

MCP	Infectio	Storage	Shelf	3.00	7.00
Control	NI	0.00		7.5	14.0
		2.00		38.6	52.3
		4.00		15.2	7.7
		8.00		12.4	24.5
		16.00		7.7	25.1
		24.00		19.2	21.6
	PE	0.00		10.2	0.5
		2.00		14.1	19.6
		4.00		14.7	11.4
		8.00		16.3	25.1
		16.00		24.7	14.2
		24.00		12.6	12.2
MCP	NI	0.00		19.4	8.8
		2.00		42.8	1.8
		4.00		38.1	5.3
		8.00		21.2	69.2
		16.00		43.0	5.1
		24.00		27.4	14.8
	PE	0.00		21.0	12.4
		2.00		14.3	13.2
		4.00		5.4	27.4
		8.00		19.3	37.9
		16.00		17.9	11.8
		24.00		18.3	13.5

Infectio	Harvest	Storage	Shelf	3.00	7.00
PE	Early	0.00		41.1	37.6
		2.00		33.9	17.0
		4.00		90.1	99.8
		8.00		47.0	60.5
		16.00		22.1	77.7
		24.00		37.0	10.4
	Late	0.00		24.1	48.7
		2.00		71.8	10.8
		4.00		54.4	15.1
		8.00		49.1	101.1
		16.00		7.0	20.9
		24.00		30.4	31.0
	Mid	0.00		39.8	101.0
		2.00		33.2	51.2
		4.00		15.7	41.9
		8.00		43.3	0.3
		16.00		69.4	63.0
		24.00		58.3	83.9
PEW	Early	0.00		72.6	27.0
		2.00		15.0	5.1
		4.00		15.7	22.9
		8.00		19.6	24.4
		16.00		42.5	61.1
		24.00		33.2	-10.7
	Late	0.00		24.0	16.8
		2.00		52.2	9.7
		4.00		21.3	31.5
		8.00		51.6	6.0
		16.00		39.7	41.1
		24.00		80.8	94.9
	Mid	0.00		58.7	54.6
		2.00		14.5	35.0
		4.00		97.8	109.3
		8.00		50.7	-64.6
		16.00		78.8	38.1
		24.00		34.1	80.9

MCP	Infectio	Harvest	Storage	Shelf	3.00	7.00
Control	PE	Early	0.00		48.1	47.8
			2.00		66.2	14.0
			4.00		17.6	6.9
			8.00		2.9	102.9
			16.00		9.7	75.3
			24.00		70.4	10.5
		Late	0.00		25.0	33.4
			2.00		111.3	4.7
			4.00		64.3	9.2
			8.00		41.2	101.8
			16.00		-0.7	0.8
			24.00		22.3	27.1
		Mid	0.00		41.7	83.6
			2.00		60.7	41.8
			4.00		24.4	76.2
			8.00		-4.2	4.8
			16.00		33.6	26.2
			24.00		67.2	63.1
	PEW	Early	0.00		67.5	31.4
			2.00		29.6	23.7
			4.00		3.8	7.4
			8.00		-22.7	37.4
			16.00		29.5	85.2
			24.00		8.1	-32.1
		Late	0.00		12.3	3.9
			2.00		34.4	4.0
			4.00		27.4	27.4
			8.00		29.8	1.4
			16.00		4.1	48.9
			24.00		98.5	146.6
		Mid	0.00		62.7	30.2
			2.00		9.4	15.8
			4.00		106.5	140.8
			8.00		-2.1	-97.6
			16.00		34.3	35.4
			24.00		8.3	28.9
MCP	PE	Early	0.00		34.1	27.4
			2.00		1.5	19.9
			4.00		162.6	192.7
			8.00		91.0	18.1
			16.00		34.5	80.1
			24.00		3.7	10.4
		Late	0.00		23.3	64.0
			2.00		32.3	16.9
			4.00		44.5	21.1
			8.00		57.0	100.4
			16.00		14.8	41.0
			24.00		38.4	34.9
		Mid	0.00		37.9	118.4
			2.00		5.6	60.7
			4.00		7.1	7.6
			8.00		90.9	-4.2
			16.00		105.1	99.8
			24.00		49.4	104.8
	PEW	Early	0.00		77.7	22.7
			2.00		0.4	-13.6
			4.00		27.6	38.5
			8.00		61.9	11.3
			16.00		55.6	37.0
			24.00		58.4	10.7
		Late	0.00		35.7	29.7
			2.00		70.1	15.4
			4.00		15.1	35.6
			8.00		73.5	10.6
			16.00		75.3	33.3
			24.00		63.1	43.2
		Mid	0.00		54.7	79.0
			2.00		19.5	54.2
			4.00		89.1	77.7
			8.00		103.6	-31.7
			16.00		123.2	40.9
			24.00		59.9	132.9

Standard errors of means

Table	MCP	Infectio	Harvest	Storage
rep.	216	216	144	72
d.f.	221	221	221	221
e.s.e.	2.99	2.99	3.66	5.18

Table	Shelf	MCP Infectio	MCP Harvest	Infectio Harvest
rep.	216	108	72	72
d.f.	221	221	221	221
e.s.e.	2.99	4.23	5.18	5.18

Table	MCP Storage	Infectio Storage	Harvest Storage	MCP Shelf
rep.	36	36	24	108
d.f.	221	221	221	221
e.s.e.	7.33	7.33	8.97	4.23

Table	Infectio Shelf	Harvest Shelf	Storage Shelf	MCP Infectio Harvest
rep.	108	72	36	36
d.f.	221	221	221	221
e.s.e.	4.23	5.18	7.33	7.33

Table	MCP Infectio Storage	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Shelf
rep.	18	12	12	54
d.f.	221	221	221	221
e.s.e.	10.36	12.69	12.69	5.98

Table	MCP Harvest Shelf	Infectio Harvest Shelf	MCP Storage Shelf	Infectio Storage Shelf
rep.	36	36	18	18
d.f.	221	221	221	221
e.s.e.	7.33	7.33	10.36	10.36

Table	Harvest Storage Shelf	MCP Infectio Harvest Storage	MCP Infectio Harvest Shelf	MCP Infectio Storage Shelf
rep.	12	6	18	9
d.f.	221	221	221	221
e.s.e.	12.69	17.95	10.36	14.66

Table	MCP Harvest Storage Shelf	Infectio Harvest Storage Shelf	MCP Infectio Harvest Storage Shelf
rep.	6	6	3
d.f.	221	221	221
e.s.e.	17.95	17.95	25.38

(Not adjusted for missing values)

Standard errors of differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	216	216	144	72
d.f.	221	221	221	221
s.e.d.	4.23	4.23	5.18	7.33

Table	Shelf	MCP Infectio	MCP Harvest	Infectio Harvest
rep.	216	108	72	72
d.f.	221	221	221	221
s.e.d.	4.23	5.98	7.33	7.33

Table	MCP Storage	Infectio Storage	Harvest Storage	MCP Shelf
rep.	36	36	24	108
d.f.	221	221	221	221
s.e.d.	10.36	10.36	12.69	5.98

Table	Infectio Shelf	Harvest Shelf	Storage Shelf	MCP Infectio Harvest
rep.	108	72	36	36
d.f.	221	221	221	221
s.e.d.	5.98	7.33	10.36	10.36

Table	MCP Infectio Storage	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Shelf
rep.	18	12	12	54
d.f.	221	221	221	221
s.e.d.	14.66	17.95	17.95	8.46

Table	MCP Harvest Shelf	Infectio Harvest Shelf	MCP Storage Shelf	Infectio Storage Shelf
rep.	36	36	18	18
d.f.	221	221	221	221
s.e.d.	10.36	10.36	14.66	14.66

Table	Harvest Storage Shelf	MCP Infectio Harvest Storage	MCP Infectio Harvest Shelf	MCP Infectio Storage Shelf
rep.	12	6	18	9
d.f.	221	221	221	221
s.e.d.	17.95	25.38	14.66	20.73

Table	MCP Harvest Storage Shelf	Infectio Harvest Storage Shelf	MCP Infectio Harvest Storage Shelf
rep.	6	6	3
d.f.	221	221	221
s.e.d.	25.38	25.38	35.90

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	216	216	144	72
d.f.	221	221	221	221
l.s.d.	8.34	8.34	10.21	14.44

Table	Shelf	MCP Infectio	MCP Harvest	Infectio Harvest
rep.	216	108	72	72
d.f.	221	221	221	221
l.s.d.	8.34	11.79	14.44	14.44

Table	MCP Storage	Infectio Storage	Harvest Storage	MCP Shelf
rep.	36	36	24	108
d.f.	221	221	221	221
l.s.d.	20.42	20.42	25.01	11.79

Table	Infectio Shelf	Harvest Shelf	Storage Shelf	MCP Infectio Harvest
rep.	108	72	36	36
d.f.	221	221	221	221
l.s.d.	11.79	14.44	20.42	20.42

Table	MCP Infectio Storage	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Shelf
rep.	18	12	12	54
d.f.	221	221	221	221
l.s.d.	28.88	35.37	35.37	16.67

Table	MCP Harvest Shelf	Infectio Harvest Shelf	MCP Storage Shelf	Infectio Storage Shelf
rep.	36	36	18	18
d.f.	221	221	221	221
l.s.d.	20.42	20.42	28.88	28.88

Table	Harvest Storage Shelf	MCP Infectio Harvest Storage	MCP Infectio Harvest Shelf	MCP Infectio Storage Shelf
rep.	12	6	18	9
d.f.	221	221	221	221
l.s.d.	35.37	50.02	28.88	40.84

Table	MCP Harvest Storage Shelf	Infectio Harvest Storage Shelf	MCP Infectio Harvest Storage Shelf
rep.	6	6	3
d.f.	221	221	221
l.s.d.	50.02	50.02	70.75

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
221	43.97	104.6

Chlorogenic acid

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	453.	453.	0.23	0.632
Infectio	1	3860.	3860.	1.96	0.163
Harvest	2	17011.	8505.	4.32	0.014
Storage	5	62367.	12473.	6.34	<.001
Shelf	1	841.	841.	0.43	0.514
MCP.Infectio	1	134.	134.	0.07	0.795
MCP.Harvest	2	8048.	4024.	2.04	0.132
Infectio.Harvest	2	6778.	3389.	1.72	0.181
MCP.Storage	5	20083.	4017.	2.04	0.074
Infectio.Storage	5	6096.	1219.	0.62	0.685
Harvest.Storage	10	75463.	7546.	3.83	<.001
MCP.Shelf	1	3400.	3400.	1.73	0.190
Infectio.Shelf	1	120.	120.	0.06	0.805
Harvest.Shelf	2	11932.	5966.	3.03	0.050
Storage.Shelf	5	18830.	3766.	1.91	0.093
MCP.Infectio.Harvest	2	2369.	1184.	0.60	0.549
MCP.Infectio.Storage	5	8177.	1635.	0.83	0.529
MCP.Harvest.Storage	10	52922.	5292.	2.69	0.004
Infectio.Harvest.Storage	9(1)	26867.	2985.	1.52	0.143
MCP.Infectio.Shelf	1	393.	393.	0.20	0.656
MCP.Harvest.Shelf	2	10216.	5108.	2.59	0.077
Infectio.Harvest.Shelf	2	1966.	983.	0.50	0.608
MCP.Storage.Shelf	5	12057.	2411.	1.22	0.298
Infectio.Storage.Shelf	5	17396.	3479.	1.77	0.121
Harvest.Storage.Shelf	10	29348.	2935.	1.49	0.144
MCP.Infectio.Harvest.Storage	9(1)	20552.	2284.	1.16	0.322
MCP.Infectio.Harvest.Shelf	2	2707.	1354.	0.69	0.504
MCP.Infectio.Storage.Shelf	5	7933.	1587.	0.81	0.547
MCP.Harvest.Storage.Shelf	5(5)	23899.	4780.	2.43	0.036
Infectio.Harvest.Storage.Shelf	3(7)	5261.	1754.	0.89	0.447
MCP.Infectio.Harvest.Storage.Shelf	-6(16)	1310.			
Residual	221(67)	435133.	1969.		
Total	334(97)	718543.			

Tables of means

Grand mean 32.9

MCP	Control	MCP
	31.9	34.0

Infectio	PE	PEW
	30.0	35.9

Harvest	Early	Late	Mid
	38.6	24.2	36.1

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	25.1	41.9	24.7	55.7	22.1	28.2

Shelf	3.00	7.00
	34.3	31.6

MCP	Infectio	PE	PEW
Control		28.4	35.5
MCP		31.5	36.4

MCP	Harvest	Early	Late	Mid
Control		37.6	28.4	29.8
MCP		39.6	19.9	42.4

Infectio	Harvest	Early	Late	Mid			
PE		33.0	18.2	38.7			
PEW		44.2	30.2	33.5			
MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		11.5	46.4	18.7	61.2	22.0	31.9
MCP		38.8	37.4	30.6	50.2	22.2	24.6
Infectio	Storage	0.00	2.00	4.00	8.00	16.00	24.00
PE		22.4	35.5	20.4	53.5	26.6	21.3
PEW		27.9	48.2	28.9	57.9	17.6	35.1
Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		41.0	39.8	16.9	71.2	24.9	37.6
Late		4.2	54.9	27.5	30.0	-5.6	34.1
Mid		30.1	30.9	29.6	65.9	47.0	13.0
MCP	Shelf	3.00	7.00				
Control		36.1	27.7				
MCP		32.6	35.4				
Infectio	Shelf	3.00	7.00				
PE		31.9	28.0				
PEW		36.8	35.1				
Harvest	Shelf	3.00	7.00				
Early		32.8	44.3				
Late		30.9	17.5				
Mid		39.3	32.8				
Storage	Shelf	3.00	7.00				
0.00		34.6	15.7				
2.00		49.5	34.2				
4.00		31.4	18.0				
8.00		49.9	61.5				
16.00		16.9	27.4				
24.00		23.8	32.7				

	Infectio	PE			PEW		
MCP	Harvest	Early	Late	Mid	Early	Late	Mid
Control		28.5	22.0	34.6	46.6	34.9	24.9
MCP		37.4	14.4	42.8	41.7	25.5	42.1

MCP	Infectio	Storage	0.00	2.00	4.00	8.00	16.00
Control	PE		11.6	47.1	10.4	58.0	24.4
	PEW		11.4	45.7	27.0	64.4	19.6
MCP	PE		33.2	24.0	30.4	49.0	28.9
	PEW		44.4	50.7	30.9	51.4	15.6

MCP	Infectio	Storage	24.00
Control	PE		18.9
	PEW		44.8
MCP	PE		23.7
	PEW		25.5

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		7.1	44.1	8.2	93.9	29.6
	Late		-14.7	73.8	30.9	36.8	-0.3
	Mid		42.0	21.2	17.0	52.8	36.7
MCP	Early		74.9	35.5	25.5	48.4	20.2
	Late		23.1	36.0	24.1	23.3	-10.8
	Mid		18.3	40.6	42.3	79.0	57.2

MCP	Harvest	Storage	24.00
Control	Early		42.5
	Late		44.2
	Mid		8.9
MCP	Early		32.8
	Late		24.0
	Mid		17.1

Infectio	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
PE	Early		38.7	24.3	27.6	58.3	24.6
	Late		5.5	41.2	10.0	33.2	-10.5
	Mid		23.0	41.2	23.6	69.0	65.7
PEW	Early		43.3	55.3	6.1	84.0	25.3
	Late		3.0	68.5	45.0	26.8	-0.6
	Mid		37.3	20.6	35.7	62.8	28.2

Infectio	Harvest	Storage	24.00
PE	Early		24.4
	Late		29.9
	Mid		9.7
PEW	Early		50.9
	Late		38.3
	Mid		16.2

	Infectio	PE		PEW	
MCP	Shelf	3.00	7.00	3.00	7.00
Control		32.2	24.6	40.1	30.8
MCP		31.6	31.5	33.5	39.3

	Harvest	Early		Late		Mid	
MCP	Shelf	3.00	7.00	3.00	7.00	3.00	7.00
Control		27.8	47.3	42.2	14.7	38.4	21.2
MCP		37.8	41.3	19.5	20.3	40.3	44.5

	Harvest	Early		Late		Mid	
Infectio	Shelf	3.00	7.00	3.00	7.00	3.00	7.00
PE		26.7	39.3	23.5	12.9	45.5	31.9
PEW		38.9	49.4	38.3	22.1	33.2	33.7

MCP	Storage	Shelf	3.00	7.00
Control	0.00		33.7	-10.8
	2.00		60.4	32.4
	4.00		27.2	10.3
	8.00		53.3	69.0
	16.00		14.9	29.1
	24.00		27.3	36.4
MCP	0.00		35.4	42.1
	2.00		38.7	36.0
	4.00		35.6	25.7
	8.00		46.5	53.9
	16.00		18.8	25.6
	24.00		20.3	28.9

Infectio	Storage	Shelf	3.00	7.00
PE	0.00		34.1	10.7
	2.00		47.0	24.1
	4.00		22.2	18.6
	8.00		59.9	47.1
	16.00		15.6	37.7
	24.00		12.6	30.1
PEW	0.00		35.0	20.7
	2.00		52.1	44.2
	4.00		40.5	17.4
	8.00		40.0	75.8
	16.00		18.2	17.1
	24.00		35.0	35.2

Harvest	Storage	Shelf	3.00	7.00
Early	0.00		36.6	45.4
	2.00		48.8	30.8
	4.00		15.1	18.6
	8.00		48.2	94.2
	16.00		25.4	24.4
	24.00		22.7	52.6
Late	0.00		26.3	-17.9
	2.00		74.3	35.5
	4.00		34.9	20.2
	8.00		39.3	20.7
	16.00		-23.2	12.1
	24.00		33.9	34.3
Mid	0.00		40.7	19.5
	2.00		25.6	36.2
	4.00		44.1	15.2
	8.00		62.3	69.5
	16.00		48.4	45.5
	24.00		14.9	11.1

MCP	Infectio	Harvest	Storage	0.00	2.00	4.00	8.00
Control	PE	Early		0.4	47.1	7.0	63.9
		Late		-10.7	58.0	10.8	52.3
		Mid		44.9	36.1	13.4	57.8
	PEW	Early		13.8	41.1	9.4	123.9
		Late		-18.8	89.5	51.0	21.2
		Mid		39.0	6.3	20.6	47.9
MCP	PE	Early		77.0	1.5	48.3	52.6
		Late		21.6	24.4	9.3	14.1
		Mid		1.0	46.3	33.7	80.3
	PEW	Early		72.9	69.5	2.8	44.2
		Late		24.7	47.5	38.9	32.5
		Mid		35.5	35.0	50.9	77.7

MCP	Infectio	Harvest	Storage	16.00	24.00
Control	PE	Early		30.3	22.4
		Late		-7.6	29.1
		Mid		50.4	5.2
	PEW	Early		28.9	62.6
		Late		7.0	59.3
		Mid		23.0	12.5
MCP	PE	Early		18.9	26.4
		Late		-13.3	30.6
		Mid		81.1	14.2
	PEW	Early		21.6	39.2
		Late		-8.3	17.3
		Mid		33.4	20.0

MCP	Infectio	Harvest	Shelf	3.00	7.00
Control	PE	Early		17.5	39.5
		Late		30.2	13.7
		Mid		48.7	20.6
	PEW	Early		38.1	55.2
		Late		54.2	15.6
		Mid		28.0	21.8
MCP	PE	Early		35.9	39.0
		Late		16.8	12.1
		Mid		42.2	43.3
	PEW	Early		39.7	43.7
		Late		22.3	28.6
		Mid		38.5	45.7

MCP	Infectio	Storage	Shelf	3.00	7.00
Control	PE	0.00		34.1	-11.0
		2.00		68.2	26.0
		4.00		9.1	11.7
		8.00		68.4	47.5
		16.00		8.6	40.1
		24.00		4.5	33.3
	PEW	0.00		33.3	-10.6
		2.00		52.5	38.8
		4.00		45.3	8.8
		8.00		38.2	90.5
		16.00		21.2	18.1
		24.00		50.1	39.5
MCP	PE	0.00		34.0	32.3
		2.00		25.8	22.3
		4.00		35.3	25.5
		8.00		51.3	46.7
		16.00		22.5	35.2
		24.00		20.7	26.8
	PEW	0.00		36.8	52.0
		2.00		51.7	49.7
		4.00		35.8	25.9
		8.00		41.8	61.1
		16.00		15.1	16.1
		24.00		20.0	31.0

MCP	Harvest	Storage	Shelf	3.00	7.00
Control	Early	0.00		7.0	7.3
		2.00		52.4	35.8
		4.00		6.7	9.7
		8.00		50.3	137.5
		16.00		26.1	33.0
		24.00		24.3	60.7
	Late	0.00		20.6	-50.0
		2.00		89.6	58.0
		4.00		46.0	15.8
		8.00		60.5	13.0
		16.00		-17.4	16.8
		24.00		54.0	34.5
	Mid	0.00		73.6	10.4
		2.00		39.0	3.4
		4.00		28.8	5.2
		8.00		49.1	56.6
		16.00		36.0	37.4
		24.00		3.6	14.1
MCP	Early	0.00		66.3	83.6
		2.00		45.1	25.9
		4.00		23.5	27.5
		8.00		46.0	50.8
		16.00		24.7	15.8
		24.00		21.2	44.4
	Late	0.00		32.0	14.3
		2.00		58.9	13.0
		4.00		23.7	24.5
		8.00		18.0	28.5
		16.00		-29.1	7.5
		24.00		13.8	34.2
	Mid	0.00		7.9	28.6
		2.00		12.2	69.0
		4.00		59.5	25.1
		8.00		75.5	82.5
		16.00		60.8	53.7
		24.00		26.1	8.0

Infectio	Harvest	Storage	Shelf	3.00	7.00
PE	Early	0.00		32.0	45.4
		2.00		35.8	12.7
		4.00		20.7	34.6
		8.00		44.2	72.3
		16.00		15.2	33.9
		24.00		12.2	36.6
	Late	0.00		27.6	-16.7
		2.00		58.8	23.5
		4.00		9.6	10.4
		8.00		55.1	11.2
		16.00		-34.2	13.3
		24.00		24.0	35.8
	Mid	0.00		42.6	3.4
		2.00		46.3	36.1
		4.00		36.3	10.8
		8.00		80.3	57.8
		16.00		65.7	65.8
		24.00		1.6	17.8
PEW	Early	0.00		41.2	45.5
		2.00		61.7	48.9
		4.00		9.5	2.7
		8.00		52.1	116.0
		16.00		35.6	14.9
		24.00		33.3	68.5
	Late	0.00		24.9	-19.0
		2.00		89.7	47.4
		4.00		60.1	29.9
		8.00		23.4	30.2
		16.00		-12.2	11.0
		24.00		43.7	32.9
	Mid	0.00		38.9	35.7
		2.00		5.0	36.3
		4.00		52.0	19.5
		8.00		44.3	81.3
		16.00		31.1	25.3
		24.00		28.1	4.3

MCP	Infectio	Harvest	Storage	Shelf	3.00	7.00
Control	PE	Early	0.00		-1.3	2.1
			2.00		61.5	32.6
			4.00		-2.9	16.9
			8.00		37.0	90.8
			16.00		10.4	50.1
			24.00		0.3	44.4
		Late	0.00		19.9	-41.3
			2.00		73.7	42.3
			4.00		8.3	13.2
			8.00		90.5	14.1
			16.00		-36.0	20.7
			24.00		24.9	33.4
		Mid	0.00		83.6	6.3
			2.00		69.3	2.9
			4.00		21.8	5.0
			8.00		77.8	37.7
			16.00		51.3	49.5
			24.00		-11.6	22.1
	PEW	Early	0.00		15.2	12.5
			2.00		43.3	38.9
			4.00		16.2	2.6
			8.00		63.6	184.2
			16.00		41.9	16.0
			24.00		48.3	76.9
		Late	0.00		21.2	-58.8
			2.00		105.5	73.6
			4.00		83.8	18.3
			8.00		30.6	11.9
			16.00		1.2	12.9
			24.00		83.1	35.5
		Mid	0.00		63.5	14.6
			2.00		8.8	3.8
			4.00		35.8	5.4
			8.00		20.3	75.5
			16.00		20.7	25.3
			24.00		18.9	6.1
MCP	PE	Early	0.00		65.2	88.7
			2.00		10.1	-7.2
			4.00		44.3	52.2
			8.00		51.5	53.8
			16.00		20.0	17.7
			24.00		24.1	28.8
		Late	0.00		35.3	7.8
			2.00		44.0	4.7
			4.00		10.9	7.6
			8.00		19.7	8.4
			16.00		-32.5	5.8
			24.00		23.2	38.1
		Mid	0.00		1.5	0.5
			2.00		23.3	69.3
			4.00		50.7	16.6
			8.00		82.7	77.8
			16.00		80.1	82.1
			24.00		14.8	13.5
	PEW	Early	0.00		67.3	78.4
			2.00		80.1	59.0
			4.00		2.8	2.8
			8.00		40.6	47.8
			16.00		29.4	13.8
			24.00		18.3	60.1
		Late	0.00		28.7	20.7
			2.00		73.8	21.3
			4.00		36.4	41.4
			8.00		16.3	48.6
			16.00		-25.6	9.1
			24.00		4.3	30.3
		Mid	0.00		14.3	56.7
			2.00		1.1	68.8
			4.00		68.2	33.6
			8.00		68.3	87.1
			16.00		41.5	25.3
			24.00		37.4	2.5

Standard errors of means

Table	MCP	Infectio	Harvest	Storage
rep.	216	216	144	72
d.f.	221	221	221	221
e.s.e.	3.02	3.02	3.70	5.23

Table	Shelf	MCP Infectio	MCP Harvest	Infectio Harvest
rep.	216	108	72	72
d.f.	221	221	221	221
e.s.e.	3.02	4.27	5.23	5.23

Table	MCP Storage	Infectio Storage	Harvest Storage	MCP Shelf
rep.	36	36	24	108
d.f.	221	221	221	221
e.s.e.	7.40	7.40	9.06	4.27

Table	Infectio Shelf	Harvest Shelf	Storage Shelf	MCP Infectio Harvest
rep.	108	72	36	36
d.f.	221	221	221	221
e.s.e.	4.27	5.23	7.40	7.40

Table	MCP Infectio Storage	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Shelf
rep.	18	12	12	54
d.f.	221	221	221	221
e.s.e.	10.46	12.81	12.81	6.04

Table	MCP Harvest Shelf	Infectio Harvest Shelf	MCP Storage Shelf	Infectio Storage Shelf
rep.	36	36	18	18
d.f.	221	221	221	221
e.s.e.	7.40	7.40	10.46	10.46

Table	Harvest Storage Shelf	MCP Infectio Harvest Storage	MCP Infectio Harvest Shelf	MCP Infectio Storage Shelf
rep.	12	6	18	9
d.f.	221	221	221	221
e.s.e.	12.81	18.12	10.46	14.79

Table	MCP Harvest Storage Shelf	Infectio Harvest Storage Shelf	MCP Infectio Harvest Storage Shelf
rep.	6	6	3
d.f.	221	221	221
e.s.e.	18.12	18.12	25.62

(Not adjusted for missing values)

Standard errors of differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	216	216	144	72
d.f.	221	221	221	221
s.e.d.	4.27	4.27	5.23	7.40

Table	Shelf	MCP Infectio	MCP Harvest	Infectio Harvest
rep.	216	108	72	72
d.f.	221	221	221	221
s.e.d.	4.27	6.04	7.40	7.40

Table	MCP Storage	Infectio Storage	Harvest Storage	MCP Shelf
rep.	36	36	24	108
d.f.	221	221	221	221
s.e.d.	10.46	10.46	12.81	6.04

Table	Infectio Shelf	Harvest Shelf	Storage Shelf	MCP Infectio Harvest
rep.	108	72	36	36
d.f.	221	221	221	221
s.e.d.	6.04	7.40	10.46	10.46

Table	MCP Infectio Storage	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Shelf
rep.	18	12	12	54
d.f.	221	221	221	221
s.e.d.	14.79	18.12	18.12	8.54

Table	MCP Harvest Shelf	Infectio Harvest Shelf	MCP Storage Shelf	Infectio Storage Shelf
rep.	36	36	18	18
d.f.	221	221	221	221
s.e.d.	10.46	10.46	14.79	14.79

Table	Harvest Storage Shelf	MCP Infectio Harvest Storage	MCP Infectio Harvest Shelf	MCP Infectio Storage Shelf
rep.	12	6	18	9
d.f.	221	221	221	221
s.e.d.	18.12	25.62	14.79	20.92

Table	MCP Harvest Storage Shelf	Infectio Harvest Storage Shelf	MCP Infectio Harvest Storage Shelf
rep.	6	6	3
d.f.	221	221	221
s.e.d.	25.62	25.62	36.23

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	216	216	144	72
d.f.	221	221	221	221
l.s.d.	8.41	8.41	10.31	14.57

Table	Shelf	MCP Infectio	MCP Harvest	Infectio Harvest
rep.	216	108	72	72
d.f.	221	221	221	221
l.s.d.	8.41	11.90	14.57	14.57

Table	MCP Storage	Infectio Storage	Harvest Storage	MCP Shelf
rep.	36	36	24	108
d.f.	221	221	221	221
l.s.d.	20.61	20.61	25.24	11.90

Table	Infectio Shelf	Harvest Shelf	Storage Shelf	MCP Infectio Harvest
rep.	108	72	36	36
d.f.	221	221	221	221
l.s.d.	11.90	14.57	20.61	20.61

Table	MCP Infectio Storage	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Shelf
rep.	18	12	12	54
d.f.	221	221	221	221
l.s.d.	29.15	35.70	35.70	16.83

Table	MCP Harvest Shelf	Infectio Harvest Shelf	MCP Storage Shelf	Infectio Storage Shelf
rep.	36	36	18	18
d.f.	221	221	221	221
l.s.d.	20.61	20.61	29.15	29.15

Table	Harvest Storage Shelf	MCP Infectio Harvest Storage	MCP Infectio Harvest Shelf	MCP Infectio Storage Shelf
rep.	12	6	18	9
d.f.	221	221	221	221
l.s.d.	35.70	50.49	29.15	41.22

Table	MCP Harvest Storage Shelf	Infectio Harvest Storage Shelf	MCP Infectio Harvest Storage Shelf
rep.	6	6	3
d.f.	221	221	221
l.s.d.	50.49	50.49	71.40

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation *****

d.f.	s.e.	cv%
221	44.37	134.7

Epicatechin

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	219.0	219.0	0.26	0.610
Infectio	1	6300.8	6300.8	7.52	0.007
Harvest	2	1679.0	839.5	1.00	0.369
Storage	5	15099.4	3019.9	3.60	0.004
Shelf	1	2476.2	2476.2	2.95	0.087
MCP.Infectio	1	5050.9	5050.9	6.03	0.015
MCP.Harvest	2	472.0	236.0	0.28	0.755
Infectio.Harvest	2	2659.9	1330.0	1.59	0.207
MCP.Storage	5	5872.2	1174.4	1.40	0.225
Infectio.Storage	5	17993.9	3598.8	4.29	<.001
Harvest.Storage	10	34624.8	3462.5	4.13	<.001
MCP.Shelf	1	453.5	453.5	0.54	0.463
Infectio.Shelf	1	358.5	358.5	0.43	0.514
Harvest.Shelf	2	25581.1	12790.6	15.26	<.001
Storage.Shelf	5	13990.8	2798.2	3.34	0.006
MCP.Infectio.Harvest	2	313.9	156.9	0.19	0.829
MCP.Infectio.Storage	5	11838.7	2367.7	2.82	0.017
MCP.Harvest.Storage	10	28530.1	2853.0	3.40	<.001
Infectio.Harvest.Storage	9(1)	35184.7	3909.4	4.66	<.001
MCP.Infectio.Shelf	1	4617.7	4617.7	5.51	0.020
MCP.Harvest.Shelf	2	3006.3	1503.2	1.79	0.169
Infectio.Harvest.Shelf	2	2812.7	1406.3	1.68	0.189
MCP.Storage.Shelf	5	4060.2	812.0	0.97	0.438
Infectio.Storage.Shelf	5	15183.2	3036.6	3.62	0.004
Harvest.Storage.Shelf	10	18487.2	1848.7	2.21	0.018
MCP.Infectio.Harvest.Storage	9(1)	8681.5	964.6	1.15	0.328
MCP.Infectio.Harvest.Shelf	2	493.5	246.7	0.29	0.745
MCP.Infectio.Storage.Shelf	5	2488.5	497.7	0.59	0.705
MCP.Harvest.Storage.Shelf	5(5)	6111.6	1222.3	1.46	0.205
Infectio.Harvest.Storage.Shelf	3(7)	4792.6	1597.5	1.91	0.129
MCP.Infectio.Harvest.Storage.Shelf	-6(16)	147.2			
Residual	221(67)	185236.4	838.2		
Total	334(97)	345517.3			

Tables of means

Grand mean 24.7

MCP	Control	MCP
	24.0	25.4

Infectio	PE	PEW
	28.5	20.9

Harvest	Early	Late	Mid
	22.5	27.3	24.2

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	17.6	23.2	32.6	24.0	32.1	18.5

Shelf	3.00	7.00
	27.1	22.3

MCP	Infectio	PE	PEW
Control		24.4	23.6
MCP		32.6	18.2

MCP	Harvest	Early	Late	Mid
Control		20.6	26.4	24.9
MCP		24.4	28.2	23.6

Infectio	Harvest	Early	Late	Mid			
PE		29.1	27.9	28.5			
PEW		15.9	26.7	20.0			
MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		14.8	27.4	28.3	19.4	36.5	17.3
MCP		20.4	19.1	36.9	28.5	27.8	19.6
Infectio	Storage	0.00	2.00	4.00	8.00	16.00	24.00
PE		16.7	36.3	45.2	22.8	33.7	16.3
PEW		18.5	10.2	20.1	25.1	30.6	20.7
Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		27.0	31.4	34.9	5.9	27.8	8.2
Late		4.9	15.0	40.5	31.7	45.6	26.0
Mid		21.0	23.4	22.6	34.3	23.0	21.2
MCP	Shelf	3.00	7.00				
Control		27.4	20.6				
MCP		26.8	24.0				
Infectio	Shelf	3.00	7.00				
PE		31.8	25.2				
PEW		22.3	19.4				
Harvest	Shelf	3.00	7.00				
Early		18.4	26.7				
Late		40.5	14.1				
Mid		22.4	26.1				
Storage	Shelf	3.00	7.00				
0.00		20.5	14.8				
2.00		17.4	29.1				
4.00		39.1	26.2				
8.00		28.0	19.9				
16.00		42.8	21.4				
24.00		14.7	22.2				

	Infectio	PE			PEW		
MCP	Harvest	Early	Late	Mid	Early	Late	Mid
Control		23.0	23.2	26.9	18.3	29.5	22.9
MCP		35.3	32.5	30.1	13.5	23.9	17.1

MCP	Infectio	Storage	0.00	2.00	4.00	8.00	16.00
Control	PE		9.7	44.7	29.6	20.6	32.4
	PEW		20.0	10.1	27.1	18.3	40.6
MCP	PE		23.8	28.0	60.8	25.0	34.9
	PEW		17.1	10.2	13.1	32.0	20.6
MCP	Infectio	Storage	24.00				
Control	PE		9.2				
	PEW		25.4				
MCP	PE		23.4				
	PEW		15.9				

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		20.2	45.2	23.1	1.1	29.5
	Late		-9.3	3.9	36.8	35.8	56.6
	Mid		33.6	33.1	25.1	21.4	23.4
MCP	Early		33.8	17.5	46.7	10.6	26.1
	Late		19.2	26.1	44.1	27.6	34.6
	Mid		8.4	13.7	20.0	47.3	22.6
MCP	Harvest	Storage	24.00				
Control	Early		4.7				
	Late		34.4				
	Mid		12.8				
MCP	Early		11.6				
	Late		17.6				
	Mid		29.7				

Infectio	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
PE	Early		40.3	50.9	55.7	2.4	23.6
	Late		3.0	12.6	60.3	32.3	46.7
	Mid		6.9	45.5	19.6	33.7	30.8
PEW	Early		13.6	11.9	14.2	9.3	32.0
	Late		6.9	17.3	20.6	31.1	44.6
	Mid		35.1	1.3	25.5	34.9	15.2

Infectio	Harvest	Storage	24.00
PE	Early		1.8
	Late		12.4
	Mid		34.7
PEW	Early		14.6
	Late		39.6
	Mid		7.8

	Infectio	PE		PEW	
MCP	Shelf	3.00	7.00	3.00	7.00
Control		32.0	16.8	22.8	24.3
MCP		31.6	33.6	21.9	14.4

	Harvest	Early		Late		Mid	
MCP	Shelf	3.00	7.00	3.00	7.00	3.00	7.00
Control		13.8	27.4	41.9	10.8	26.4	23.4
MCP		22.9	25.9	39.0	17.4	18.4	28.8

	Harvest	Early		Late		Mid	
Infectio	Shelf	3.00	7.00	3.00	7.00	3.00	7.00
PE		22.6	35.6	44.9	10.8	27.8	29.2
PEW		14.1	17.7	36.0	17.4	16.9	23.0

MCP	Storage	Shelf	3.00	7.00
Control	0.00		22.0	7.6
	2.00		17.1	37.7
	4.00		34.9	21.8
	8.00		28.1	10.7
	16.00		47.2	25.8
	24.00		15.0	19.6
MCP	0.00		19.0	21.9
	2.00		17.7	20.5
	4.00		43.2	30.6
	8.00		27.8	29.1
	16.00		38.5	17.1
	24.00		14.4	24.9

Infectio	Storage	Shelf	3.00	7.00
PE	0.00		22.8	10.7
	2.00		22.2	50.4
	4.00		61.7	28.6
	8.00		29.6	16.1
	16.00		46.8	20.6
	24.00		7.8	24.8
PEW	0.00		18.2	18.9
	2.00		12.5	7.9
	4.00		16.4	23.8
	8.00		26.4	23.8
	16.00		38.9	22.3
	24.00		21.7	19.6

Harvest	Storage	Shelf	3.00	7.00
Early	0.00		17.4	36.5
	2.00		13.8	49.0
	4.00		38.3	31.5
	8.00		7.6	4.1
	16.00		24.6	31.0
	24.00		8.6	7.8
Late	0.00		28.3	-18.4
	2.00		24.8	5.1
	4.00		60.2	20.7
	8.00		35.1	28.4
	16.00		72.4	18.9
	24.00		22.1	29.9
Mid	0.00		15.8	26.2
	2.00		13.4	33.3
	4.00		18.7	26.4
	8.00		41.3	27.4
	16.00		31.6	14.4
	24.00		13.4	29.0

MCP	Infectio	Harvest	Storage	0.00	2.00	4.00	8.00
Control	PE	Early		26.5	65.0	24.3	2.9
		Late		-9.1	4.1	46.3	35.0
		Mid		11.6	65.0	18.3	24.0
	PEW	Early		13.9	25.5	21.9	-0.7
		Late		-9.5	3.7	27.4	36.7
		Mid		55.6	1.3	31.9	18.8
MCP	PE	Early		54.1	36.8	87.1	2.0
		Late		15.1	21.1	74.3	29.6
		Mid		2.1	25.9	20.9	43.5
	PEW	Early		13.4	-1.7	6.4	19.2
		Late		23.3	31.0	13.8	25.6
		Mid		14.6	1.4	19.2	51.0

MCP	Infectio	Harvest	Storage	16.00	24.00
Control	PE	Early		18.1	1.1
		Late		52.3	10.8
		Mid		26.9	15.8
	PEW	Early		40.9	8.4
		Late		60.9	58.0
		Mid		20.0	9.7
MCP	PE	Early		29.0	2.5
		Late		41.0	13.9
		Mid		34.7	53.6
	PEW	Early		23.1	20.8
		Late		28.3	21.3
		Mid		10.4	5.8

MCP	Infectio	Harvest	Shelf	3.00	7.00
Control	PE	Early		17.8	28.1
		Late		46.7	-0.3
		Mid		31.4	22.5
	PEW	Early		9.8	26.8
		Late		37.2	21.9
		Mid		21.4	24.3
MCP	PE	Early		27.4	43.1
		Late		43.2	21.8
		Mid		24.3	35.9
	PEW	Early		18.4	8.6
		Late		34.9	12.9
		Mid		12.4	21.7

MCP	Infectio	Storage	Shelf	3.00	7.00
Control	PE	0.00		24.8	-5.4
		2.00		30.2	59.2
		4.00		45.1	14.1
		8.00		38.5	2.7
		16.00		47.4	17.5
		24.00		5.9	12.6
	PEW	0.00		19.3	20.7
		2.00		4.0	16.3
		4.00		24.6	29.5
		8.00		17.8	18.7
		16.00		47.0	34.2
		24.00		24.2	26.5
MCP	PE	0.00		20.7	26.8
		2.00		14.3	41.6
		4.00		78.3	43.2
		8.00		20.6	29.4
		16.00		46.2	23.7
		24.00		9.7	37.0
	PEW	0.00		17.2	17.0
		2.00		21.0	-0.6
		4.00		8.2	18.1
		8.00		35.0	28.9
		16.00		30.8	10.5
		24.00		19.1	12.7

MCP	Harvest	Storage	Shelf	3.00	7.00
Control	Early	0.00		16.9	23.5
		2.00		20.0	70.4
		4.00		27.1	19.2
		8.00		-3.9	6.1
		16.00		21.5	37.4
		24.00		1.4	8.0
	Late	0.00		19.0	-37.6
		2.00		6.4	1.4
		4.00		56.4	17.3
		8.00		53.0	18.6
		16.00		85.2	28.1
		24.00		31.6	37.2
	Mid	0.00		30.2	37.1
		2.00		24.8	41.5
		4.00		21.2	29.0
		8.00		35.2	7.5
		16.00		34.9	12.0
		24.00		12.1	13.5
MCP	Early	0.00		17.9	49.6
		2.00		7.6	27.5
		4.00		49.5	43.9
		8.00		19.2	2.1
		16.00		27.6	24.6
		24.00		15.8	7.5
	Late	0.00		37.5	0.9
		2.00		43.3	8.8
		4.00		64.1	24.0
		8.00		17.1	38.1
		16.00		59.5	9.8
		24.00		12.6	22.6
	Mid	0.00		1.5	15.3
		2.00		2.1	25.2
		4.00		16.2	23.9
		8.00		47.3	47.2
		16.00		28.3	16.8
		24.00		14.8	44.6

Infectio	Harvest	Storage	Shelf	3.00	7.00
PE	Early	0.00		24.6	56.0
		2.00		23.3	78.5
		4.00		65.4	45.9
		8.00		2.0	2.9
		16.00		19.1	28.0
		24.00		1.3	2.3
	Late	0.00		31.6	-25.6
		2.00		17.4	7.8
		4.00		94.5	26.1
		8.00		43.2	21.4
		16.00		77.0	16.3
		24.00		6.0	18.7
	Mid	0.00		12.1	1.6
		2.00		25.9	65.0
		4.00		25.3	13.9
		8.00		43.5	24.0
		16.00		44.2	17.4
		24.00		16.0	53.4
PEW	Early	0.00		10.3	17.0
		2.00		4.3	19.5
		4.00		11.1	17.2
		8.00		13.2	5.3
		16.00		30.0	34.0
		24.00		15.9	13.2
	Late	0.00		24.9	-11.1
		2.00		32.3	2.4
		4.00		26.0	15.2
		8.00		26.9	35.4
		16.00		67.7	21.5
		24.00		38.3	41.0
	Mid	0.00		19.6	50.7
		2.00		1.0	1.7
		4.00		12.1	39.0
		8.00		39.0	30.8
		16.00		18.9	11.4
		24.00		10.8	4.7

MCP	Infectio	Harvest	Storage	Shelf	3.00	7.00
Control	PE	Early	0.00		22.8	30.3
			2.00		34.4	95.5
			4.00		35.0	13.6
			8.00		1.8	4.0
			16.00		11.5	24.7
			24.00		1.5	0.6
		Late	0.00		29.8	-48.1
			2.00		7.0	1.2
			4.00		78.7	13.9
			8.00		68.2	1.7
			16.00		87.0	17.7
			24.00		9.6	12.0
		Mid	0.00		21.7	1.6
			2.00		49.1	80.9
			4.00		21.7	14.8
			8.00		45.5	2.4
			16.00		43.7	10.1
			24.00		6.4	25.2
	PEW	Early	0.00		11.0	16.7
			2.00		5.6	45.3
			4.00		19.1	24.8
			8.00		-9.6	8.2
			16.00		31.6	50.1
			24.00		1.3	15.5
		Late	0.00		8.1	-27.1
			2.00		5.8	1.5
			4.00		34.2	20.6
			8.00		37.9	35.4
			16.00		83.4	38.5
			24.00		53.7	62.4
		Mid	0.00		38.6	72.6
			2.00		0.5	2.0
			4.00		20.6	43.2
			8.00		25.0	12.6
			16.00		26.0	13.9
			24.00		17.7	1.8
MCP	PE	Early	0.00		26.4	81.8
			2.00		12.2	61.4
			4.00		95.9	78.2
			8.00		2.3	1.8
			16.00		26.8	31.3
			24.00		1.1	4.0
		Late	0.00		33.4	-3.1
			2.00		27.9	14.4
			4.00		110.3	38.3
			8.00		18.1	41.0
			16.00		67.0	14.9
			24.00		2.3	25.5
		Mid	0.00		2.4	1.7
			2.00		2.7	49.1
			4.00		28.8	13.0
			8.00		41.5	45.5
			16.00		44.7	24.7
			24.00		25.7	81.5
	PEW	Early	0.00		9.5	17.4
			2.00		2.9	-6.4
			4.00		3.1	9.6
			8.00		36.0	2.4
			16.00		28.4	17.9
			24.00		30.5	11.0
		Late	0.00		41.7	4.8
			2.00		58.7	3.3
			4.00		17.8	9.8
			8.00		16.0	35.3
			16.00		52.1	4.6
			24.00		22.9	19.6
		Mid	0.00		0.5	28.8
			2.00		1.4	1.3
			4.00		3.6	34.8
			8.00		53.0	49.0
			16.00		11.9	9.0
			24.00		4.0	7.6

Standard errors of means

Table	MCP	Infectio	Harvest	Storage
rep.	216	216	144	72
d.f.	221	221	221	221
e.s.e.	1.97	1.97	2.41	3.41

Table	Shelf	MCP Infectio	MCP Harvest	Infectio Harvest
rep.	216	108	72	72
d.f.	221	221	221	221
e.s.e.	1.97	2.79	3.41	3.41

Table	MCP Storage	Infectio Storage	Harvest Storage	MCP Shelf
rep.	36	36	24	108
d.f.	221	221	221	221
e.s.e.	4.83	4.83	5.91	2.79

Table	Infectio Shelf	Harvest Shelf	Storage Shelf	MCP Infectio Harvest
rep.	108	72	36	36
d.f.	221	221	221	221
e.s.e.	2.79	3.41	4.83	4.83

Table	MCP Infectio Storage	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Shelf
rep.	18	12	12	54
d.f.	221	221	221	221
e.s.e.	6.82	8.36	8.36	3.94

Table	MCP Harvest Shelf	Infectio Harvest Shelf	MCP Storage Shelf	Infectio Storage Shelf
rep.	36	36	18	18
d.f.	221	221	221	221
e.s.e.	4.83	4.83	6.82	6.82

Table	Harvest Storage Shelf	MCP Infectio Harvest Storage	MCP Infectio Harvest Shelf	MCP Infectio Storage Shelf
rep.	12	6	18	9
d.f.	221	221	221	221
e.s.e.	8.36	11.82	6.82	9.65

Table	MCP Harvest Storage Shelf	Infectio Harvest Storage Shelf	MCP Infectio Harvest Storage Shelf
rep.	6	6	3
d.f.	221	221	221
e.s.e.	11.82	11.82	16.72

(Not adjusted for missing values)

Standard errors of differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	216	216	144	72
d.f.	221	221	221	221
s.e.d.	2.79	2.79	3.41	4.83

Table	Shelf	MCP Infectio	MCP Harvest	Infectio Harvest
rep.	216	108	72	72
d.f.	221	221	221	221
s.e.d.	2.79	3.94	4.83	4.83

Table	MCP Storage	Infectio Storage	Harvest Storage	MCP Shelf
rep.	36	36	24	108
d.f.	221	221	221	221
s.e.d.	6.82	6.82	8.36	3.94

Table	Infectio Shelf	Harvest Shelf	Storage Shelf	MCP Infectio Harvest
rep.	108	72	36	36
d.f.	221	221	221	221
s.e.d.	3.94	4.83	6.82	6.82

Table	MCP Infectio Storage	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Shelf
rep.	18	12	12	54
d.f.	221	221	221	221
s.e.d.	9.65	11.82	11.82	5.57

Table	MCP Harvest Shelf	Infectio Harvest Shelf	MCP Storage Shelf	Infectio Storage Shelf
rep.	36	36	18	18
d.f.	221	221	221	221
s.e.d.	6.82	6.82	9.65	9.65

Table	Harvest Storage Shelf	MCP Infectio Harvest Storage	MCP Infectio Harvest Shelf	MCP Infectio Storage Shelf
rep.	12	6	18	9
d.f.	221	221	221	221
s.e.d.	11.82	16.72	9.65	13.65

Table	MCP Harvest Storage Shelf	Infectio Harvest Storage Shelf	MCP Infectio Harvest Storage Shelf
rep.	6	6	3
d.f.	221	221	221
s.e.d.	16.72	16.72	23.64

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	216	216	144	72
d.f.	221	221	221	221
l.s.d.	5.49	5.49	6.72	9.51

Table	Shelf	MCP Infectio	MCP Harvest	Infectio Harvest
rep.	216	108	72	72
d.f.	221	221	221	221
l.s.d.	5.49	7.76	9.51	9.51

Table	MCP Storage	Infectio Storage	Harvest Storage	MCP Shelf
rep.	36	36	24	108
d.f.	221	221	221	221
l.s.d.	13.45	13.45	16.47	7.76

Table	Infectio Shelf	Harvest Shelf	Storage Shelf	MCP Infectio Harvest
rep.	108	72	36	36
d.f.	221	221	221	221
l.s.d.	7.76	9.51	13.45	13.45

Table	MCP Infectio Storage	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Shelf
rep.	18	12	12	54
d.f.	221	221	221	221
l.s.d.	19.02	23.29	23.29	10.98

Table	MCP Harvest Shelf	Infectio Harvest Shelf	MCP Storage Shelf	Infectio Storage Shelf
rep.	36	36	18	18
d.f.	221	221	221	221
l.s.d.	13.45	13.45	19.02	19.02

Table	Harvest Storage Shelf	MCP Infectio Harvest Storage	MCP Infectio Harvest Shelf	MCP Infectio Storage Shelf
rep.	12	6	18	9
d.f.	221	221	221	221
l.s.d.	23.29	32.94	19.02	26.90

Table	MCP Harvest Storage Shelf	Infectio Harvest Storage Shelf	MCP Infectio Harvest Storage Shelf
rep.	6	6	3
d.f.	221	221	221
l.s.d.	32.94	32.94	46.59

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
221	28.95	117.3

p-coumin

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	10.256	10.256	5.33	0.022
Infectio	1	0.002	0.002	0.00	0.974
Harvest	2	3.387	1.694	0.88	0.416
Storage	5	26.128	5.226	2.71	0.021
Shelf	1	11.204	11.204	5.82	0.017
MCP.Infectio	1	4.990	4.990	2.59	0.109
MCP.Harvest	2	1.451	0.725	0.38	0.686
Infectio.Harvest	2	45.149	22.574	11.73	<.001
MCP.Storage	5	30.332	6.066	3.15	0.009
Infectio.Storage	5	27.691	5.538	2.88	0.015
Harvest.Storage	10	59.557	5.956	3.09	0.001
MCP.Shelf	1	26.449	26.449	13.74	<.001
Infectio.Shelf	1	0.024	0.024	0.01	0.911
Harvest.Shelf	2	13.841	6.921	3.60	0.029
Storage.Shelf	5	20.199	4.040	2.10	0.067
MCP.Infectio.Harvest	2	24.522	12.261	6.37	0.002
MCP.Infectio.Storage	5	27.072	5.414	2.81	0.017
MCP.Harvest.Storage	10	18.090	1.809	0.94	0.498
Infectio.Harvest.Storage	9(1)	50.724	5.636	2.93	0.003
MCP.Infectio.Shelf	1	2.095	2.095	1.09	0.298
MCP.Harvest.Shelf	2	4.839	2.419	1.26	0.287
Infectio.Harvest.Shelf	2	6.463	3.231	1.68	0.189
MCP.Storage.Shelf	5	8.171	1.634	0.85	0.516
Infectio.Storage.Shelf	5	37.820	7.564	3.93	0.002
Harvest.Storage.Shelf	10	20.165	2.016	1.05	0.405
MCP.Infectio.Harvest.Storage	9(1)	6.629	0.737	0.38	0.943
MCP.Infectio.Harvest.Shelf	2	10.947	5.474	2.84	0.060
MCP.Infectio.Storage.Shelf	5	26.055	5.211	2.71	0.021
MCP.Harvest.Storage.Shelf	5(5)	23.226	4.645	2.41	0.037
Infectio.Harvest.Storage.Shelf	3(7)	19.280	6.427	3.34	0.020
MCP.Infectio.Harvest.Storage.Shelf	-6(16)	1.318			
Residual	221(67)	425.422	1.925		
Total	334(97)	788.738			

Tables of means

Grand mean 1.208

MCP	Control	MCP
	1.054	1.362

Infectio	PE	PEW
	1.210	1.206

Harvest	Early	Late	Mid
	1.266	1.083	1.275

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	0.934	0.901	1.398	1.602	1.243	1.170

Shelf	3.00	7.00
	1.047	1.369

MCP	Infectio	PE	PEW
Control		1.164	0.944
MCP		1.257	1.468

MCP	Harvest	Early	Late	Mid
Control		1.178	0.938	1.046
MCP		1.354	1.228	1.505

Infectio	Harvest	Early	Late	Mid
PE		1.567	0.636	1.427
PEW		0.965	1.530	1.123

MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		1.174	0.690	0.954	1.092	1.287	1.129
MCP		0.695	1.113	1.842	2.112	1.199	1.212

Infectio	Storage	0.00	2.00	4.00	8.00	16.00	24.00
PE		0.979	1.013	1.734	1.236	1.429	0.871
PEW		0.889	0.790	1.063	1.968	1.057	1.469

Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		1.515	1.047	1.468	1.266	1.663	0.637
Late		0.595	0.987	1.204	1.854	0.302	1.556
Mid		0.693	0.670	1.522	1.686	1.764	1.317

MCP	Shelf	3.00	7.00
Control		1.140	0.968
MCP		0.954	1.771

Infectio	Shelf	3.00	7.00
PE		1.042	1.379
PEW		1.052	1.360

Harvest	Shelf	3.00	7.00
Early		1.174	1.358
Late		1.099	1.067
Mid		0.869	1.682

Storage	Shelf	3.00	7.00
0.00		0.984	0.884
2.00		0.980	0.823
4.00		1.233	1.563
8.00		1.417	1.787
16.00		0.660	1.826
24.00		1.009	1.332

	Infectio	PE			PEW		
MCP	Harvest	Early	Late	Mid	Early	Late	Mid
Control		1.261	0.688	1.542	1.095	1.188	0.550
MCP		1.874	0.584	1.312	0.835	1.871	1.697

MCP	Infectio	Storage	0.00	2.00	4.00	8.00	16.00
Control	PE		1.248	0.739	1.132	1.335	1.486
	PEW		1.100	0.640	0.776	0.849	1.088
MCP	PE		0.711	1.286	2.335	1.138	1.372
	PEW		0.679	0.940	1.350	3.087	1.026

MCP	Infectio	Storage	24.00
Control	PE		1.043
	PEW		1.215
MCP	PE		0.700
	PEW		1.724

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		1.906	0.532	1.107	1.258	1.482
	Late		0.838	1.015	0.914	1.088	0.456
	Mid		0.777	0.521	0.841	0.929	1.922
MCP	Early		1.124	1.562	1.829	1.275	1.844
	Late		0.352	0.959	1.494	2.619	0.147
	Mid		0.608	0.818	2.204	2.443	1.606

MCP	Harvest	Storage	24.00
Control	Early		0.782
	Late		1.318
	Mid		1.286
MCP	Early		0.492
	Late		1.794
	Mid		1.348

Infectio	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
PE	Early		1.864	1.861	2.498	0.683	1.914
	Late		0.326	0.469	1.259	1.548	-0.401
	Mid		0.749	0.708	1.443	1.478	2.773
PEW	Early		1.167	0.233	0.438	1.850	1.411
	Late		0.864	1.506	1.149	2.160	1.005
	Mid		0.636	0.632	1.601	1.893	0.755

Infectio	Harvest	Storage	24.00
PE	Early		0.584
	Late		0.617
	Mid		1.413
PEW	Early		0.691
	Late		2.496
	Mid		1.221

	Infectio	PE		PEW	
MCP	Shelf	3.00	7.00	3.00	7.00
Control		1.312	1.015	0.969	0.920
MCP		0.771	1.743	1.136	1.799

	Harvest	Early		Late		Mid	
MCP	Shelf	3.00	7.00	3.00	7.00	3.00	7.00
Control		1.354	1.002	1.063	0.814	1.005	1.087
MCP		0.994	1.715	1.135	1.321	0.733	2.276

	Harvest	Early		Late		Mid	
Infectio	Shelf	3.00	7.00	3.00	7.00	3.00	7.00
PE		1.373	1.762	0.817	0.455	0.936	1.919
PEW		0.975	0.955	1.380	1.679	0.802	1.444

MCP	Storage	Shelf	3.00	7.00
Control	0.00		1.328	1.020
	2.00		1.003	0.377
	4.00		1.142	0.766
	8.00		1.396	0.787
	16.00		0.805	1.768
	24.00		1.170	1.088
MCP	0.00		0.641	0.749
	2.00		0.957	1.269
	4.00		1.324	2.361
	8.00		1.439	2.786
	16.00		0.514	1.883
	24.00		0.847	1.576

Infectio	Storage	Shelf	3.00	7.00
PE	0.00		0.831	1.128
	2.00		0.933	1.092
	4.00		1.685	1.782
	8.00		1.515	0.958
	16.00		0.397	2.460
	24.00		0.888	0.854
PEW	0.00		1.137	0.641
	2.00		1.026	0.554
	4.00		0.780	1.345
	8.00		1.320	2.616
	16.00		0.922	1.191
	24.00		1.129	1.810

Harvest	Storage	Shelf	3.00	7.00
Early	0.00		1.436	1.595
	2.00		0.935	1.159
	4.00		1.386	1.550
	8.00		1.396	1.137
	16.00		1.345	1.980
	24.00		0.545	0.730
Late	0.00		0.717	0.474
	2.00		1.554	0.420
	4.00		1.562	0.847
	8.00		1.623	2.085
	16.00		-0.197	0.800
	24.00		1.334	1.779
Mid	0.00		0.801	0.585
	2.00		0.450	0.890
	4.00		0.751	2.294
	8.00		1.234	2.138
	16.00		0.831	2.697
	24.00		1.147	1.488

MCP	Infectio	Harvest	Storage	0.00	2.00	4.00	8.00
Control	PE	Early		2.143	0.812	1.870	0.884
		Late		0.525	0.652	0.658	1.574
		Mid		1.076	0.752	0.869	1.545
	PEW	Early		1.670	0.252	0.344	1.631
		Late		1.151	1.378	1.170	0.602
		Mid		0.478	0.290	0.812	0.312
MCP	PE	Early		1.584	2.911	3.127	0.482
		Late		0.127	0.285	1.860	1.521
		Mid		0.421	0.663	2.018	1.411
	PEW	Early		0.664	0.213	0.532	2.068
		Late		0.577	1.634	1.128	3.717
		Mid		0.795	0.974	2.389	3.475

MCP	Infectio	Harvest	Storage	16.00	24.00
Control	PE	Early		1.338	0.517
		Late		0.018	0.701
		Mid		3.102	1.910
	PEW	Early		1.625	1.047
		Late		0.894	1.935
		Mid		0.743	0.663
MCP	PE	Early		2.491	0.650
		Late		-0.821	0.532
		Mid		2.445	0.917
	PEW	Early		1.197	0.334
		Late		1.115	3.057
		Mid		0.767	1.780

MCP	Infectio	Harvest	Shelf	3.00	7.00
Control	PE	Early		1.583	0.938
		Late		0.840	0.537
		Mid		1.514	1.571
	PEW	Early		1.124	1.066
		Late		1.286	1.091
		Mid		0.496	0.603
MCP	PE	Early		1.163	2.586
		Late		0.794	0.374
		Mid		0.357	2.268
	PEW	Early		0.825	0.844
		Late		1.475	2.268
		Mid		1.108	2.285

MCP	Infectio	Storage	Shelf	3.00	7.00
Control	PE	0.00		1.011	1.485
		2.00		1.033	0.445
		4.00		1.378	0.886
		8.00		2.104	0.565
		16.00		1.129	1.842
		24.00		1.218	0.867
	PEW	0.00		1.645	0.555
		2.00		0.972	0.308
		4.00		0.905	0.646
		8.00		0.687	1.010
		16.00		0.481	1.694
		24.00		1.122	1.309
MCP	PE	0.00		0.652	0.770
		2.00		0.834	1.739
		4.00		1.993	2.677
		8.00		0.926	1.350
		16.00		-0.335	3.078
		24.00		0.559	0.841
	PEW	0.00		0.630	0.728
		2.00		1.080	0.800
		4.00		0.655	2.044
		8.00		1.952	4.221
		16.00		1.364	0.689
		24.00		1.136	2.311

MCP	Harvest	Storage	Shelf	3.00	7.00
Control	Early	0.00		2.371	1.442
		2.00		0.705	0.360
		4.00		1.272	0.942
		8.00		1.774	0.741
		16.00		1.304	1.660
		24.00		0.696	0.868
	Late	0.00		0.921	0.755
		2.00		1.647	0.383
		4.00		1.099	0.730
		8.00		1.350	0.826
		16.00		-0.108	1.020
		24.00		1.466	1.170
	Mid	0.00		0.691	0.863
		2.00		0.656	0.387
		4.00		1.055	0.626
		8.00		1.063	0.794
		16.00		1.219	2.625
		24.00		1.346	1.227
MCP	Early	0.00		0.500	1.748
		2.00		1.166	1.958
		4.00		1.500	2.158
		8.00		1.017	1.533
		16.00		1.386	2.301
		24.00		0.393	0.592
	Late	0.00		0.512	0.193
		2.00		1.461	0.458
		4.00		2.025	0.963
		8.00		1.895	3.343
		16.00		-0.287	0.581
		24.00		1.202	2.387
	Mid	0.00		0.910	0.306
		2.00		0.244	1.392
		4.00		0.447	3.961
		8.00		1.404	3.482
		16.00		0.444	2.768
		24.00		0.948	1.749

Infectio	Harvest	Storage	Shelf	3.00	7.00
PE	Early	0.00		1.585	2.142
		2.00		1.498	2.225
		4.00		2.367	2.629
		8.00		0.916	0.450
		16.00		1.350	2.479
		24.00		0.521	0.646
	Late	0.00		0.381	0.271
		2.00		0.639	0.299
		4.00		2.058	0.460
		8.00		2.218	0.878
		16.00		-1.075	0.272
		24.00		0.681	0.552
	Mid	0.00		0.529	0.969
		2.00		0.663	0.752
		4.00		0.631	2.255
		8.00		1.411	1.545
		16.00		0.917	4.630
		24.00		1.463	1.364
PEW	Early	0.00		1.287	1.047
		2.00		0.373	0.093
		4.00		0.405	0.471
		8.00		1.875	1.824
		16.00		1.340	1.482
		24.00		0.569	0.813
	Late	0.00		1.052	0.676
		2.00		2.469	0.542
		4.00		1.066	1.233
		8.00		1.028	3.292
		16.00		0.680	1.329
		24.00		1.986	3.005
	Mid	0.00		1.073	0.200
		2.00		0.237	1.027
		4.00		0.870	2.332
		8.00		1.056	2.731
		16.00		0.746	0.763
		24.00		0.831	1.612

MCP	Infectio	Harvest	Storage	Shelf	3.00	7.00
Control	PE	Early	0.00		2.325	1.960
			2.00		1.004	0.620
			4.00		2.131	1.608
			8.00		1.706	0.062
			16.00		1.760	0.917
			24.00		0.571	0.462
		Late	0.00		0.155	0.895
			2.00		0.902	0.403
			4.00		0.916	0.400
			8.00		2.505	0.644
			16.00		-0.358	0.393
			24.00		0.919	0.484
		Mid	0.00		0.554	1.599
			2.00		1.192	0.313
			4.00		1.088	0.650
			8.00		2.102	0.989
			16.00		1.987	4.216
			24.00		2.163	1.657
	PEW	Early	0.00		2.417	0.923
			2.00		0.406	0.099
			4.00		0.412	0.275
			8.00		1.842	1.421
			16.00		0.848	2.403
			24.00		0.822	1.273
		Late	0.00		1.688	0.615
			2.00		2.392	0.363
			4.00		1.281	1.059
			8.00		0.196	1.009
			16.00		0.143	1.646
			24.00		2.014	1.855
		Mid	0.00		0.829	0.126
			2.00		0.120	0.461
			4.00		1.022	0.602
			8.00		0.024	0.600
			16.00		0.452	1.034
			24.00		0.529	0.797
MCP	PE	Early	0.00		0.844	2.325
			2.00		1.992	3.830
			4.00		2.603	3.651
			8.00		0.127	0.837
			16.00		0.940	4.041
			24.00		0.470	0.831
		Late	0.00		0.607	-0.353
			2.00		0.376	0.194
			4.00		3.200	0.520
			8.00		1.930	1.112
			16.00		-1.791	0.150
			24.00		0.444	0.620
		Mid	0.00		0.504	0.339
			2.00		0.134	1.192
			4.00		0.175	3.861
			8.00		0.720	2.102
			16.00		-0.154	5.044
			24.00		0.763	1.071
	PEW	Early	0.00		0.157	1.170
			2.00		0.339	0.086
			4.00		0.398	0.666
			8.00		1.908	2.228
			16.00		1.832	0.561
			24.00		0.316	0.353
		Late	0.00		0.417	0.738
			2.00		2.546	0.721
			4.00		0.850	1.406
			8.00		1.860	5.574
			16.00		1.218	1.012
			24.00		1.959	4.154
		Mid	0.00		1.316	0.274
			2.00		0.355	1.593
			4.00		0.718	4.061
			8.00		2.088	4.861
			16.00		1.041	0.493
			24.00		1.132	2.427

Standard errors of means

Table	MCP	Infectio	Harvest	Storage
rep.	216	216	144	72
d.f.	221	221	221	221
e.s.e.	0.0944	0.0944	0.1156	0.1635

Table	Shelf	MCP Infectio	MCP Harvest	Infectio Harvest
rep.	216	108	72	72
d.f.	221	221	221	221
e.s.e.	0.0944	0.1335	0.1635	0.1635

Table	MCP Storage	Infectio Storage	Harvest Storage	MCP Shelf
rep.	36	36	24	108
d.f.	221	221	221	221
e.s.e.	0.2312	0.2312	0.2832	0.1335

Table	Infectio Shelf	Harvest Shelf	Storage Shelf	MCP Infectio Harvest
rep.	108	72	36	36
d.f.	221	221	221	221
e.s.e.	0.1335	0.1635	0.2312	0.2312

Table	MCP Infectio Storage	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Shelf
rep.	18	12	12	54
d.f.	221	221	221	221
e.s.e.	0.3270	0.4005	0.4005	0.1888

Table	MCP Harvest Shelf	Infectio Harvest Shelf	MCP Storage Shelf	Infectio Storage Shelf
rep.	36	36	18	18
d.f.	221	221	221	221
e.s.e.	0.2312	0.2312	0.3270	0.3270

Table	Harvest Storage Shelf	MCP Infectio Harvest Storage	MCP Infectio Harvest Shelf	MCP Infectio Storage Shelf
rep.	12	6	18	9
d.f.	221	221	221	221
e.s.e.	0.4005	0.5664	0.3270	0.4625

Table	MCP Harvest Storage Shelf	Infectio Harvest Storage Shelf	MCP Infectio Harvest Storage Shelf
rep.	6	6	3
d.f.	221	221	221
e.s.e.	0.5664	0.5664	0.8010

(Not adjusted for missing values)

Standard errors of differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	216	216	144	72
d.f.	221	221	221	221
s.e.d.	0.1335	0.1335	0.1635	0.2312

Table	Shelf	MCP Infectio	MCP Harvest	Infectio Harvest
rep.	216	108	72	72
d.f.	221	221	221	221
s.e.d.	0.1335	0.1888	0.2312	0.2312

Table	MCP Storage	Infectio Storage	Harvest Storage	MCP Shelf
rep.	36	36	24	108
d.f.	221	221	221	221
s.e.d.	0.3270	0.3270	0.4005	0.1888

Table	Infectio Shelf	Harvest Shelf	Storage Shelf	MCP Infectio Harvest
rep.	108	72	36	36
d.f.	221	221	221	221
s.e.d.	0.1888	0.2312	0.3270	0.3270

Table	MCP Infectio Storage	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Shelf
rep.	18	12	12	54
d.f.	221	221	221	221
s.e.d.	0.4625	0.5664	0.5664	0.2670

Table	MCP Harvest Shelf	Infectio Harvest Shelf	MCP Storage Shelf	Infectio Storage Shelf
rep.	36	36	18	18
d.f.	221	221	221	221
s.e.d.	0.3270	0.3270	0.4625	0.4625

Table	Harvest Storage Shelf	MCP Infectio Harvest Storage	MCP Infectio Harvest Shelf	MCP Infectio Storage Shelf
rep.	12	6	18	9
d.f.	221	221	221	221
s.e.d.	0.5664	0.8010	0.4625	0.6540

Table	MCP Harvest Storage Shelf	Infectio Harvest Storage Shelf	MCP Infectio Harvest Storage Shelf
rep.	6	6	3
d.f.	221	221	221
s.e.d.	0.8010	0.8010	1.1328

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	216	216	144	72
d.f.	221	221	221	221
l.s.d.	0.2631	0.2631	0.3222	0.4557

Table	Shelf	MCP Infectio	MCP Harvest	Infectio Harvest
rep.	216	108	72	72
d.f.	221	221	221	221
l.s.d.	0.2631	0.3721	0.4557	0.4557

Table	MCP Storage	Infectio Storage	Harvest Storage	MCP Shelf
rep.	36	36	24	108
d.f.	221	221	221	221
l.s.d.	0.6445	0.6445	0.7893	0.3721

Table	Infectio Shelf	Harvest Shelf	Storage Shelf	MCP Infectio Harvest
rep.	108	72	36	36
d.f.	221	221	221	221
l.s.d.	0.3721	0.4557	0.6445	0.6445

Table	MCP Infectio Storage	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Shelf
rep.	18	12	12	54
d.f.	221	221	221	221
l.s.d.	0.9114	1.1163	1.1163	0.5262

Table	MCP Harvest Shelf	Infectio Harvest Shelf	MCP Storage Shelf	Infectio Storage Shelf
rep.	36	36	18	18
d.f.	221	221	221	221
l.s.d.	0.6445	0.6445	0.9114	0.9114

Table	Harvest Storage Shelf	MCP Infectio Harvest Storage	MCP Infectio Harvest Shelf	MCP Infectio Storage Shelf
rep.	12	6	18	9
d.f.	221	221	221	221
l.s.d.	1.1163	1.5787	0.9114	1.2890

Table	MCP Harvest Storage Shelf	Infectio Harvest Storage Shelf	MCP Infectio Harvest Storage Shelf
rep.	6	6	3
d.f.	221	221	221
l.s.d.	1.5787	1.5787	2.2325

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
221	1.3874	114.8

HPLC Data

Non Infected & *Botrytis cinerea*
3 d infection

Analysis of variance

Benzoic acid

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	10855.	10855.	10.05	0.002
Infectio	1	8130.	8130.	7.53	0.007
Harvest	2	3998.	1999.	1.85	0.162
Storage	5	4303.	861.	0.80	0.554
MCP.Infectio	1	70.	70.	0.06	0.800
MCP.Harvest	2	12935.	6467.	5.99	0.003
Infectio.Harvest	2	4892.	2446.	2.26	0.108
MCP.Storage	5	6934.	1387.	1.28	0.276
Infectio.Storage	5	15923.	3185.	2.95	0.015
Harvest.Storage	10	21355.	2135.	1.98	0.042
MCP.Infectio.Harvest	2	10422.	5211.	4.82	0.010
MCP.Infectio.Storage	5	2878.	576.	0.53	0.751
MCP.Harvest.Storage	9(1)	32412.	3601.	3.33	0.001
Infectio.Harvest.Storage	8(2)	12467.	1558.	1.44	0.186
MCP.Infectio.Harvest.Storage	0(10)	15011.			
Residual	117(27)	126385.	1080.		
Total	175(40)	250284.			

Tables of means

Grand mean 18.3

MCP	Control	MCP
	11.2	25.4

Infectio	BC	NI
	12.2	24.4

Harvest	Early	Late	Mid
	15.7	14.9	24.4

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	15.5	20.6	16.5	20.5	25.3	11.3

MCP	Infectio	BC	NI
Control		5.6	16.8
MCP		18.7	32.1

MCP	Harvest	Early	Late	Mid
Control		15.6	11.5	6.5
MCP		15.7	18.2	42.2

Infectio	Harvest	Early	Late	Mid
BC		16.0	7.2	13.3
NI		15.4	22.5	35.4

MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		7.9	12.5	9.8	18.5	7.4	11.1
MCP		23.1	28.6	23.3	22.5	43.3	11.5

Infectio	Storage	0.00	2.00	4.00	8.00	16.00	24.00
BC		17.6	0.4	6.4	23.9	25.4	-0.7
NI		13.4	40.7	26.6	17.1	25.3	23.3

Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		14.2	36.7	6.6	17.8	13.7	5.0
Late		11.5	12.3	30.4	11.0	10.6	13.4
Mid		20.8	12.7	12.6	32.8	51.8	15.5

	Infectio	BC			NI		
MCP	Harvest	Early	Late	Mid	Early	Late	Mid
Control		6.7	8.7	1.6	24.5	14.4	11.4
MCP		25.2	5.8	25.0	6.2	30.6	59.4

MCP	Infectio	Storage	0.00	2.00	4.00	8.00	16.00
Control	BC		8.3	-13.5	4.3	24.7	7.1
	NI		7.5	38.6	15.2	12.4	7.7
MCP	BC		26.8	14.4	8.5	23.2	43.6
	NI		19.4	42.8	38.1	21.8	43.0

MCP	Infectio	Storage	24.00
Control	BC		2.9
	NI		19.2
MCP	BC		-4.3
	NI		27.4

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		3.3	58.3	4.3	13.9	7.5
	Late		14.0	3.4	5.0	18.4	7.0
	Mid		6.5	-24.1	20.0	23.3	7.7
MCP	Early		25.0	15.1	8.9	21.6	19.8
	Late		9.0	21.2	55.9	3.6	14.1
	Mid		35.2	49.5	5.1	42.3	96.0

MCP	Harvest	Storage	24.00
Control	Early		6.2
	Late		21.3
	Mid		5.7
MCP	Early		3.8
	Late		5.6
	Mid		25.2

Infectio	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
BC	Early		23.2	13.6	9.7	28.0	18.7
	Late		11.4	10.7	3.0	13.5	10.7
	Mid		18.1	-23.0	6.5	30.3	46.7
NI	Early		5.1	59.8	3.5	7.5	8.6
	Late		11.7	13.9	57.9	8.5	10.4
	Mid		23.6	48.4	18.6	35.3	57.0

Infectio	Harvest	Storage	24.00
BC	Early		2.4
	Late		-5.8
	Mid		1.3
NI	Early		7.6
	Late		32.7
	Mid		29.7

MCP	Infectio	Harvest	Storage	0.00	2.00	4.00	8.00
Control	BC	Early		4.2	5.2	2.5	18.5
		Late		9.2	5.2	4.9	25.2
		Mid		11.5	-50.9	5.6	30.3
	NI	Early		2.3	111.4	6.2	9.4
		Late		18.9	1.6	5.1	11.7
		Mid		1.4	2.7	34.5	16.3
MCP	BC	Early		42.2	22.0	17.0	37.5
		Late		13.6	16.2	1.0	1.8
		Mid		24.6	4.8	7.5	30.3
	NI	Early		7.9	8.2	0.7	5.7
		Late		4.5	26.2	110.7	5.3
		Mid		45.8	94.2	2.7	54.4

MCP	Infectio	Harvest	Storage	16.00	24.00
Control	BC	Early		6.7	2.9
		Late		3.2	4.4
		Mid		11.5	1.5
	NI	Early		8.4	9.5
		Late		10.7	38.3
		Mid		3.8	9.9
MCP	BC	Early		30.7	1.9
		Late		18.2	-16.0
		Mid		81.9	1.0
	NI	Early		8.9	5.7
		Late		10.1	27.1
		Mid		110.1	49.5

Standard errors of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
e.s.e.	3.16	3.16	3.87	5.48

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
e.s.e.	4.47	5.48	5.48	7.75

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
e.s.e.	7.75	9.49	7.75	10.96

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
e.s.e.	13.42	13.42	18.98

(Not adjusted for missing values)

Standard errors of differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
s.e.d.	4.47	4.47	5.48	7.75

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
s.e.d.	6.33	7.75	7.75	10.96

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
s.e.d.	10.96	13.42	10.96	15.49

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
s.e.d.	18.98	18.98	26.84

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
l.s.d.	8.86	8.86	10.85	15.34

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
l.s.d.	12.53	15.34	15.34	21.70

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
l.s.d.	21.70	26.57	21.70	30.68

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
l.s.d.	37.58	37.58	53.15

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
117	32.87	179.6

Catechin

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	45.	45.	0.03	0.871
Infectio	1	35504.	35504.	20.91	<.001
Harvest	2	908.	454.	0.27	0.766
Storage	5	13926.	2785.	1.64	0.155
MCP.Infectio	1	11757.	11757.	6.92	0.010
MCP.Harvest	2	6331.	3165.	1.86	0.160
Infectio.Harvest	2	10122.	5061.	2.98	0.055
MCP.Storage	5	12003.	2401.	1.41	0.224
Infectio.Storage	5	9675.	1935.	1.14	0.344
Harvest.Storage	10	27487.	2749.	1.62	0.110
MCP.Infectio.Harvest	2	6189.	3094.	1.82	0.166
MCP.Infectio.Storage	5	15153.	3031.	1.78	0.121
MCP.Harvest.Storage	9(1)	54139.	6015.	3.54	<.001
Infectio.Harvest.Storage	8(2)	18325.	2291.	1.35	0.226
MCP.Infectio.Harvest.Storage	0(10)	6909.			
Residual	117(27)	198683.	1698.		
Total	175(40)	389157.			

Tables of means

Grand mean 35.7

MCP	Control	MCP
	36.2	35.3

Infectio	BC	NI
	22.9	48.6

Harvest	Early	Late	Mid
	34.4	34.2	38.6

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	31.1	28.9	33.4	44.8	27.7	48.6

MCP	Infectio	BC	NI
Control		30.8	41.6
MCP		15.1	55.5

MCP	Harvest	Early	Late	Mid
Control		29.1	41.9	37.6
MCP		39.7	26.5	39.7

Infectio	Harvest	Early	Late	Mid
BC		29.4	22.4	17.0
NI		39.4	46.0	60.3

MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		34.2	32.9	21.9	53.6	33.9	40.7
MCP		28.0	24.9	44.9	36.0	21.4	56.4

Infectio	Storage	0.00	2.00	4.00	8.00	16.00	24.00
BC		17.0	14.7	20.3	40.4	21.6	23.6
NI		45.2	43.1	46.5	49.2	33.7	73.6

Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		42.0	34.3	6.9	45.8	14.7	62.5
Late		19.1	22.8	43.8	55.8	29.7	34.1
Mid		32.1	29.6	49.6	32.9	38.6	49.1

	Infectio	BC			NI		
MCP	Harvest	Early	Late	Mid	Early	Late	Mid
Control		30.8	31.3	30.1	27.3	52.5	45.1
MCP		27.9	13.6	3.8	51.5	39.4	75.6

MCP	Infectio	Storage	0.00	2.00	4.00	8.00	16.00
Control	BC		23.9	21.5	34.5	52.1	29.3
	NI		44.4	44.3	9.4	55.2	38.5
MCP	BC		10.1	7.9	6.2	28.7	13.9
	NI		46.0	41.9	83.6	43.3	29.0

MCP	Infectio	Storage	24.00
Control	BC		23.3
	NI		58.1
MCP	BC		23.8
	NI		89.1

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		51.7	31.5	26.0	37.6	13.8
	Late		18.6	33.3	19.6	82.6	39.2
	Mid		32.2	33.8	20.3	40.6	48.5
MCP	Early		32.3	37.1	-12.2	54.0	15.5
	Late		19.6	12.3	68.0	29.0	20.2
	Mid		32.1	25.3	78.9	25.1	28.6

MCP	Harvest	Storage	24.00
Control	Early		13.7
	Late		58.2
	Mid		50.3
MCP	Early		111.4
	Late		9.9
	Mid		48.0

Infectio	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
BC	Early		27.0	12.2	13.0	67.7	12.3
	Late		17.9	16.0	30.6	35.0	27.0
	Mid		5.9	15.8	17.4	18.5	25.5
NI	Early		56.9	56.5	0.8	23.9	17.1
	Late		20.3	29.5	57.0	76.6	32.4
	Mid		58.4	43.3	81.8	47.2	51.7

Infectio	Harvest	Storage	24.00
BC	Early		44.0
	Late		8.1
	Mid		18.6
NI	Early		81.1
	Late		60.0
	Mid		79.7

MCP	Infectio	Harvest	Storage	0.00	2.00	4.00	8.00
Control	BC	Early		36.5	6.0	51.0	63.8
		Late		24.1	28.3	20.8	56.5
		Mid		11.0	30.1	31.6	35.9
	NI	Early		66.9	57.0	1.0	11.4
		Late		13.1	38.2	18.4	108.7
		Mid		53.3	37.5	8.9	45.3
MCP	BC	Early		17.5	18.4	-25.0	71.6
		Late		11.8	3.7	40.4	13.5
		Mid		0.9	1.6	3.2	1.1
	NI	Early		47.0	55.9	0.6	36.5
		Late		27.5	20.9	95.6	44.5
		Mid		63.4	49.0	154.6	49.1

MCP	Infectio	Harvest	Storage	16.00	24.00
Control	BC	Early		14.2	13.5
		Late		30.0	28.0
		Mid		43.6	28.6
	NI	Early		13.4	14.0
		Late		48.4	88.3
		Mid		53.5	72.0
MCP	BC	Early		10.3	74.5
		Late		24.1	-11.7
		Mid		7.3	8.6
	NI	Early		20.8	148.2
		Late		16.3	31.6
		Mid		49.8	87.4

Standard errors of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
e.s.e.	3.97	3.97	4.86	6.87

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
e.s.e.	5.61	6.87	6.87	9.71

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
e.s.e.	9.71	11.90	9.71	13.74

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
e.s.e.	16.82	16.82	23.79

(Not adjusted for missing values)

Standard errors of differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
s.e.d.	5.61	5.61	6.87	9.71

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
s.e.d.	7.93	9.71	9.71	13.74

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
s.e.d.	13.74	16.82	13.74	19.43

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
s.e.d.	23.79	23.79	33.65

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
l.s.d.	11.11	11.11	13.60	19.24

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
l.s.d.	15.71	19.24	19.24	27.20

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
l.s.d.	27.20	33.32	27.20	38.47

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
l.s.d.	47.12	47.12	66.64

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
117	41.21	115.3

Chlorogenic acid

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	77.	77.	0.01	0.905
Infectio	1	23448.	23448.	4.38	0.039
Harvest	2	2333.	1167.	0.22	0.805
Storage	5	13261.	2652.	0.50	0.779
MCP.Infectio	1	1078.	1078.	0.20	0.654
MCP.Harvest	2	1252.	626.	0.12	0.890
Infectio.Harvest	2	8821.	4410.	0.82	0.441
MCP.Storage	5	22097.	4419.	0.83	0.534
Infectio.Storage	5	46245.	9249.	1.73	0.134
Harvest.Storage	10	64437.	6444.	1.20	0.296
MCP.Infectio.Harvest	2	12449.	6224.	1.16	0.316
MCP.Infectio.Storage	5	15642.	3128.	0.58	0.712
MCP.Harvest.Storage	9(1)	68782.	7642.	1.43	0.184
Infectio.Harvest.Storage	8(2)	45485.	5686.	1.06	0.395
MCP.Infectio.Harvest.Storage	0(10)	12411.			
Residual	117(27)	626435.	5354.		
Total	175(40)	907369.			

Tables of means

Grand mean 29.8

MCP	Control	MCP
	30.4	29.2

Infectio	BC	NI
	19.4	40.2

Harvest	Early	Late	Mid
	34.3	26.5	28.7

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	30.0	44.5	22.7	29.6	20.0	32.2

MCP	Infectio	BC	NI
Control		17.8	43.1
MCP		21.0	37.4

MCP	Harvest	Early	Late	Mid
Control		36.6	23.7	31.0
MCP		32.0	29.3	26.4

Infectio	Harvest	Early	Late	Mid
BC		22.2	24.6	11.4
NI		46.4	28.4	45.9

MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		38.0	57.8	14.0	25.7	29.1	17.9
MCP		22.0	31.2	31.3	33.5	10.9	46.5

Infectio	Storage	0.00	2.00	4.00	8.00	16.00	24.00
BC		25.2	2.7	18.2	33.7	9.8	26.7
NI		34.8	86.2	27.1	25.4	30.2	37.6

Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		29.8	97.1	22.8	32.7	3.5	19.9
Late		21.4	25.5	20.1	35.7	15.0	41.1
Mid		38.8	10.8	25.1	20.2	41.5	35.5

	Infectio	BC			NI		
MCP	Harvest	Early	Late	Mid	Early	Late	Mid
Control		11.5	24.1	17.7	61.6	23.3	44.3
MCP		32.8	25.1	5.2	31.2	33.5	47.6

MCP	Infectio	Storage	0.00	2.00	4.00	8.00	16.00
Control	BC		28.4	-1.2	11.8	38.8	12.3
	NI		47.6	116.8	16.3	12.6	45.8
MCP	BC		22.0	6.7	24.6	28.7	7.3
	NI		22.0	55.7	37.9	38.3	14.6

MCP	Infectio	Storage	24.00
Control	BC		16.5
	NI		19.3
MCP	BC		37.0
	NI		55.9

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		25.3	156.1	17.7	27.7	-10.3
	Late		22.9	32.0	11.9	31.6	15.5
	Mid		65.8	-14.8	12.6	17.8	82.0
MCP	Early		34.3	38.1	27.9	37.8	17.2
	Late		19.9	19.1	28.2	39.9	14.6
	Mid		11.9	36.4	37.7	22.7	1.0

MCP	Harvest	Storage	24.00
Control	Early		2.9
	Late		28.2
	Mid		22.6
MCP	Early		36.9
	Late		54.0
	Mid		48.5

Infectio	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
BC	Early		38.5	12.4	29.7	48.1	-13.8
	Late		23.5	19.4	14.1	34.6	13.8
	Mid		13.8	-23.5	10.7	18.4	29.4
NI	Early		21.1	181.8	15.8	17.4	20.7
	Late		19.4	31.7	26.1	36.9	16.2
	Mid		63.9	45.1	39.5	22.0	53.7

Infectio	Harvest	Storage	24.00
BC	Early		18.2
	Late		42.3
	Mid		19.7
NI	Early		21.6
	Late		39.9
	Mid		51.4

MCP	Infectio	Harvest	Storage	0.00	2.00	4.00	8.00
Control	BC	Early		21.2	22.2	19.2	43.8
		Late		26.9	27.0	4.2	42.0
		Mid		37.2	-52.8	11.9	30.5
	NI	Early		29.4	290.1	16.2	11.6
		Late		18.9	36.9	19.7	21.1
		Mid		94.5	23.3	13.2	5.0
MCP	BC	Early		55.7	2.6	40.3	52.4
		Late		20.0	11.8	23.9	27.2
		Mid		-9.6	5.8	9.5	6.4
	NI	Early		12.9	73.5	15.5	23.2
		Late		19.9	26.5	32.5	52.6
		Mid		33.3	67.0	65.9	39.0

MCP	Infectio	Harvest	Storage	16.00	24.00
Control	BC	Early		-37.9	0.7
		Late		5.6	38.7
		Mid		69.3	10.0
	NI	Early		17.3	5.2
		Late		25.4	17.7
		Mid		94.8	35.1
MCP	BC	Early		10.3	35.7
		Late		22.0	45.9
		Mid		-10.5	29.3
	NI	Early		24.1	38.1
		Late		7.1	62.1
		Mid		12.6	67.6

Standard errors of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
e.s.e.	7.04	7.04	8.62	12.20

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
e.s.e.	9.96	12.20	12.20	17.25

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
e.s.e.	17.25	21.12	17.25	24.39

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
e.s.e.	29.87	29.87	42.25

(Not adjusted for missing values)

Standard errors of differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
s.e.d.	9.96	9.96	12.20	17.25

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
s.e.d.	14.08	17.25	17.25	24.39

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
s.e.d.	24.39	29.87	24.39	34.49

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
s.e.d.	42.25	42.25	59.74

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
l.s.d.	19.72	19.72	24.15	34.16

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
l.s.d.	27.89	34.16	34.16	48.30

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
l.s.d.	48.30	59.16	48.30	68.31

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
l.s.d.	83.67	83.67	118.32

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
117	73.17	245.4

Epicatechin

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	0.1	0.1	0.00	0.985
Infectio	1	4016.6	4016.6	10.27	0.002
Harvest	2	502.7	251.3	0.64	0.528
Storage	5	4661.4	932.3	2.38	0.042
MCP.Infectio	1	1.5	1.5	0.00	0.951
MCP.Harvest	2	366.1	183.0	0.47	0.627
Infectio.Harvest	2	168.4	84.2	0.22	0.807
MCP.Storage	5	1500.1	300.0	0.77	0.575
Infectio.Storage	5	5390.8	1078.2	2.76	0.022
Harvest.Storage	10	6714.0	671.4	1.72	0.085
MCP.Infectio.Harvest	2	1690.3	845.1	2.16	0.120
MCP.Infectio.Storage	5	305.6	61.1	0.16	0.978
MCP.Harvest.Storage	9(1)	10535.8	1170.6	2.99	0.003
Infectio.Harvest.Storage	8(2)	5723.4	715.4	1.83	0.078
MCP.Infectio.Harvest.Storage	0(10)	2151.3			
Residual	117(27)	45759.7	391.1		
Total	175(40)	84335.8			

Table of Means

Grand mean 15.4

MCP	Control	MCP
	15.4	15.3

Infectio	BC	NI
	11.1	19.7

Harvest	Early	Late	Mid
	13.3	16.9	15.9

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	12.3	14.0	18.7	17.4	7.8	22.1

MCP	Infectio	BC	NI
Control		11.2	19.6
MCP		10.9	19.7

MCP	Harvest	Early	Late	Mid
Control		14.7	17.3	14.2
MCP		11.9	16.5	17.7

Infectio	Harvest	Early	Late	Mid
BC		7.9	12.6	12.7
NI		18.7	21.2	19.2

MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		9.7	18.7	16.5	15.1	8.4	23.9
MCP		14.8	9.4	21.0	19.6	7.1	20.2

Infectio	Storage	0.00	2.00	4.00	8.00	16.00	24.00
BC		11.5	9.3	13.0	20.1	3.9	8.5
NI		13.0	18.8	24.5	14.6	11.6	35.6

Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		6.2	17.8	7.9	21.3	12.6	13.8
Late		21.6	9.3	19.5	14.3	5.3	31.1
Mid		8.9	15.0	28.7	16.5	5.3	21.3

	Infectio	BC			NI		
MCP	Harvest	Early	Late	Mid	Early	Late	Mid
Control		5.6	13.8	14.0	23.7	20.7	14.4
MCP		10.1	11.4	11.3	13.6	21.6	24.0

MCP	Infectio	Storage	0.00	2.00	4.00	8.00	16.00
Control	BC		7.6	13.6	11.0	19.7	3.4
	NI		11.9	23.8	21.9	10.6	13.5
MCP	BC		15.4	5.1	15.0	20.6	4.4
	NI		14.2	13.7	27.0	18.6	9.8

MCP	Infectio	Storage	24.00				
Control	BC		11.8				
	NI		36.0				
MCP	BC		5.3				
	NI		35.2				

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		5.7	24.3	9.0	13.4	10.0
	Late		11.4	8.0	18.9	19.4	3.4
	Mid		12.1	23.7	21.5	12.7	11.9
MCP	Early		6.6	11.4	6.9	29.1	15.3
	Late		31.9	10.5	20.2	9.3	7.3
	Mid		5.8	6.3	35.8	20.3	-1.3

MCP	Harvest	Storage	24.00
Control	Early		25.6
	Late		42.7
	Mid		3.5
MCP	Early		2.0
	Late		19.5
	Mid		39.2

Infectio	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
BC	Early		7.1	4.8	8.6	21.1	2.2
	Late		13.3	3.3	16.5	20.4	5.5
	Mid		14.1	19.9	13.8	18.8	4.0
NI	Early		5.2	30.9	7.3	21.4	23.1
	Late		30.0	15.3	22.6	8.3	5.2
	Mid		3.8	10.1	43.5	14.1	6.6

Infectio	Harvest	Storage	24.00
BC	Early		3.4
	Late		16.6
	Mid		5.6
NI	Early		24.2
	Late		45.6
	Mid		37.0

MCP	Infectio	Harvest	Storage	0.00	2.00	4.00	8.00
Control	BC	Early		1.9	8.2	6.6	13.3
		Late		3.6	4.1	19.7	26.3
		Mid		17.2	28.4	6.6	19.3
	NI	Early		9.5	40.5	11.4	13.4
		Late		19.2	11.9	18.0	12.4
		Mid		6.9	18.9	36.4	6.0
MCP	BC	Early		12.3	1.5	10.6	28.9
		Late		22.9	2.4	13.3	14.5
		Mid		10.9	11.4	21.0	18.3
	NI	Early		1.0	21.3	3.3	29.4
		Late		40.8	18.7	27.1	4.2
		Mid		0.7	1.3	50.6	22.2

MCP	Infectio	Harvest	Storage	16.00	24.00
Control	BC	Early		-2.3	5.9
		Late		0.5	28.8
		Mid		12.1	0.6
	NI	Early		22.4	45.2
		Late		6.3	56.6
		Mid		11.7	6.3
MCP	BC	Early		6.7	0.8
		Late		10.6	4.4
		Mid		-4.1	10.6
	NI	Early		23.8	3.2
		Late		4.0	34.5
		Mid		1.5	67.7

Standard errors of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
e.s.e.	1.90	1.90	2.33	3.30

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
e.s.e.	2.69	3.30	3.30	4.66

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
e.s.e.	4.66	5.71	4.66	6.59

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
e.s.e.	8.07	8.07	11.42

(Not adjusted for missing values)

Standard errors of differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
s.e.d.	2.69	2.69	3.30	4.66

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
s.e.d.	3.81	4.66	4.66	6.59

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
s.e.d.	6.59	8.07	6.59	9.32

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
s.e.d.	11.42	11.42	16.15

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
l.s.d.	5.33	5.33	6.53	9.23

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
l.s.d.	7.54	9.23	9.23	13.06

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
l.s.d.	13.06	15.99	13.06	18.46

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
l.s.d.	22.61	22.61	31.98

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
117	19.78	128.7

p-coumin

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
MCP	1	1.887	1.887	0.55	0.459
Infectio	1	8.207	8.207	2.40	0.124
Harvest	2	6.511	3.255	0.95	0.389
Storage	5	23.259	4.652	1.36	0.244
MCP.Infectio	1	6.341	6.341	1.86	0.176
MCP.Harvest	2	4.144	2.072	0.61	0.547
Infectio.Harvest	2	4.290	2.145	0.63	0.535
MCP.Storage	5	4.430	0.886	0.26	0.934
Infectio.Storage	5	23.106	4.621	1.35	0.247
Harvest.Storage	10	84.417	8.442	2.47	0.010
MCP.Infectio.Harvest	2	24.471	12.235	3.58	0.031
MCP.Infectio.Storage	5	36.772	7.354	2.15	0.064
MCP.Harvest.Storage	9(1)	49.542	5.505	1.61	0.120
Infectio.Harvest.Storage	8(2)	34.356	4.294	1.26	0.273
MCP.Infectio.Harvest.Storage	0(10)	14.165			
Residual	117(27)	399.633	3.416		
Total	175(40)	633.880			

Tables of means

Grand mean 1.41

MCP	Control	MCP
	1.32	1.50

Infectio	BC	NI
	1.21	1.60

Harvest	Early	Late	Mid
	1.58	1.17	1.48

Storage	0.00	2.00	4.00	8.00	16.00	24.00
	1.13	1.17	1.00	1.53	1.72	1.89

MCP	Infectio	BC	NI
Control		0.95	1.68
MCP		1.48	1.53

MCP	Harvest	Early	Late	Mid
Control		1.63	1.12	1.20
MCP		1.53	1.22	1.76

Infectio	Harvest	Early	Late	Mid
BC		1.20	1.00	1.45
NI		1.95	1.34	1.52

MCP	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Control		1.06	1.26	0.77	1.48	1.40	1.93
MCP		1.20	1.09	1.24	1.59	2.04	1.86

Infectio	Storage	0.00	2.00	4.00	8.00	16.00	24.00
BC		1.21	0.56	1.17	1.63	1.42	1.30
NI		1.04	1.79	0.84	1.44	2.03	2.49

Harvest	Storage	0.00	2.00	4.00	8.00	16.00	24.00
Early		1.12	1.95	1.18	1.65	0.80	2.77
Late		0.86	1.09	1.36	1.26	0.90	1.55
Mid		1.40	0.48	0.48	1.70	3.48	1.36

	Infectio	BC			NI		
MCP	Harvest	Early	Late	Mid	Early	Late	Mid
Control		0.67	0.76	1.42	2.58	1.48	0.99
MCP		1.72	1.23	1.48	1.33	1.20	2.05

MCP	Infectio	Storage	0.00	2.00	4.00	8.00	16.00
Control	BC		1.25	0.48	0.65	1.82	1.17
	NI		0.86	2.04	0.88	1.14	1.64
MCP	BC		1.17	0.63	1.68	1.45	1.67
	NI		1.22	1.55	0.80	1.73	2.41

MCP	Infectio	Storage	24.00				
Control	BC		0.33				
	NI		3.53				
MCP	BC		2.28				
	NI		1.44				

MCP	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
Control	Early		0.85	2.68	0.38	1.05	0.87
	Late		0.61	0.89	1.42	1.86	0.67
	Mid		1.72	0.22	0.50	1.52	2.67
MCP	Early		1.39	1.22	1.97	2.25	0.72
	Late		1.11	1.30	1.30	0.66	1.12
	Mid		1.09	0.74	0.45	1.87	4.28

MCP	Harvest	Storage	24.00
Control	Early		3.93
	Late		1.28
	Mid		0.57
MCP	Early		1.61
	Late		1.82
	Mid		2.14

Infectio	Harvest	Storage	0.00	2.00	4.00	8.00	16.00
BC	Early		1.65	0.75	1.52	1.94	0.31
	Late		0.88	0.76	1.17	1.16	0.67
	Mid		1.11	0.15	0.81	1.80	3.28
NI	Early		0.59	3.15	0.84	1.36	1.28
	Late		0.84	1.43	1.54	1.36	1.12
	Mid		1.70	0.81	0.14	1.59	3.67

Infectio	Harvest	Storage	24.00
BC	Early		1.03
	Late		1.33
	Mid		1.54
NI	Early		4.51
	Late		1.77
	Mid		1.17

MCP	Infectio	Harvest	Storage	0.00	2.00	4.00	8.00
Control	BC	Early		1.26	0.39	0.36	1.35
		Late		0.71	0.83	0.72	1.85
		Mid		1.79	0.23	0.88	2.24
	NI	Early		0.43	4.97	0.41	0.74
		Late		0.51	0.94	2.13	1.87
		Mid		1.64	0.22	0.12	0.80
MCP	BC	Early		2.04	1.11	2.67	2.52
		Late		1.06	0.69	1.63	0.48
		Mid		0.42	0.08	0.73	1.35
	NI	Early		0.75	1.32	1.27	1.97
		Late		1.16	1.92	0.96	0.84
		Mid		1.76	1.40	0.17	2.39

MCP	Infectio	Harvest	Storage	16.00	24.00
Control	BC	Early		0.32	0.35
		Late		0.21	0.24
		Mid		2.97	0.38
	NI	Early		1.42	7.50
		Late		1.12	2.33
		Mid		2.38	0.76
MCP	BC	Early		0.30	1.70
		Late		1.13	2.42
		Mid		3.59	2.70
	NI	Early		1.15	1.52
		Late		1.12	1.22
		Mid		4.97	1.58

Standard errors of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
e.s.e.	0.178	0.178	0.218	0.308

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
e.s.e.	0.252	0.308	0.308	0.436

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
e.s.e.	0.436	0.534	0.436	0.616

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
e.s.e.	0.755	0.755	1.067

(Not adjusted for missing values)

Standard errors of differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
s.e.d.	0.252	0.252	0.308	0.436

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
s.e.d.	0.356	0.436	0.436	0.616

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
s.e.d.	0.616	0.755	0.616	0.871

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
s.e.d.	1.067	1.067	1.509

(Not adjusted for missing values)

Least significant differences of means

Table	MCP	Infectio	Harvest	Storage
rep.	108	108	72	36
d.f.	117	117	117	117
l.s.d.	0.498	0.498	0.610	0.863

Table	MCP Infectio	MCP Harvest	Infectio Harvest	MCP Storage
rep.	54	36	36	18
d.f.	117	117	117	117
l.s.d.	0.704	0.863	0.863	1.220

Table	Infectio Storage	Harvest Storage	MCP Infectio Harvest	MCP Infectio Storage
rep.	18	12	18	9
d.f.	117	117	117	117
l.s.d.	1.220	1.494	1.220	1.725

Table	MCP Harvest Storage	Infectio Harvest Storage	MCP Infectio Harvest Storage
rep.	6	6	3
d.f.	117	117	117
l.s.d.	2.113	2.113	2.989

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

d.f.	s.e.	cv%
117	1.848	131.1