Department of Environment and Agriculture

Blossom thinning and managing bitter pit, storage life and fruit quality in organically grown apples

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: .....

Date: 12-June-2015 .....

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Abstract

Fruit quality is important for both growers and consumers, however excessive crop load and physiological disorders such as bitter pit and superficial scald adversely affect apple fruit quality at harvest, during storage and transportation. The quality of fruit, physiological disorders including bitter pit in apple depends on various pre- and post-harvest factors. The present study focused on evaluating the effect of blossom thinning to reduce crop load and optimisation of fruit quality by using organic chemicals. Efficacy of different concentrations of various organic calcium and boron sources were examined on reducing bitter pit and superficial scald as well as maintaining storage life and fruit quality of organically grown apples. Amongst different spray treatments of 5% of lime sulphur (LS) alone and in combination with different types of oils (3%) such as canola oil, fish oil and olive oil at 75% of bloom stage, lime sulphur in combination with olive oil was most effective in reducing fruit set (11.74%), improving fruit size, skin colour, SCC, ascorbic acid levels and lower mean leaf scorch (2.25%) in organically grown 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apples. LS applied twice at 25% bloom and at 75% bloom and once at 75% bloom appear to be the most effective treatments in reducing fruit set, fruit retention and improving fruit size, weight and skin colour in all cultivars. However, double application of LS at 25% bloom and at 75% bloom resulted in higher leaf scorch (14.45%) than LS applied once at 75% bloom (12.08%) in all cultivars. All concentrations of LS (1% - 4%) significantly ($P \leq 0.05$) reduced fruit set, however, the spray application of 3-4% LS alone was most effective in reducing fruit set (7-11%) but caused higher leaf scorch (13-14%) compared to control and other treatments. Spray application of LS (4%) in combination with olive oil (3%) appears to be effective treatment in blossom thinning and reducing fruit set (11%) which also improved fruit quality and reduced leaf scorching compared to LS spray alone in both 'Gala' and 'Cripps Pink^{TM}' apple. While the higher concentrations of LS (3-4%) alone or with 3% of olive oil showed a decreasing trend in firmness, SSC and TA following cold storage for 60, 90 and 120 days in 'Cripps Pink', and 'Gala' apples and the percentage of fruit weight loss increased following a cold storage period.

Four pre-harvest spray applications of Biomin® Calcium (1, 2 or 3kg/ha) and Biomin® Boron (1 or 2 kg/ha) alone and in combination significantly increased

calcium and B concentration in leaves and fruit respectively in comparison to the control in 'Gala' and ''Cripps Pink^{TM}' apples. However, the application of Ca (3kg) alone was most effective in increasing the levels of calcium in leaves and fruit and was also effective in decreasing the incidence of bitter pit and superficial scald; increasing fruit firmness and improved fruit quality at commercial harvest following 60, 90 and 120 days of cold storage in 'Gala' and 'Cripps Pink^{TM}' apple fruit. However, the highest incidence of bitter pit and superficial scald were observed when apple trees were sprayed with 2 kg boron.

In conclusion, the spray application of 4% LS in combination with 3% olive oil and synertrol oil (0.05%) as a surfactant at 75% full bloom stage was effective in reducing fruit set and crop load; increasing the fruit size; and improving fruit colour on 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apples. Four pre-harvest sprays of emulsion containing Biomin® calcium (3 kg/ha) and synertrol oil (0.05%) as a surfactant, commencing from 30 days after full bloom stage at 25 day intervals reduced bitter pit and superficial scald; as well as maintaining fruit quality of organically grown 'Gala' and ''Cripps Pink^{TM}' apples in up to 90 days of cold storage.

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List of symbols and abbreviations

×	Multiply / interaction between
+	Plus
-	Minus
>	Greater than
<	Less than
≥	Greater than or equal to
≤	Less than or equal to
±	Plus minus
/	Divide
=	Equal to
~	Approximately
´	Minute(s)
°	Degree
°C	Degree celcious
%	Per cent
α	Alpha
β	Beta
λ	Lambda
β-Gal	*β*-galactosidase
Δ	Changes of
µg	Microgram(s)
µL	Microlitre(s)
µM	Micromolar(s)
µm	Micrometre(s)
µmol	Micromole(s)
1-MCP	1-Methylcyclopropene
2,4-D	2,4-Dichlorophenoxyacetic acid
3-Meox	3-methylene oxindole
A$	Australian dollar
ABA	Abscisic acid
ABS	Australian Bureau of Statistics

ACC	1-aminocyclopropane-1-carboxylic acid
ACO	1-aminocyclopropane-1-carboxylic acid oxidase or ACC oxidase
ACS	1-aminocyclopropane-1-carboxylic acid synthase or ACC synthase
a.i.	Active ingredient
AI	Acid invertase
ANOVA	Analysis of variance
AU	Absorbance units
AVG	Aminoethoxyvinylglycine
BL	Brassinolide
BC	Break colour
BRs	Brassinosteroids
BSTFA	*N,O*-Bis (trimethylsilyl)trifluoroacetamide
Ca	Calcium
$CaCl_2$	Calcium chloride
C*	Chroma
CA^1	Controlled atmosphere
CA^2	California
CAS	Castasterone
C_2H_4	Ethylene
cm	Centimetre(s)
Co.	Company
CO_2	Carbon dioxide / respiration
CO	Canola oil
conc.	Concentration
$CoCl_2$	Cobalt chloride
Corp.	Corporation
cv.	Cultivar
d	Day(s)
DAFB	Days after full bloom
DAFI	Days after flower induction
DAFS	Days after fruit set
dH_2O	Distilled water
DM	Dry matter

DPH	Day prior to harvest
DPPH	2, 2-diphenyl-1-picryl-hydrazyl
Dr.	Doctor
DTT	Dithiotheritol
e	Exponential
E	East
EC	Enzyme commission
EDTA	Ethylenediamine tetra-acetic acid disodium
i.e.	That is
EFE	Ethylene-forming enzyme
e.g.	For example
EGase	*Endo*-1,4-*β*-D-glucanase
Endo-PG	*Endo*-polygalacturonic acid
Epi-BL	Epibrassinolide
Eq.	Equivalent
et al.	Et alia
EU	European Union
Exo-PG	*Exo*-polygalacturonic acid
f	Force
FAOSTAT	Food and Agriculture Organisation Statistic
$FeSO_4$	Ferrous sulphate
FID	Flame ionization detector
FR	Full-red
FO	Fish oil
FW	Fresh weight
g	Gram(s)
g	Gravity
G	Green
GC	Gas chromatograph
GA	Gibberellic acid
h	Hour(s)
H	Hydrogen
h°	Hue angle

ha	Hectare(s)
HCl	Hydrochloric acid
$HgCl_2$	Mercury chloride
H_2O	Water
H_2O_2	Hydrogen peroxide
Homo-BL	Homobrassinolide
HPLC	High performance liquid chromatography
HWT	Hot water treatments
IAA	Indole-3-acetic acid
Inc.	Incorporated
Intl.	International
kg	Kilogram(s)
KOH	Potassium hydroxide
kPa	Kilo pascals
L	Litre(s)
L*	Lightness
lat.	Latitude
LDPE	Low-density polyethylene
LS	lime sulphur
LSD	Least significant difference
Ltd.	Limited
long.	Longitude
m	Metre(s)
M	Molar
MA	Massachusetts
MACC	1-malonyl aminocyclopropane-1-carboxylic acid
MAP	Modified Atmospheres Packaging
MeOH	Methanol
3-Meox	3-methylene oxindole
MG	Mature green
mg	Milligram(s)
$MgCO_3$	Magnesium carbonate
min	Minute(s)

MJ	Methyl jasmonate
mL	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar(s)
mmol	Millimole(s)
MPE	Microperforated polyethylene
MS	Mass Spectrometry
Mt	Metric tonnes
N	Newton(s)
N	Normality
n	Number of sample
N_2	Nitrogen
NA	Not available
NAA	Naphthalene acetic acid
NaCl	Sodium chloride
NaF	Sodium fluoride
$NaHSO_3$	Sodium hydrogen sulphite
NaOH	Sodium hydroxide
NaOCl	Sodium hypochlorite
NCED	9-cis-epoxycarotenoid dioxygenase
ND	Not detected
NDGA	Nordihydroguaiaretic acid
ng	Nanogram(s)
NIR	Near Infra Red
nL	Nanolitre(s)
nm	Nanometre(s)
Nmm	Newton millimetre(s)
nmol	Nanomole(s)
NO	Nitric oxide
NC	North Carolina
NS	Not significant
NSW	New South Wales
NT	Northern Territory

O_2	Oxygen
OR	Orange
OO	Olive oil
P	Probability
PA	Pennsylvania
pA	Peak area
psi	Pounds per square inch
PCIB	α(p-Chlorophenoxy)isobutyric acid
PC	Pre-climacteric
PE	Pectin esterase
PEPC	Phosphoenol pyruvate carboxylase
PG	Polygalacturonic acid
pH	Symbol denoting hydrogen ion in a solution
PI	Pink
pmol	Picomole(s)
PL	Pectate lyase
PLP	Pyridoxal-5-phosphate
POD	Peroxidase
ppb	Part per billion (10^{-9})
ppm	Part per million (10^{-6})
Prof.	Professor
psi	Pounds per square inch
Pty.	Proprietary
PVP	Polyvinylpyrolidone
PVPP	Polyvinylpyrolidone phosphate
QLD	Queensland
®	Registered
r	Correlation coefficient
rcf	Relative centrifugation force
RH	Relative humidity
RI	Refractive index
ROS	Radical oxygen species
RP	Ripening period

rpm	Rounds per minute
RR	Red ripe
S	South
s	Second(s)
SAM	S-adenosyl methionine
SAMDC	S-adenosyl methionine decarboxylic acid
SAS	Statistical Analysis System
SCO_2	Rate of CO_2 for sample
S.E.	Standard error
SI	Sucrose invertase
SLV	Scanning Laser Virometry
SNP	Sodium nitroprusside
SP	Storage period
sp.	Species
SS	Sucrose synthase
SSC	Soluble solids concentration
St.	Saint
$StdCO_2$	Rate of CO_2 for standard
T	Treatment
TA	Titratable acidity
tan	Tangent
TAPP	Tanzania Agriculture Productivity Program
TEAC	Trolox Equivalent Antioxidant Activity
TM	Trademark
TPA	Texture Profile Analyser
Trolox	6-hydroxy-2, 5, 7, 8-tetramethychroman-2-carboxylic acid
UK	United Kingdom
USA	United States of America
US$	United States dollar
UV	Ultra-violet
V	Viscosity
VA	Virginia
VIC	Victoria

VIS	visible
Vol.	Volume(s)
vs.	Versus
v/v	Volume by volume
WA	Western Australia
WAFB	Weeks after full bloom
WAFF	Weeks after first flowers
WAPF	Weeks after post flowering
WPH	Weeks prior harvest
MV	Millivolt(s)
w/v	Weight by volume
XF	Xtend® film

Chapters 1

General Introduction

Apple (*Malus domestica* Borkh.) is one of the most popular fruit worldwide and is produced in temperate climates. Asia is the largest apple producer in the world, followed by Europe, North America, South America, Africa and Australia. The Australian apple industry contributed only 1% of the world total production in 2007 (FAO, 2014). However, apple production is still imperative to the Australian apple industry where the estimated gross value for export in 2011-12 was AU$ 7.2 million (Department of Agriculture and Food Western Australia, 2014). Organic production of fruit is gaining impetus and occupies 116,000 ha in the world and organic apples are grown on 35,268 ha, followed by apricots with a production area of 10,683 ha (Kirby and Granatstein, 2010). Apple is grown throughout various states of Australia over approximately 12,258 ha. In respect to apple growing, Victoria has the largest area (4,279 ha) followed by New South Wales (2,455 ha), Queensland (1,571 ha), Western Australia (1,423 ha), South Australia (1,306 ha) and Tasmania (1,224 ha) (Australia Bureau of Statistics, 2014). The three most common varieties of apples produced in Australia are 'Cripps Pink^{TM}' (Pink Lady) (60,500 tonnes), 'Granny Smith' (58,600 tonnes) and 'Gala' (39,500 tonnes). Most apple industries in Western Australia are concentrated around Donnybrook, Manjimup, Dwellingup and the Perth Hills. The 'Cripps Pink^{TM}' cultivar is the main variety grown contributing 34% of the state's apple production, followed by 'Gala' (17%) and 'Granny Smith' (17%) (Australia Bureau of Statistics, 2014). Organic apple is grown in all states of Australia with over 60 certified growers (McCoy, 2007). In Western Australia, organic apple production is in an infant stage with annual production of 300 tonnes only (McCoy, 2007).

Thinning apple fruit is a pre-requisite in the production of high quality fruit (Link, 1973). Crop load has a major impact on apple fruit quality and the regularity of bearing (Link, 2000). Ayala and Andrade, (2009) reported that regulation of crop load can be achieved by several methods such as hand thinning, chemical thinning, mechanical thinning, artificial spur extinction (removal of complete spur from the branch) or bud thinning. Hand thinning is expensive and time consuming (Childers,

1983). Chemical-free flower and fruit thinning reduces chemical input in integrated fruit production as well as reducing production costs and alternate bearing in biological (organic) apple orchards (Bertschinger et al., 1998). Fallahi and Greene (2010) reported early thinning of apple fruit as an important operation because of its impact on fruit size and next season's flower bud initiation. Chemical thinning continues to be the most important practice in modern apple production (Looney, 1986). Yuan and Greene (2000) found that spray application of 6-benzyladenine at 100 mg L^{-1} effectively thinned apple fruit and increased fruit size. The spray application of carbaryl (1000 mg L^{-1}), ethephon (474 mg L^{-1}) and naphthalene acetic acid (58 mg L^{-1}) on 'Golden Delicious' apples reduced auxin transport to fruitlets hence leading to thinning (Ebert and Bangerth, 1982). The application of Tergitol TMN-6 (0.75% to 1.25%) at 75 to 80% bloom was effective in blossom thinning of 'Rome Beauty' apples (Fallahi and Greene, 2010). Spray application of lime sulphur (LS) (up to 4%) at 85% full bloom reduces fruit set (Guak et al., 2004). Stopar (2004) also reported a severe thinning with 3% lime sulphur (CaS_x) in 'Golden Delicious' apple to reduce the crop load and increase the fruit weight. Blossom thinning using organic chemicals to reduce crop load for improving fruit quality is a major challenge for organically grown apples. Manual thinning is tedious, time consuming and very expensive. Presently, there are only a few methods and agents allowed for certified organic horticulture (Stopar, 2008). Most experiments to regulate fruit set in organic horticulture are based on two strategies including injuring flowers (Ju et al., 2001; Pfeiffer and Ruess, 2002) and mechanical reduction in the number of flower buds (Roche and Masseron, 2002). Efficacy of several products such as mineral oil, corn oil, rape oil, olive oil, vinegar, sodium bicarbonate, sodium salt and lime sulphur has been tested to damage flowers (Alegre and Alins, 2005). The efficacy of organic bloom thinners under Australian conditions on the reduction of fruit set, fruit retention as well as maintaining storage life and fruit quality of organically grown apples is yet to be investigated.

Calcium (Ca) plays an extremely important role in plant growth and development; and for maintaining and modulating various cell functions (Hanson, 1984; Palta, 1996). Ca is essential to maintain membrane stability and is an integral part of the cell wall where it provides rigidity. Ca also plays an important role as an

intracellular secondary messenger (Poovaiah and Reddy, 1993). Ca treatments can maintain fruit quality by reducing softening (Mason, et al., 1975). In addition, the Ca increases cell wall strength and thickness by forming cross-links within the pectin polysaccharide matrix (Easterwood, 2002).

Among different nutritional disorders in apple, bitter pit is a major one (Witney et al., 1991) which is related to calcium deficiency (Ferguson et al., 1979; Simons & Chu, 1982; Perring, 1986; Raese, 1989; Cocucci et al., 1990; Fallahi et al., 1990; Siddiqui & Bangerth, 1993). Bangerth (1979) also reported that the incidence of bitter pit in apple is commonly associated with low calcium concentrations in the fruit. Later on, Ferguson and Watkins (1983) also reported that apple fruit tissues are sensitive to bitter pit with low calcium concentrations. Neilsen and Neilsen (2002) found 4-5 calcium (0.7%) sprays applied at post-bloom stages significantly increased calcium concentration in the apple fruit thus reducing incidence of bitter pit. Calcium plays an important role in reducing incidence of bitter pit in apple (Moor et al., 2005). However, no research work has been reported on the effects of different concentration of organic calcium on reducing the incidence of bitter pit in organically grown apple fruit in Australia.

One of the most significant physiological disorders is superficial scald that affects various fruit such as apples and pears (Emongor et al., 1994). It is characterized by irregular brown patches of damaged cells developing under the cuticle, thus adversely affecting the quality and value of the apple fruit (Paliyath et al., 1997). Apple scald appears during marketing or after storage (Soria et al., 1999) and is influenced by several factors including cultivar, stage of maturity; and growing and storage conditions (Diamantidis et al., 2002). Nutrient partitioning during different stages of apple fruit growth and development may be of importance to scald development within the storage environment. Sharples (1980) reported that calcium affects cell metabolism and structure, not only conferring greater resistance to changes preceding softening, fungal invasion and the development of disorders, but also delays the general rate of senescence of the tissues. Ca also plays a role in determining fruit quality (Raese and Drake, 2000). O'Loughlin and Jotic (1978) found that spray applications of calcium (different concentrations) made a significant

reduction in the incidence of internal breakdown and superficial scald on apple fruit. Calcium propionate preserves the structural integrity of the cells and acts as a food preservative, thus may increase shelf life (Quiles et al., 2007). Hayat et al (2005) has reported that apples treated with calcium chloride (2%) have an extended storage life and consumer acceptability after 60 days of cold storage. The fruit treated with calcium lactate exhibited an acceptable shelf life of 17 days following cold storage (Pereira, 2010). Emongor et al (1994) reported that apples with low calcium levels often develop more scald than those with higher levels. However, the effect of organic calcium on superficial scald and bitter pit incidence in organically grown apple fruit in Australia had not yet been investigated. Therefore the current research was conducted with the following objectives.

The main objectives of the research were:

1. To investigate the effects of spray application of lime sulphur alone and in combination with different types of oils (canola oil, fish oil and olive oil) on blossom thinning, fruit set, fruit retention, leaf scorch and fruit quality in organically grown commercial cultivars 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apples.
2. To examine the effects of number and time of spray applications of lime sulphur (LS) at different blossom stages on bloom thinning, fruit set, fruit retention, leaf scorch and fruit quality in organically grown commercial cultivars 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apples.
3. To explore the effects of spray applications of different concentrations of lime sulphur alone and in combination with organic olive oil on blossom thinning, fruit set, fruit retention, leaf scorch and fruit quality in organically grown 'Gala' and 'Cripps Pink^{TM}' apples.
4. To evaluate the effects of different concentrations of LS alone or in combination with organic olive oil on cold storage life and fruit quality including fruit firmness, weight loss, soluble solids, titratable acidity and ascorbic acid in 'Gala' and 'Cripps Pink^{TM}' apples.
5. To investigate the effects of post-bloom spray applications of different concentration of organic calcium alone or followed by application of organic

boron on Ca and B uptake into the fruit and leaf, incidence of bitter pit, and superficial scald; and quality at harvest and following cold storage of organically grown ‘Gala’ and ‘Cripps Pink^{TM}’ apples.

Chapter 2

General Review of Literature

2.1. Introduction

Apple (*Malus domestica* Borkh.) along with orange and banana, dominate the world market as one of the most popular fruits.

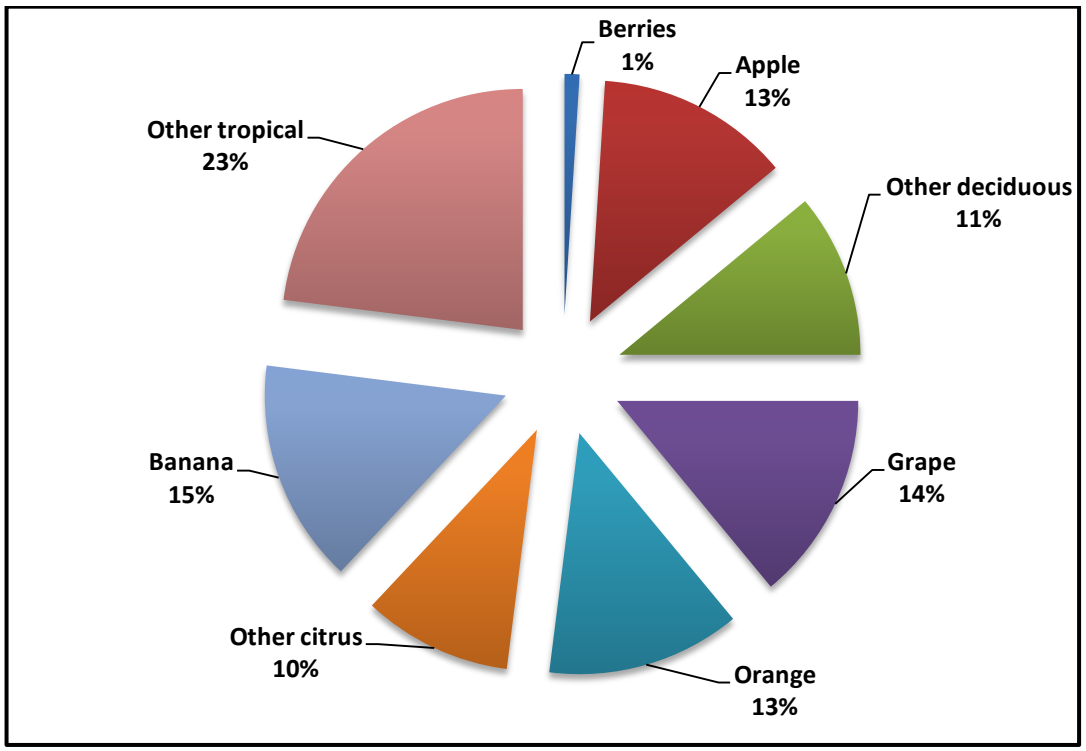

Figure 2.1. World fruit production **(**Kirby and Granatstein, 2010).

Global apple production is dominated by China, which produces five times more than the USA, its closest producing competitor. France, Turkey and Italy follow in that order, and are the major European producers, and these are followed closely by other European and South American countries. Australian production is less than 1% of the world total (Fig 2.2) (FAO, 2014).

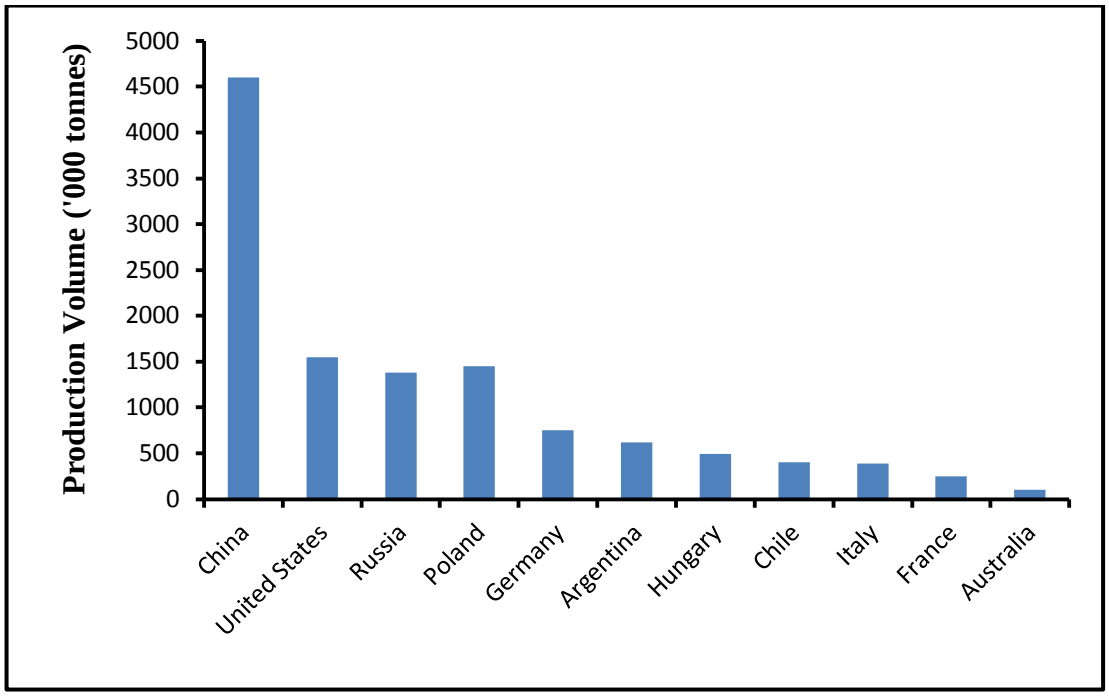

Figure 2.2. Apple production in top ten producing countries and Australia in 2012. Source: FAO (2014).

2.1.1. World organic fruit production:

Organic agriculture is expanding worldwide, driven by consumer demand in world markets, as well as its claimed potential to address resource conservation, food security, and farm income issues in developing countries (Granatstein et al., 2010). Organic crops, especially fruits and vegetables, are being promoted as a critical part of a healthy diet that can help to avoid problems such as obesity, diabetes, and heart diseases. Not surprisingly, consumers interested in healthy diets are often also attracted to organic foods (Hartman et al., 2006) and thus organic horticultural crops play a prominent role in consumer purchases. Organic production is continually improving, particularly according to the worldwide annual survey conducted by the Research Institute for Organic Agriculture (FiBL) and the International Federation of Organic Agriculture Movements (FiBL/IFOAM, 2010). Production of fruit is gaining impetus and occupies 116,000 ha in the world with organic apple grown on 35,268 ha, followed by apricot production area of 10,683 ha (Kirby and Granatstein, 2010) (Fig 2.2).

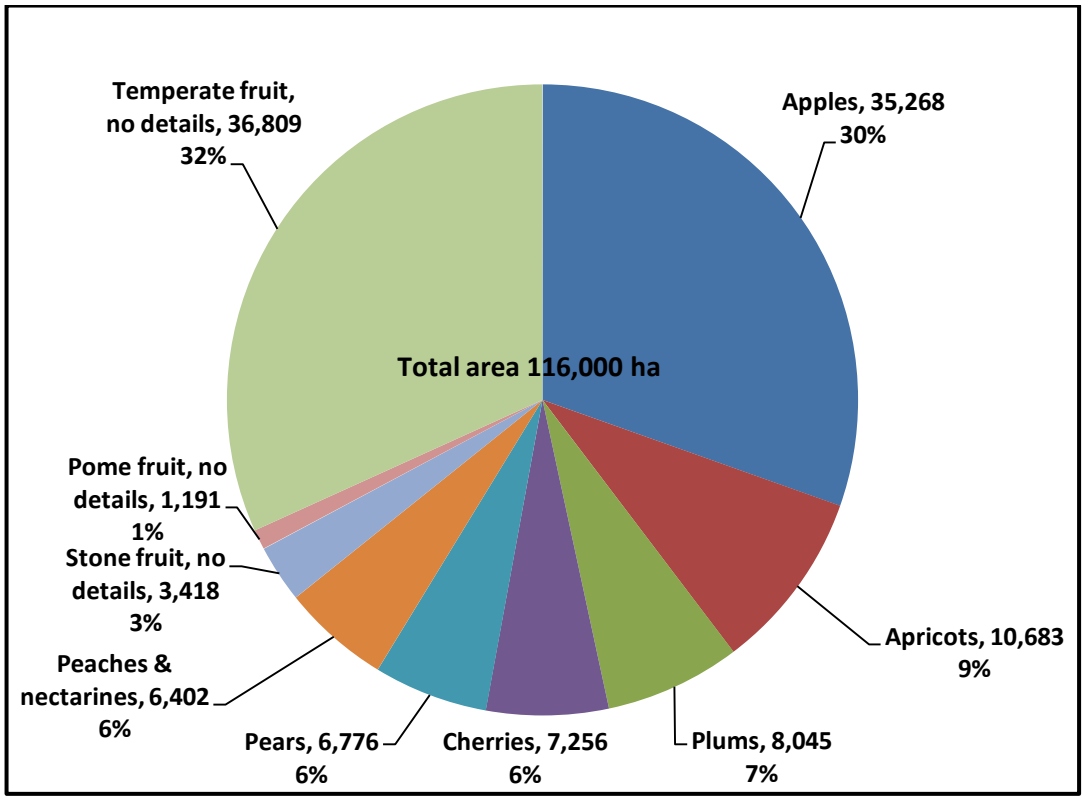

Figure 2.3. World organic temperate tree fruit area 2008 **(** FiBL/IFOAM survey, CDFA 2008; WSDA 2008)

2.1.2. Apple production in Australia

Apple is grown throughout various states of Australia over approximately 12,258 ha. Victoria has largest area of apple production (4,279 ha) followed by New South Wales (2,455 ha), Queensland (1,571 ha), Western Australia (1,423 ha), South Australia (1,306 ha) and Tasmania (1,224 ha) (Figure 2.4) (Australia Bureau of Statistics 2014). The three most common varieties of apples produced in Australia are 'Cripps PinkTM' (Pink Lady^{TM}) (60,500 tonnes), 'Granny Smith' (58,600 tonnes) and 'Gala' (39,500 tonnes) (Figure 2.5) (Australian Bureau of Statistics, 2014).

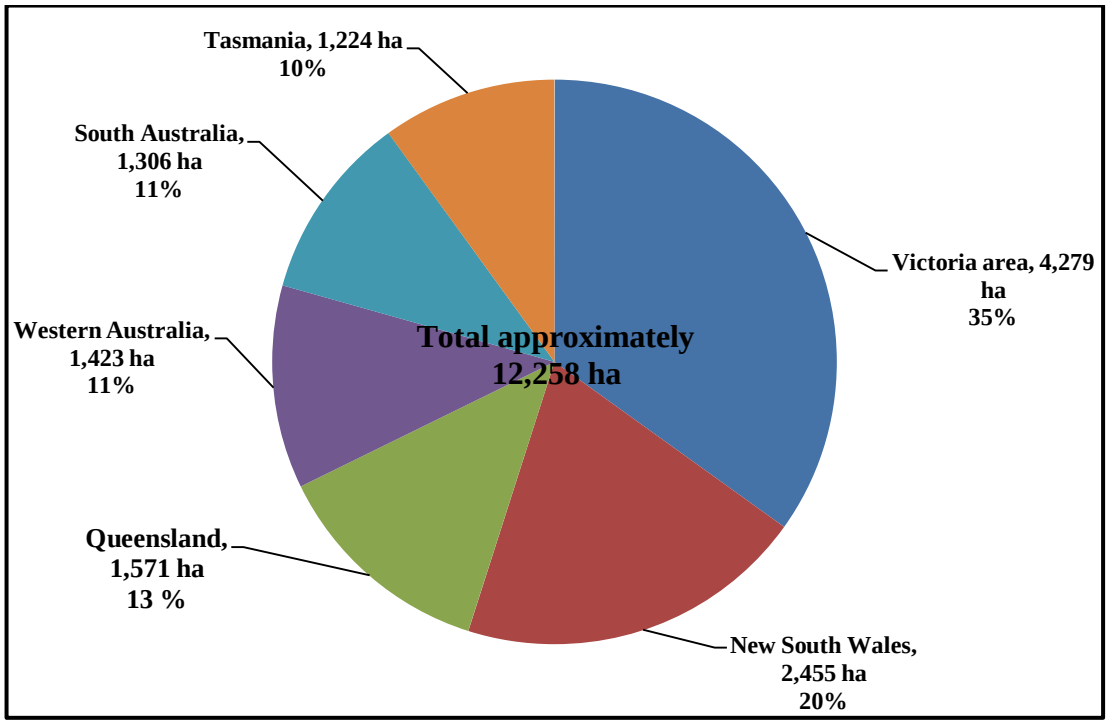

Figure 2.4. Apple production in different states of Australia **(**Australian Bureau of Statistics, 2014)

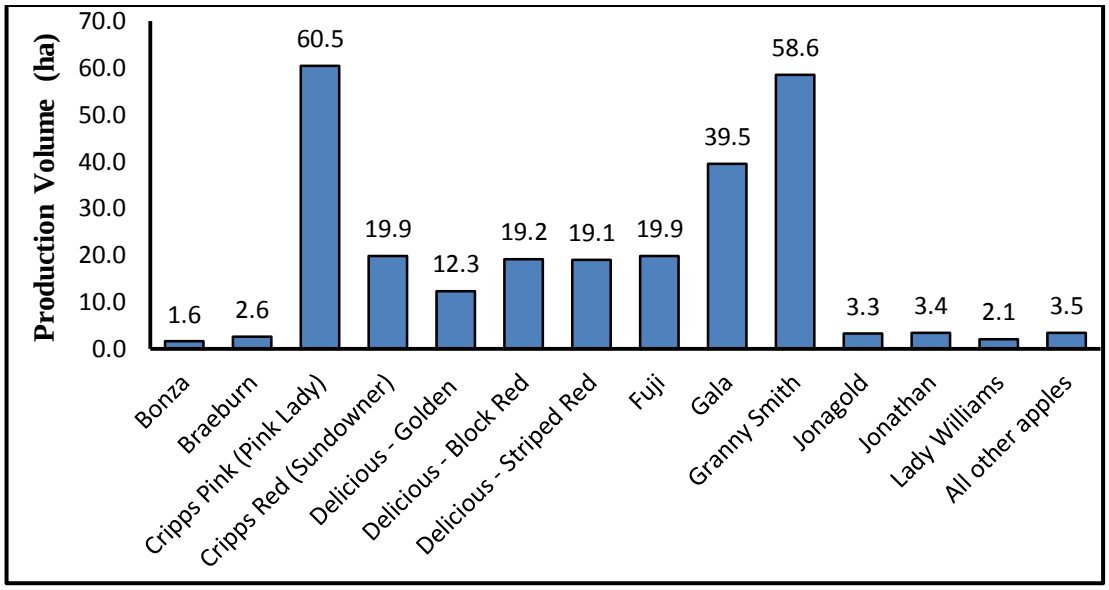

Figure 2.5. Production of different apple cultivars in Australia (Australian Bureau of Statistics, 2014)

2.1.3. Apple production in Western Australia

The Western Australian apple industry is concentrated around Donnybrook, Manjimup, Dwellingup and the Perth Hills. 'Cripps Pink™' is the main cultivar grown, thus contributing 34% of the state's apple production, followed by 'Gala' (17%) and 'Granny Smith' (17%). (Australian Bureau of Statistics, 2014). Organic apple is grown in all states of Australia with over 60 certified growers (McCoy, 2007). In Western Australia, organic apple production is in an infant stage with annual production of 300 tonnes (McCoy, 2007).

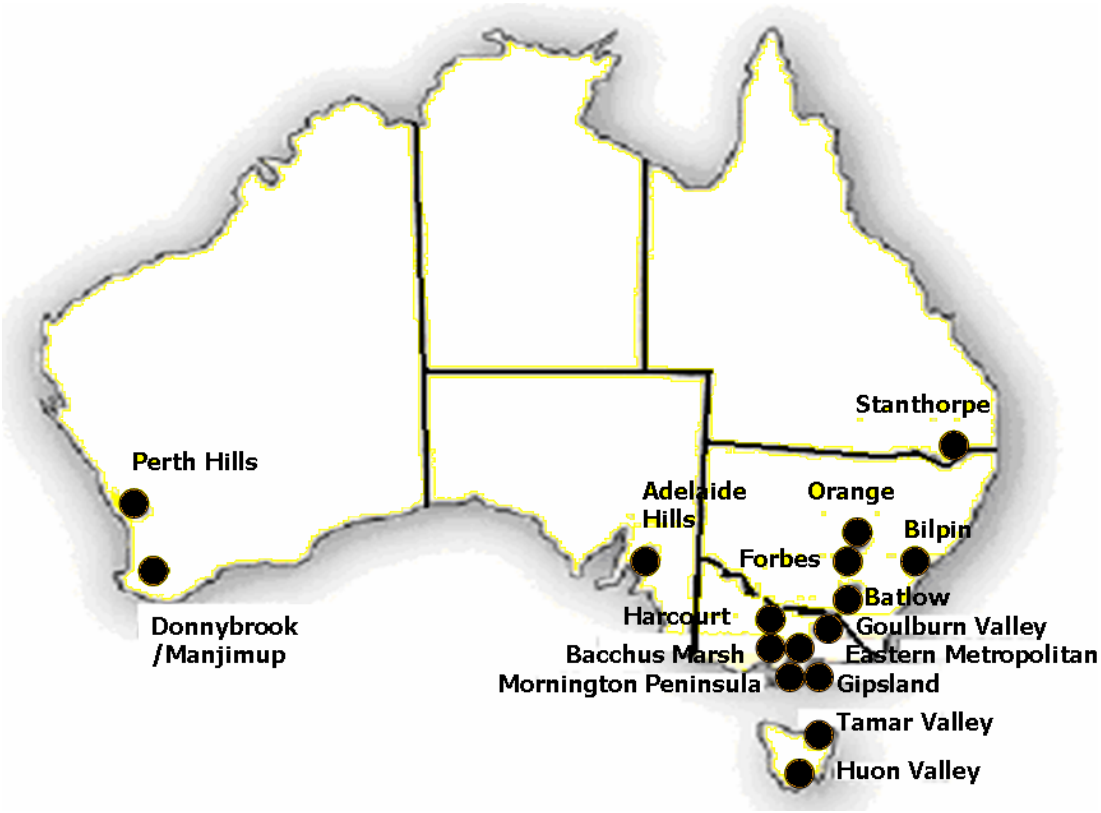

Figure 2.6. Map of major apple growing areas in Australia (Plant Health Australia, 2014).

2.2. Fruit Quality

2.2.1. Fruit size and weight

Fruit size is the result of the combination of number of cells and cell size (Smith, 1950; Bain and Robertson, 1951; Martin et al., 1964; Sugiura et al., 1995). Reducing fruit numbers at, or soon after, flowering has an effect on reducing competition for photosynthates among developing fruit, which may allow remaining individual fruit to develop greater cell numbers. Smith (1950); Martin et al. (1964); Sugiura et al. (1995) and Webster (1997) have reported that size differences in fruit are primarily due to differences in the number and individual size of cells within the fruit cortex and pith. Marguery and Sangwan (1993) found that cell division began a few days later on the younger wood because of the later blooming time and the mitotic period stopped simultaneously on both ages of wood (40-50 days after full bloom (DAFB) on the 2nd year wood). They concluded that fruits from one-year-old wood were smaller than other fruits because they had fewer cells, probably due to later flower opening and pollination. Many tree physiological factors influence fruit cell number and size. Crop load, time and severity of thinning, tree/soil water relationships, tree vigour, tree nutritional status and stress all impact on number of cells within the fruit and individual cell size, and thus affect final fruit size (Westwood et al., 1967; Forshey and Elfving, 1977; Faust, 1989; Boucher, 1995; Tromp, 1997; Dris et al., 1999; Warrington et al., 1999;). Fruit size and fruit weight are closely correlated, both being inversely related to fruit retention (Stanley et al., 2000)

2.2.2. Weight loss

Fresh fruits commonly release water vapour into the surrounding atmosphere by transpiration. Transpiration by fruit involves the diffusion of water vapour from the fruit into the surrounding environment. Fruits also exchange CO_2 for oxygen as a result of respiration. The weight loss of fruit is principally due to the loss of water in transpiration and to a lesser extent to the loss of carbon in the respiration process. Total weight loss from fruit is most often expressed as a percentage of the original weight. Excessive weight loss in fruit can result in a shrivelled appearance caused by decreased turgidity and can render it unsaleable. Only a 5% loss of apple weight may cause a shrivelled appearance (Hatfield and Knee, 1988). Wilcke (1992) described

weight loss being cultivar dependent with 'Golden Delicious', one of the more susceptible varieties, losing 5% on a fresh weight basis after 100 days of cold storage and attributed this high loss in cold storage to ventilation. In controlled atmosphere (CA) storage, the loss of weight was lower, about 2% in 100 days. Carnauba or shellac based wax coatings, commonly applied to apples to improve appearance, have also been linked to extended shelf-life by reducing water loss, respiration rates and retardation of fruit ripening (Saftner, 1999; Bai et al., 2002).

2.2.3. Fruit firmness

Fruit firmness is one of the most important characteristics of apple quality (DeEll et al., 2001). When pome fruits such as apples ripen, the cementing material calcium pectate between the cells dissolves (Kingston, 1992). Decreasing firmness as maturity approaches is well-documented in apples (Harker et al., 1997). However, firmness is being used as an internal quality criterion by fruit storage facilities and wholesalers rather than as a maturity index. Jones et al. (1998) have suggested that firmness is related to both the size and number of cells within the fruit. Large cell size generally means softer fruit. Firmness of fruit can be maintained by increasing cell numbers while keeping cell size to a minimum (Martin et al., 1964). Some other factors such as seasonal and orchard variability, tree vigour, fruit size, nitrogen and calcium levels in the fruit and use of growth regulators are also known to influence firmness of apple fruit (Little, 1999). Robinson et al. (1983) suggested that this increase in fruit firmness with increasing spur age was a result of the decrease in fruit size associated with increased spur age or delayed maturity.

2.2.4. Soluble solids concentration (SSC)

As apples mature, starch is converted to sugars. This increase in sugars renders the fruit much sweeter and therefore more acceptable to consumers (Kingston, 1992). SSC tends to increase as apple fruit ripen (Blankenship and Unrath, 1988). As with firmness, SSC is increasingly being used as a quality criterion by wholesalers. Sugar level depends on the leaf to fruit ratio, hence anything that increases leaf size and optimises photosynthesis throughout the canopy will aid in accumulation of sugars in the fruit (Kupferman, 2003). Collins (2003) noted that total sugars content can be

influenced by a range of factors such as irrigation, nutrition, weather and position of the fruit on the tree.

2.2.5. Titratable acidity (TA)

Malic acid is the predominant acid contributing to the TA in apple fruit. During the greater metabolic activity that occurs during ripening, organic acids decline since they are respired. Acids are generally considered a reserve source of energy to the fruit (Wills et al., 1989). Consumer acceptability of apples in European countries is closely correlated to acid content (Blanpied and Blak, 1977).

2.2.6. Ascorbic acid

Apple fruit is known as one of the major sources of ascorbic acid (vitamin C) which is very important in human nutrition. However, the ascorbic acid concentration in the fruit juice was higher at the early fruit development stage and decreased at the ripening stage (Ladaniya, 2007). Ascorbic acid of fruit juices is readily oxidized and lost during staying of the juices, at rates depending on the conditions of fruit storage. It is evident therefore that the quality of any fruit juice and its value as a source of vitamin C depends on its content and its rate of loss upon staying. Majority of reports are on determining the ascorbic acid content in fruit juices (Finley & Duang, 1981; Karayannis & Farasoglou, 1987; Ozgur & Sungur, 1995) but only a few are aimed at determining the amounts of ascorbic acid lost from different fruit juices under different storage conditions (Haddad, 1977; Jova & Yankov, 1986).

2.2.7. Apple fruit colour

Apple fruit colour is due to the blending of various amounts of different pigments including plastid-based pigments such as chlorophylls and carotenoids and also vacuole-based pigments such as anthocyanins and flavonols (Lancaster et al., 1997; Lancaster, 1992). Chlorophylls are responsible for the green background colour and are located in the chloroplast, while carotenoids responsible for the yellow background colour are located in the chromoplast (Lancaster, 1992). Meanwhile, the vacuole-based pigments, anthocyanins, are responsible for the red colour and production is controlled by genetics, environment and cultural practices (Jackson, 2003; Lancaster, 1992; Whale et al., 2008). These pigments change

continuously during the maturation and ripening of apple fruit (Knee, 1972; Whale and Singh, 2007).

2.2.8. Leaf damage

Healthy early season canopy of spur leaves and later addition of bourse leaves is essential for fruit set, fruit growth and quality (Proctor and Palmer, 1991). Various reports claim that desiccating chemicals are becoming increasingly popular as chemical thinning agents, but their use is coupled with foliar damage (Irving et al., 1989; Southwick et al., 1996; Bound and Jones, 1997). Although Bound and Jones (1997, 2004) reported blossom thinning by desiccating chemicals affected some fruit quality attributes, the impact of leaf damage caused by these desiccants on fruit quality is not known. Following removal of whole leaves from spurs or bourse shoots, Proctor and Palmer (1991) found no effect of early season defoliation on mean fruit weight. Several studies relating to foliar feeding by pests have shown that leaf damage impacts on both size and quality of the fruit. Lakso et al. (1996) reported reduced fruit growth rates in Starkrimson 'Delicious' trees following European red mite injury of leaves. Marini et al. (1994) and Francesconi et al. (1996) reported greater decreases in fruit size, colour and SSC in damaged trees with heavy crops than in lightly cropped trees.

2.3. Effects of blossom thinning on fruit quality

Thinning of apples is an important cultural practice in modern orchard management of intensive apple growing (Deckers et al., 2010). Fallahi, and Willemsen (2002) have reported that the blossom thinners are caustic and reduce fruit set by damaging different flower parts, including anthers, stigma, style, and pollen tubes, and thus prevent fertilization. The caustic blossom thinners cause damage to blossoms and/or pollen which prevents fertilization of some flowers (Southwick, 1996). Byers and Lyons (1984) have reported that the caustic thinners can be applied from pink to full bloom; the greatest response has been when applications are made near bloom. However, Byers et al., (2003) have suggested that the best time for thinning may be approximately two weeks after bloom. Forshey and Elfving (1977) and Myers (1990) reported that thinning increases leaf:fruit ratio in apple trees. The trees with low yield had a higher leaf:fruit ratio which led to a higher

accumulation of photosynthates in the fruit, thus increasing the fruit weight (Fallahi and Simons, 1996). Thinning of flowers or fruitlets improves fruit quality and returns bloom and has become standard practice in the growing of many fruit crops (Wertheim 1998). Byers et al. (2003) noted that the bloom thinning increased crop value one to three times because of increased fruit size, yield and price. Moreover, the bloom thinning can maximize the tree's capacity to allocate sufficient photosynthates to fruit when the leaf:fruit ratio is low early in the growing season (Southwick, 1996). As well, blossom thinning influences the partitioning of carbohydrates, and affects the induction and differentiation of the floral buds (González-Rossia et al., 2006). A reduction in the number of fruitlets or flowers on the tree by blossom thinning will result in an increase in the fruit size (Wertheim, 1997, Dennis, 2000) and quality of the remaining fruit (Wertheim, 1997, Costa and Vizzotto, 2000). Blossom thinning is desirable to achieve maximum fruit size (Jones et al. 1992b; McArtney et al. 1996) and the only way this can be achieved economically is by using chemicals. Johnson (1992) also mentioned that thinning has increased susceptibility to physiological storage disorders. However, Hansen (1997) has reported that blossom thinning may increase fruit weight and size which may be detrimental to other aspects of fruit quality. Early thinning of apples is important because of its impact on fruit size and next season flower bud initiation (Fallahi and Willemsen, 2002). Frank (1998) noted that thinning whether mechanical or chemical prevents the development of some fruits, allowing the remainder to become larger and more marketable.

2.3.1. Effect of chemical thinning on fruit quality

Most studies relating crop load to fruit quality involve the use of thinning chemicals. Over 50 years of research has shown that chemical thinning regulates crop load in apple and other fruit trees (Link, 1967; Wertheim, 1974; Flore, 1978; Williams and Edgerton, 1981; Jones et al., 1988; Bound et al., 1993a; 1993b; Greene, 1993b; Byers, 1997). Chemical thinners include caustic materials, growth regulators and photosynthetic inhibitors (Southwick et al., 2008; González-Rossia et al., 2007). Chemical thinning can be used at bloom or shortly thereafter to reduce current season crop load (Wertheim, 1997) or during the flower induction phase to reduce flower density and therefore crop load in the following season (Southwick et al., 1995).

Thinning chemicals in pome fruit have become limited in number (Damerow and Blanke 2009). This is due to various reasons, e.g. carbaryl is harmful to a wide spectrum of insects and water organisms (Wertheim, 1997), naphthyl acetic acid (NAA) is being phased out in many European countries (Damerow and Blanke, 2009) and the renewal costs of registration is high and exceeds the return (Wertheim, 1997). In addition, the efficacy of the few available chemicals is temperature dependent (Damerow and Blanke, 2009). Other disadvantages include that the chemical thinners give inconsistent results (Baugher et al. 2009) due to variable responses to weather conditions, flower dynamics and tree age (Hehnena et al., 2011). However, chemical thinning of the flowers or the fruits on apple trees is a standard measure in modern apple growing with the aim to improve crop regularity, improve fruit size and to break the natural biannual bearing tendency (Deckers et al., 2010). There are many factors which have to be taken into account for consistent thinning with chemicals such as site, cultivar, spray volume, timing of application and temperature (Marini, 2004). Effective chemical fruit thinning in apples would reduce production costs and increase fruit size (Osborne et al., 2006). Byers et al. (2003) reported that chemical blossom thinning can result in a 7 to 30% increase in peach fruit size and yield when compared to hand thinning fruit 40-50 days after full bloom. The use of naphthalene acetic acid (NAA), a synthetic auxin, as a blossom thinner, gained acceptance in the 1950's and 1960's. Another synthetic auxin, napthaleneacetamide (NAD), was found to be suitable for post-bloom thinning of many commercial cultivars of apple (Westwood and Batjer, 1960). In the past, apple trees were often treated with Elgethol during full bloom, followed by a post-bloom application of a fruit thinner such as 1-naphthyl N-methylcarbamate (carbaryl) with or without (NAA) (Williams and Edgerton, 1981). Webster and Holland (1993) found that sprays of highly phytotoxic chemicals at blossom time achieve this effect by killing the flowers, but may also damage other parts of the tree. Spraying of high volume ammonium thiosulphate (ATS) at blossom time has thinned peaches (Byers et al. 1986; Baroni et al., 1998) and has been used in plums (Webster and Holland, 1993). Fallahi et al. (1990) applied hydrogen cyanamide (Dormex, 50% a.i.) on 'Florida Prince' peach at "pink bloom" and observed reduced numbers of open blossoms, thus reduced fruit set. Dormex was also found to be an effective blossom thinner for plums (Fallahi et al., 1992), apples (Fallahi, 1997; Fallahi et al., 1997),

and peaches (Fallahi, 1997). Williams (1994) has tested pelargonic acid (Thinex, 60% a.i.) at 0.25% formulation (v/v) at full bloom and found reduced fruit set in 'Delicious' apple. Yuan and Greene (2000) found that a spray application of 6-benzylaminopurin (100 mg L^{-1}) effectively thinned apple fruit and increased size. Similarly, a spray application of carbaryl (1000 mg L^{-1}), ethephon (474 mg L^{-1}) and naphthalene acetic acid (58 mg L^{-1}) on 'Golden Delicious' apples reduced auxin transport to fruitlets - leading to thinning (Ebert and Bangerth, 1982). The chemical thinners available in Australia include lime sulphur, ammoniumthiosulfate (ATS), ethephon (Bayer), Flordimex (Nufarm) and 6-benzyladenine (6-BA, MaxCel (Valent), Globaryl (Globachem) or Exilis (Fine)) (Damegrow and Blanke, 2009). Bound and Jones (2004) assessed the effect of ATS on apple fruit skin colour and reported that higher concentration of ATS causes high levels of foliar damage and bud death.

Most trials confirmed that the post-bloom sprays of BA increased fruit size, weight (Bound et al., 1991, 1993; Elfving, 1989; Elfving and Cline, 1993a,b; Greene et al., 1992; McLaughlin and Greene, 1984) and flesh firmness (Greene and Autio, 1989; Greene et al., 1990). Greene et al. (1992) reported that spray of BA on apple trees increased fruit weight independent of its effects on reducing crop load, when applied directly to the fruit. Cell division may be stimulated in the fruit when BA is applied directly (Denne, 1960; Schechter et al., 1993a; MacArthur and Wetmore, 1941) and as a result, fruit firmness, weight and size of fruit may increase due to an increase in cell number (Greene et al. 1990).

SSC and the length to diameter ratio of apples also increase with BA treatments (Elfving, 1989; Elfving and Cline, 1993a, b; Greene and Autio, 1989 and Greene et al., 1990). The increased SSC may be due to the change in the leaf to fruit ratio as more leaves support each individual fruit (Westwood, 1993).

2.3.2. Effects of time of thinning on fruit quality

Timing of chemical blossom thinning is a very important factor influencing its effectiveness in thinning in both apple and peach. Time of thinning has been used as a guide to fruit quality improvement for more than 50 years (Tukey, 1965; Batjer etal., 1968; Donoho, 1968; Gerin et al., 1972; Leuty, 1973). Early flower/fruit

removal usually results in the largest fruit at harvest and the greatest return bloom in the subsequent year (Fallahi and Greene, 2010; Batjer and Hoffman, 1951; Way, 1967; Preston and Quinlan, 1968; Knight, 1978; Knight and Spencer, 1987). Auchter and Roberts (1934) reported the best time to reduce the fruit set with chemicals is during apple bloom. Similarly, Jones et al (1990) reported that fruit weight of Red Fuji is dependent on the timing and concentration of chemicals applied for thinning. The best time to spray for blossom thinning is when the king bloom is open and fertilized, and only one side bloom is open but not fertilized (Fallahi and Willemsen, 2002). Donoho (1964) and Tukey (1962) have suggested to apply chemical thinning agents when king fruit diameter is between 8 and 12 mm. The application of Tergitol TMN-6 (0.75% to 1.25%) at 75 to 80% bloom stage was effective in blossom thinning of 'Rome Beauty' apples (Fallahi and Greene, 2010). The caustic chemical blossom thinner is gaining in popularity, but timing of application is critical and can be difficult in practice, in addition to uncertainty regarding the efficacy of thinning (Bound and Jones, 2004; Whiting et al., 2006). Bound et al. (2013) have reported that chemical blossom thinning is becoming more extensive, but many Australian growers are aware of thinning during the bloom period because of the potential risk of over-thinning.

2.3.3. Use of lime sulphur (LS)

Fruit thinning with LS at full bloom has been recommended by Kvale (1978, 1985) which has a caustic effect on the flowers (Kvale and Ystaas, 1969). There is conflicting evidence on the impact of LS on fruit set and yield. Many trial works on LS have given inconsistent results from year to year and it has been suggested that this may be attributable to the different sensitivity of flowers at different stages of their development (Byers and Lyons, 1986). Jones et al. (1990) found sprays with LS at full bloom on 'Red Delicious' apple trees produced higher fruit weight (150g) compared to control. Alegre and Alins (2007) and Weibel et al. (2004) did not achieve flower thinning effect by spraying LS (2%), while Warlop and Libourel (2002) noted thinning effect of LS. Guak et al. (2004) found that spray application of LS (up to 4%) at 85% full bloom stage reduced fruit set in 'Golden Delicious' apples. Stopar (2004) reported a spray application of 3% lime sulphur (CaSx) to

'Golden Delicious' apple trees resulted in severe flower thinning, consequently reducing crop load and increasing fruit weight. However, Osborne et al. (2006) did not find significant reduction of fruit set while they examined LS (1.0% or 3.0%) plus Crocker's fish oil, but the high rate of LS alone reduced crop load and yield.

2.4. Use of thinning agents in organic apple production

The regulation of crop load to improve fruit quality is one of the major challenges in organic apple production. As a prelude, manual thinning is tedious, time consuming and very expensive. Presently, there are only a few methods and agents allowed for certified organic horticulture. However, synthetic chemical thinning agents or plant hormones for crop regulation are not permitted to be used in certified organic apple production. Most experiments to regulate fruit set in organic horticulture are based on two strategies including injuring flowers (Ju et al., 2001; Pfeiffer and Ruess, 2002) and mechanical reduction in the number of flower buds (Roche and Masseron, 2002). Efficacy of several products such as mineral oil, corn oil, rape oil, olive oil, vinegar, sodium bicarbonate, sodium salt and lime sulphur has been tested to damage flowers (Alegre and Alins, 2005). Nevertheless, none of these products have been tested under Australian climatic conditions.

Lime sulphur (LS) has long been used by organic apple growers applied during bloom to reduce the number of viable flowers (Edwards, 1998). Lime sulphur and different types of oils have been used by organic growers in some countries which have achieved partly satisfying thinning. Stopar (2004) investigated some blossom thinners for possible use in organic apple production on ten-year-old 'Golden Delicious' trees. Some natural oils such as sunflower, soybean, corn, and fish oil, as well as sulphur lime have been tested for bloom thinning in organic apple which burn weaker flowers (Melland, 1998; Pfeiffer and Ruess, 2002; Stopar, 2004; Warlop et al., 2003; Fallahi and Fallahi, 2006). Sometimes, thinning of flowers/fruitlets is caused by substances such as table salt, soap, molasses, dextrin and others (Stopar, 2004; Embree and Foster, 1999; Weibel and Walther, 2003), and substances that cover the stigma, thus preventing or hampering pollen germination and pollen-tube growth, such as Safer-Soap (potassium salts of fatty acids), PEG-1000($HO(-CH_2CH_2O-)NH$), anti-stress (acrylic polymers), nutri-safe (N,O-carboxy-methylchitosan), biofilm (alkylaryl-polyethoxy ethanol + fatty acids + phosphatic

acids + isopropanol) (Bertshinger et al., 1998; Embree and Foster, 1999). Fallahi (2006) used lime sulphur in combination with fish oil and found this to be effective organic blossom thinners for apples and peaches. The effectiveness of organic bloom thinners under Australian conditions on flower/fruit thinning along with their effects on fruit quality and cold storage life have not yet been investigated.

2.5. Calcium

'Ca' analysis as a prediction tool for bitter pit has various potential problems. Differences in results for the same fruit samples analysed in different laboratories have been reported (Holland et al., 1975; Marcelle, 1990a). The sample size and position of fruit within a tree canopy further complicates the accuracy of the results of such samples (Ferguson et al., 1979). In addition, the time of sampling, tissues being sampled and interpretation of these results as well as extrapolation of the results to orchard level can potentially reduce the accuracy of this method. Success in accurate prediction of the number of pitted fruit varied. In spite of all of the above, this is still the most widely accepted method for bitter pit prediction. 'Ca' content of apple peel samples has been correlated at harvest leaf 'Ca' by Drake et al. (1974) with varying results. Results were promising when compared to correlations between leaf and whole fruit 'Ca', but too much variation was caused by crop load. Shear (1972) reported a regression coefficient of 0.49 for a prediction of cork spot in 'York Imperial', using only the 'Ca' concentrations of fruit cortex at harvest in a 2^{nd} degree polynomial regression. The prediction with 'Ca' concentrations of leaves, collected two weeks prior to harvest, had a regression coefficient of 0.52, also with a 2^{nd} degree polynomial regression. Wills et al. (1976) compared results regarding 'Ca' concentration thresholds for bitter pit predictions from New Zealand to those from the rest of the world and emphasised that this is only possible in uniform orchards. Alternatively, Zavalloni et al. (2001) reported a continuous linear increase in 'Ca' from about 40 days after full bloom until harvest for different cultivars such as Braeburn, Fuji and Golden Delicious in Italy which confirmed earlier findings by Rogers and Batjer (1954), Wilkinson (1968), Tromp (1979), Jones et al. (1983) and Tomala et al. (1989) who reported fruit Ca uptake until harvest.

2.6. Bitter pit and nutrition

Bitter pit is the main nutrient related disorder in apple and remains one of the main problems in the apple industry around the world. Several studies have noted the relationship between the application of fertilizers and bitter pit within a growing season and observed that the incidence of bitter pit in apple is commonly associated with a low calcium concentration (Witney et al. 1991; Bangerth, 1979; Moor et al., 2005; Ferguson et al., 1979; Perring and Pearson, 1987; and Ferguson and Watkins, 1989). Later on, Ferguson and Watkins (1983) also reported that apple tissues are sensitive to bitter pit with low calcium concentrations. Reduced levels of calcium in fruit flesh are correlated with a higher risk of bitter pit development (Ferguson and Watkins, 1983). Neilsen and Neilsen (2002) found 4-5 calcium (0.7%) sprays commencing at post-bloom stages significantly increased calcium concentration in apple fruit and reduced bitter pit. Nitrogen content increases the apple fruit's susceptibility to bitter pit (Waller, 1980). Sharples (1980) also noted that the fruit from tree cultivation which had a lower nitrogen content ripened more slowly and were less susceptible to rotting and breakdown. Ca gave the best positive correlation with bitter pit from all minerals tested, but the K/Ca ratio was more highly positively correlated with bitter pit. Cooper and Bangerth (1976) also suggested a prediction for bitter pit in apples with the Mg/Ca ratio. As this ratio decreased, fruit behaved as Ca deficient fruit, irrespective of the cause, being a lack of Ca or increase in Mg. According to Autio et al (1986), the Ca and K concentration of fruit accounted for 49% of the variance in bitter pit. Ferguson et al. (1979) also found an acceptable association between the predicted and actual bitter pit when fruit were classified into five Ca categories. The bitter pit developed predominantly where fruitlet Ca content was low, which was also confirmed in fruit with low Ca content at harvest (Turner and Hill, 1998). Ferguson and Watkins (1992) also noted that crop load affects the mineral concentration and thus bitter pit. A variation in crop load significantly altered K, Ca, Ca/Mg and Ca/K of 'Cox's Orange Pippin' fruit. Although adding crop load as a variable on its own was not significant, the Ca x crop load interaction was significant. The study also indicated that light cropping trees produced fruit with less Ca, higher K and a higher bitter pit incidence, regardless of fruit size. Relatively high fruit Ca content at harvest can reduce the incidence of bitter pit considerably. Foliar Ca applications at pre-harvest stage increase the Ca content of fruit and reduce

bitter pit (Beyers, 1963; Terblanche et al., 1970; Ferguson et al., 1987, Hewett and Watkins, 1991 and Yuri et al., 2002). Foliar Ca applications highly successful cases, to a substantial number with effect on Ca concentration of fruit or bitter pit control (Carbo et al., 1988; Hewitt & Watkins, 1991; Le Grange et al., 1998). Rapid uptake and penetration of Ca into fruit occurs mainly during the first four weeks after full bloom or between six and 14 weeks after full bloom, followed by a decline until harvest (Quinlan, 1969; Ferguson et al., 1987; Faust, 1989; Cline et al., 1991; Casero et al., 2002; Schlegel and Schönherr, 2002). In Chile, Venegas (1994) evaluated time and number of Ca applications on 'Granny Smith' and reported that more applications result in less bitter pit and no significant differences were found when the same number of sprays were applied at different times, starting from 42, 83 and 130 weeks after full bloom. Yuri et al. (2002) also reported that applications of Ca after full bloom have a greater influence on increasing Ca levels of the fruit than the late applications. In British Columbia, Neilsen and Neilsen (2002) have recommended four to five foliar applications ($CaCl_2$) at 10 day intervals from approximately 40 days after full bloom to control bitter pit in 'Fuji', 'Jonagold' and 'Gala' apple. Late applications in July were also more effective than those in August for elimination of bitter pit in 'Braeburn', a cultivar that is susceptible to bitter pit, before harvest (Neilsen and Neilsen, 2002). In Spain, Carbo et al. (1998) found that increasing the frequency of the applications from every 20 to every 10 days was more successful in increasing fruit Ca content than by advancing the date of the first foliar application by 10 days from 40 to 30 days after full bloom. In South Africa most studies favour late season (from 70 days after full bloom) Ca applications and for 'Golden Delicious', 20 days after full bloom (Beyers, 1963) was found to be too early and February (harvest) too late for efficient control of bitter pit in the Elgin area. Only three to five foliar Ca applications at fortnightly intervals from 70 days after full bloom for 'Golden Delicious' can ensure effective control of bitter pit when it's incidence is less than 16 percent in the orchard (Terblanche et al., 1970, 1974, 1975). When higher bitter pit percentages occurred, the efficiency of control with foliar applications decreased (Wooldridge and Joubert 1997).

2.7. Apple scald and nutrition

Nutrient partitioning during different stages of apple fruit growth and development may play an important role in scald development within the storage environment. Wills and Scott (1981) have reported that the concentration of nitrogen, potassium, magnesium and calcium in apples at pre- and post-harvest can affect fruit size and storage life. Calcium plays a very important role in plant growth, development, resisting softening, fungal invasion, development of disorders and delays the senescence of the tissues (Sharples, 1980; Raese and Drake, 2000). Apples with low calcium levels often develop more scald than with higher levels (Emongor et al., 1994; William, 1993). Spray applications of calcium (4 to 6) onto apple trees are suggested for significant reduction in the incidence of internal breakdown and superficial scald (Loughlin and Jotic, 1978; Neilsen and Neilsen, 2002). Calcium sprays on the apple have improved the fruit's finish, reduced cork spot, reduce rot, shown less superficial scald, greener fruit skin colour, whiter flesh and more juiciness (Raese and Drake, 2000). Phosphorus content in apple fruit also influences scald susceptibility and phosphorus fertilization is reported to decrease α-farnesene content in apple fruit tissue (Emongor et al., 1994; Yogaratman and Sharples, 1982). Wills and Scott (1981) noted opposite effects of calcium and potassium on this disorder.

2.8. Effects of Ca on fruit quality

Fruit quality has been affected and most often correlated with pre-harvest fruit mineral nutrients especially Ca (Fallahi et al., 2010). Ca acts as an intracellular secondary messenger (Poovaiah and Reddy, 1993). Enhancing the calcium content of apple fruits can be very beneficial in maintaining fruit quality during storage by reducing softening (Mason, et al., 1975), internal breakdown and respiration (Bangerth et al., 1972), bitter pit (Reid and Padfield, 1975), ethylene production (Sams and Conway, 1984), and decay (Conway and Sams, 1983). Ca spray application improved fruit quality such fruit finish, juiciness, red skin colour, texture and fruit firmness as well as reduced incidence of scald and bitter pit in apple fruit (Thomas and Stephen, 2000). When fruit size is large and trees are excessively vigorous, Ca concentrations in the apple fruits are diluted and N:Ca ratios become high. This may contribute to high incidence of fruit disorders and poor fruit quality

(Fallahi et al., 1985a,b; Fallahi et al., 1997a; Raese and Drake, 1993). Positive correlation with concentrations of Ca in fruit and firmness and incidence of injury in apple fruit were observed by Beavers et al. (1994) and Raese et al. (1999).

2.9. Effects of long term storage on fruit quality

The responses of fruit to imposed storage conditions, principally low temperature, low O_2 and high CO_2, may well depend on pre-harvest growing conditions (Ferguson et al., 1999a). Long term controlled atmosphere (CA) storage of apple fruit has been reviewed by Bai et al., (2009). But this is not a focus of current research. In terms of specific disorders, there are a range of low temperature disorders which can be connected back to the condition of the fruit at harvest. The pre-harvest factors which modify fruit responses to storage conditions are important in optimizing product storage quality (Ferguson et al. 1999a). Apple is a climacteric fruit in which the post-harvest processes such as fruit softening, loss of acidity and conversion of starch into sugars continues during maturation and ripening. Many of these changes will reduce the consumer acceptability and consequently reduce net income of apple industries (Little and Hollmes, 2000). Cold storage is the most common method used in extending storage and shelf life of various fruits, thus making fruits available throughout the year. Low temperature storage reduces respiration and water loss (Paull, 1999). However, some apple cultivars are susceptible to low temperature storage, which causes chilling injury (Bai et al., 2009). The recommended cold storage temperature can be as high as 4°C, but depends on cultivar and growing region (Bai et al., 2009; Kupferman, 2003). The 'Pink Lady' apple fruit requires cooling down slowly to 0.5° to 1°C over 5 to 7 days, while 'Braeburn' and 'Fuji' require cooling to 1°C for 2 to 3 weeks (Bai et al., 2009). Some apple fruit such as 'Pink Lady' is suitable to store at 0°C for 60 to 120 days and also for 7 to 14 days shelf life at 20°C (Gualanduzzi et al., 2005). Fruit quality of some cultivars decreased after 180 days in cold storage due to the loss of juiciness, crispness and also increased mealiness (Gualanduzzi et al., 2005). Treating the apple fruit with calcium propionate preserves the structural integrity of the fruit cells and increases their shelf life (Quiles et al., 2007). Hayat et al. (2005) have claimed that apples treated with calcium chloride (2%) have an extended shelf life and consumer acceptability after 60 days of cold storage. The fruit treated with calcium lactate were

more acceptable after 17 days of storage (Pereira et al., 2010). However, there is no information available on the effects of organic calcium on storage life and maintenance of quality in organically grown apple fruit.

Chapter 3

Materials and Methods

3.1. Plant materials

The experiments were conducted on four commercially important cultivars of apples 'Cripps Pink^{TM}', 'Jazz' and 'Granny Smith' grafted on rootstock M26 and 'Gala' on rootstock MM06 apple trees of 15 years of age at the Newton Brothers Orchards in Manjimup (latitude 34°14´South, longitude 116°8´East) Western Australia. Mean temperature and monthly mean rainfall of the experimental site during 2011 to 2014 are presented in figure 3.1 and 3.2. The trees were spaced 7.5 m between rows and 2.5 m within rows in the North-South orientation. All the experimental organically grown apple trees received similar cultural practices including nutrition, irrigation and plant protection except varying experimental treatments. The experimental site has a loam soil. The fruit were harvested at commercial maturity in the morning of the day of collection. Fruit were transported to the Horticulture Research Laboratory, Technology Park, Curtin University, WA, by using an air conditioned (15 ± 1 C^{o}) vehicle.

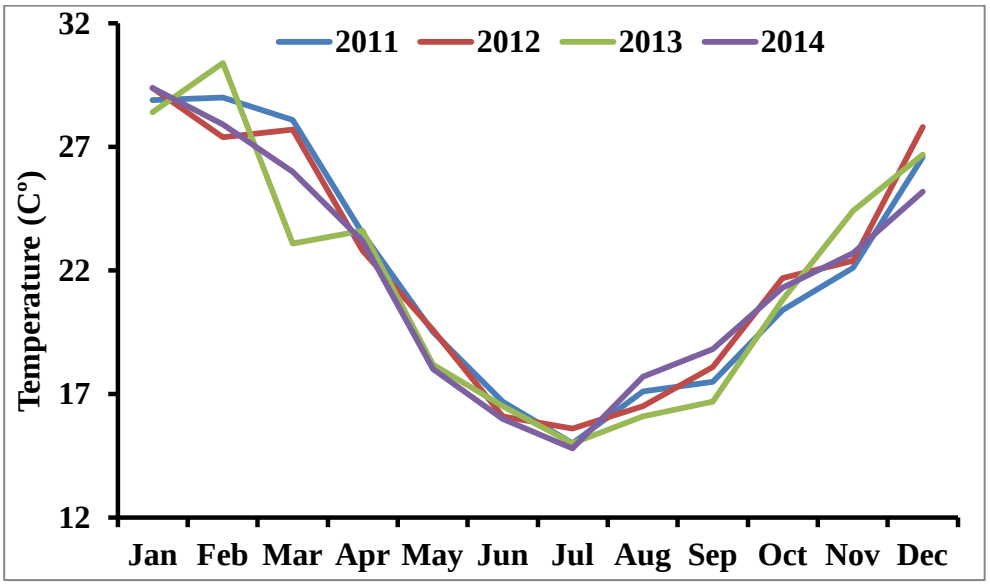

Figure 3.1. Mean temperature at Manjimup during three consecutive years (2011, 2012, 2013 and 2014) of investigations. Source: Bureau of Meteorology (2015) http://www.bom.gov.au/climate/data

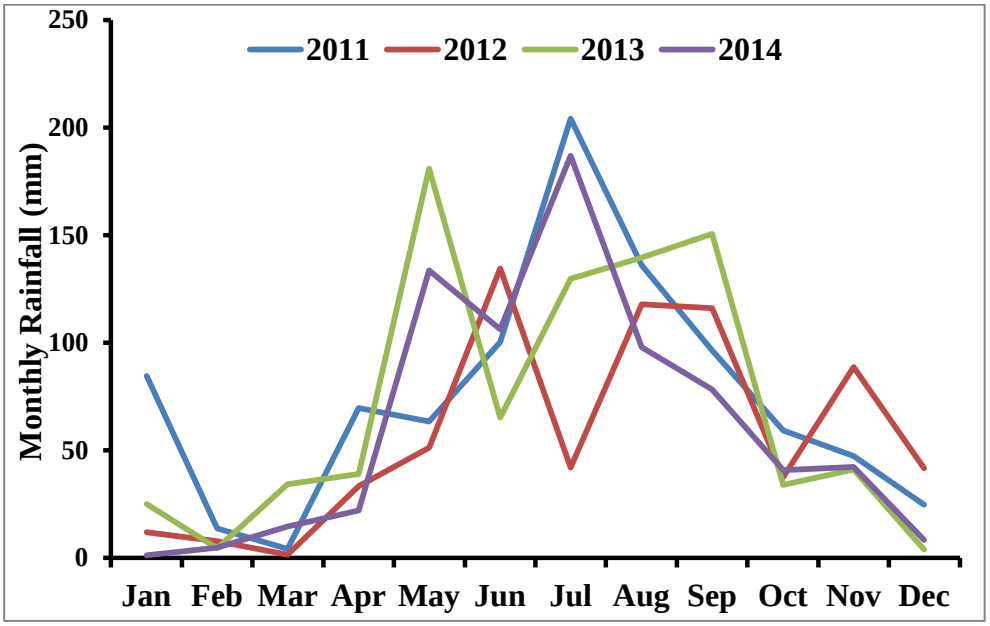

Figure 3.2. Monthly mean temperature at Manjimup during three consecutive years (2011, 2012, 2013 and 2014) of investigations. Source: Bureau of Meteorology (2015) http://www.bom.gov.au/climate/data

3.4. Organic Chemicals

3.4.1. Lime Sulphur (LS)

Lime sulphur (active constituent 200gL^{-1} sulphur (S) as polysulfide sulphur was sourced from Richgro, Jandakot, Western Australia.

3.4.2. Oils

- Canola oil was purchased from Cookers Bulk Oil System, Western Australia.
- Fish oil (Tuna oil) was purchased from Big John's Premium™ Western Australia.
- Organic olive oil was purchased from Cypressa, UK.

3.4.3. Calcium (Ca)

Organic calcium (Biomin® Calcium) manufactured by JH Biotech, Inc, Australia, was used in the experiments. Chemical constituents: true amino Chelates Calcium (Ca) 15% and amino acid Nitrogen (N) 3.6%.

3.4.4. Boron (B)

The organic boron (Biomin® Boron) used in the experiments was also manufactured by JH Biotech, Inc, Australia. Chemical constituents: true amino Chelates Boron (B) 15%.

3.4.5. Surfactant Synertrol oil, a surfactant was purchased from Organic Crop Production, Pty Ltd, Lilyfield, NSW, Australia.

3.5. Spray application

An aqueous emulsion containing different concentrations of lime sulphur alone and in combination with different types of oils, Biomin® calcium or Biomin® boron and synertrol oil as a surfactant were sprayed onto the whole trees using asprayer (The Selecta Trolleypak Mk II, Acacia Ridge, Australia).

3.6. Cold storage

Fruit were harvested from various experimental trees. Fruit for cold storage were packed in cardboard boxes in trays for individual apples. Fruit quality was assessed following varying cold storage (0.5 ± 0.5°C) periods plus 10 days of simulated shelf conditions (21 ± 1ºC). Ten fruit were included in each replication and replicated three/four times. Various fruit quality parameters including bitter pit, superficial scald, fruit weight loss, firmness, TA, SSC, SSC/TA ratio and ascorbic acid concentration were assessed following various cold storage periods plus 10 days of simulated shelf conditions (21 ± 1ºC). During the cold storage and simulated shelf conditions period the temperature was tracked at 15 minutes intervals using TinyTag data loggers (Gemini Data Logger Ltd, South Sussex, UK).

3.7. Nutrient analysis

3.7.1. Collection of samples

Fully developed six-month old spring flush leaves (25/tree) from non-fruiting shoots and five fruit per tree were randomly collected from different locations of canopy for nutrient analysis. The leaves and fruit from each tree were collected from unshaded positions at about 1.50 m height at the north, east, south and west points of the tree. All leaves and fruit collected were free of damage from insects or diseases.

3.7.2. Determination of mineral concentrations

Following washing, the leaves and pulp of fruit were dried in an oven at 60°C ± 2°C for 72 hours and milled and sieved through a 1 mm screen.

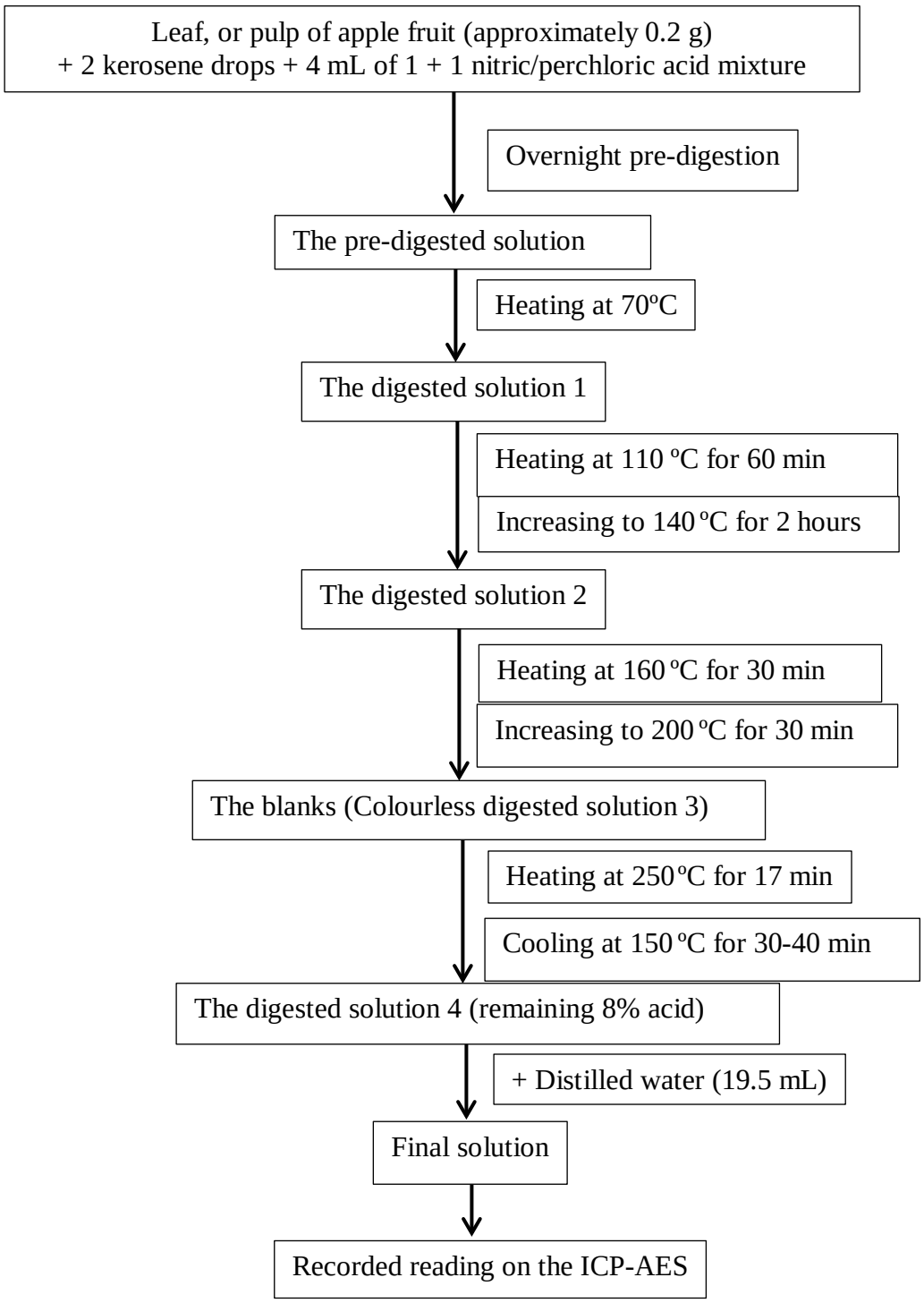

Figure 3.3. Flow chart of mineral analysis from leaf, pulp of apple (McQuaker et al., 1979; Pham, 2009).

3.8. Pre-harvest parameters

3.8.1. Fruit set (%)

Three branches per tree were tagged prior to the application of different spray treatments. Total numbers of flowers per branch were recorded. Numbers of fruit set on each branch was counted 26 days after application of blossom thinner treatments. Fruit set was calculated as percentage from each branch using the following formula-

$$\text{Fruit set (\%)} = \frac{\text{Total numbers of fruit set}}{(\text{Total numbers of flowers}) \text{ x } 100}$$

3.8.2. Fruit retention (%)

Three branches per tree were randomly tagged and total numbers of fruit on each branch were counted after fruit set (26 days after application of blossoms thinner treatments). At harvest, total numbers of fruit per tagged branch were counted again. Fruit retention was expressed as percentage by using following formula-

$$\text{Fruit retention (\%)} = \frac{\text{Numbers of fruit at harvest}}{(\text{Numbers of fruit set}) \text{ x } 100}$$

3.8.3. Leaf scorch (%)

Three branches per tree were randomly tagged and total numbers of leaves on each branch were counted before applying lime sulphur treatments. Numbers of scorched leaves from three branches was counted 30 days after the application of different spray treatments. Leaf scorch was expressed as percentage-

$$\text{Leaves scorched (\%)} = \frac{\text{Numbers of scorched leaves}}{(\text{Total numbers of leaves before spray}) \text{ x } 100}$$

3. 9. Post-harvest parameters

3. 9. 1. Bitter pit and superficial scald index

Bitter pit and superficial scald on apple fruit were recorded using a visual peel damage rating scale (0-10) at commercial harvest and after cold storage periods plus 10 days of simulated shelf conditions (21 ± 1ºC). The severity of incidence was recorded using a rating scale from 0 to 10. 0 = no visible injury, 1 = slight injury, 5 = moderate injury, 10 = severe injury. The index was calculated by using the following formula (Pesis et al., 2010).

$$\text{Scald or Bitter pit index} = \sum_0^{10} \frac{(\text{index level}) \times (\text{fruits at this level})}{\text{total no of fruits}}$$

3. 9. 2. Determination of fruit size (mm)

Fruit size was determined as a diameter of the fruit. Fruit size of ten randomly selected fruit per replication was measured using a digital Vernier calliper following harvest.

3. 9. 3. Determination of fruit weight (g)

Fruit weight was recorded from ten randomly selected fruit per replication by using a digital balance (A&D Limited, Tokyo, Japan). Average fruit weight was expressed as grams (g) per fruit.

3. 9. 4. Determination of loss of fruit weight (%)

In cold storage experiments, ten randomly selected fruit were weighed prior to commencement of cold storage (initial weight). The final weight of ten fruit was recorded following various cold storage periods plus 10 days of simulated shelf conditions (21 ± 1ºC). The loss of fruit weight was expressed as percentage of fresh weight against initial weight at harvest (Ahmad et al., 2013) by using the following formula-

$$\text{Weight loss (\%)} = \frac{(\text{Initial weight} - \text{Final weight}) \times 100}{\text{Initial weight}}$$

3. 9. 5. Determination of fruit firmness (N)

Fruit firmness was determined by using a texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Hampshire, UK), equipped with a horizontal square base table (15cm × 15cm) and interfaced to a personal computer with Nexygen® software following the methods explained earlier by Singh et al. (2009). Ten randomly selected fruit per replicate were used for this test after a small slice (< 2 mm thick) of fruit skin was removed and the firmness was recorded on the two opposite sides of the equatorial region of individual fruit by puncturing a 7/16 inch Magness Taylor probe, with a 500 N load cell on. The crosshead speed, depth, trigger and compression were maintained at 100 mm min^{-1}, 7.5 mm, 1 N and 75%, respectively, for firmness (Fig. 3. 4 and 3. 5). Fruit firmness was expressed as Newtons (N).

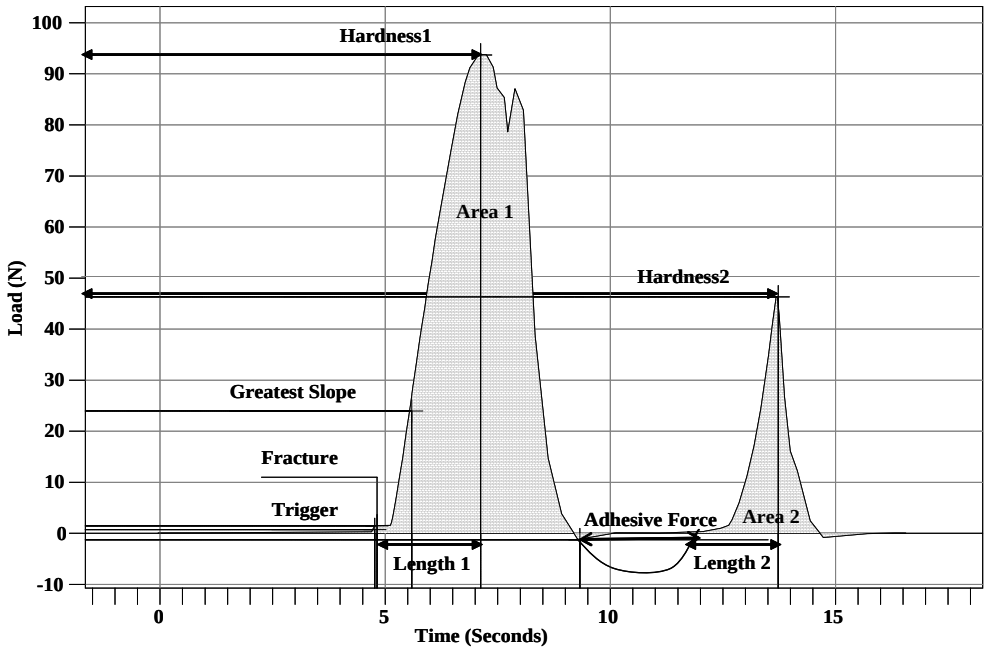

Fig. 3.4. A typical presentation of a profile of rheological properties of fruit sample using texture profile analyser (TPA) (Zaharah, 2011)

Fig. 3.5. Analysing rheological profile of apple fruit using a texture profile analyser.

3. 9. 6. Surface skin colour: HunterLab ColorFlex

The 'Gala' and 'Cripps Pink^{TM}' apple fruit colour was recorded using a ColorFlex $45^{o}/0^{o}$ Spectrophotometer (HunterLab ColorFlex, Hunter Associates Inc. Reston, Virginia, U.S.A.). Colour was measured at each of two equatorial opposite sides of every fruit. Fruit colour data were expressed in L*, a* and b* values (Figure 3. 6) (Hunter, 1975). L* represented the lightness coefficient which ranges from 0 (black) to 100 (white). a* ranges from -60 to +60, which indicates red (+60) and green (-60) colour. Meanwhile b* ranges from -60 to +60, which indicates as yellow (+60) and blue (-60) colours. a* and b* were further used to calculate hue angle ($h^{o} = \tan^{-1}$ b*/a*) for colour interpretation. Chroma (C*) corresponded to the intensity or colour saturation, in which low values represent dull colour while high values represent vivid colour. Chroma was calculated from $(a^{*2}+b^{*2})^{½}$. Hue angle (h^{o}) represented red-purple (0^{o}), yellow (90^{o}), bluish green (180^{o}) and blue (270^{o}) (McGuire, 1992). Fruit colour data was expressed in L* (lightness), a* and b*, while hue angle and chroma using formula explained (McGuire, 1992).

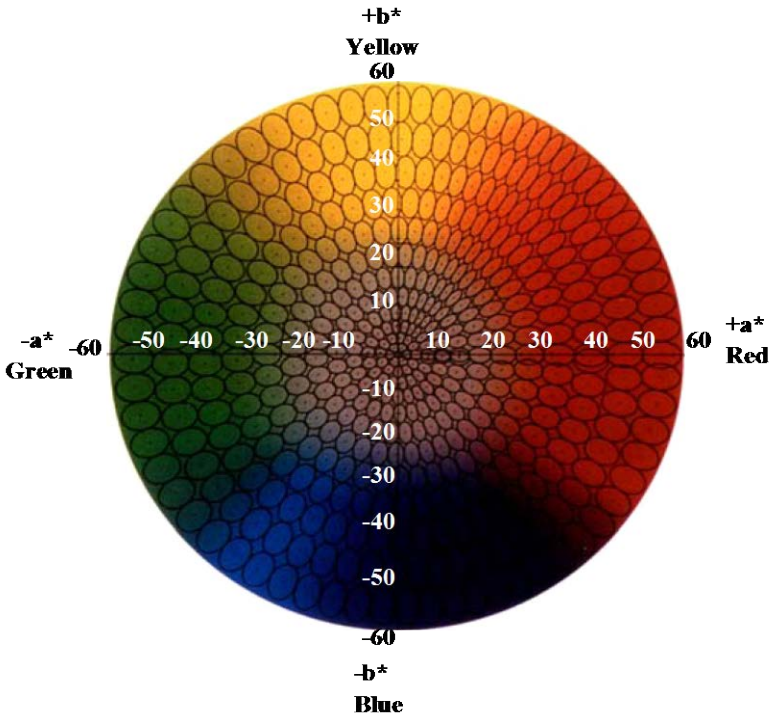

Fig. 3. 6. Colour chart – Commission International de L' Eclairage (CIE) L*, a* and b* (HunterLab, 1998).

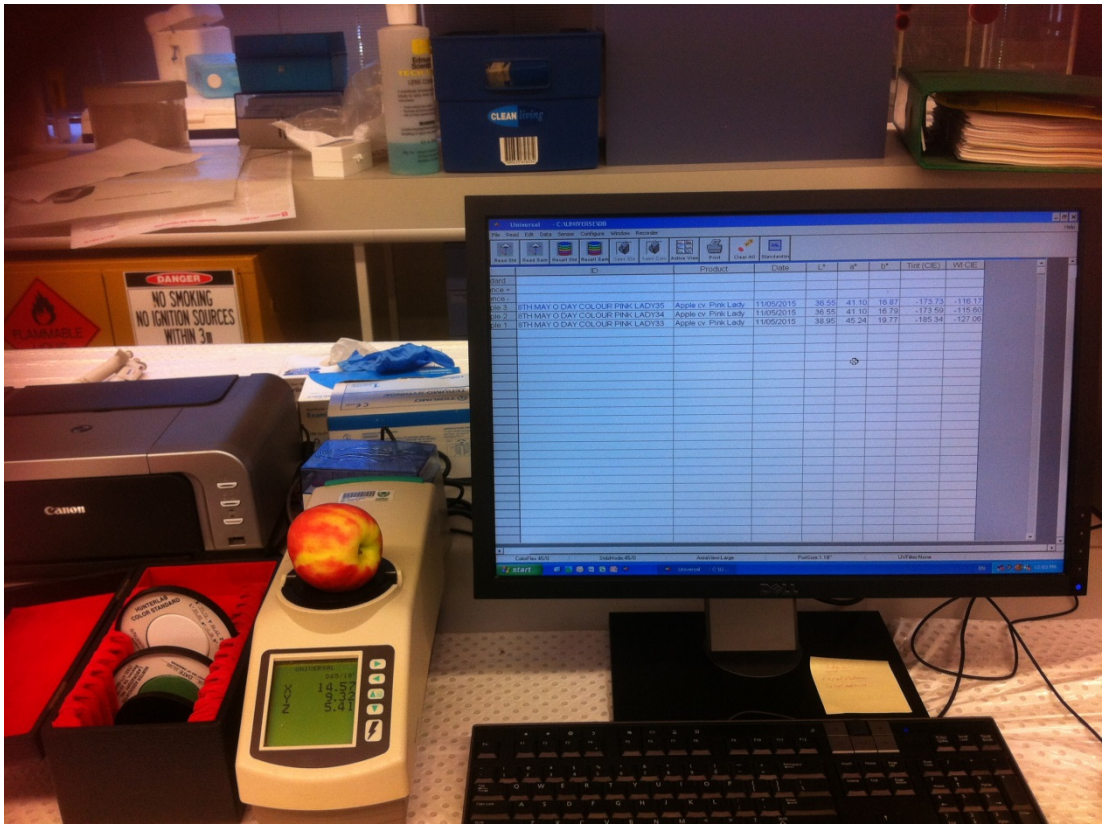

Fig. 3. 7. Analysing colorimetric profile of apple fruit using ColorFlex $45^{o}/0^{o}$ Spectrophotometer.

3. 9. 7. Soluble solids concentration (SSC)

A Panasonic juice extractor (Model MJ-66PR, Matsushita Electric Ind. Co. Ltd., Kadoma, Osaka, Japan) was used to extract juice from the blended composite of apple slices from ten fruit randomly selected in each replication. SSC was determined by recording the refractive index of the juice using an infra-red digital refractometer (Atago-Palette PR-101, Atago Co., Ltd., Itabashi-Ku, Tokyo, Japan). SSC was expressed in percentage (%).

3. 9. 8. Titratable acidity (TA)

To determine TA, freshly extracted juice (10 ml) was diluted with 20 ml dH_2O. Aliquot (5 ml) was titrated against 0.1 N NaOH solutions using 3 drops of phenolphthalein as an indicator to a pink colour end point. The TA was expressed as % malic acid and calculated as follows-

$$\text{Titratable acidity (malic acid \%)} = \frac{0.0067 \text{ X Total volume (30 mL) X } 100}{\text{Volume of aliquot (5mL) X Juice (10 mL)}}$$

Where,

0.0067 = Milli-equivalent weight of malic acid

30 = Total volume (mL)

10 = Extract juice sample (mL)

5 = Volume of aliquot (mL)

3. 9. 9. SSC/TA ratio

SSC: TA ratio was calculated by dividing SSC with corresponding TA value.

3. 9.10. Determination of ascorbic acid

Ascorbic acid concentration was estimated using the method previously described by Malik and Singh (2005) and Pham (2009) with some modifications as detailed in Figure 3.4. Freshly extracted apple juice (5 ml) was mixed with 25 ml 6% metaphosphoric acid containing (0.18%) disodium salt ethylenediaminetetraacetic acid (EDTA) then homogenised and centrifuged at 3220× g for 15 minutes using a centrifuge (Eppendorf Centrifuge 5810 R, Hamburg, Germany). The supernatant (400 µl) was mixed with 200 µl of 3% metaphosphoric acid, 1.4 ml dH_2O, and diluted with 200 µl folin reagent (Folin:dH_2O, 1:5 v/v). Disposable cuvettes (2 ml)

were used to record the absorbance of the mixed sample after 15 min at 760 nm wave length using an UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, Dunmow, Essex, UK). Ascorbic acid concentration was calculated by using standard curve of L-ascorbic acid and expressed as mg ascorbic acid per 100 ml fresh juice.

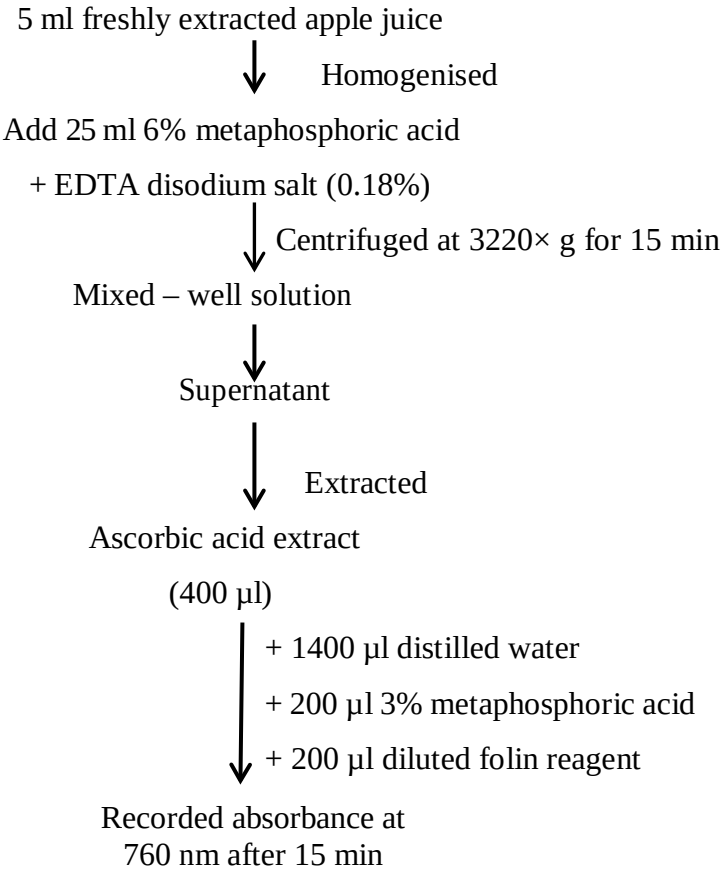

Figure 3.8. Flow chart for the estimation of concentration of ascorbic acid in apple juice, (Malik and Singh, 2005; Pham, 2009).

3. 10. Statistical analysis

The experimental data were subjected to one or two-way analysis of variance (ANOVA) depending upon experiment using GenStat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK) software. The effects of various treatments and their interactions for different parameters were assessed within ANOVA. Least significance difference (LSD) was calculated following significant F-test ($P \leq 0.05$). All the assumptions of analysis were checked to ensure validity of ANOVA.

Chapter 4

Efficacy of lime sulphur alone and in combination with different types of oils on blossom thinning and fruit quality of organically grown apple

Abstract

Organic apple cultivation constitutes 30% of the world's organic temperate tree fruits in area. Blossom thinning improves apple fruit quality, but plant growth regulators cannot be used in organic apple production. Hand thinning is tedious, laborious and not economical. The effects of spray applications of lime sulphur (LS) alone and in combination with different types of oils (3%) such as canola oil, fish oil and olive oil on blossom thinning or fruit set, fruit retention, size and quality in organically grown apples were investigated. An aqueous emulsion containing LS (5%) alone and in combination with canola oil, fish oil and olive oil (each at 3%) and 0.05% synertrol oil as a surfactant was sprayed onto whole trees until runoff at 75% bloom stage in 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple trees. Unsprayed trees served as control. All treatments had significantly reduced fruit set as compared to the control. Spray applications of LS (5%) in combination with olive oil (3%) was most effective in reducing fruit set (11.74%) coupled with lower mean leaf scorch (2.25%). The combination of LS (5%) and olive oil (3%) reduced the leaf scorching compared to spraying LS alone or in combination with canola oil or fish oil. Spray application of LS in combination with olive oil appears to be the most effective treatment in reducing fruit set and leaf scorching. This treatment also reduced fruit retention and improved fruit size, increased mean SCC and ascorbic acid levels. It also improved fruit skin colour in 'Gala' and 'Cripps Pink^{TM}', and increased firmness slightly in 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple.

4. 1. Introduction

Apple (*Malus domestica* Borkh.) is usually produced in temperate climates. However, low chill apple cultivars are also grown in the warmer parts of the world, including the Mediterranean regions of California, South Africa and Australia (Mackay et al., 1994). China dominates the global apple production, producing more than five times than the USA, its closest apple producing competitor followed by France, Turkey and Italy. Australian apple production is less than 1% of the world production (FAO, 2014). The world apple production area in 2012 was an estimated 157,725.35 ha with production of 76,378,738 tonnes (FAO, 2014). Apple is grown on 12,258 ha throughout Australia. Victoria has the largest area of apple cultivation in Australia (4,279 ha) followed by New South Wales (2,455 ha), Queensland (1,571 ha), Western Australia (1,423 ha), South Australia (1,306 ha) and Tasmania (1,224 ha) (Australia Bureau of Statistics, 2014). The three most common cultivars of apples produced in Australia are 'Cripps Pink™' (Pink Lady™) (60,500 tonnes), 'Granny Smith' (58,600 tonnes) and 'Gala' (39,500 tonnes). Most of the apple orchards in Western Australia are concentrated around Donnybrook, Manjimup, Dwellingup and the Perth Hills. 'Cripps Pink™' apple is the main cultivar grown in Western Australia, contributing 34% of the state's apple production, followed by 'Gala' (17%) and 'Granny Smith' (17%) (Australia Bureau of Statistics, 2014).

Organic fruit production is gaining impetus and currently occupies an area of 116,000 ha under cultivation worldwide. Organic apples are grown on 35,268 ha, followed by apricots with a production area of 10,683 ha (Kirby and Granatstein, 2010). Organic apple is grown in all states of Australia with over 60 certified growers (McCoy, 2007). In Western Australia, organic apple production is still in its infancy with an annual production of 300 tonnes (McCoy, 2007). Organically grown fruit command higher returns as consumers of organic food will pay more to receive quality organic produce.

Thinning of apple fruit is a pre-requisite in the production of high quality fruit (Link, 1973). Crop load has a major impact on apple fruit quality and the regularity of bearing (Link, 2000). Chemical-free flower and fruit thinning reduces chemical input into integrated fruit production as a means to regulate alternate bearing in biological (organic) apple orchards (Bertschinger et al., 1998). Fallahi and Greene

(2010) reported early thinning of apple fruit was important because of its impact on fruit size and next season's flower bud initiation. Chemical thinning continues to be the most important practice in modern conventional apple production (Looney, 1986). Yuan and Greene (2000) reported that a spray application of 6-benzyladenine (100 $mg1^{-1}$) effectively thinned apple fruit and increased fruit size. The spray application of carbaryl (1000 mg L^{-1}), ethephon (474 mg L^{-1}) and naphthalene acetic acid (58 mg L^{-1}) on 'Golden Delicious' apples reduced auxin transport to fruitlets, leading to thinning (Ebert and Bangerth, 1981). The application of Tergitol TMN-6 (0.75% to 1.25%) at 75 to 80% bloom stage was effective in blossom thinning of 'Rome Beauty' apples (Fallahi and Greene, 2010). Guak et al. (2004) found that spray application of LS (up to 4%) at 85% full bloom stage reduced fruit set. Stopar (2004) reported a spray application of 3% lime sulphur (CaSx) to 'Golden Delicious' apple trees resulted in severe flower thinning, consequently reducing crop load and increasing fruit weight.

One of the major challenges in organic apple production is the regulation of crop load to improve fruit quality. Manual thinning is tedious, time consuming and very expensive. Presently, there are only a few methods and agents allowed for certified organic horticulture. However, synthetic chemical thinning agents or plant hormones for crop regulation are not permitted to be used in certified organic apple production. Most experiments to regulate fruit set in organic horticulture are based on two strategies including injuring flowers (Ju et al., 2001; Pfeiffer and Ruess, 2002) and mechanical reduction in the number of flower buds (Roche and Masseron, 2002). Efficacy of several products such as mineral oil, corn oil, rape oil, olive oil, vinegar, sodium bicarbonate, sodium salt and lime sulphur has been tested to damage flowers (Alegre and Alins, 2007). Nevertheless, none of these products have been tested under Australian climatic conditions.

Lime sulphur and different types of oils have been used by organic growers in some countries which have achieved partly satisfying thinning. Stopar (2004) investigated some blossom thinners for possible use in organic apple production on ten-year-old 'Golden Delicious' trees. The effectiveness of organic bloom thinners under Australian conditions on flower/fruit thinning along with their effects on fruit quality have not yet been investigated. This study was undertaken to investigate the effects of spray application of high concentration of lime sulphur alone and in

combination with different types of oils such as canola oil, fish oil and olive oil at 3% concentration, on fruit set, fruit retention, leaf scorch and fruit quality in organically grown apples.

4. 2. Materials and Methods

4. 2. 1. Plant materials

The experiment was conducted on three commercially important cultivars of apples in 2011-2012. Fifteen-year-old 'Cripps Pink^{TM}' and 'Granny Smith' grafted on rootstock M26 and 'Gala' on rootstock MM06 apple trees grown at Newton Brothers Orchards at Manjimup (lat. 34°14´S, long. 116°8´E) Western Australia were used. The trees were spaced 7.5 m between rows and 2.5 m within rows in the north-south orientation. All the experimental trees received similar cultural practices including nutrition, irrigation and plant protection (McCoy, 2007) except varying experimental treatments.

4. 2. 2. Organic Chemicals

Lime sulphur (active constituent 200gL^{-1} sulphur (s) as polysulfide sulphur was sourced from Richgro, Jandakot, Western Australia. Organic oils such as canola, fish and olive oil were also purchased from Western Australia. Synertrol oil, a surfactant was purchased from Organic Crop Production, Pty Ltd, Lilyfield, NSW, Australia.

4. 2. 3. Treatments and experimental design

The trees were sprayed with an aqueous emulsion containing lime sulphur (5%) alone or in combination with different types of oils (3%) canola oil, fish oil or olive oil and 0.5% synertrol as a surfactant. The experimental trees were sprayed at 75% bloom stage. The emulsion was sprayed onto the entire experimental apple trees including 'Cripps PinkTM' , 'Granny Smith' and 'Gala' using a sprayer (The Selecta Trolleypak Mk II, Acacia Ridge, Australia) until runoff. Control trees were kept unsprayed. The experiment was laid out by following two-factor (treatments and cultivars) factorial randomised block design with four replicates. Single trees were treated as one experimental unit.

Prior to applying the spray treatments, three branches per tree were randomly tagged and the total number of flowers on each branch was counted. Fruit set (%) retention (%), leaf scorch (%), fruit size, weight and colour were recorded. SSC, total acidity (TA), SSC:TA ratio as well as ascorbic acid were determined from the apple fruit juice.

4. 2. 3. Observations recorded

4. 2. 3.1. Fruit set (%)

Number of fruit set from three tagged branches was counted 26 days after the application of blossom thinner treatments as explained in Chapter 3, Section 3.8.1.

4. 2. 3.1.2. Fruit retention (%)

Total number of fruit on each tagged branch was counted at fruit set and again one week before commercial harvest. Percent fruit retention was calculated as outlined in Chapter 3, Section 3.8.2.

4. 2. 3.1.3. Leaf scorch (%)

Total number of leaves on each branch was counted before the application of treatments. The number of leaves scorched from the three branches was counted 30 days after spray treatment as explained in Chapter 3, Section 3.8.3.

4. 3. Fruit quality

4. 3. 1. Fruit size (mm)

Fruit size was measured in mm as the diameter using a digital Vernier calliper from ten randomly selected fruit in each replication as described in Chapter 3, Section 3.9.2.

4. 3. 2. Fruit weight (g)

Fruit weight was calculated by weighing ten fruit per replication by using a digital balance (A&D Limited, Tokyo, Japan) and average weight was calculated as grams (g) per fruit.

4. 3. 3. Fruit firmness (N)

Fruit firmness from ten randomly selected fruit was determined using an electronic pressure tester (Model EPT-1 pressure tester, Lake City Technical products Inc., Kelowna, BC, Canada) as outlined in Chapter 3, Section 3.9.5. Average fruit firmness was expressed as newtons (N).

4. 3. 4. Fruit surface skin colour

Fruit colour from ten randomly selected fruit per replication was measured on the fruit surface ('Cripps Pink^{TM}' and 'Gala' only) using a HunterLab ColorFlex 458/08 Spectrophotometer (HunterLab ColorFlex, Hunter Associates Inc., Reston, VA, USA) as L*, a* and b*. The data were expressed as Commission Internationale de L'Eclairage (CIE) lightness (L*, a*, b*), chroma (C*) and hue angle (h°) as outlined in Chapter 3, Section 3.9.6.

4. 3. 5. Soluble solids concentration, titratable acidity and SSC/TA ratio

Soluble solids concentration (SSC) from the fresh fruit juice was recorded using a digital refractometer as described in Chapter 3, Section 3.9.7. Titratable acidity (TA) was determined by titrating the fruit juice with 0.1 N NaOH following the method outlined in Chapter 3, Section 3.9.8. SSC/TA ratio was calculated by dividing SSC with the corresponding TA value as detailed in Chapter 3, Section 3.9.9.

4. 3. 6. Ascorbic acid

The concentration of ascorbic acid from the juice was determined following the methods outlined by Jagota and Dani (1982) and Malik and Singh (2005) with some modifications as described in Chapter 3, Section 3.9.10. The concentration of ascorbic acid was expressed as mg ascorbic acid per 100 ml fresh juice.

4. 4. Statistical analysis

The experimental data were analysed using two-way (treatments and cultivars) analysis of variance (ANOVA) using GenStat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK). The effects of various treatments and their interactions for different parameters were assessed

within ANOVA and least significance differences (LSD) were calculated following significant F-test ($P \leq 0.05$). All the assumptions of analysis were checked to ensure validity of statistical analysis.

4. 5. Results

Single spray of lime sulphur alone and in combination with different types of oils (3%) (Canola oil, fish oil or olive oil) were applied at 75% bloom to 'Gala' 'Granny Smith' and 'Cripps Pink^{TM}' apple trees. All the treatments differed significantly in reducing the fruit set and fruit retention in all cultivars. Results obtained from this experiment have been explained and discussed below.

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4. 5. 1. Fruit set, retention and leaf scorch

All the lime sulphur spray treatments alone and in combination with different types of oils significantly ($P \leq 0.05$) reduced fruit set, retention and leaf scorch in comparison to the control in 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apples (Table 4. 1). When averaged over different cultivars, all the lime sulphur spray treatments alone and in combination with different types of oils significantly ($P \leq 0.05$) reduced mean fruit set, retention and leaf scorch in comparison to the control (Fig. 4. 1). The interaction between the treatments and the cultivars was found to be significant only for fruit set and leaf scorch. When averaged over treatments, mean fruit set and leaf scorch differ significantly among the cultivars, whilst mean fruit retention did not differ significantly. 'Gala' exhibited significantly higher leaf scorch (12.87%) as compared to 'Granny Smith' and 'Cripps Pink^{TM}' (Table 4. 1). A spray application of lime sulphur (5%) alone and in combination with olive oil (3%) resulted in the significantly lowest mean fruit set (12.12% and 11.74 % respectively) (Fig. 4. 1a) and mean fruit retention (23.73%) (Fig. 1b), as compared to control and all other treatments. Mean leaf scorch percentage was significantly lowest (2.25%) with a spray application of lime sulphur (5%) and in combination with olive oil (3%) as compared to all other treatments except the spray application of 5% lime sulphur alone (Fig. 4. 1c). In general, single spray application of lime sulphur (5%) alone and in combination with olive oil (3%) resulted in lower fruit set, retention and leaf scorch as compared to the spray of lime sulphur (5%) alone or in combination with canola oil or fish oil.

Table 4. 1. Effects of spray application of lime sulphur (LS, 5%) alone and in combination with (3%) canola oil (CO), fish oil (FO) or olive oil (OO) at 75 % blossom stage on fruit set (%), fruit retention (%) and leaf scorch (%) in 'Gala', 'Granny Smith' and' Cripps Pink' apple trees.

		Treatments (T)						LSD ($P \leq 0.05$)		
Parameters	Cultivar (Cv)	Control	L.S (5%)	L.S (5%) + CO (3%)	L.S (5%) + FO (3%)	L.S (5%) + OO (3%)	Mean (cv)	T	Cv	T x Cv
Fruit Set (%)	Gala	37.56	12.97	20.8	26.11	15.49	22.59A	4.47	3.47	7.75
	Granny Smith	26.65	10.55	7.88	10.15	8.81	12.81B			
	Cripps Pink	43.11	12.84	29.03	19.68	10.92	23.12A			
	Mean (T)	35.77A	12.12C	19.24B	18.65B	11.74 C				
Fruit retention (%)	Gala	63.81	34.12	45.72	40.93	22.81	41.48	8.42	NS	NS
	Granny Smith	67.57	31.11	40.69	38.24	24.49	40.42			
	Cripps Pink	66.58	36.88	37.27	34.72	23.9	39.87			
	Mean (T)	65.99A	34.04B	41.23 B	37.96 B	23.73 C				
Leaf scorch (%)	Gala	0	5.14	28.2	30.06	0.95	12.87A	4.94	3.83	8.56
	Granny Smith	0	3.87	6.99	7.69	2.17	4.14B			
	Cripps Pink	0	3.76	1.04	5.58	3.64	2.80B			
	Mean (T)	0	4.26B	12.08A	14.45A	2.25B				

Any two means within a column and a row followed by different letters are significantly different at $P < 0.05$. NS = not significant, n for fruit set = four replicates (200 - 300 flowers per tree replicate, n for fruit retention= four replicates (150 - 250 fruit set per tree replicate), n for leaf scorch = four replicates (3 branches per tree replicate)

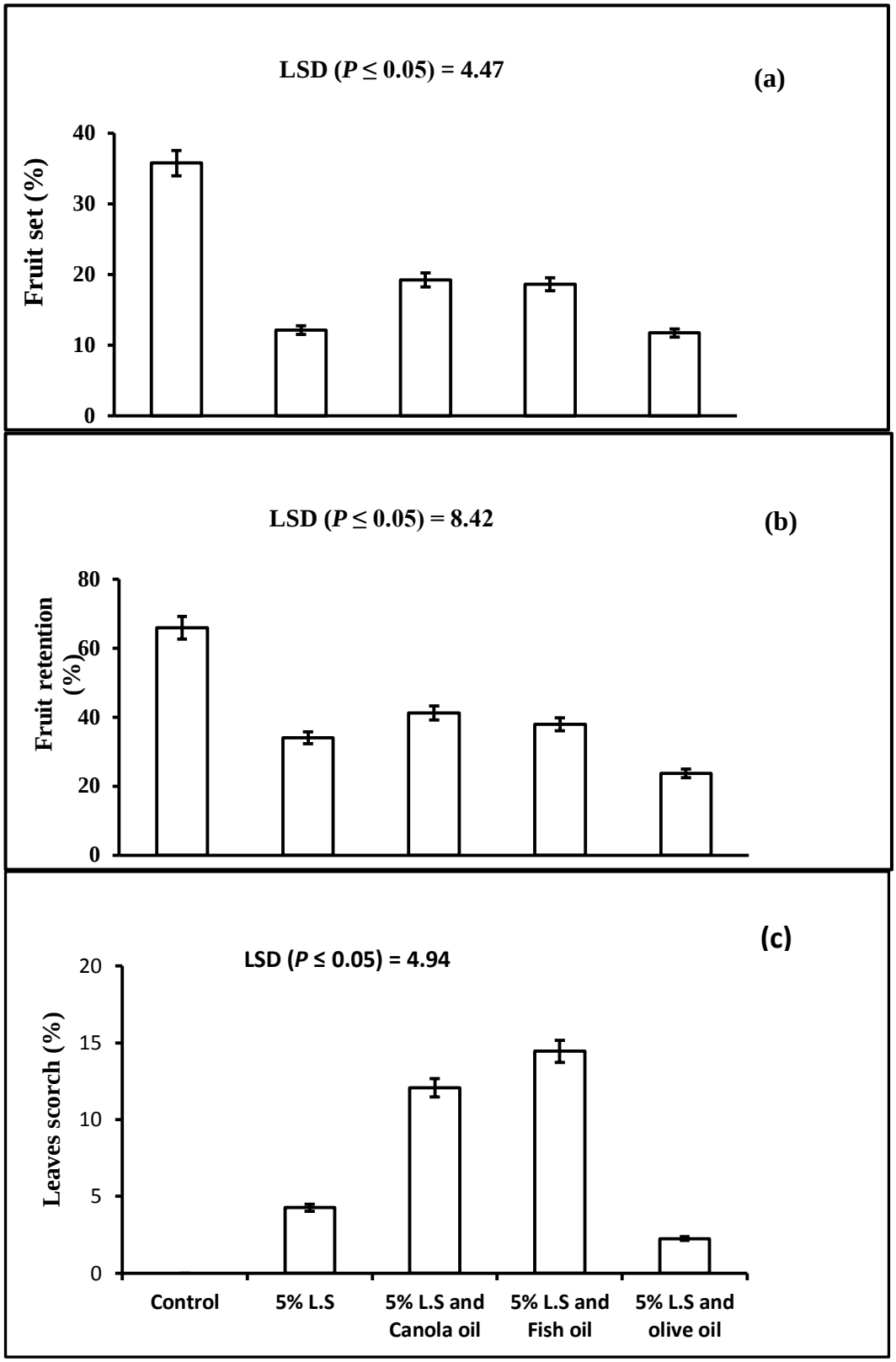

Figure 4. 1. Effects of spray application of lime sulphur (LS 5%) alone and in combination with (3%) canola oil (CO), fish oil (FO) or olive oil (OO) at 75% blossom stage on mean fruit set (%) (a), fruit retention (%) (b) and leaf scorching (%) (c). Vertical bars represent standard error.

4. 5. 2. Fruit size, weight and firmness

All treatments of lime sulphur alone and in combination with different types of oils sprayed on 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple trees showed significant ($P \leq 0.05$) increase in fruit size, weight and firmness in comparison to the control fruit at commercial harvest. When averaged over the three cultivars, the largest fruit size (67.68 and 66.68 mm) was noted when apple trees were sprayed with 5% lime sulphur in combination with 3% olive oil or fish oil respectively, whilst 5% lime sulphur in combination with olive oil resulted in highest fruit weight (148g) (Figs. 4. 2a and b). There was a direct linear negative relationship (r = - 0.26, y = - 0.1006x + 66.238) between per cent fruit set and fruit size as regulated by the spray applications of lime sulphur (LS 5%) alone and in combination with different types of oils (Fig. 4. 3a). Trees sprayed with 5% lime sulphur in combination with olive oil or lime sulphur alone produced fruit with higher average weight (148g and 138g respectively) (Fig.4. 2b). There was a direct linear negative relationship (r = -0.35, y = 0.7217x +145.7) between percent fruit set and fruit weight as influenced by the application of lime sulphur (LS, 5%) alone and in combination with different types of oils (Fig. 4. 3b). Fruit firmness increased significantly when trees were sprayed with 5% lime sulphur in combination with olive oil (94.35 N) or canola oil (90.63 N) compared to all other treatments (Fig. 4. 2 c). Irrespective of the treatment, there were significant differences among cultivars for fruit size, fruit weight and firmness. The mean fruit firmness was highest (96.33 N) in 'Gala' followed by 'Cripps Pink^{TM}' and 'Granny Smith' (88.93 N and 82.25 N respectively). The interaction between the cultivars and the treatments differed significantly for fruit size and firmness (Table 4. 2).

Table 4. 2. Effects of spray application of lime sulphur (LS, 5%) alone, in combination (3%) with canola oil (CO), fish oil (FO) or olive oil (OO) at 75% blossom stage on fruit size, fruit weight and fruit firmness in 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple fruit at commercial harvest.

		Treatments (T)						LSD ($P \leq 0.05$)		
Parameters	Cultivar (Cv)	Control	L.S (5%)	L.S (5%) + CO	L.S (5%) + FO	L.S (5%) + OO	Mean (cv)	T	Cv	T x Cv
Fruit Size (mm)	Gala	59.16	60.28	61.31	59.43	61.89	60.41B	1.56	1.18	2.644
	Granny Smith	64.37	67.37	65.99	70.13	70.93	67.73A			
	Cripps Pink	63.83	68.92	64.91	69.66	70.22	67.50A			
	Mean (T)	62.41 D	65.52 C	64.07 C	66.68AB	67.68 A				
Fruit Weight (g)	Gala	111	115	112	104	122	113B	8.0	6. 0	NS
	Granny Smith	139	146	153	151	160	150A			
	Cripps Pink	126	152	139	142	162	144A			
	Mean (T)	126 C	138 B	135 A	132 BC	148 A				
Firmness (N)	Gala	86.87	92.36	100.08	97.28	105.08	96.33A	3.512	2.72	6.083
	Granny Smith	78.15	84.4	81.85	79.28	87.55	82.25C			
	Cripps Pink	89.03	86.9	89.98	88.3	90.43	88.93B			
	Mean (T)	84.68 C	87.89 BC	90.63 B	88.28 BC	94.35 A				

Any two means within a column and row followed by different letters are significantly different using LSD at $P < 0.05$. NS = not significant, n = four replicates (10 fruit per replicate)

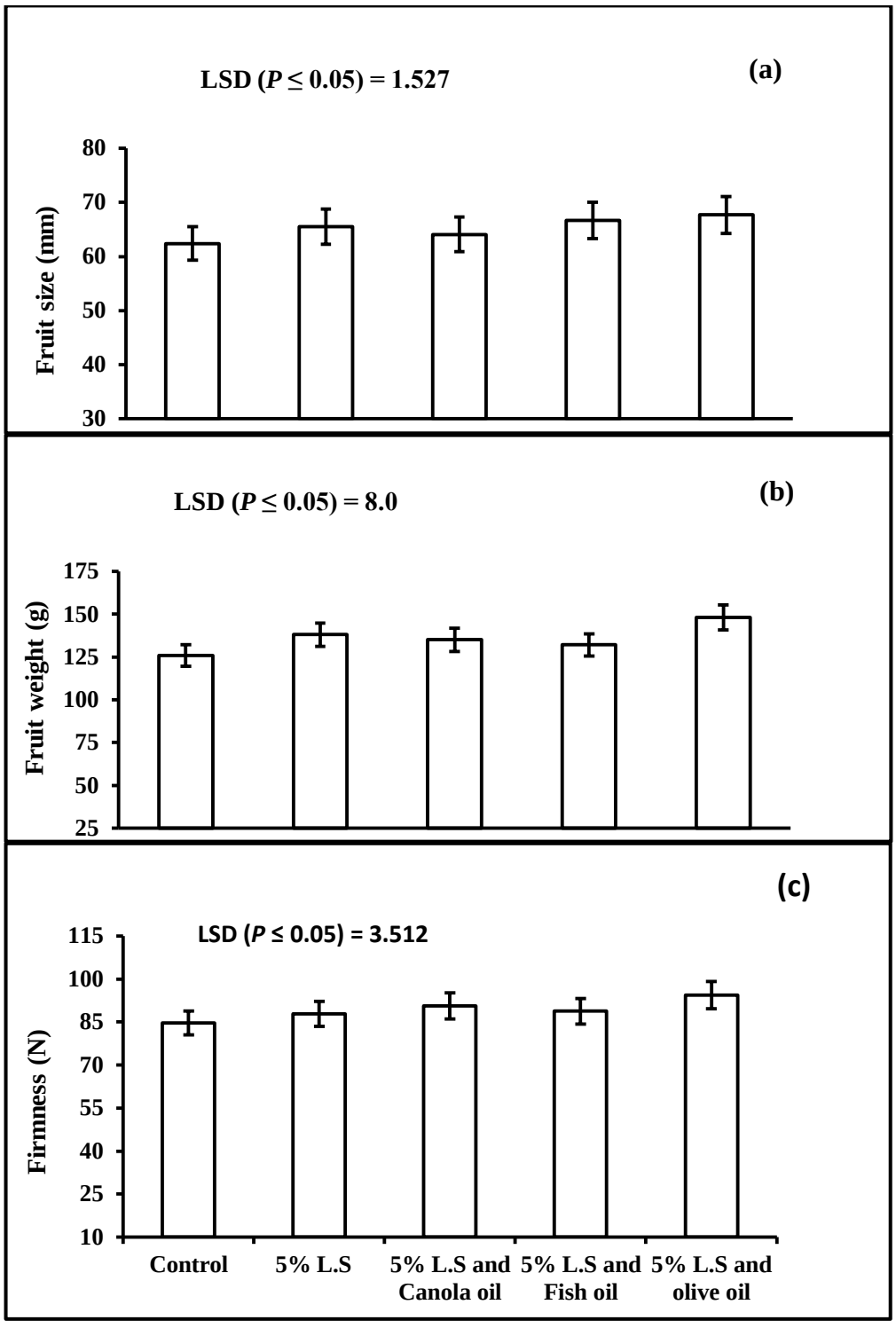

Figure 4. 2. Effects of spray application of lime sulphur (LS 5%) alone and in combination with (3%) canola oil (CO), fish oil (FO) or olive oil (OO) at 75% blossom stage on mean fruit size (a), fruit weight (b) and fruit firmness (c). Vertical bars represent standard error.

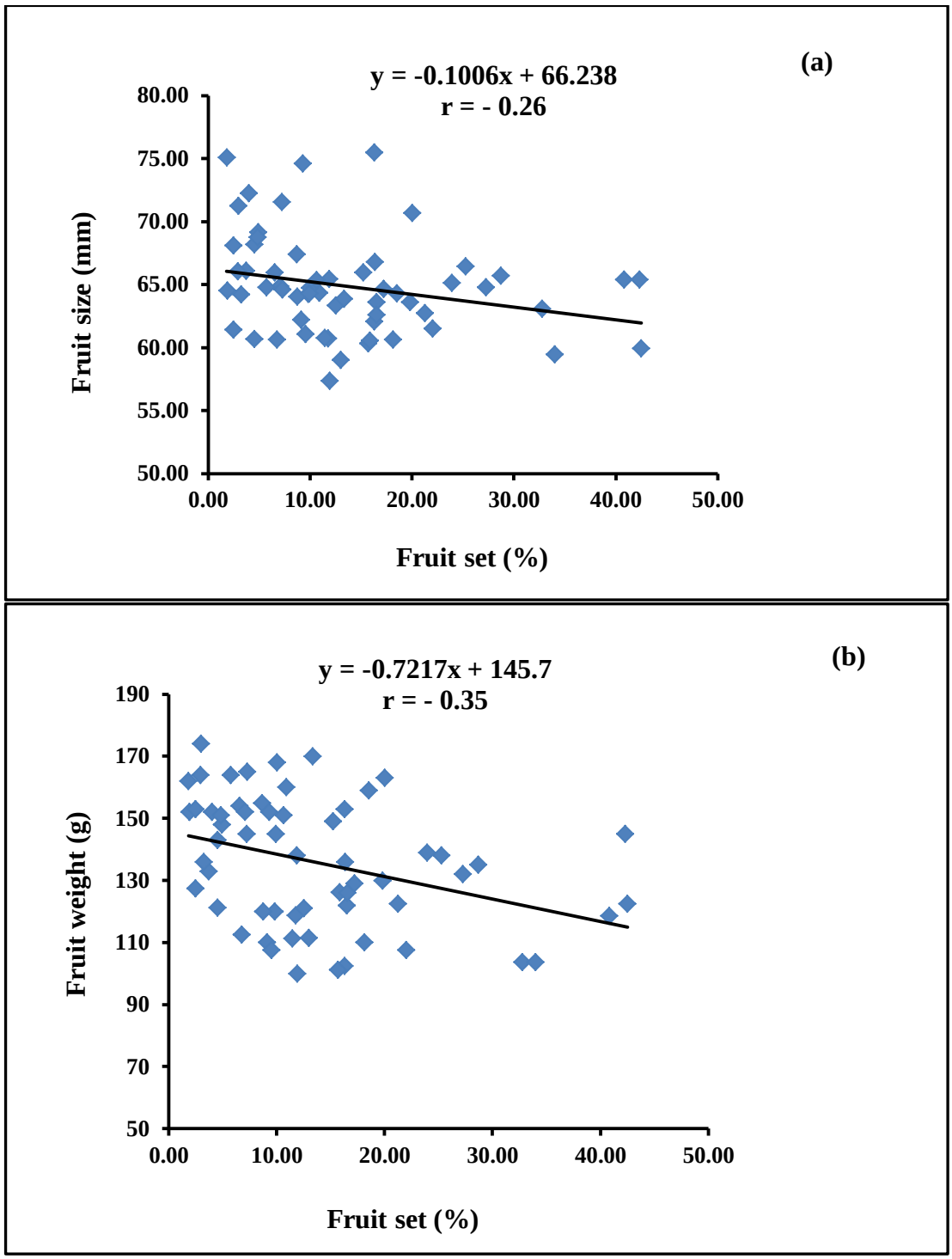

Figure 4. 3. Relationships between percentage fruit set and fruit size (mm) (a) and percentage fruit set and fruit weight (b) as regulated by the spray applications of lime sulphur (LS, 5%) alone and in combination with (3%) canola oil (CO), fish oil (FO) or olive oil (OO) at 75% blossom stage in 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple trees.

4. 5. 3. Skin colour

Fruit skin colour was recorded from 'Gala' and 'Cripps Pink^{TM}' though not from 'Granny Smith'. The bloom thinning treatments significantly ($P \leq 0.05$) influenced the skin colour of 'Gala' and 'Cripps Pink^{TM}' apple fruit. However, the mean values of L*, a*, b*, Chroma (*C) and hue angle (h) were lower on the fruit treated with lime sulphur (5%) alone and in combination with different types of oils compared to the control in both cultivars (Fig 4. 4). The mean values L* and h° were lower on fruit treated with lime sulphur (5%) in combination with olive oil compared to all others treatments, whilst the mean a*, b* and chroma were highest on the fruit treated with lime sulphur (5%) in combination with 3% fish oil (figs. 4. 4a and e). Irrespective of the treatments, the mean L*, a*, *C and h° values differed significantly between both cultivars. The mean values (L* and h° were higher in 'Cripps Pink^{TM}' apple fruit (50.50 L* and 34.21°h respectively) than 'Gala' apple fruit, whilst the mean values (a*, *C) were higher in 'Gala' apple fruit (39.85 a* and 46.54 *C respectively) than 'Cripps Pink^{TM}' apple fruit. The interactions between the cultivars and the treatments for L*, a*, b*, *C and h° were found to be significant (Table. 4. 3).

Table 4. 3. Effects of spray application of lime sulphur (LS 5%) alone, in combination (3%) with canola oil (CO), fish oil (FO) or olive oil (OO) at 75% blossom stage on fruit colour in 'Gala' and 'Cripps Pink™' (CP) apple fruit.

		Treatments (T)						LSD ($P \leq 0.05$)		
Colour (chromaticity)	Cultivar (cv)	Control	LS	LS+CO	LS+FO	LS+OO	Mean cv	T	Cv	T x Cv
L*	Gala	48.04	40.92	47.42	46.85	29.8	42.61 B	3.515	2.223	4.971
	Cripps Pink	55.24	50.41	52.25	46.66	47.92	50.50 A			
	Mean (T)	51.64 A	45.67 C	49.84 AB	46.75 C	38.86 D				
a*	Gala	36.79	40.09	37.58	38.4	46.36	39.85 A	3.189	2.017	4.510
	Cripps Pink	41.04	35.26	36.26	29.01	39.22	36.13 B			
	Mean (T)	38.92 B	37.59 B	36.92 B	33.71 C	42.79 A				
b*	Gala	24.45	18.97	23.92	28.75	21.25	23.47	1.637	NS	2.315
	Cripps Pink	26.47	24.52	24.06	22.91	20.42	23.67			
	Mean (T)	25.46 AB	21.75 C	23.99 B	25.83 A	20.83 C				
*C	Gala	42.68	44.47	44.85	54.6	45.63	46.54 A	2.236	1.414	3.162
	Cripps Pink	45.99	43.61	44.14	45.82	40.04	43.92 B			
	Mean (T)	44.33 B	44.04 B	44.50 B	50.21 A	42.84 B				
h°	Gala	32.51	25.2	32.61	31.76	30	30.42 B	3.832	2.424	5.420
	Cripps Pink	43.28	35.79	34.39	30.82	26.77	34.21 A			
	Mean (T)	37.89 A	30.49 C	33.50 B	31.29 BC	28.39 C				

Any two means within a column and row followed by different letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (10 fruit per replicate)

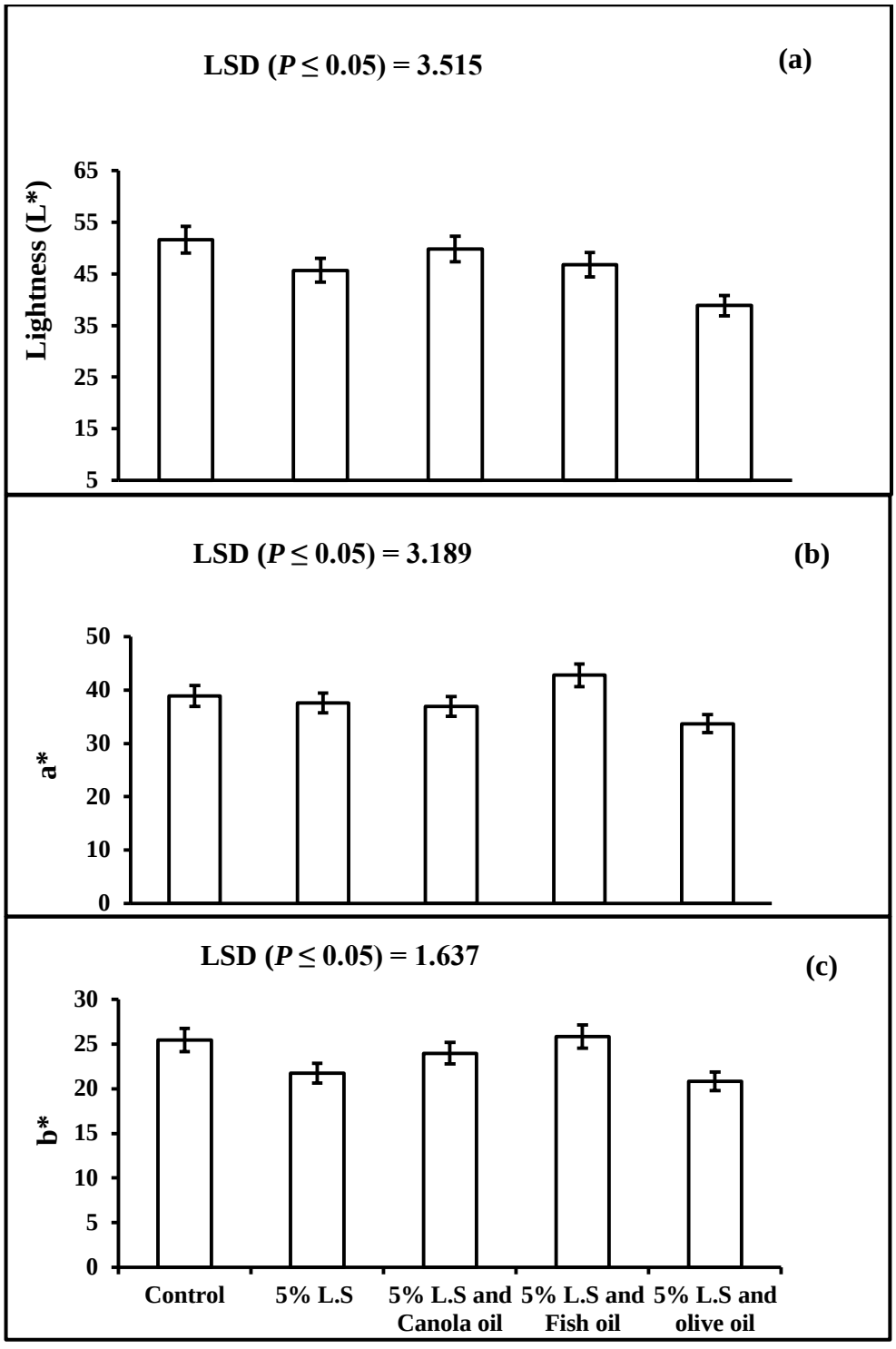

Figure 4. 4. Changes in mean chromaticity (L*, a*, b*) influenced with the spray of lime sulphur (LS, 5%) alone, in combination with (3%) canola oil (CO), fish oil (FO) or olive oil (OO) applied at 75% blossom stage. Vertical bars represent standard error.

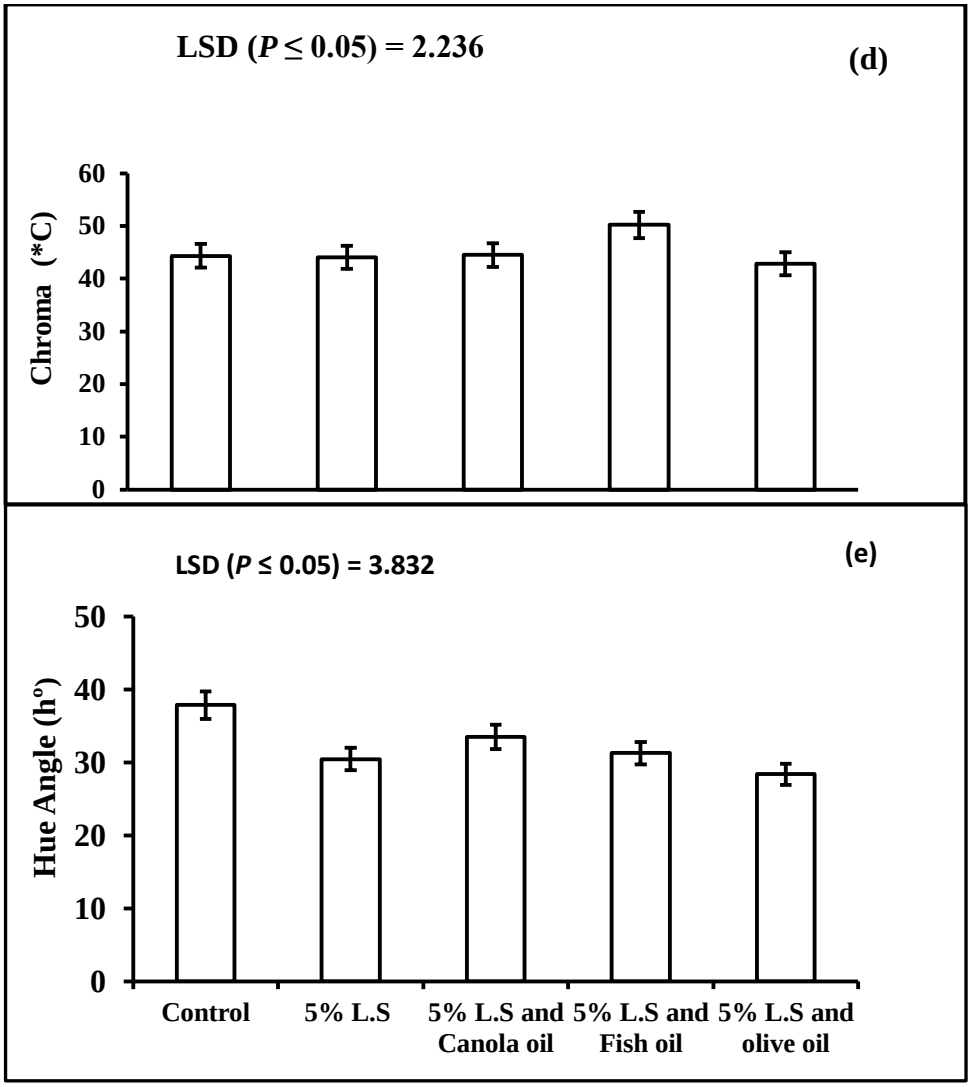

Figure 4. 4. Changes in chromaticity (C*, and h^{o}) influenced with the spray of lime sulphur (LS, 5%) alone, in combination with (3%) canola oil (CO), fish oil (FO) or olive oil (OO) applied at 75% blossom stage. Vertical bars represent standard error.

4. 5. 4. TA, SSC, SSC:TA ratio and ascorbic acid

All the treatments of lime sulphur alone and in combination with different types of oils sprayed on 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple trees significantly ($P \leq 0.05$) affected mean SSC, titratable acidity (TA) and ascorbic acid in apple juice as compared to control. The SSC:TA ratio in juice did not differ significantly ($P \leq 0.05$) among treatments (Fig. 4. 5c). Mean SSC was significantly ($P \leq 0.05$) higher when the trees were sprayed with lime sulphur in combination with olive oil (13.51%) or canola oil (13.28%) as compared to untreated trees (Fig.4. 5a). The mean TA was significantly higher in the fruit harvested from the trees sprayed with 5% lime sulphur alone and in combination with different oils compared to the control (Fig. 4. 5b). The trees treated with the lime sulphur (5%) in combination with olive oil showed higher mean levels of ascorbic acid (37.50 mg.100 ml^{-1} FJ) compared to all other treatments and control trees (34.87 mg.100 ml^{-1} FJ) (Fig.4. 5d). When averaged across the treatments, SSC, TA and ascorbic acid differ significantly among cultivars. SSC was higher in 'Gala' and 'Cripps Pink^{TM}' apple (13.24% and 13.84% respectively) than 'Granny Smith' (11.31%). When averaged over treatments, mean TA was higher in the juice of 'Cripps Pink^{TM}' and 'Granny Smith' apple (2.44% and 2.12%) compared to 'Gala' (1.85%). SSC:TA ratio was significantly higher in 'Gala' (7.166) compared to the SSC:TA ratio of 'Granny Smith' and 'Cripps Pink^{TM}' (5.39 and 5.69 respectively) apple. The interaction between the cultivars and different treatments were found to be significant for SSC, TA and ascorbic acid Table (4. 4).

Table 4. 4. Effects of spray applications of lime sulphur (LS 5%) alone, in combination (3%) with canola oil (CO), fish oil (FO) or olive oil (OO) at 75% blossom stage on soluble solids concentration (SSC), titratable acidity (TA), SSC:TA ratio and levels of ascorbic acid in the juice of 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple fruit at commercial harvest.

		Treatments (T)						LSD ($P \leq 0.05$)		
Parameters	Cultivar (Cv)	Control	L.S (5%)	L.S (5%) + CO	L.S + FO	L.S + OO	Mean (cv)	T	Cv	T x Cv
SSC (%)	Gala	12.2	11.33	14.65	12.55	15.48	13.24A	0.706	0.547	1.222
	Granny Smith	10.58	11.68	11	12.05	11.25	11.31B			
	Cripps Pink	13	14.03	14.2	14.18	13.8	13.84A			
	Mean (T)	11.93 C	12.34 BC	13.28 A	12.93 AB	13.51 A				
Titratable acidity (% malic acid)	Gala	1.65	1.75	1.98	1.73	2.15	1.85C	0.166	0.128	0.287
	Granny Smith	1.88	2.15	2.05	2.42	2.08	2.12B			
	Cripps Pink	1.92	2.5	2.52	2.6	2.35	2.44A			
	Mean (T)	1.92 B	2.13 A	2.18 A	2.25 A	2.19 A				
SSC:TA ratio	Gala	7.403	6.479	7.451	7.297	7.202	7.166A	NS	0.278	NS
	Granny Smith	5.646	5.48	5.404	5.025	5.43	5.397B			
	Cripps Pink	5.861	5.631	5.64	5.473	5.882	5.697B			
	Mean (T)	6.303	5.864	6.165	5.932	6.171				
Ascorbic acid ($mg \cdot 100ml^{-1}$ FJ)	Gala	32.65	35.82	48.35	44.37	49.06	42.05A	2.367	1.833	4.100
	Granny Smith	32.23	34.3	28.44	31.58	30.45	31.40C			
	Cripps Pink	39.74	32.87	31.93	32.26	33	33.96B			
	Mean (T)	34.87 B	34.33 B	36.24 AB	36.07 AB	37.50 A				

Any two means within a column followed by different letters are significantly different using LSD at $P < 0.05$. NS = not significant, n = four replicates (10 fruit per replicate). FJ = fruit juice.

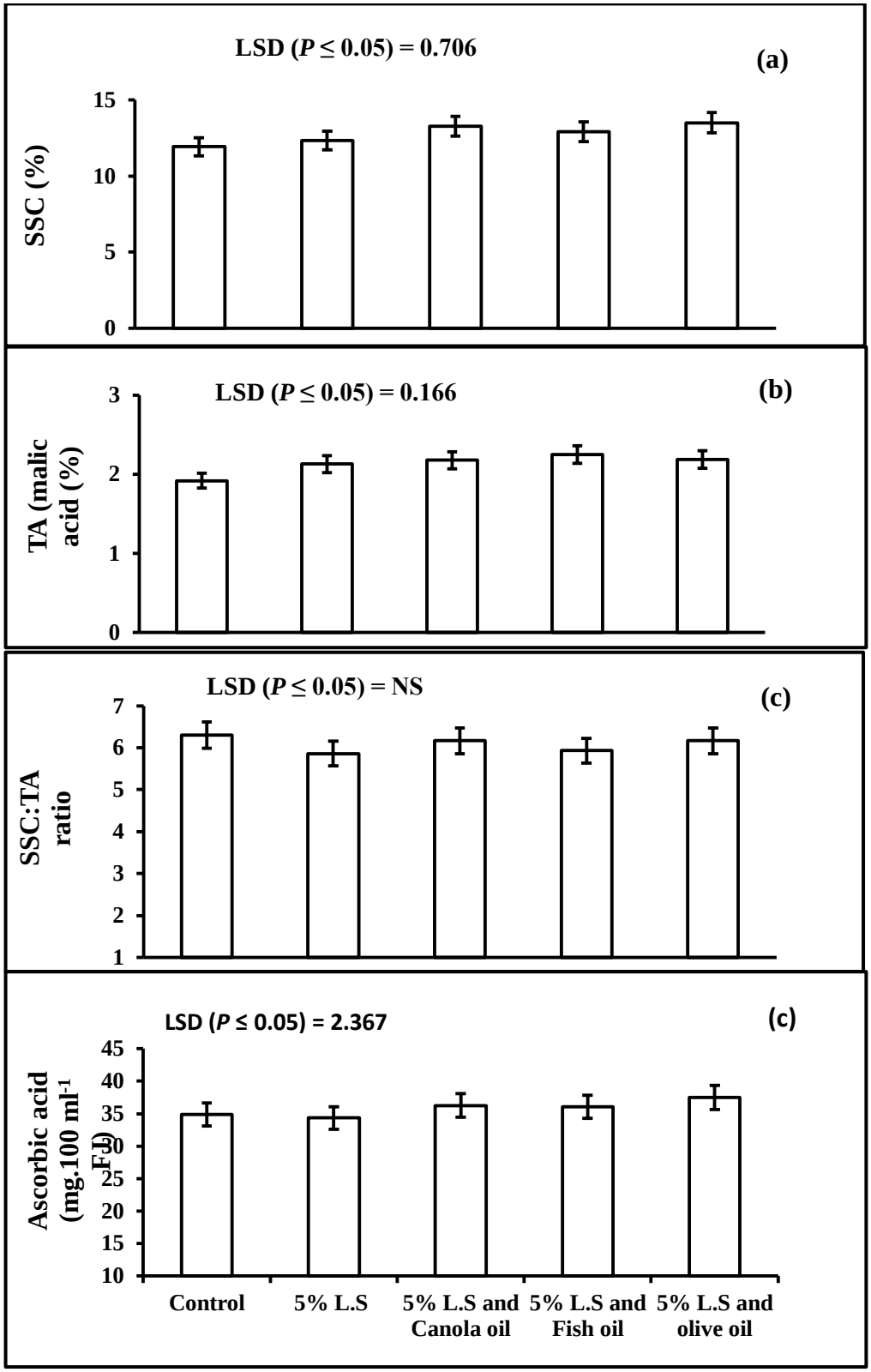

Figure 4. 5. Changes in mean soluble solids concentration (SSC), titratable acidity (TA), SSC:TA ratio and ascorbic acid as influenced with the spray applications of lime sulphur (LS, 5%) alone, in combination with (3%) canola oil (CO), fish oil (FO) or olive oil (OO) applied at 75% blossom stage. Vertical bars represent standard error.

4. 6. Discussion

Flower and fruit thinning of apples is an important cultural practice in the production of high quality fruit. Crop load, a quantitative parameter used by industry, is generally defined as the number of fruit per tree (Jones et al., 1992). Most studies relating crop load to fruit quality include the use of thinning chemicals. Chemical thinning of fruit is used effectively in stone fruit and apple to reduce production costs and increase fruit size (Fallahi et al., 2006). Organic management practices exclude chemical pesticide and fertilizer inputs in fruit production and use naturally derived products as defined by organic certification programs. Organic apple growers have long used lime sulphur (LS) applied during bloom to reduce the number of viable flowers (Edwards, 1998). Lime sulphur and oil products have been used by organic growers in some countries and achieve partly satisfying thinning results (Weibe et al., 2008). Byers (2003) reported chemical products may act as blossom thinners by decreasing pollen viability or fertility (i.e., act as pollenicides). They may also decrease stigmatic surface receptivity, or interfere with pollen-pistil interactions at the stigmatic surface or during pollen tube growth in the pistil (Weibe et al., 2008).

The data obtained from the current experiments are in agreement with earlier reports suggesting that fruit set was significantly reduced with chemical blossom thinning than without chemical thinning. Lime sulphur alone or in combination with different types of oils is most effective as a blossom thinner when applied at 75% bloom (Table 4. 1). However, all the lime sulphur spray treatments alone and in combination with different types of oils significantly ($P \leq 0.05$) reduced the fruit set, fruit retention and leaf scorch in comparison to the control in cultivars ‘Gala’, ‘Granny Smith’ and ‘Cripps Pink^{TM}’ (Table 4. 1). The application of lime sulphur resulted in scalding of petals and other flower parts; and thus possibly hampered effective fertilisation and consequently reduced fruit set in apple. It may also be argued that the reduced fruit set on trees treated with LS alone or in combination with oils can possibly be partially attributed to the reduction in pollen viability and germination (Table 4. 1). Earlier, Myraa et al., (2011) also reported that the application of lime sulphur at blossom time reduced percent pollen germination which ultimately reduces the fruit set in apple.

Similarly, LS (4%) application at 85 per cent bloom stage has been reported to reduce fruit set on 'Gala' and 'Fuji' apple where by reducing the crop load. Lime sulphur (5%) in combination with 3% olive oil also resulted in the least fruit set parent compared to control and all other treatments, while, the control trees exhibited the highest fruit set. Spray application of lime sulphur in combination with olive oil had significantly ($P \leq 0.05$) reduced leaf scorch in 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apples compared to control and lime sulphur alone (Table 4. 1). This finding signifies the potential role of lime sulphur in burning of leaves of apple trees. The exact mechanism whereby olive oil in combination with LS reduces leaf burning caused by lime sulphur is yet to be investigated in detail.

The thinning treatments with LS significantly ($P \leq 0.05$) influenced fruit size, weight and fruit firmness compared to the control (Table 4. 2). The improved fruit size and weight may be ascribed to increased availability of photosynthates from the leaves to the fruit due to reduced number of fruit with the thinning treatments. A direct linear negative s relationship (r = - 0.26, y = -0.1006x + 66.238) between per cent fruit set and fruit size as regulated by the spray applications of lime sulphur (LS, 5%) alone and in combination with different types of oils (Fig. 4. 3a) and between percent fruit set and fruit weight (r = -0.35, y = 0.7217x +145.7) (Fig. 3b) also supports the experimental findings. Fruit size is the result of the combination of number of cells and cell size (Smith 1950; Bain and Robertson 1951; Martin et al. 1964; Sugiura et al. 1995). Reducing fruit numbers at, or soon after, flowering has an effect on reducing competition for resources between fruit, which may allow remaining individual fruit to develop greater cell numbers. Fruit size and fruit weight are closely correlated, both being inversely related to fruit retention. Lime sulphur (5%) in combination with olive oil reduced fruit set and fruit retention leading to largest fruit size with highest weight and higher fruit firmness. This observation is also supported by earlier reports by Stanley et al. (2000), Stopar et al. (2002), Guak et al. (2004), Osborne et al. (2005) and Fallahi, E. B. Fallahi. (2006) who explained that lower fruit load also gives individual fruit a greater share of resources allowing cells to increase to the maximum size. The mean fruit firmness decreased with increasing retention, supporting the results of Garriz et al. (2000) who found that fruit flesh firmness was significantly lower in 'Braeburn' trees with high crop loads than in trees with moderate or low crop loads. Link (2000) suggested that the supply

of carbohydrate for cell wall synthesis is lowered which may result in reduced fruit firmness in heavily cropped trees. Though the trends relating to firmness and fruit retention were investigated in the current study, low bloom thinning produced softer fruit than trees thinned at high bloom thinning. Jones et al. (1997b) also reported increased firmness with reduced crop load following chemical thinning of 'Pink Lady' and 'Jonagold' with ethephon and benzyladenine.

The bloom thinning treatments significantly ($P \leq 0.05$) improved the skin colour of 'Gala' and 'Cripps Pink^{TM}' apple fruit. This is represented by the mean values of L* and (h) being lower in fruit treated with lime sulphur (5%) in combination with olive oil compared to all others treatments (Table 4. 3). The mean a*, b* and Chroma (*C) were highest on the fruit treated with lime sulphur (5%) in combination with 3% fish oil (figure 4. 4a and b).

Lime sulphur applied alone or in combination with different types of oils at bloom-time substantially influenced SSC, TA and ascorbic acid in apples fruit juice at harvest (Table 4. 4). In all cultivars, SSC, TA and ascorbic acid increased with increasing bloom thinning. Similarly, Guak et al., (2004) also reported higher soluble solids and titratable acidity in 'Gala' and 'Fuji' apples with high bloom thinning. The current results are also in agreement with the findings of Stopar et al. (2002) who reported that fruit from low-cropping trees exhibited significantly higher percentages of soluble solids and better flesh firmness compared with the high cropping trees. A possible explanation for this result is that high bloom thinning causes fruit to mature earlier than low thinning, particularly when combined with the increased soluble solids observed in high bloom thinning.

In conclusion, a spray application of lime sulphur (5%) alone or in combination with olive oil at 75% full bloom on 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple trees reduced fruit set and ultimately increased the fruit size, firmness, SSC, TA and improved fruit colour and minimised leaf scorch.

Chapter 5

Effects of number and time of spray applications of lime sulphur on blossom thinning and fruit quality in apple

Abstract

To determine the effects of number and time of application of lime sulphur (LS) on bloom thinning and fruit quality, organically grown 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple trees were treated with LS at 5% concentration once at 25% or 75% bloom or twice (25% + 75% bloom). An aqueous emulsion containing 5% concentration of lime sulphur (LS) and 0.05% synertrol oil as a surfactant was sprayed at different blossom stages (25% bloom, 75% bloom and double application at 25% bloom and at 75% bloom) onto whole trees of 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' until runoff. Unsprayed trees were kept as control. The experimental layout was a randomised block design with two-factor factorial, with single trees as one treatment unit with four replicates. Fruit set was recorded 26 days after spraying. Fruit retention was recorded two weeks prior to harvest. Double spray application of LS at 25% bloom and at 75% bloom and single application of LS at 75% bloom were most effective in reducing fruit set (16.61-19.74%) coupled with higher leaf scorching (14.45-12.08%) respectively in all cultivars. In conclusion, the results suggest that LS applied twice (at 25% bloom and at 75% bloom) and LS applied once at 75% bloom appear to be the most effective treatments in reducing fruit set, fruit retention and improving fruit size, weight and skin colour in 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple.

5. 1. Introduction

Blossom thinning is one of the most important cultural practices in apple growing for improving fruit quality (Looney, 1993). Apple blossom thinning improves fruit size, colour, and fruit quality at harvest, and promotes return bloom (Childers et al., 1995). Later on, Link (2000) also noted that crop load has a major impact on apple fruit quality and the regularity of bearing.

Crop load is a quantitative parameter used by industry, and is generally defined as the number of fruit per tree (Jones et al., 1992). Usually flower or fruitlet thinning with usage of chemicals is being practised for reducing crop load consequently improving apple fruit size and other quality parameters. Such practice of reducing crop load is coupled with reduced total fruit yield, however, the remaining fruit have higher value because of increased fruit size whereby compensating profit for the reduced yield (Forshey, 1986). Chemical-free flower and fruit thinning reduces chemical input in integrated fruit production as well as reducing production costs and alternate bearing in biological (organic) apple orchards (Bertschinger et al., 1998).

Chemical thinning continues to be the most important practice in modern apple production (Looney, 1986). Knight (1986) regarded the timing of chemical application as critical in order to achieve effective thinning for many apple cultivars. Spray application of carbaryl at petal fall gave the best improvement in apple fruit size (Knight 1986). Spray applications of 6-benzylaminopurine (BA), napthalene acetic acid (NAA), and carbaryl reduced fruit set and yield per tree, but increased fruit size, percent dry weight, soluble solids concentration and promoted return bloom (Paull, 1994). Similarly, Yuan and Greene (2000) reported that spray application of 6-benzylaminopurine (100 $mg.L^{-1}$) effectively thinned apple fruit and increased fruit size. The major objective of fruit thinning is to increase fruit size, therefore there is a negative correlation between fruit size and fruit number (Forshey and Elfving, 1977).

The use of NAA, a synthetic auxin, as a blossom thinner, gained acceptance in the 1950's and 1960's. Another synthetic auxin, napthaleneacetamide (NAD), was found to be suitable for post-bloom thinning of many commercial cultivars of apple (Westwood and Batjer, 1960). The spray applications of carbaryl (1000 mg L^{-1}), ethephon (474 mg L^{-1}) and NAA (58 mg L^{-1}) on 'Golden Delicious' apples reduced

auxin transport to fruitlets which resulted in fruitlet thinning (Ebert and Bangerth, 1982). The spray application of Tergitol TMN-6 (0.75% to 1.25%) at 75 to 80% bloom was effective in blossom thinning of 'Rome Beauty' apples (Fallahi and Greene, 2010). Guak et al. (2004) found that spray application of lime sulphur (LS, up to 4%) at 85% full bloom reduces fruit set. Stopar (2004) also reported a spray application of 3% lime sulphur (CaSx) resulted in severe thinning, consequently reduced crop load and increased fruit weight in 'Golden Delicious' apples. While the effects of crop load on fruit weight and size, as well as on return bloom is well documented, there is, however, limited information available on how time of thinning impacts fruit set, fruit retention and fruit quality.

Timing of chemical blossom thinning is a very important factor influencing its effectiveness in thinning in both apple and peach. It is essential that blossom thinners be applied when partial flowers have fertilised. In apples the best time to spray for blossom thinning, is when the king bloom is open and fertilized, and only one side bloom is open but not fertilized (Fallahi and Willemsen, 2002). The early chemical thinning of apples has been shown to produce larger fruit (Way, 1967; Knight and Spencer, 1987). Mean fruit weight of 'Red Fuji' is dependent on the timing and concentration of chemical application for thinning (Jones et al., 1990). 'Red Delicious' apple trees sprayed with LS at full bloom produced higher fruit weight (150g) compared to control (Jones et al., 1990). Fallahi and Greene (2010) reported early thinning of apple fruit is important because of its impact on fruit size and flower bud initiation in the following season. Organic and integrated apple management systems offer alternative practices that address environmental concerns (Conacher and Conacher, 1998). Organic management practices exclude chemical pesticides and fertilizer inputs and use naturally derived products as defined by organic certification programs. One of the main challenges in organic apple growing is the regulation of the crop load and improving fruit quality; and additionally reducing labour costs for manual thinning. Currently, there are only a few methods and agents allowed for certified organic agriculture. However, chemical and synthetic thinning agents or plant hormones for crop regulation are not allowed for certified organic apple production. Most experiments to regulate fruit set in organic agriculture are based on two strategies: mechanical reduction in the number of flower buds (Roche and Masseron, 2002) and injuring flowers (Ju et al., 2001; Pfeiffer and

Ruess, 2002; Warlop and Libourel, 2002). The efficacy of several products such as mineral oil, corn oil, rape oil, olive oil, vinegar, sodium bicarbonate, sodium salt and lime sulphur have been tested to damage flowers. However, none of these products have been tested in apples under Australian climatic conditions.

Organic apple growers have long used lime sulphur (LS) applied during bloom to reduce the number of viable flowers (Edwards, 1998). Lime sulphur and oil products have been used by organic growers in some countries and achieve partly satisfying thinning results - but is not a recurring practice. Stopar (2004) investigated some blossom thinners for possible use in organic apple production on ten-year-old 'Golden Delicious' trees. A strong thinning (over-thinning) and consequently an accelerated fruit growth occurred, when lime sulphur (3% CaSx) was applied at full bloom (Stopar, 2004). The efficacy of time and number of spray applications of organic bloom thinners under Australian conditions on blossom/fruit thinning along with their effects on fruit quality have not yet been investigated. Therefore, the effects of number and time of spray applications of lime sulphur (LS) at different blossom stages on blossom thinning, fruit set, fruit retention, leaf scorch and fruit quality in organically grown commercial cultivars 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apples were investigated.

5. 2. Materials and Methods

5. 2. 1. Plant materials

The experiment was conducted on 15-year-old apple trees including cultivar 'Cripps Pink^{TM}' and 'Granny Smith' on rootstock M26 and 'Gala' on rootstock MM06 at Newton Brothers Orchards at Manjimup (lat. 34°14 S, long. 116°8 E) Western Australia. The trees were spaced 7.5 m between rows and 2.5 m within rows in the North-South orientation and soil type was gravelly loam.

5. 2. 2. Organic Chemicals

Lime sulphur (LS) (active constituent 200g/L sulphur (s) as polysulfide sulphur) sourced from Richgro, Jandakot, Western Australia and synertrol oil (active constituent 832g/L emulsifiable vegetable oil) purchased from Organic Crop Production, Pty Ltd, Lilyfield, NSW, Australia.

5. 2. 3. Treatments and experimental design

The trees were sprayed with an aqueous emulsion containing lime sulphur (5 %) and 0.5% synertrol as a surfactant. The experimental trees were sprayed at different times of bloom (single spray at 25% bloom or 75% bloom; or two sprays one at 25% bloom followed by a second application at 75% bloom). The emulsion was sprayed onto 'Cripps Pink', 'Granny Smith' and 'Gala' whole trees until runoff by using a sprayer (The Selecta Trolleypak Mk II, Acacia Ridge, Australia). Control trees were kept unsprayed. The experiment was laid out by following two factors (treatments and cultivars) factorial randomised block design with four replicates. Single trees were treated as one experimental unit. Three branches per tree were randomly tagged on the tree. The total numbers of flowers on each branch previously tagged were counted prior to applying spray treatments. Fruit set (%) retention (%), leaf scorch (%), fruit size, weight and colour were recorded. Soluble solids concentration (SSC), titratable acidity (TA) and SSC:TA ratio were determined from the juice of apple fruit harvest at commercial maturity.

5. 2. 4. Observations recorded

5. 2. 4. 1. Fruit set (%)

Number of fruit set from three tagged branches was counted 26 days after the application of various treatments and expressed as percentage fruit set as explained in Chapter 3, Section 3.8.1.

5. 2. 4. 2. Fruit retention (%)

Total number of fruit on each tagged branch was first counted at the fruit set (26 days after application of treatments) and again, one week before commercial harvest. Percentage fruit retention was calculated as outlined in Chapter 3, Section 3.8.2.

5. 2. 4. 3. Leaf scorch (%)

Total numbers of leaves on each tagged branch were counted before the spray application of different treatments. The number of leaves that were scorched was counted 30 days after spray treatment and expressed as percentage leaf scorch as explained in Chapter 3, Section 3.8.3.

5. 2. 5. Fruit quality

5. 2. 5. 1. Fruit size (mm)

Fruit size was measured in mm as the diameter of the fruit using a digital Vernier calliper from ten randomly selected fruit in each replication as described in Chapter 3, Section 3.9.2.

5. 2. 5. 2. Fruit weight (g)

Fruit weight was calculated by weighing ten fruit per replication by using a digital balance (A&D Limited, Tokyo, Japan) and average weight was calculated as grams (g) per fruit as described in Chapter 3, Section 3.9.3.

5. 2. 5. 3. Fruit firmness (N)

Fruit firmness from ten randomly selected fruit was determined using an electronic pressure tester (Model EPT-1 pressure tester, Lake City Technical Products Inc., Kelowna, BC, Canada) as outlined in Chapter 3, Section 3.9.5. Fruit firmness was expressed as N.

5. 2. 5. 4. Surface skin colour: HunterLab ColorFlex

Fruit colour from ten randomly selected fruit was measured on the fruit surface using a HunterLab ColorFlex 458/08 Spectrophotometer (HunterLab ColorFlex, Hunter Associates Inc., Reston, VA, USA) as L*, a* and b*. The data were expressed as Commission Internationale de L'Eclairage (CIE) lightness (L*,a* and b*), chroma (C*) and hue angle (h°) as outlined in Chapter 3, Section 3.9.6.

5. 2. 5. 5. SSC, TA and SSC/TA ratio

Titratable acidity (TA) was determined by titrating the juice with 0.1 N NaOH following the method outlined in Chapter 3, Section 3.9.7. SSC was determined from juice using a digital refractometer as described in Chapter 3, Section 3.9.8. SSC/TA ratio was calculated by dividing SSC with the corresponding TA value as detailed in Chapter 3, Section 3.9.10.

5. 2. 5. 6. Ascorbic acid

The concentration of ascorbic acid from juice was determined following the method described by Jagota and Dani (1982) and Malik and Singh (2005) with some

modifications. The concentration of ascorbic acid was expressed as mg ascorbic acid per 100 ml fresh juice as outlined in Chapter 3, Section 3.9.10.

5. 3. Statistical analysis

The experimental data were subjected to two-way (treatments and cultivars) analysis of variance (ANOVA) using GenStat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK). The effects of various treatments and their interactions for different parameters were assessed within ANOVA and least significance differences (LSD) were calculated following significant F-test ($P \leq 0.05$). The validity of statistical analysis was ensured by checking all the assumptions of ANOVA.

5. 4. Results

5. 4. 1. Fruit set, fruit retention and leaf scorch

Spray applications of LS at different blossom stages significantly ($P \leq 0.05$) reduced mean fruit set, retention, and increased leaf scorch in 'Gala', 'Cripps Pink^{TM}' and 'Granny Smith' apple trees compared to control (Table 5. 1). The reduction in fruit set and retention were more pronounced when trees were treated with LS either as a single spray at 75% full bloom or when LS was applied at 25% followed by its second spray at 75% full bloom stage (Fig. 5. 1a). Spray applications of LS at 25% bloom followed by its second spray at 75% bloom and single spray LS at 75% bloom resulted in significantly lower fruit retention (33.15 and 35.05% respectively) as compared to the other treatments (Fig. 5. 1b). Irrespective of the treatments, there were no significant differences among cultivars for fruit set and retention (Table 5. 1). Leaf scorching was significantly highest (14.45%) with a spray of double application LS at 25% bloom and at 75% blooms as compared to control and when LS was sprayed at 25% full bloom (Table 5. 1 and Fig. 5. 1c). Mean fruit set and fruit retention did not differ significantly among the three cultivars, whilst mean fruit leaf scorch differed significantly. 'Gala' exhibited significantly higher leaf scorch (9.43%) as compared to 'Granny Smith' and 'Cripps Pink'. The interaction between the treatments and the cultivars was found to be significant for all the parameters (Table 5. 1).

Table 5. 1. Effects of number and time of spray applications of lime sulphur (LS) at different blossom stages (25%, 25-75% and 75% bloom) on fruit set (%), fruit retention (%) and leaf scorching (%) in' Gala', 'Granny Smith' and 'Cripps Pink™' apple trees.

		Treatment (T)					LSD ($P \leq 0.05$)		
Parameters	Cultivar (Cv)	Control	5% LS at 25% bloom	5% LS at 25and at 75% bloom	5% L S at 75% bloom	Mean (Cv)	T	Cv	T x Cv
Fruit Set (%)	Gala	65.85	44.83	11.93	15.18	34.44	3.35	NS	5.80
	Granny Smith	56.89	41.96	19.6	23.53	35.49			
	Cripps Pink	61.67	39.87	18.31	20.52	35.09			
	Mean (T)	61.47a	42.22b	16.61c	19.74c				
Fruit retention (%)	*Gala*	*67.3*	*49.5*	*23.1*	*40.6*	*45.1*	*6.38*	*NS*	*11.04*
	Granny Smith	61.3	52.5	39	36.6	47.3			
	Cripps Pink	61	48.3	37.4	28	43.7			
	Mean (T)	63.22a	50.09b	33.15c	35.05c				
Leaf scorch (%)	Gala		7.89	5.14	24.69	9.43 A	3.67	3.18	6.35
	Granny Smith	0	5.2	3.76	16.47	6.36 B			
	Cripps Pink	0	1.44	3.76	6.71	2.98 C			
	Mean (T)	0	4.26 b	14.45 a	12.08 a				

Any two means within a column followed by different uppercase letters and any two means within a row followed by different lowercase letters are significantly different at $P < 0.05$. NS = not significant, n (fruit Set) = four replicates (200 - 300 flowers per tree replicate), n (fruit retention) = four replicates (150 - 250 fruit set per tree replicate), n (leaf scorch) = four replicates (3 branches per tree replicate).

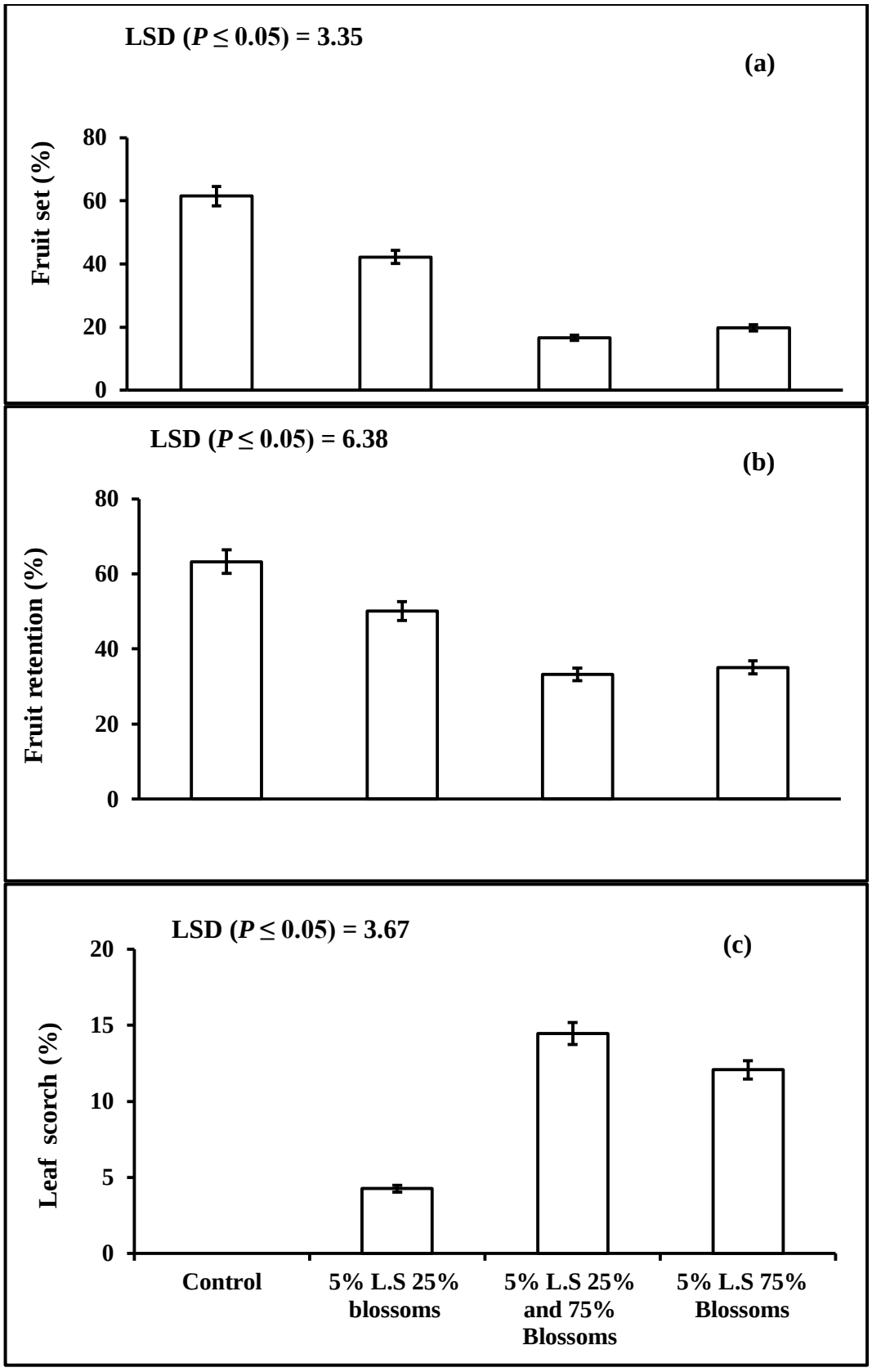

Figure 5. 1. Changes in mean fruit set (a), fruit retention (b) and leaf scorching (c) as influenced by the spray applications of lime sulphur (LS) at different blossom stages (25%, 25-75% and 75% bloom). Vertical bars represent standard error.

5. 4. 2. Fruit size, weight and firmness

All treatments of different time of blossom stages and number of sprays of LS applied on 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple trees showed significant ($P \leq 0.05$) increase in fruit size, weight and firmness in comparison to the control fruit except, the single spray 5% LS at 25% bloom. LS sprayed first at 25% bloom stage followed by second application at 75% bloom stage or LS sprayed once at 75% bloom stage significantly improved fruit size, fruit weight and fruit firmness in 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apples at harvest compared to the other two treatments (Table 5. 2). When averaged across the cultivars, the largest fruit size (67.41-65.69 mm) (Fig. 5. 2a), highest weight (145.1-143.2g) (Fig. 5. 2b) and highest firmness (97.32-93.02 N) (Fig. 5. 2c) were noted in apple trees sprayed with LS twice (once at 25% bloom and again 75% bloom) and when LS was applied once at 75% bloom respectively. There were no statistical differences between control and a single LS (5%) spray at 25% bloom stage. However, there was a direct linear negative significant relationship ($r = - 0.343$ $y = - 0.0547x + 67.16$) between percentage fruit set and fruit size (Fig. 5. 3a). Also, There was a direct linear negative significant relationship ($r = - 0.532$ $y = - 0.4739 x + 151.25$) between percentage fruit set and fruit weight (Fig. 5. 3b). Mean fruit size was largest (66.13 mm) in 'Cripps Pink^{TM}' followed by 'Granny Smith' and 'Gala' (65.44 mm and 64.17 mm respectively). Irrespective of the treatment, there were no significant differences in fruit size and fruit weight between 'Cripps Pink^{TM}' and 'Granny Smith' apples. These cultivars exhibited higher fruit size and weight as compared to cultivar 'Gala'. However, 'Gala' and 'Cripps Pink^{TM}' apples were significantly firmer (92.13 and 91.08 N respectively) compared to 'Granny Smith' (88.22N) apples at commercial harvest (Table 5. 2). The interaction between the cultivars and the treatments also differed significantly only for fruit size (Table 5. 2).

Table 5. 2 . Effects of number and time of spray applications of lime sulphur (LS) at different blossom stages (25%, 25-75 % and 75% bloom) on fruit size, weight, and firmness of 'Gala', 'Granny Smith' and 'Cripps Pink™'.

		Treatments (T)					LSD ($P \leq 0.05$)		
Parameters	Cultivar (Cv)	Control	5% L. S at 25% bloom	5% L. S at 25 and at 75% bloom	5% L. S at 75% bloom	Mean (Cv)	T	Cv	T x Cv
Fruit Size (mm)	Gala	65.79	63.68	66.57	60.63	64.17 B	1.38	1.20	2.39
	Granny Smith	63.45	64.96	65.82	67.52	65.44 A			
	Cripps Pink	61.91	63.83	69.84	68.92	66.13 A			
	Mean (T)	63.72c	64.16c	67.41a	65.69b				
Fruit Weight (g)	Gala	112.4	120.3	137.9	125.9	124.2 B	9.84	8.53	NS
	Granny Smith	132.0	133.7	140.7	145.7	138.1 A			
	Cripps Pink	123.7	128.5	156.7	158.0	141.7 A			
	Mean (T)	122.7 b	127.5 b	145.1 a	143.2 a				
Firmness (N)	Gala	86.89	89.33	99.22	93.06	92.13 A	2.54	2.20	NS
	Granny Smith	80.65	82.67	95.17	94.4	88.22 B			
	Cripps Pink	86.87	88.27	97.55	91.6	91.08 A			
	Mean (T)	84.81 c	86.76 c	97.32 a	93.02 b				

Any two means within a column followed by different uppercase letters and any two means within a row followed by different lowercase letters are significantly different at $P \leq 0.05$. NS = not significant, n = four replicates (10 fruit per replicate).

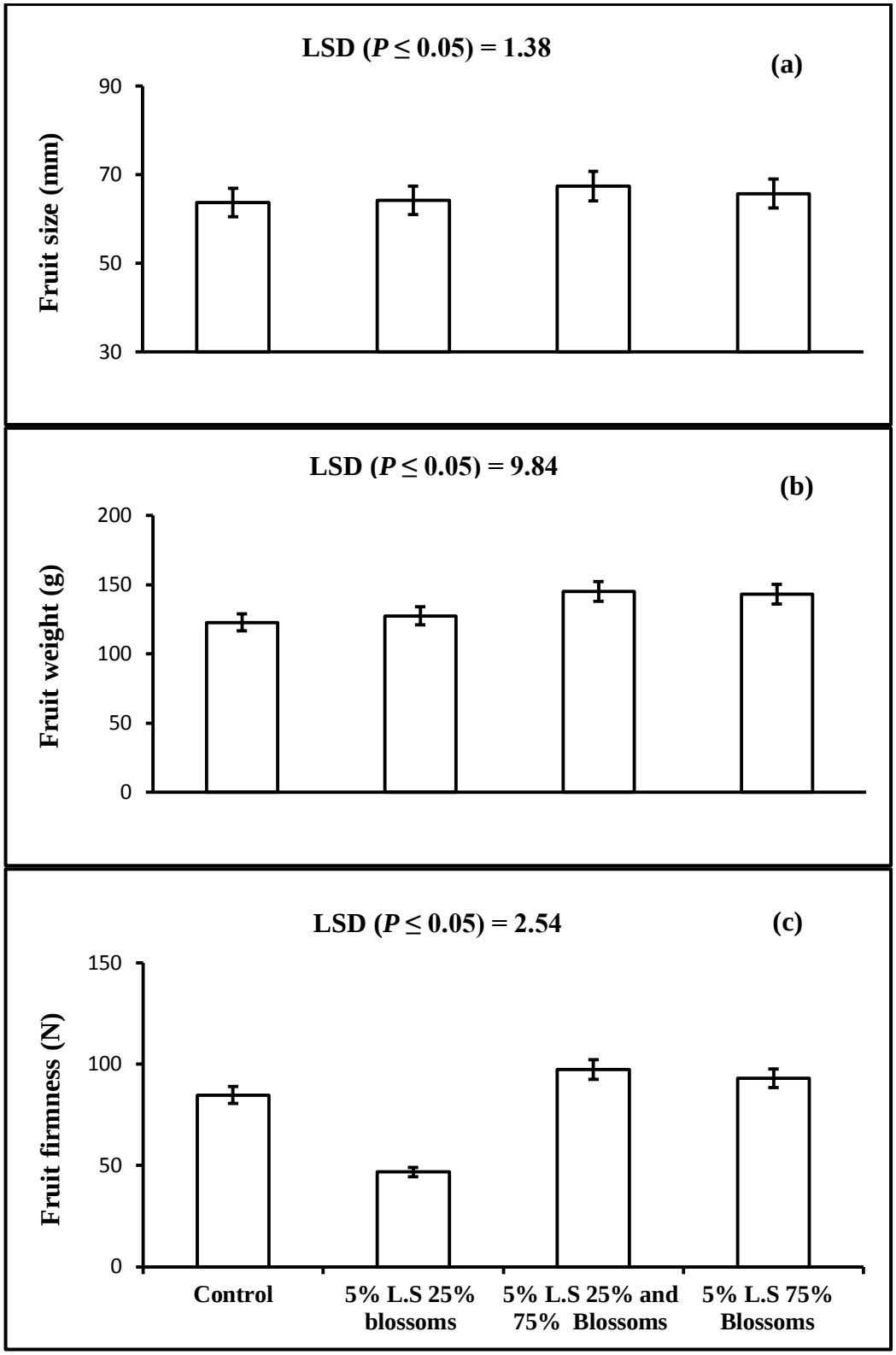

Figure 5. 2. Changes in mean fruit size (a), fruit weight (b) and fruit firmness (c) as influenced by the number of spray applications of lime sulphur (LS) and different bloom stages (25%, 25-75% and 75% bloom). Vertical bars represent standard error.

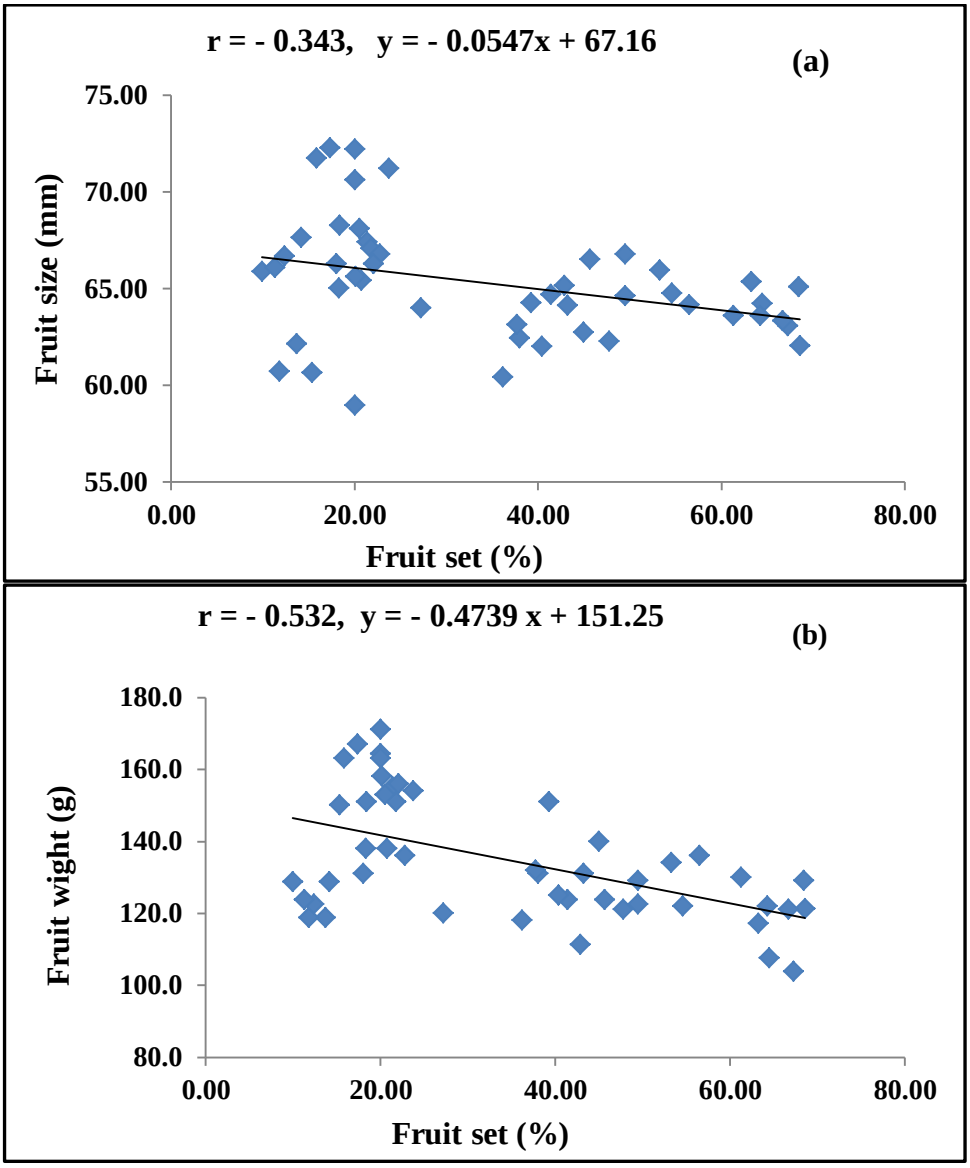

Figure 5. 3. Relationship between percentage fruit set and fruit size (a) and percentage fruit set and fruit weight (b) as regulated by the number of spray applications of lime sulphur and different blossom stages (25%, 25-75% and 75% blossom) in 'Gala' and 'Cripps Pink^{TM}' apple trees.

5. 4. 3. Skin colour

Fruit colour parameters were recorded only from coloured cultivars 'Gala' and 'Cripps Pink^{TM}' not from green coloured fruit cultivar 'Granny Smith'. Fruit skin colour of 'Gala' and 'Cripps Pink^{TM}' apples was significantly ($P \leq 0.05$) affected by number of LS sprays and their time of application at different bloom stages (Table 5. 3). The mean values L*, a*, and hue angle were lower on the fruit treated with two spray applications of LS, first at 25% bloom followed by second at 75% bloom stage in both cultivars, (Figs. 5. 4a, b and e). Lower hue angles in 'Gala' and 'Cripps Pink^{TM}' apples indicate redder fruit colour. Mean b* and chroma (saturation of colour) were lower in trees that received a single spray application of LS at 25% bloom and untreated trees. On the other hand, the mean L*, a*, and hue angle were higher in the fruit from untreated trees and fruit treated with a single spray application of LS at 25% bloom in both cultivars (Figure 5. 4a, b and e and Table 5. 3). Irrespective of the treatments, mean L*, a*, chroma and hue angle of fruit differed significantly between both cultivars. Mean L* and H° values were higher in 'Cripps Pink^{TM}' apple fruit (49.61 and 33.78 respectively) than 'Gala' apple fruit (44.07 and 28.97 respectively). Meanwhile, mean a* and chroma values were higher in 'Gala' apple fruit (40.41 and 46.47 respectively) than 'Cripps Pink^{TM}' apple fruit (36.04 and 43.77 respectively). The interactions between the cultivars and the treatments for L*, a*, b*, chroma and hue angle were found be significant (Table. 5. 3).

Table 5. 3. Effects of number and time of spray applications of lime sulphur (LS) at different blossom stages in 'Gala' and 'Cripps Pink^{TM}' apple.

		Treatments (T)					LSD ($P \leq 0.05$)		
Colour Parameters	Cultivar (Cv)	Control	5% LS at 25% bloom	5% LS at 25-75% bloom	5% LS at 75% bloom	Mean cv	T	Cv	T x Cv
Lightness (L*)	Gala	46.85	45.57	42.93	40.92	44.07 B	3.6	2.54	5.09
	Cripps Pink	46.66	53.39	47.98	50.41	49.61 A			
	Mean (T)	46.75 ab	49.48 a	45.46 b	45.67 b				
a*	Gala	36.79	45.22	39.55	40.09	40.41 A	3.44	2.43	4.87
	Cripps Pink	41.04	39.0	29.03	35.1	36.04 B			
	Mean (T)	38.92 ab	42.11 a	34.29 c	37.59 bc				
b*	Gala	21.25	20.34	29.79	18.97	22.59	2.42	NS	3.42
	Cripps Pink	20.42	25.55	21.89	24.52	23.09			
	Mean (T)	20.83 B	22.95 B	25.84 A	21.75 B				
Chroma (C*)	Gala	42.69	44.54	54.25	44.40	46.47 A	2.073	1.47	2.93
	Cripps Pink	45.99	40.31	45.18	43.61	43.77 B			
	Mean (T)	44.34 b	42.42 b	49.72 a	44.01 a				
Hue Angle (h^o)	Gala	33.39	27.29	30	25.20	28.97 B	5.18	3.66	7.32
	Cripps Pink	30.05	42.52	26.77	35.79	33.78 A			
	Mean (T)	31.71 ab	34.91 a	28.39 b	30.49 ab				

Any two means within a column followed by different uppercase letters and any two means within a row followed by different lowercase letters are significantly different at $P < 0.05$. NS = not significant, n = four replicates (10 fruit per replicate).

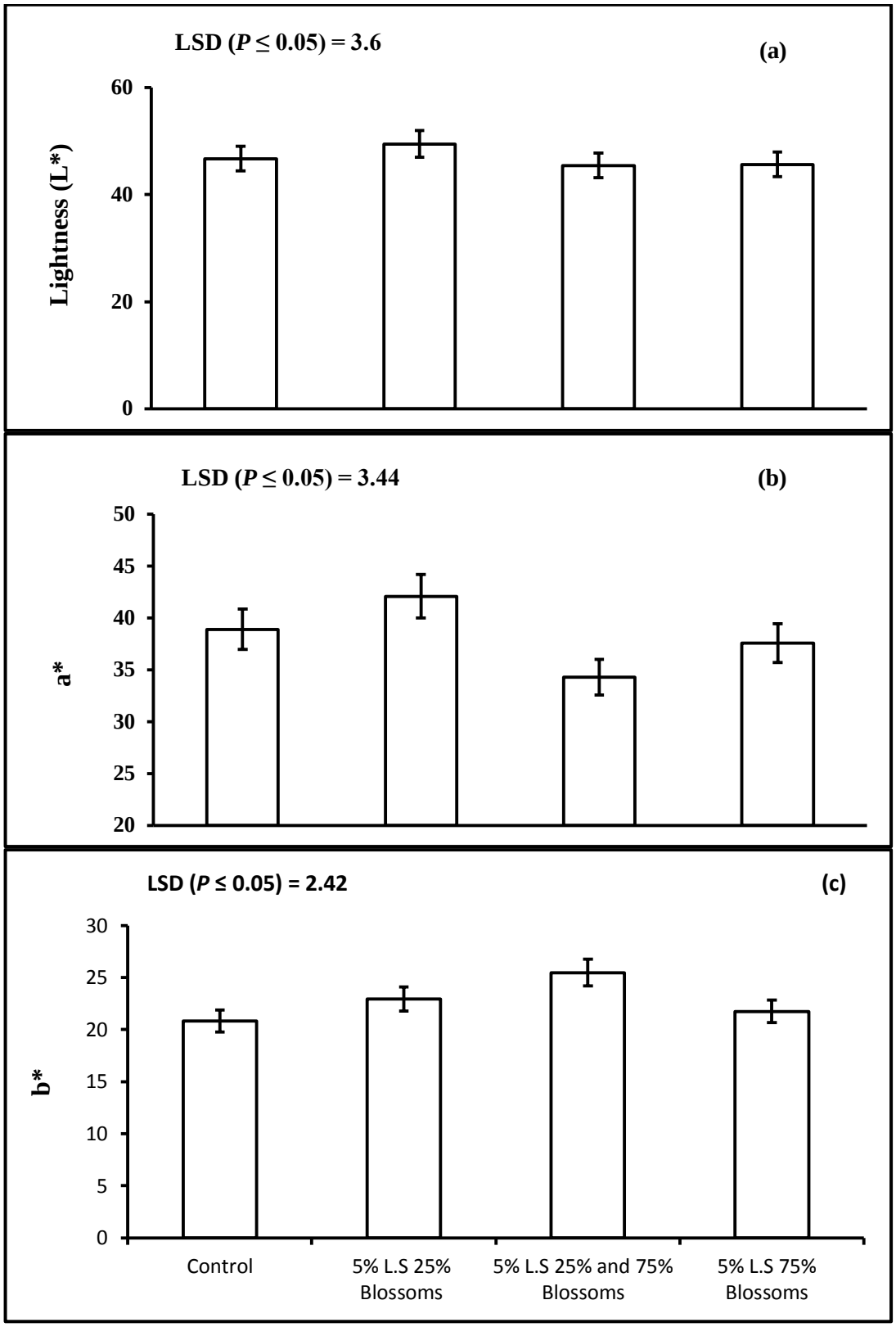

Figure 5. 4. Changes in mean chromaticity (L*, a*, b*) of fruit skin influenced with the number and time of spray applications of lime sulphur at different blossom stages. Vertical bars represent standard error.

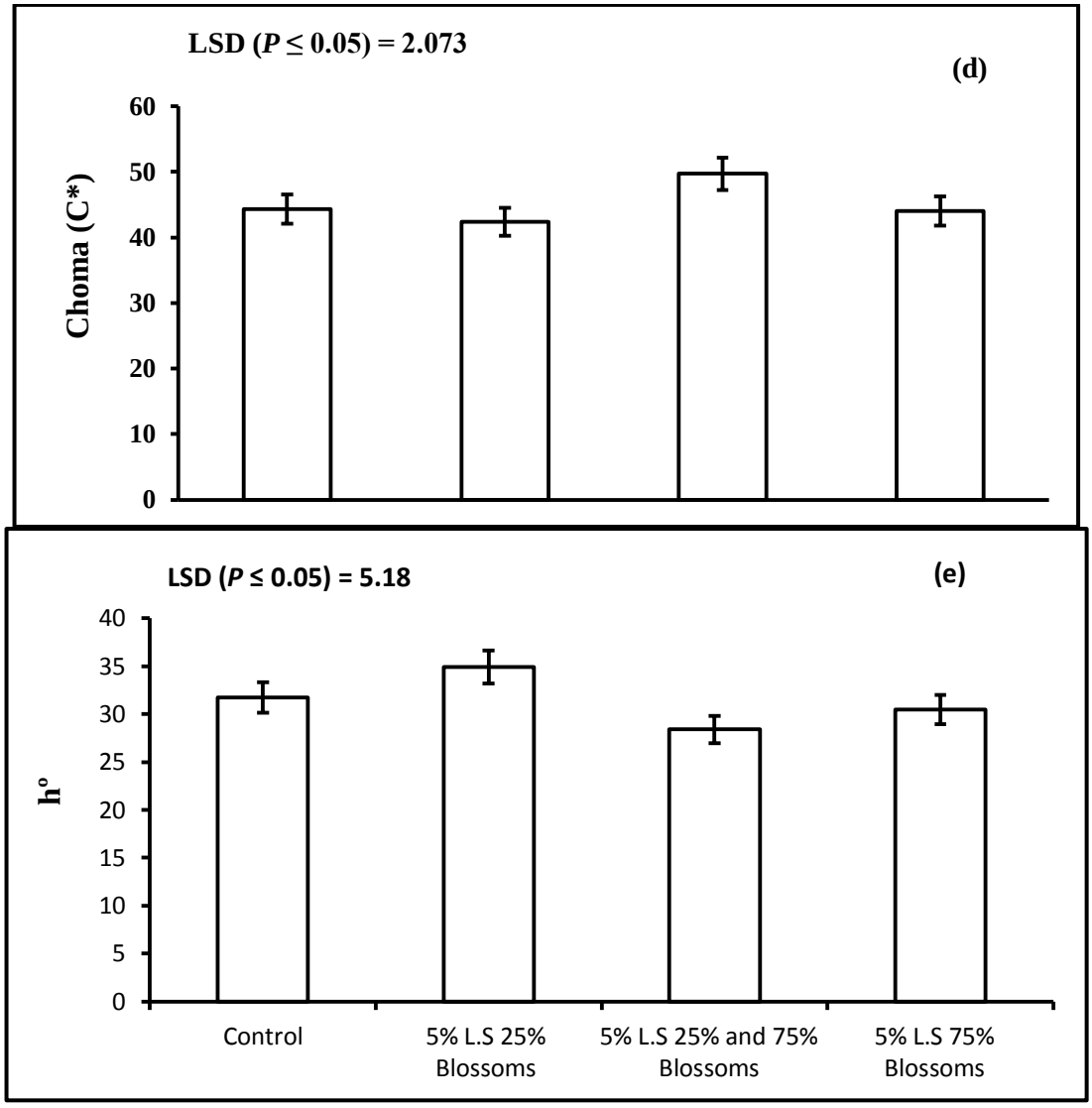

Figure 5. 4. Changes in chromaticity (C*, and h^{o}) influenced with the number and time of spray applications of lime sulphur at different bloom stages. Vertical bars represent standard error.

5. 4. 4. TA, SSC, SSC:TA ratio and ascorbic acid

LS treatments did not significantly ($P \leq 0.05$) affect SSC, SSC:TA ratio and levels of ascorbic acid in the juice of 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apples as compared to control (Table 5. 4). TA was significantly higher in trees sprayed with LS at 75% bloom once (2.13%) and also when LS was applied twice (25% bloom and again at 75% bloom - 2.15%) as compared to control (1.92%) (Table 5. 4 and Fig. 5. 5 b). When averaged over the treatments, mean SSC, TA and SSC:TA ratio differed significantly among cultivars. However, ascorbic acid levels did not differ significantly between the three cultivars (Table 5. 4). SSC was highest in 'Cripps Pink^{TM}' apple (13.53 %) compared to the other two cultivars. TA was higher in the juice of 'Cripps Pink^{TM}' and 'Granny Smith' apple (2.46 % and 2.08 % respectively) compared to 'Gala' (1.64 %). SSC:TA ratio was significantly higher in 'Gala' (7.05) compared to 'Granny Smith' and 'Cripps Pink^{TM}' (5.54-5.53 respectively) apples. The interaction between the cultivars and different treatments was significant only for ascorbic acid content Table (5. 4).

Table 5. 4 . Effects of number and time of spray applications of lime sulphur (LS) at different blossom stages on soluble solids concentration (SSC), titratable acidity (TA), SSC:TA ratio and ascorbic acid levels in the juice of 'Gala', 'Granny Smith' and 'Cripps Pink™' apple

	Treatments (T)						LSD ($P \leq 0.05$)		
	Cultivar (Cv)	Control	5% L S at 25% bloom	5% L. S at 25-75% bloom	5% L.S at 75% bloom	Mean (Cv)	T	Cv	T x Cv
SSC (%)	Gala	12.2	11.3	11.25	11.32	11.52 B			
	Granny Smith	10.57	11.4	11.85	11.67	11.32 B			
	Cripps Pink	13	13.52	13.57	14.02	13.53 A	NS	0.599	NS
	Mean (T)	11.92	12.07	12.22	12.34				
TA (malic acid %)	Gala	1.65	1.63	1.52	1.75	1.64 C			
	Granny Smith	1.88	2	2.27	2.15	2.08 B			
	Cripps Pink	2.23	2.48	2.65	2.5	2.46 A	0.18	0.15	NS
	Mean (T)	1.92 b	2.03 ab	2.15 a	2.13 a				
SSC:TA ratio	Gala	7.40	6.98	7.34	6.48	7.05 A			
	Granny Smith	5.65	5.70	5.34	5.48	5.54 B			
	Cripps Pink	5.86	5.49	5.15	5.63	5.53 B	NS	0.338	NS
	Mean (T)	6.30 a	6.06 ab	5.94 ab	5.86 b				
Ascorbic acid ($mg \cdot 100ml^{-1}$ FW)	Gala	32.65	34.56	38.93	35.82	35.49			
	Granny Smith	32.23	36.47	36.73	34.30	34.93	NS	NS	4.68
	Cripps Pink	39.74	35.20	35.24	32.87	35.76			
	Mean (T)	34.87 a	35.41 a	36.96 a	34.33 a				

Any two means within a column followed by different uppercase letters and any two means within a row followed by different lowercase letters are significantly different at $P < 0.05$. NS = not significant, n = four replicates (10 fruit per replicate).

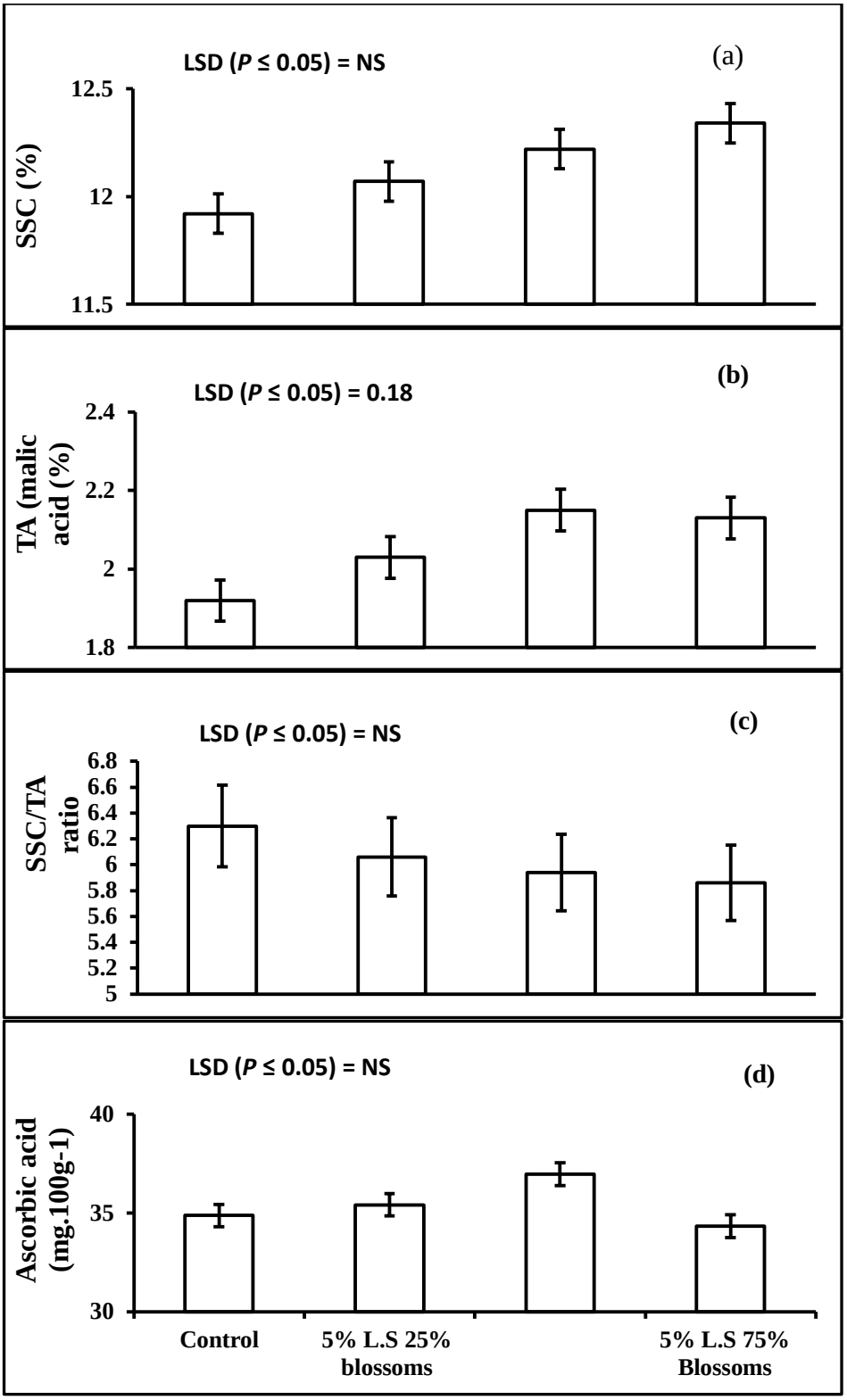

Figure 5. 5. Changes in mean soluble solids concentration (SSC) (a), titratable acidity (TA) (b), SSC:TA ratio (c) and ascorbic acid (d) levels as influenced by the number of sprays and time of applications of lime sulphur (LS) at different bloom stages. Vertical bars represent standard error.

5. 5. Discussion

Lime sulphur (LS) is commonly used in horticulture as a fungicide to control various fungal diseases. It is composed of lime (calcium hydroxide) and inorganic sulphur and is used to control diseases such as peach leaf curl, plum pockets and black spot in roses (Hazeu et al., 1988; Lanasa et al., 2004). However, sulphur is toxic to some plants and lime is used to reduce its toxicity (Hazeu et al., 1988 and Hell, 1997). Blossom thinning is the most important technique in apple growing for improving fruit quality (Looney, 1993). LS spray application has significantly reduced fruit set and fruit retention in all apple cultivars tested as compared to control (Table 5. 1). These results conform with earlier findings that 4% LS applied at 85% full bloom reduced fruit set and fruit retention in 'Gala' and 'Fuji' apple (Guak et al., 2002; Guak et al., 2004; Osborne, 2006). The reduction in fruit set and fruit retention in my study were more pronounced with double application of LS (first at 25% bloom second at 75% bloom stage) and also single application of LS when applied at later bloom stage (75%) (Table 5. 1). Reduced fruit set may be attributed to higher blossom thinning. Similarly, Fallahi et al., (2004) reported that double applications of blossom thinners such as ammonium thiosulfate caused higher blossom thinning in 'Fuji' apples. Time of application of various blossoms thinner is a very important factor influencing the effectiveness of blossom thinning in both apple and peach. In apples, the best time to spray for blossom thinning, is when the king flower (central dominant blossom in the cluster) is open and fertilized, and only one side bloom is open but not fertilized (Fallahi and Willemsen, 2002). Therefore, the reduction in fruit set and fruit retention observed with LS sprayed at 75% bloom (either as a single or as a double spray) could be ascribed to impeding fertilisation in the flowers. Similarly, earlier Myraa et al., (2011) reported that spray application of LS at blossom time reduced pollen germination, which ultimately reduces the fruit set.

Post-bloom thinning must be implemented during a critical period of fruit development. Timing of post-bloom sprays on apple has been based on fruit size and/or days after bloom (Williams, 1994). Chemical thinning is applied during post-bloom time period because it is the best time for carbohydrate demand by the actively growing fruits. Paull (1994) noted that BA is an effective thinner in several

apple cultivars, at a range of concentrations and timings. Fruit set in 'Delicious' was reduced with a 25 mg-litre1 BA spray applied before full bloom (Masabni, 1988). Lime sulphur (LS) is most effective as a blossom thinner when applied at 80% bloom. All treatments of LS had a significant ($P \leq 0.05$) effect on leaf scorching in 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apples compared to control (Fig 5. 1c). Higher percentage of leaf scorching was observed in the trees of all cultivars treated with double application LS at 25% bloom and at 75% bloom. This finding indicates that LS is responsible for the burning of leaves on the apple tree, whilst reducing fruit set and retention. Southwick et al., (1996) reported that application of Armothin 5% reduced fruit set in peach, although some phytotoxicity symptoms (slight leaf yellowing and burning and young shoot dieback in canopy interior) were observed without affecting the yield or fruit quality.

The thinning of blossoms, rather than developing fruitlets maximizes the ability to adjust the fruit-to-leaf ratio, a method particularly desirable in early ripening peach cultivars with a short fruit developmental period and fruit sizing problems (Byers and Lyons, 1984; Havis, 1962). Also, Southwick et al., (1996) confirmed that bloom thinning maximizes the tree's capacity to allocate sufficient photosynthates to fruit when the leaf-to-fruit ratio is increased in earlier fruit growth stages.

All the blossom thinning treatments improved fruit size compared to the control (Table 5. 2). Double application LS at 25% bloom and at 75% bloom and single application of LS at 75% bloom reduced fruit set and retention leading to larger fruit size with higher weight compared to a single spray of LS at 25% bloom and untreated trees (Table 5. 2). Earlier reports also suggested that reduction of crop load with blossom thinning treatments resulted in improved fruit weight and size in apple (Guak et al., 2004; Osborne et al., 2006; Fallahi and Green 2010; Link, 2000). Stanley et al., (2000) also reported that lower fruit load allows individual fruit a greater share of resources thereby allowing cells to increase to the maximum size. There is a negative relationship between fruit set and fruit number (Fig 5. 3a) and similarly between fruit set and fruit weight (Fig 5. 3b). This has been confirmed by many researchers and is one of the main reasons for using fruit thinning in apple orchards (Wertheim 1997) and (Neilsen et al., 2001). Increasing the crop load

increased the number of small fruit on the tree and decreased the average fruit size and weight (Stopar et al., 2002). Fruit size is affected by cell division and differences in fruit size are mainly due to differences in the number and individual size of cells within the fruit cortex and pith (Smith, 1950; Martin et al., 1964; Sugiura et al., 1995; Webster 1997). Webster (1997) reported that cell numbers are determined within the first few weeks of fruit development. The characteristic size for each cultivar is determined primarily by the degree of cell multiplication occurring after pollination (Smith, 1950). Therefore timing the application of blossom thinners such as LS is critical and this study suggests that application at 75% bloom is beneficial. Factors affecting cell number and cell size of apples are economically important because they will determine final fruit size (Westwood et al., 1967). All LS treatments at different blossom stages significantly influenced fruit firmness (Table 5. 2), titratable acidity (TA) and SSC:TA ratio (Table 5. 4) in 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple. SSC and concentration of ascorbic acid in apple juice at harvest did not differ significantly ($P \leq 0.05$) in all cultivars. Fruit firmness, SSC:TA ratio and titratable acidity increased with double application of LS, first at 25% bloom and second at 75% bloom and with a single application of lime sulphur at 75% bloom. These results are similar to that reported by Guak et al., (2004) where they found increased fruit firmness, juice soluble solids and titratable acidity in 'Gala' and 'Fuji' apples treated with high LS concentrations. Furthermore, Garriz et al., (2000) also found that fruit firmness was significantly lower in 'Braeburn' trees carrying high crop loads than in trees with moderate or low crop loads. Therefore, improved fruit firmness observed in my experiment with LS treatments at 75% bloom may be attributed to reduced crop load. Jones et al. (1997b) also reported increased firmness with reduced crop load following chemical thinning in 'Pink Lady' and 'Jonagold' apples with ethephon and benzylaminopurine (BA). However, Link, (2000) suggested that the reduced firmness often observed in heavily cropped trees could be due to limited carbohydrate supply for cell wall synthesis. In the current study, bloom thinning produced softer fruit than trees with less thinning. It is possible that high bloom thinning resulted in earlier fruit maturity and increased soluble solids than those treated with lower bloom thinning.

On the other hand, bloom thinning treatments with LS significantly improved ($P \leq 0.05$) the skin colour of 'Gala' and 'Cripps Pink^{TM}' apple fruit. Mean values L*,

a* and hue angle (h°) were lower in fruit treated with double application of LS at 25% bloom and at 75% bloom in both cultivars compared to control and all other treatments indicating that fruit were more red. Meanwhile, the mean b* and chroma were highest on the fruit treated with a single application of LS at 75% bloom in both cultivars compared to control and all others treatments (Table 5. 3). Spark, (2007) reported that apple trees that bear larger fruit develop colour earlier and also reach maturity earlier. Trees sprayed with LS at 75% bloom (either once or twice) resulted in larger size fruit (Table 5. 2) which could indicate that these fruit matured earlier and hence the improvement in colour.

In conclusion, both double applications of 5% LS first at 25% bloom and second at 75% bloom and a single application of LS at 75% bloom on 'Gala', 'Granny Smith' and 'Cripps Pink^{TM}' apple trees reduced fruit set and increased the fruit size, improved fruit colour, while reduced firmness due to slightly over-ripened fruit. Single spray application of lime sulphur (5% at 75% bloom) improved apple fruit size, colour and lessened leaf scorch than its double spray, while also more cost effective and hence improving crop value under Western Australian conditions.

Chapter 6

Efficacy of different concentrations of lime sulphur alone or in combination with olive oil on blossom thinning and fruit quality in organically grown 'Cripps Pink™' and 'Gala' apples

Abstract

To determine the effects of different concentrations of lime sulphur (LS) alone or in combination with organic olive oil on blossom thinning, fruit retention, fruit size and fruit quality, organically grown, 'Gala' and 'Cripps Pink™' apple trees were sprayed with an aqueous emulsion containing different concentrations (1, 2, 3 or 4%) of LS alone or in combination with olive oil (3%) and 0.05% synertrol oil as a surfactant. Aqueous emulsions were sprayed onto whole trees until runoff at 75% bloom stage. Unsprayed trees were kept as control. All LS treatments significantly ($P \leq 0.05$) reduced fruit set. Spray applications of LS (3 - 4%) alone were most effective in reducing fruit set (7-11%) but caused higher leaf scorch (13-14%) compared to control and other treatments. The combination of LS and olive oil reduced leaf scorching compared to LS spray alone. Spray application of LS (4%) in combination with olive oil (3%) appears to be the most effective treatment in blossom thinning and reducing fruit set in 'Gala' and 'Cripps Pink™' apple trees (14.9% and 15.6% respectively) with lower percentage of leaf scorching (10.1% and 12.1%) compared to LS alone. This treatment also improved fruit size, increased concentrations of individual and total sugars as well as organic acids in the juice of both cultivars and improved fruit skin colour. However, fruit firmness was reduced which may be attributed to slightly over-ripened fruit in both 'Gala' and 'Cripps Pink™' apple.

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6. 1. Introduction

Blossom thinning in apples is one of the most important management practices a commercial grower is required to do to produce high quality fruit. Blossom thinning may increase value of the crop one to three times because of increased fruit size, yield and price (Byers et al., 2003). Crop load has a major impact on apple fruit quality and the regularity of bearing (Link, 2000). Heavy crop load has negative effects on fruit quality and on the initiation of flower buds in the following season (Pfeiffer and Englert, 2003). According to Southwick et al., (1996) bloom thinning can maximize the tree's capacity to allocate sufficient resources to fruit when the leaf-to-fruit ratio is low early in the growing season. Meanwhile, Byers and Lyons, (1984) reported with caustic thinners applied from pink to full bloom stage, the greatest response has been when applications are made near bloom. Chemicals such as hydrogen cyanamide (Dormex), sulfcarbamide (Wilthin), pelargonic acid (Thinex), endothalic acid (Entohal) and ammonium thiosulfate have been investigated as potential blossom thinners in apples with satisfactory results in thinning and fruit set in 'Delicious' and 'Rome' apples (Fallahi et al., 2004; Fallahi and Willemsen, 2002; Fallahi and Green, 2010). Chemical thinning continues to be the most important practice in modern apple production (Looney, 1986). Effective chemical fruit thinning in apples would reduce production costs and increase fruit size (Osborne et al., 2006). Byers et al., (2003) reported that blossom thinning can result in a 7 to 30% increase in peach fruit size and yield when compared to hand thinning fruit 40-50 days after full bloom. Yuan and Greene, (2000) found that a spray application of 6-benzyladenine (100 $mg1^{-1}$) effectively thinned apple fruit and increased the size. The spray application of carbaryl (1000 mg L^{-1}), ethephon (474 mg L^{-1}) and naphthalene acetic acid (58 mg L^{-1}) on 'Golden Delicious' apples reduced auxin transport to fruitlets - leading to thinning (Ebert and Bangerth, 1982). The application of Tergitol TMN-6 (0.75% to 1.25%) at 75 to 80% bloom was effective in blossom thinning of 'Rome Beauty' apples (Fallahi and Greene, 2010). Guak et al. (2004) found that spray application of lime sulphur (LS) (up to 4%) at 85% bloom stage reduces fruit set. Stopar, (2004) reported a spray application of 3% lime sulphur (CaSx) to 'Golden Delicious' apple trees resulted in severe thinning, consequently reduced crop load and increased fruit weight.

In organic orchards, however, the use of chemicals or growth regulators to regulate crop load is not permitted. Chemical-free flower and fruit thinning reduces the use of chemicals in integrated fruit production and regulates alternate bearing in organic apple orchards (Bertschinger et al., 1998). The alternative to chemical thinning would involve manual thinning in organic orchards which is not a feasible management practice due to the high cost of production. Fallahi and Greene, (2010) reported early thinning of apple fruit is important because of its impact on fruit size and next season's flower bud initiation. Tromp, (2000) and Greene, (2002) suggested that fruitlets should be thinned up to a few weeks after flowering to prevent biennial bearing in apples. Hand picking of these fruitlets would then be impractical as an organic management practice. Presently, there are only a few methods and agents allowed for certified organic agriculture. In organic fruit production, fruit set is regulated by either mechanically reducing the number of flower buds (Roche and Masseron, 2002) or by injuring the flowers (Ju et al., 2001; Pfeiffer and Ruess, 2002). A number of organic compounds capable of thinning flowers and fruitlets in pome and stone fruit have been studied. These include table salt, soap, molasses, dextrin (Stopar, 2004; Embree and Foster, 1999). The mode of action of some organic compounds such as Safer-Soap (potassium salts of fatty acids), PEG-1000 (HO(-CH2CH2O-)NH), Anti-Stress (acrylic polymers), Nutri-Safe (N,O-carboxy-methylchitosan), Biofilm (alkylaryl-polyethoxy ethanol + fatty acids + phosphatic acids + isopropanol) is to hamper pollen germination and pollen-tube growth by covering the stigma (Bertshinger et al., 1998; Embree and Foster, 1999).

Apart from injuring flowers (Ju et al., 2001; Pfeiffer and Ruess, 2002) and mechanical reduction in the number of flower buds (Roche and Masseron, 2002), lime sulphur (calcium polysulphide) is considered as an organic chemical that is approved by EU legislation to be used in organic apple production. LS is an effective blossom thinning agent in apples (Bertschinger et al., 2000; Meland, 1998; Stopar, 2004). Stopar, (2004) reported over-thinning and enhanced fruit growth in ten-year old 'Golden Delicious' apples, when 3% lime sulphur (LS) as applied at full bloom stage. Guak et al., (2004) reported that 4% LS applied at 85% full bloom stage to 'Fuji' and 'Gala' trees reduced crop load and increased the number of fruiting sites with one fruit without affecting fruit quality parameters such as fruit size and fruit appearance. Other products such as corn oil, rape oil, olive oil, vinegar, sodium

bicarbonate and sodium salt have been tested in different studies as organic compounds for thinning fruit crop with mixed results (Stopar, 2004). Nevertheless, none of these products have been tested under Australian climatic conditions.

The efficacy of organic blossom thinners on flower/fruit thinning along with their effects on apple fruit quality have not yet been investigated under Australian conditions. Therefore, the objectives of this study were to investigate the effects of spray applications of different concentrations (0, 1, 2, 3 or 4%) of lime sulphur alone and in combination with organic olive oil (3%) and 0.5% synertrol oil on blossom thinning, fruit set, fruit retention as well as fruit quality in organically grown 'Gala' and 'Cripps Pink^{TM}' apples.

6. 2. Materials and Methods

6. 2. 1. Plant materials

The experiment was conducted on three commercially important cultivars of apples 'Cripps Pink^{TM}' grafted on rootstock M26 and 'Gala' on rootstock MM06 apple trees of 15 years old at the Newton Brothers Orchards in Manjimup (latitude 34°14´ South, longitude 116°8´ East) Western Australia. The trees were spaced 7.5 m between rows and 2.5 m within rows in the North-South orientation. All the experimental organically grown apple trees received similar cultural practices including nutrition, irrigation and plant protection (McCoy, 2007) except varying experimental treatments.

6. 2. 2. Organic Chemicals

Lime sulphur (active constituent 200g/L sulphur (s) as polysulfide) was sourced from Richgro, Jandakot, Western Australia. Synertrol oil was purchased from Organic Crop Production, Pty Ltd, Lilyfield, NSW, Australia. Organic olive oil was purchased from Cypressa, UK.

6. 2. 3. Treatments and experimental design

Aqueous emulsion containing different concentrations (1, 2, 3 or 4%) of LS alone or in combination with organic olive oil (3%) and 0.5% synertrol oil as a surfactant were sprayed onto whole trees. Fifteen- year old 'Cripps Pink^{TM}' and 'Gala' experimental trees were sprayed at 75% bloom stage by using a sprayer (The

Selecta Trolleypak Mk II, Acacia Ridge, Australia). Control trees were kept unsprayed. The experiment was laid out by following two-factor (treatments and cultivars) factorial randomised block design with four replicates. Single trees were treated as one experimental unit. Three branches were randomly tagged on the tree and total number of flowers on each branch was counted prior to applying spray treatments. Fruit set (%), fruit retention (%), leaf scorch (%), fruit size, fruit weight and colour were recorded. Soluble solids concentration (SSC), titratable acidity (TA), SSC:TA ratio and ascorbic acid levels were determined from the apple fruit juice following harvest.

6. 2. 4. Observations recorded

6. 2. 4. 1. Fruit set (%)

Total numbers of flowers per tagged branch were counted at time of spray application and number of fruit set per branch was counted 26 days after the application of blossom thinner treatments. Percentage fruit set was calculated as explained in Chapter 3, Section 3.8.1.

6. 2. 4. 2. Fruit retention (%)

Three branches per tree were randomly tagged and total number of fruit on each branch was counted at fruit set and later on, one week before harvest. Percentage fruit retention was calculated as outlined in Chapter 3, Section 3.8.2.

6. 2. 4. 3. Leaf scorch (%)

Three branches per tree were randomly tagged and total numbers of leaves on each branch were counted before the application of treatments. Number of scorched leaves from three branches was counted after 30 days of spray treatment and percentage leaf scorch was calculated as explained in Chapter 3, Section 3.8.3.

6. 2. 5. Fruit quality

6. 2. 5. 1. Fruit size (mm)

Fruit size was measured as the diameter using a digital Vernier calliper from ten randomly selected fruit in each replication as described in detail in Chapter 3, Section 3.9.2.

6. 2. 5. 2. Fruit weight (g)

Fruit weight was calculated by weighing ten fruit per replication by using a digital balance (A&D Limited, Tokyo, Japan) and average weight was calculated as grams (g) per fruit as explained in Chapter 3, Section 3.9.3.

6. 2. 5. 3. Fruit firmness (N)

Fruit firmness from ten randomly selected fruit was determined using an electronic pressure tester (Model EPT-1 pressure tester, Lake City Technical Products Inc., Kelowna, BC, Canada) as outlined in Chapter 3, Section 3.9.5. Fruit firmness was expressed in Newtons (N).

6. 2. 5. 4. Surface skin colour: HunterLab ColorFlex

Fruit colour from ten randomly selected fruit was measured on the fruit surface using a HunterLab ColorFlex 458/08 Spectrophotometer (HunterLab ColorFlex, Hunter Associates Inc., Reston, VA, USA) as L*, a* and b*. The data were expressed as Commission Internationale de L'Eclairage (CIE) lightness (L*, a*, b*), chroma (C*) and hue angle (h°) as outlined in Chapter 3, Section 3.9.6.

6. 2. 5. 5. SSC, TA and SSC:TA ratio

TA was determined by titrating the juice with 0.1 N NaOH following the method outlined in Chapter 3, Section 3.9.7. SSC was recorded using digital refractometer as described in Chapter 3, Section 3.9.8. SSC:TA ratio was calculated dividing SSC with the corresponding TA value as detailed in Chapter 3, Section 3.9.9.

6. 2. 5. 6. Ascorbic acid

The concentration of ascorbic acid from juice was determined following the method of Jagota and Dani (1982) and Malik and Singh (2005) with some modifications as outlined in Chapter 3, Section 3.9.10. The concentration of ascorbic acid was expressed as mg ascorbic acid per 100 ml fresh juice.

6. 3. Statistical analysis

The experimental data were subjected to two-way analysis of variance (ANOVA) using GenStat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental

Station, Rothamsted, UK). The effects of various treatments and their interactions for different parameters were assessed within ANOVA and least significance differences (LSD) were calculated following significant F-test ($P \leq 0.05$). All the assumptions of analysis were checked to ensure validity of statistical analysis.

6. 4. Results

Spray applications of different concentrations (1, 2, 3 or 4%) of LS alone and in combination with organic olive oil (3%) were applied at 75-80 % bloom stage to 'Gala' and 'Cripps Pink^{TM}' apple trees. All the treatments differed significantly in reducing the fruit set and fruit retention in all cultivars. Results obtained from this experiment have been explained and discussed as follows.

6. 4. 1. Fruit set, fruit retention and leaf scorch

Blossom thinning LS treatments alone or in combination with olive oil significantly ($P \leq 0.05$) reduced fruit set and fruit retention when compared to control in both 'Gala' and 'Cripps Pink^{TM}' apple trees (Table 6. 1). Spray application of LS (2% and 3%) with or without olive oil, significantly increased leaf scorching compared to control trees (Table 6. 1). 'Gala' and 'Cripps Pink^{TM}' trees sprayed with LS at 4% concentration alone had lowest fruit set (4.98 and 10.21% respectively) compared to all other treatments (Table 6. 1). When averaged across cultivars, the lowest mean fruit set was observed when trees were treated with 3 or 4% LS (7.60-11.04% respectively) without olive oil (Figure 6. 1a). Olive oil in combination with higher concentrations of LS (3 or 4%) appeared to increase fruit set when compared to LS treatments of 3 or 4% alone (Fig. 6. 1a). Spray application of LS (4%) alone resulted in 50.72% fruit retention whilst LS 3% + olive oil reduced fruit retention to 42.13%, however, the two treatments did not differ significantly (Fig. 6. 1b). Increasing concentrations of LS alone had no significant effects on fruit retention, however percentage fruit retention on trees treated with LS alone was significantly lower when compared to control (Table 6. 1, Fig. 6. 1b). There were no significant differences between 'Gala' and 'Cripps Pink^{TM}' apples for fruit retention with various treatments. Combining olive oil with LS appeared to reduce fruit retention, despite some treatments showing no significant differences (Table 6. 1).

The increased concentration of LS (1% to 4%) alone resulted in increased leaf scorch (1.34% to 24.32%). Leaf scorch was higher on all the trees treated with LS alone compared to the trees sprayed with a combination of LS and olive oil (Fig. 6. 1c). A spray application of LS (4%) alone caused the highest leaf scorch % compared to all other treatments (Table 6. 1). Combining LS with olive oil appears to reduce the incidence of leaf scorch compared to treatments with LS alone. Leaf scorch percentage on trees sprayed with LS (1 or 2%) in combination with olive oil were comparable to control trees (Table 6. 1). 'Cripps Pink^{TM}' apple trees exhibited significantly ($P \leq 0.05$) higher mean leaf scorch percentage compared to 'Gala' trees (Table 6. 1). The interaction between the treatments and the cultivars was found to be significant for fruit set, fruit retention and leaf scorch.

Table 6. 1. Effects of spray application of different concentrations of lime sulphur (LS) alone, or in combination with organic olive oil on fruit set (%), fruit retention (%) and leaf scorch (%) in 'Gala' and 'Cripps Pink^{TM}' apple trees.

	Fruit set (%)			Fruit retention (%)			Leaf scorch (%)		
Treatments (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	68.5	36.26	52.38 A	55.54	60.72	71.63 A	1.73	0.96	1.34 E
LS 1%	41.75	34	37.87 B	27.7	64.61	58.13 B	5.38	5.27	5.32 DE
LS 1% + Olive oil 3%	36.09	39.61	37.85 B	61.22	46.19	46.15 CD	1.71	0.47	1.09 E
LS 2 %	23.96	26.58	25.27 C	46.61	50.75	53.70 BC	9.01	6.19	7.60 CD
LS 2 % + Olive oil 3%	26.97	19.43	23.20 C	59.41	42.94	48.68 BCD	4.02	1.83	2.93 E
LS 3 %	10.47	11.61	11.04 EF	40.94	46.6	51.17 BCD	12.53	13.42	12.97 B
LS 3 % + Olive oil 3%	21.1	21.89	21.50 CD	51.67	49.77	43.77 D	8.45	8.57	8.51 CD
LS 4 %	4.98	10.21	7.60 F	44.69	39.57	50.72 BCD	31.01	17.637	24.32 A
LS 4 % + Olive oil 3%	14.9	15.56	15.23 DE	55.54	60.72	42.13 D	10.18	12.092	11.13 BC
Mean (cultivars)	27.64 a	23.91 b		50.57	53.01		9.33 a	7.381 b	
LSD ($P \leq 0.05$)				LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)		
Treatments	6.92			8.64			3.95		
Cultivars	3.26			NS			1.86		
Treatments × cultivars	9.78			12.220			5.58		

Any two means within a column followed by different uppercase letters and in a row followed by different lowercase letter are significantly different using LSD at $P \leq 0.05$. NS = not significant, n (fruit set) = four replicates (200 - 300 flowers per tree replicate, n (fruit retention) = four replicates (150 - 250 fruit set per tree replicate), n (leaves scorch) = four replicates (3 branches per tree replicate)

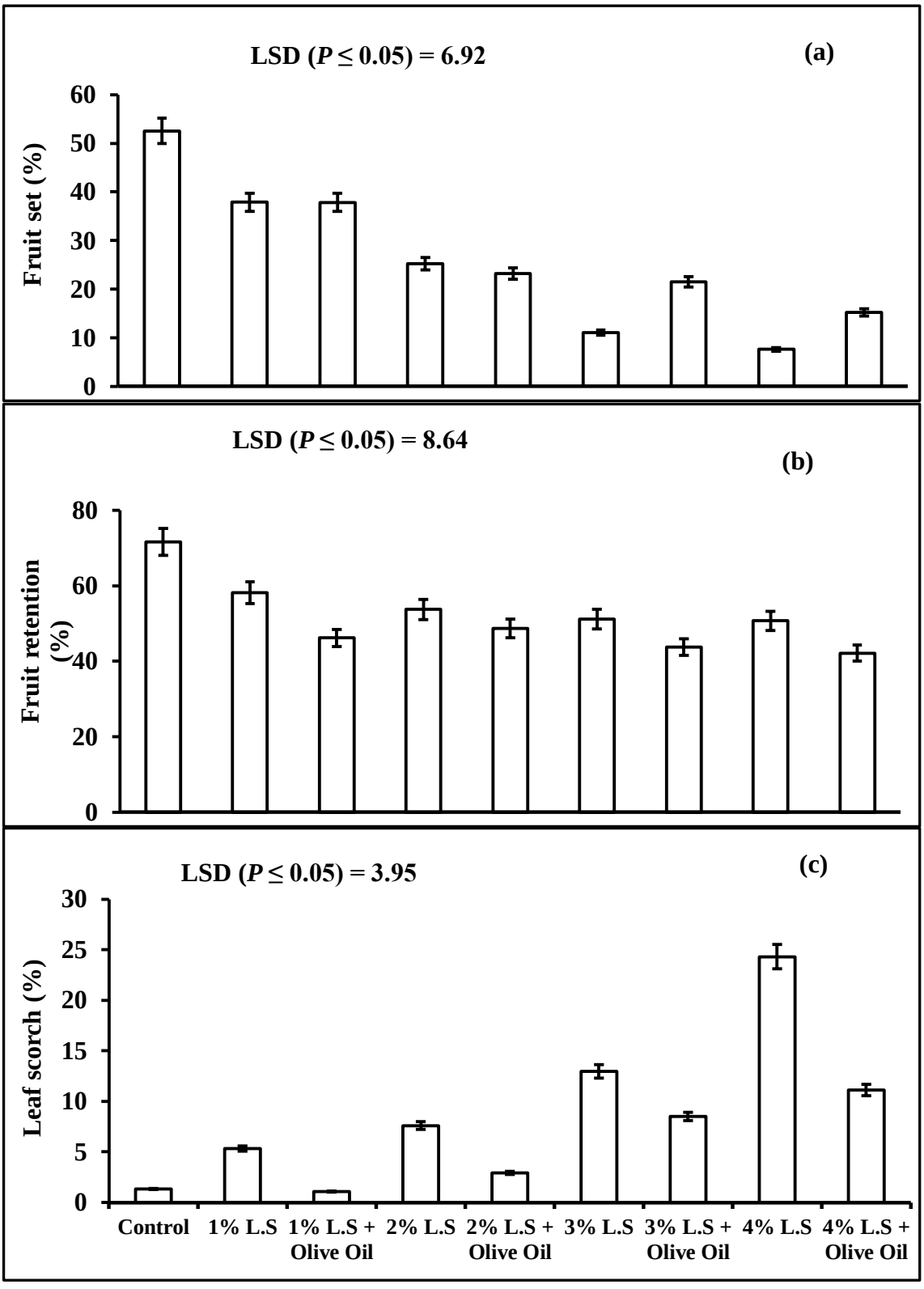

Figure 6. 1. Changes in mean fruit set (%) (a), fruit retention (%) (b) and leaf scorching (%) (c) influenced with the spray application of different concentrations of lime sulphur (LS) alone and in combination with organic olive oil applied at 75% bloom stage.

6. 4. 2. Fruit size, fruit weight and fruit firmness

LS treatments of 2, 3 and 4% alone or in combination with olive oil sprayed on 'Gala' and 'Cripps Pink^{TM}' apple trees showed significant ($P \leq 0.05$) increase in fruit size and weight in comparison to the control and trees treated with 1% LS and olive oil (Fig 6. 2a). Increasing concentrations of LS with or without olive oil appear to have increased mean fruit size in both cultivars. Mean fruit size of 'Gala' apples was significantly ($P \leq 0.05$) higher than that of 'Cripps Pink^{TM}' apples (Table 6. 2). LS treatments of 2, 3 and 4% with or without olive oil showed no significant differences for mean fruit weight, however these fruit weighed significantly more than control fruit and fruit treated with 1% LS alone and in combination with olive oil (Table 6. 2). Mean fruit weight did not differ significantly between both cultivars.

When averaged over the cultivars, the largest fruit size (69.57-68.58mm respectively) was recorded when trees were sprayed with 4% LS alone or combination with 3% olive oil and this corresponded within the highest fruit weight (134-139.7g) (Figs. 6. 2a, b). There was a direct linear negative significant relationship (r= - 0.391, y = - 0.1438x + 65.988) between percentage fruit set and fruit size (Fig. 6. 3a) regulated with different blossom thinning treatments. Similarly, there was a direct linear negative significant relationship (r= - 0.394, y = - 0.2408x + 136.39) between percentage fruit set and fruit weight (Fig. 6. 3b) regulated with different blossom thinning treatments.

Fruit firmness was significantly ($P \leq 0.05$) affected with various LS treatments (Fig. 6. 2c). Fruit firmness decreased significantly when trees were sprayed with 4% LS in combination with olive oil (80.72 N) or 3% LS in combination with olive oil (83.28 N) compared to control (93.47 N) (Fig. 6. 2c). Irrespective of the treatments, there were significant differences amongst the two cultivars for mean fruit firmness. The mean fruit firmness was higher in 'Cripps Pink^{TM}' (87.49 N) than in 'Gala' (85.15 N) (Table 6. 2). The interaction between the cultivars and the treatments also differed significantly for mean fruit size, fruit weight and firmness (Table 6. 2).

Table 6. 2. Effects of spray application of different concentrations of lime sulphur (LS) alone, or in combination with organic olive oil on fruit size, weight, and fruit firmness (N) in 'Gala' and 'Cripps Pink™' apple.

	Fruit size (mm)			Fruit weight (g)			Fruit firmness (N)		
Treatments (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	62.02	53.38	57.70f	123	123	123 cd	89.14	97.8	93.47a
LS 1%	64.11	54.84	59.48de	125	125	120.3 d	87.01	90.44	88.73b
LS 1% + Olive oil 3%	64.07	53.49	58.78ef	122	124	122.6 cd	84.52	87.43	85.98bc
LS 2 %	65.51	55.72	60.62cd	142	136	132.2 ab	89.15	84.94	87.04bc
LS 2 % + Olive oil 3%	64.6	56.84	60.72cd	134	142	133.9 ab	78.88	88.44	83.66cd
LS 3 %	67.3	59.21	63.25b	135	134	131.7 ab	85.95	85.63	85.79bc
LS 3 % + Olive oil 3%	66.9	56.78	61.84bc	132	138	130.4 bc	82.74	83.83	83.28cd
LS 4 %	71.63	67.51	69.57a	144	132	134 ab	91.18	85.24	88.21b
LS 4 % + Olive oil 3%	70.57	66.6	68.58a	131	151	139.7 a	77.82	83.63	80.72d
Mean (cultivars)	66.30 A	58.26 B		134	138		85.15 B	87.49 A	
	LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)		
Treatments	1.437			8.10			3.44		
Cultivars	0.678			NS			1.624		
Treatments × cultivars	2.033			11.41			4.871		

Any two means within a column in lowercase letter and row followed by different uppercase letters are significantly different using LSD at $P \leq 0.05$. NS = not significant, n = four replicates (10 fruit per replicate).

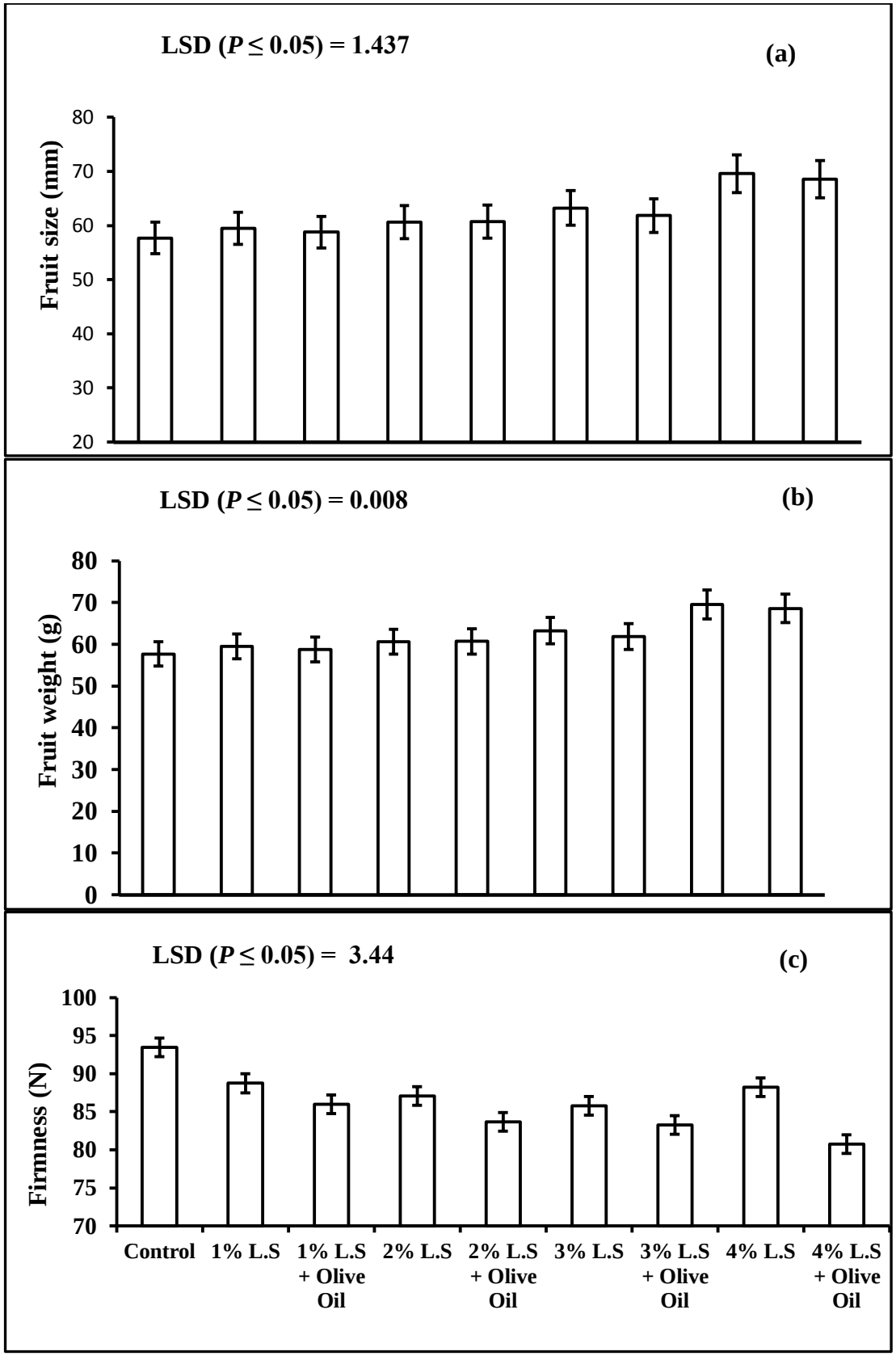

Figure 6. 2. Changes in mean fruit size (a), fruit weight (b) and fruit firmness (c) influenced with the spray application of different concentrations of lime sulphur (LS) alone and in combination with organic olive oil applied at 75% bloom stage.

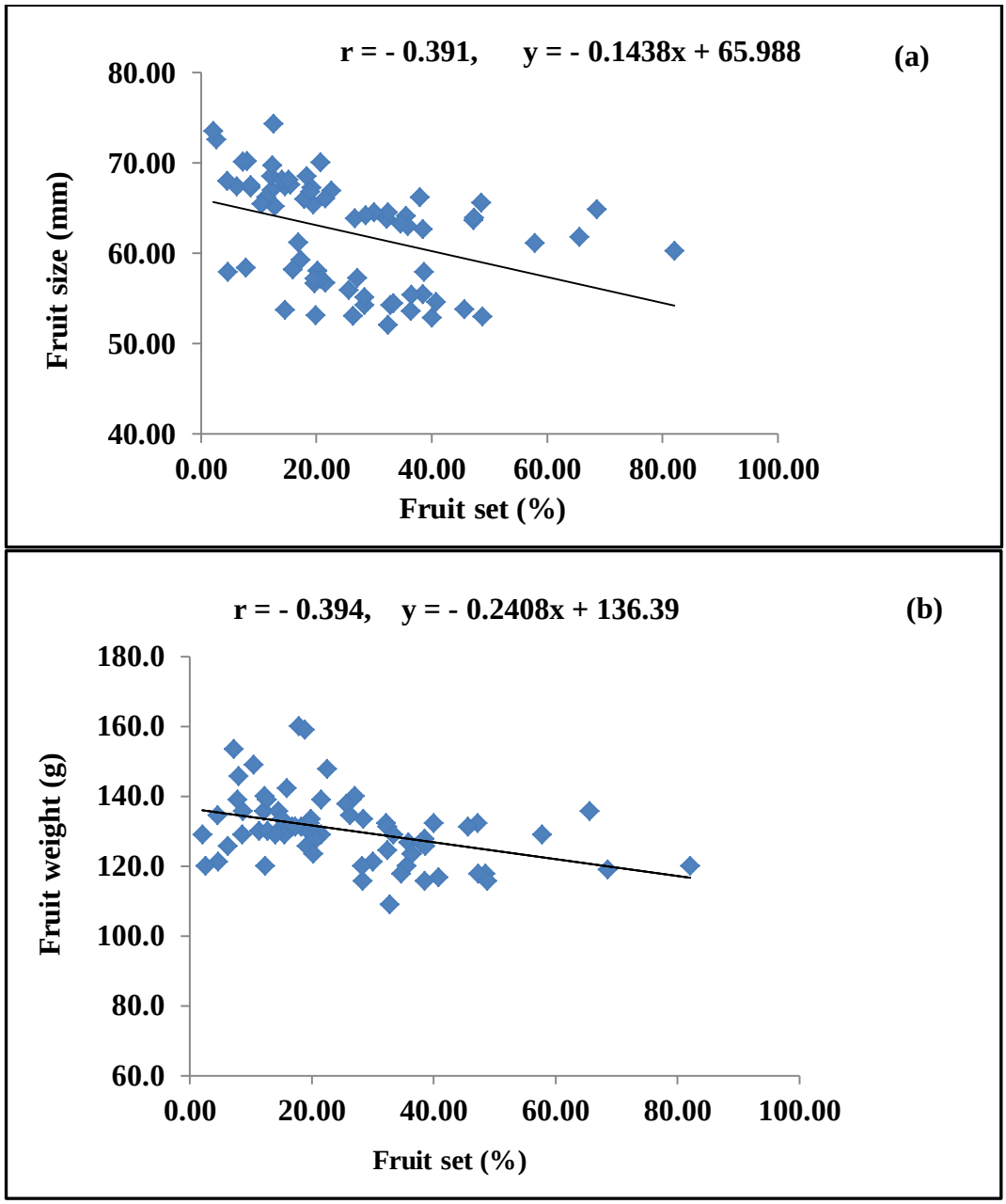

Figure 6. 3. Relationship between percentage fruit set and fruit size (mm) (a) and percentage fruit set and fruit weight (b) as regulated with spray application of different concentrations of lime sulphur alone and in combination with organic olive oil applied at 75% bloom stage in 'Gala' and 'Cripps Pink^{TM}' apple trees.

6. 4. 3. Skin colour

All blossom thinning treatments with different concentrations of LS alone or in combination with olive oil significantly ($P \leq 0.05$) affected the skin colour of 'Gala' and 'Cripps Pink^{TM}' apple fruit (Table 6. 3). Mean a*, b* and chroma (Figs. 6. 4b, c and d) values were higher in the fruit of trees treated with different concentrations of LS alone and in combination with 3% olive oil compared to the control in both cultivars. Meanwhile, mean L* and hue angle (Figs. 6. 4a and e) were lower in fruit of trees sprayed with LS alone or in combination with olive oil compared to control (Table 6. 3). The mean L* and hue angle were lower (indicating redder fruit) in fruit from trees treated with LS (4%) in combination with olive oil compared to all other treatments, whilst the mean a*, b* and chroma (or colour saturation) were highest in the fruit of trees treated with LS (4%) in combination with olive oil (Figure 6. 4a and b). Irrespective of the treatments, mean L*, a*, b*, chroma and hue angles of fruit differed significantly between both cultivars. The mean value L* and hue angle were higher in 'Cripps Pink^{TM}' apple fruit (33.54 L* and 54.33 h^{o} respectively) than 'Gala' apple fruit, whilst mean a* and chroma' were higher in 'Gala' apple (26.38 a* and 30.85 *C respectively) than 'Cripps Pink^{TM}' apple fruit. The interactions between the cultivars and the treatments for L*, a*, b*, chroma and hue angle were found be significant (Table. 6. 3 and 6. 4).

Table 6. 3. Effects of spray application of different concentrations of lime sulphur (LS) alone or in combination with organic olive oil on fruit colour in 'Gala' and 'Cripps Pink™' apple fruit.

	Lightness (L*)			a*			b*		
Treatments (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	34.52	35.74	35.13 a	23.78	21.93	11.85 b	14.8	16.66	15.73 d
LS 1%	26.85	33.47	30.16 bc	26.83	15.74	21.29 a	15.07	19.76	17.42 bc
LS 1% + Olive oil 3%	23.35	33.98	28.66 c	29.63	11.59	20.61 a	14.9	19.88	17.39 bc
LS 2 %	26.19	33.57	29.88 bc	28.81	15.55	22.18 a	14.51	18.84	16.68 cd
LS 2 % + Olive oil 3%	22.14	33.91	28.03 c	29.39	10.74	20.06 a	14.49	20.41	17.45 bc
LS 3 %	28.02	35.08	31.55 b	27.15	16.24	21.70 a	14.19	19.95	17.07 bcd
LS 3 % + Olive oil 3%	24.36	35.83	30.09 bc	26.94	10.14	18.54 a	14.74	22.51	18.63 b
LS 4 %	21.4	33.69	27.54 c	31.52	14.45	22.99 a	15.25	20.33	17.79 bc
LS 4 % + Olive oil 3%	30.25	26.6	28.42 c	13.36	10.34	22.85 a	18.19	22.26	20.23 a
Mean (cultivars)	26.34 B	33.54 A		26.38 A	14.08 B		15.13 A	20.07 B	
LSD ($P \leq 0.05$)				LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)		
Treatments	2.44			4.46			1.47		
Cultivars	1.15			2.10			0.69		
Treatments × cultivars	3.45			6.31			2.07		

Any two means within a column following lowercase letter and within a row followed by different uppercase letters are significantly different using LSD at $P \leq 0.05$, n = four replicates (10 fruit per replicate).

Table 6. 4 Effects of spray application of different concentrations of lime sulphur (LS) alone, or in combination with organic olive oil on fruit colour in 'Gala' and 'Cripps Pink™' apple fruit.

	Chroma (C*)			Hue angle (h°)		
Treatments	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	25.23	25.03	25.13 c	48.53	66.65	57.59 a
LS 1%	31.43	25.13	28.28 b	29.85	67.44	48.65 ab
LS 1% + Olive oil 3%	32.62	25.28	28.95 ab	27.46	55.09	41.27 bc
LS 2 %	33.38	24.20	28.79 ab	25.81	52.32	39.06 bc
LS 2 % + Olive oil 3%	31.41	24.13	27.77 b	27.97	59.14	43.56 bc
LS 3 %	30.75	24.27	27.51 b	27.66	57.98	42.82 bc
LS 3 % + Olive oil 3%	30.07	24.93	27.50 b	28.78	36.86	32.82 c
LS 4 %	34.37	25.51	29.94 a	26.47	55.41	40.94 bc
LS 4 % + Olive oil 3%	28.37	28.49	28.43 b	31.96	38.06	35.01 c
Mean (cultivars)	30.85 A	25.22 B		30.50B	54.33A	
LSD ($P \leq 0.05$)				LSD ($P \leq 0.05$)		
Treatments	1.37			9.98		
Cultivars	0.65			4.704		
Treatments × cultivars	1.94			14.11		

Any two means within a column following lowercase letter and within a row followed by different uppercase letters are significantly different using LSD at $P \leq 0.05$, n = four replicates (10 fruit per replicate).

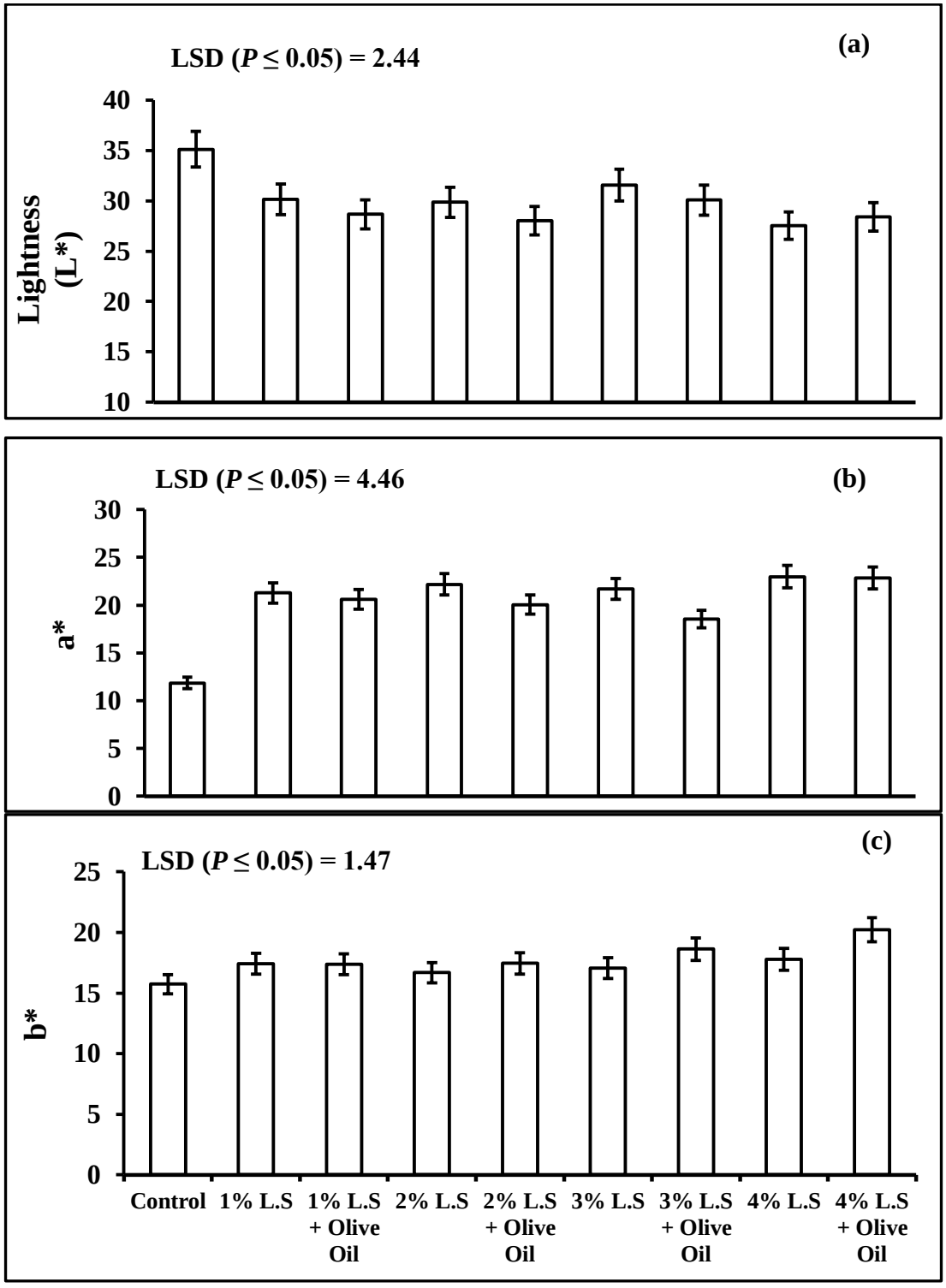

Figure 6. 4. Changes in mean lightness (L*), (a*) and (b*) as influenced with the spray application of different concentrations of lime sulphur (LS) alone and in combination with organic olive oil applied at 75% bloom stage.

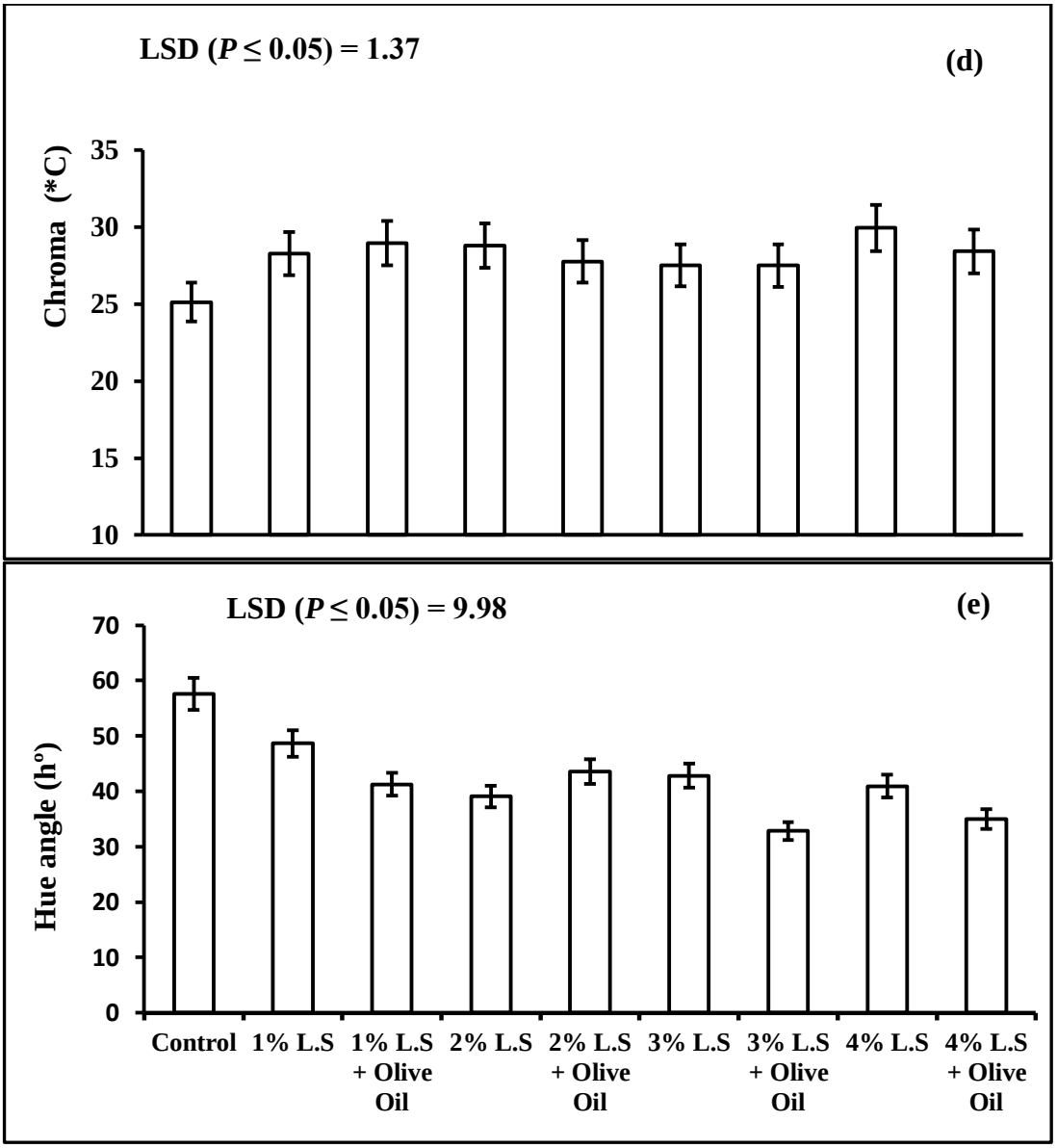

Figure 6. 4. Changes in chroma (C*) and hue angle (h°) as influenced with the spray application of different concentrations of lime sulphur (LS) alone and in combination with organic olive oil applied at 75% bloom stage.

6. 4. 4. TA, SSC, SSC:TA ratio and ascorbic acid

LS treatments alone or in combination with olive oil sprayed on 'Gala' and 'Cripps Pink^{TM}' apple trees significantly ($P \leq 0.05$) affected titratable acidity (TA) and SSC:TA ratio in apple juice as compared to control (Fig. 6. 5). The mean SSC was significantly ($P \leq 0.05$) lower in trees that were sprayed with 4% of LS alone or in combination with olive oil and 3% of LS with olive oil compared to untreated trees and trees treated with 1% or 2% LS with or without olive oil (Fig. 6. 5a). Mean TA was significantly lower (1.47-1.37% respectively) with the same treatments compared to the control (Fig. 6. 5b). 'Cripps Pink^{TM}' apples exhibited significantly ($P \leq 0.05$) higher mean SSC and TA and lower SSC:TA ratio when compared to 'Gala' (Table 6. 5). Trees sprayed with 4% LS alone recorded higher concentration of ascorbic acid (62.03 mg.100 ml^{-1}) compared to control trees and trees treated with 1% LS with or without olive oil (Fig. 6. 5c). Concentration of ascorbic acid in the fruit juice did not vary significantly amongst all the treatments and control. 'Cripps Pink^{TM}' apples had significantly higher mean concentrations of ascorbic acid compared to 'Gala' (Table 6. 5). The interaction between the cultivars and different treatments were found to significant for SSC, TA and ascorbic acid (Table 6. 5).

Table 6. 5. Effects of spray application of different concentrations of lime sulphur (LS) alone, or in combination with organic olive oil on soluble solids concentration (SSC), titratable acidity (TA), SSC:TA ratio and ascorbic acid in the juice of 'Gala' and 'Cripps Pink™' apple fruit.

	SSC (%)			(TA) (Malic acid %)			SSC/TA ratio			Ascorbic acid (mg/100 ml)		
Treatments (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	11.35	13.22	12.29a	1.52	2.27	1.90 a	7.468	5.82	6.65 e	48.84	60.5	54.67 b
LS 1%	11.2	12.75	11.97abc	1.3	1.9	1.60 b	8.684	6.74	7.71 abc	47.29	62.91	55.10 b
LS 1% + Olive oil 3%	11.08	12.83	11.95abc	1.28	1.68	1.48 bc	8.848	7.66	8.25 a	49.79	59.63	54.71 b
L. S 2 %	11.7	12.65	12.18ab	1.5	1.73	1.61 b	7.818	7.34	7.58 abc	53.94	61.02	57.48 ab
LS 2 % + Olive oil 3%	10.95	12.6	11.78bc	1.22	1.8	1.51 bc	8.954	7.06	8.01 abc	55.58	58.38	56.98 ab
LS 3 %	11	12.4	11.70c	1.45	1.77	1.61 b	7.621	7.04	7.33 cd	56.09	56.31	56.20 b
LS 3 % + Olive oil 3%	10.3	11.85	11.07d	1.25	1.5	1.38 c	8.253	7.92	8.09 ab	53.68	59.25	56.46 b
LS 4 %	10.98	11.25	11.11d	1.6	1.48	1.54 b	6.977	7.64	7.31 d	57.82	66.24	62.03 a
LS 4 % + Olive oil 3%	10.15	11.53	10.84d	1.23	1.73	1.48 bc	8.321	6.69	7.51 bcd	55.06	60.45	57.76 ab
Mean (cultivars)	10.97 B	12.34 A		1.37 B	1.76 A		8.11 A	7.10 B		53.12 B	60.52 A	
LSD ($P \leq 0.05$)				LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)		
Treatments	0.388			0.136			0.61			4.70		
Cultivars	0.183			0.064			0.29			2.213		
Treatments × cultivars	0.548			0.193			0.86			6.640		

Any two means within a column following lowercase letter and within a row followed by different uppercase letters are significantly different using LSD at $P \leq 0.05$, n = four replicates (10 fruit per replicate).

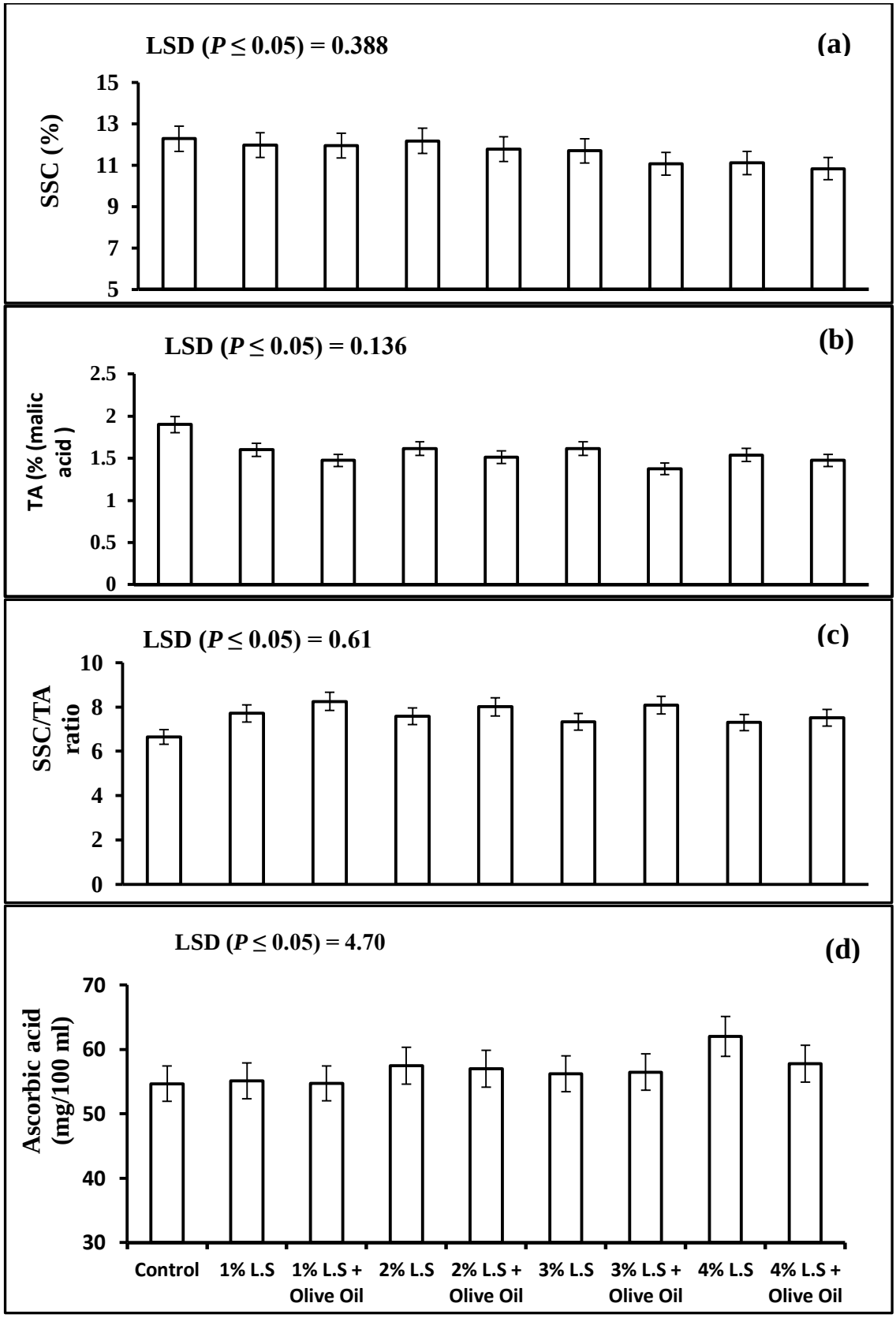

Figure 6. 5. Changes in mean soluble solids concentration, titratable acidity, SSC/TA ratio and ascorbic acid influenced with the spray application of different concentrations of lime sulphur (LS) alone and in combination with organic olive oil applied at 75% bloom stage.

6. 5. Discussion

LS spray application alone or in combination with olive oil at 75% bloom stage significantly ($P \leq 0.05$) reduced fruit set in both 'Gala' and 'Cripps Pink^{TM}' apple cultivars compared to control trees (Table 6. 1). The experimental results show that blossom thinning was achieved in 'Gala' and 'Cripps Pink^{TM}' apple trees with LS, an organic compound approved by the EU legislation for organically grown fruit. This was also confirmed by earlier findings that 4% LS applied at 85% full bloom reduced fruit set in 'Gala' and 'Fuji' apple (Guak et al., 2004; Osborne, 2006). Trees treated with LS concentrations of 3% or 4% without olive oil had the lowest fruit set while trees that received LS at 1% with and without olive oil and control trees had higher fruit set (Fig. 6. 1). According to Myraa et al., (2011) spraying LS at blossom time reduced pollen germination, which ultimately reduces the fruit set. Therefore, it may be assumed that higher concentrations of LS may potentially inhibit pollen germination or pollen tube growth, leading to reduced fruit set in 'Gala' and 'Cripps Pink^{TM}' apple trees. Previously, similar claims have been made by Bertshinger et al., (1998) and Embree and Foster, (1999) with regards to chemicals such as Safer-Soap, PEG-1000, Anti-Stress, Nutri-Safe and Biofilm.

Treatments of 4% LS alone or with olive oil resulted in the lowest fruit retention compared to control in both 'Gala' and 'Cripps Pink^{TM}' apples (Table 6. 1). Earlier findings by Guak et al., (2004) and Osborne et al., (2006) also support the findings in the current study. Application of 4% LS at 85% bloom on 'Fuji' and 'Gala' apple trees resulted in a higher proportion of fruiting sites with one fruit and a lower proportion of fruiting sites with more than two fruits (Guak et al., 2004).

The thinning of blossoms, rather than developing fruit maximizes the ability to adjust the fruit-to-leaf ratio, a method particularly desirable in early ripening peach cultivars with a short fruit developmental period and fruit sizing problems (Byers and Lyons, 1984; Havis, 1962). Also, Southwick et al., (1996) confirmed that bloom thinning can maximize the tree's capacity to allocate sufficient resources to fruit when the leaf-to-fruit ratio is increased early in the growing season. The treatments of LS alone or in combination with olive oil had a significant ($P \leq 0.05$) effect on leaf scorch in 'Gala' and 'Cripps Pink^{TM}' apples compared to control (Table 6. 1 and Fig 6. 1 c). Higher percentage of scorched leaves was observed on the trees of both

cultivars treated with the higher concentrations (3- 4 %) of LS alone. However, when LS was used in conjunction with olive oil, percentage of scorched leaves reduced significantly (Table 6. 1). This finding indicates that LS at higher concentrations is probably phytotoxic and is responsible for the burning of leaves on the trees. Stopar, (2004) similarly reported stunting of leaves in 'Golden Delicious' apples treated with 3% LS, which did not recover during the growing season. In contrast, Guak e al., (2004) reported no damage to 'Fuji' and 'Gala' apples treated with 4% LS. The results from this study indicate that when LS is combined with olive oil, the phytotoxic effect of LS on apple leaves is lessened.

Size differences in fruit are primarily ascribed to differences in the number and individual size of cells within the fruit cortex and pith (Smith, 1950; Martin et al., 1964; Sugiura et al., 1995; Webster, 1997). According to Smith, (1950), the characteristic size for each cultivar is determined primarily by the degree of cell multiplication occurring after pollination; however he stated that the relation between increase in fruit weight and cell increase was not the same for each cultivar. Webster, (1997) reported that cell numbers are determined within the first few weeks of fruit development. The cell division in the flesh (pith and cortex) of the fruit ceases about 4-6 weeks after blossom (Smith, 1950; Bain and Robertson, 1951). Fruit weight and size are closely correlated both being inversely related to fruit set (Figs. 6. 3a and 6.3b). All blossom thinning treatments with LS improved fruit size compared to the control (Table 6. 2). The highest LS concentration (4%) alone or with olive oil produced larger fruit with higher weights (Table 6. 2) which may be attributed to reduced fruit set and fruit retention on the trees receiving the same treatment. Earlier reports also suggested that reduction of crop load with blossom thinning treatments resulted in improved fruit weight and size in apple (Guak et al., 2004; Osborne et al. 2006; Fallahi and Green 2010; Link, 2000). Stanley et al., (2000) has also reported that lower fruit load also gives individual fruit a greater share of photosynthates allowing cells to increase to the maximum size. Increasing the crop load increased the number of small size fruit on the tree and decreased the average fruit size and weight (Stopar et al., 2002). This inverse relationship between fruit size and fruit number on the crown has been confirmed by many researchers and is one of the main reasons for using fruit thinning in apple orchards (Wertheim, 1997 and 2000) and (Neilsen et al., 2001). Webster, (1997) reported that competition for photosynthates

among growing fruit can be reduced by reducing fruit numbers at, or soon after flowering has a positive effect of reducing competition for photosynthates with fruit consequently remaining on the tree to increase their cell numbers. Spray applications of LS at bloom-time significantly influenced fruit firmness, SSC, TA and concentration of ascorbic acid in apple juice at harvest (Table 6. 5). In both 'Gala' and 'Cripps Pink^{TM}' apple cultivars, the fruit firmness, SSC and TS decreased with increased concentrations of LS applied, while, concentration of ascorbic acid increased marginally. Similar results have also been reported previously in 'Gala' and 'Fuji' apples treated with high LS concentrations (4%) by Guak et al., (2004), where they found reduced fruit firmness, SSC and TA. Chun et al., (2012) reported no significant effects of various LS concentrations on fruit firmness, SSC and TA in 'Fuji' and 'Hongro' apples. Garriz et al., (2000) found that fruit firmness was significantly lower in 'Braeburn' trees with higher crop loads compared to trees with moderate or low crop loads. However, Jones et al., (1997b) reported increased fruit firmness with reduced crop load following chemical thinning of 'Pink Lady' and 'Jonagold' with ethephon and BA. Link, (2000) suggested that the fruit firmness in trees that are heavily cropped may be reduced because these fruit receive limited carbohydrates for cell wall synthesis. In the current study, bloom thinning produced softer fruit than the trees with less thinning and control trees. It is possible that high bloom thinning with LS treatments resulted in earlier fruit maturation than lower bloom thinning, particularly when increased SSC values are considered in a highly thinned crop (Table 6. 5).

Bloom thinning treatments significantly ($P \leq 0.05$) influenced the skin colour of both 'Gala' and 'Cripps Pink^{TM}' apple fruit (Table 6. 4 a, b). Lightness and hue angle were lower on fruit of trees treated with higher concentration lime sulphur alone or in combination with olive oil indicating redder fruit compared to control and all other treatments. Mean a*, b* and chroma or red colour saturation were highest in the fruit of trees treated with higher concentration 3-4 of LS alone or in combination with olive oil compared to control and all others treatments (Figs. 6. 4b, c and d). This improvement in colour in part could be ascribed to advanced maturity of fruit in trees treated with high concentrations of LS. Guak et al., (2004) reported no significant effects of LS treatments on fruit colour in 'Fuji' or 'Gala' apples.

In conclusion, a spray application of 4% LS alone or in combination with olive oil at 75% bloom stage on 'Gala' apple and 'Cripps Pink^{TM}' apple trees reduced fruit set and increased the fruit size and improved fruit skin colour with minimum leaf scorch. However fruit firmness was reduced due to slightly advanced maturity of fruit from these treated trees.

Chapter 7

Effects of organic blossom thinning with lime sulphur alone or in combination with olive oil on cold storage life and quality of 'Gala' and 'Cripps Pink™' apples

Abstract

Effects of blossom thinning treatments of lime sulphur (LS) alone or in combination with organic olive oil on cold storage life and fruit quality of 'Gala' and 'Cripps Pink™' apples were investigated. Different concentrations (1, 2, 3 or 4%) of LS alone and in combination with organic olive oil (3%) and 0.05% synertrol oil as a surfactant were sprayed onto 'Gala' and 'Cripps Pink™' apple trees until runoff at 75% full bloom. Unsprayed trees were kept as control. Fruit were harvested at commercial maturity and stored at low temperature (0.5 ± 0.5 °C) for 60, 90 and 120 days plus 10 days of simulated shelf conditions (21 ± 1ºC) prior to determining fruit weight loss (%), firmness, soluble solids concentration (SSC), titratable acidity (TA), SSC/TA ratio and levels of ascorbic acid. The mean firmness, SSC and TA from control fruit were significantly ($P \leq 0.05$) higher following cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions as compared to fruit from blossom thinning treatments. Percentage of fruit weight loss was higher in cold-stored fruit 120 days plus 10 days of simulated shelf conditions between (3.33-3.85%) following by cold stored fruit 90 days plus 10 days of simulated shelf conditions between (2.22 - 3.00%) in both 'Gala' and 'Cripps Pink™' apples respectively. Spray application of higher concentrations of LS (3-4%) alone or with 3% of olive oil at blossom thinning showed a decreasing trend in firmness, SSC and TA following cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions in 'Cripps Pink™' and 'Gala' apples.

7. 1. Introduction

Flower and fruit thinning is one of the most important cultural practices performed in modern apple orchards to improve fruit quality. This practice directly influences the size of the fruit at harvest and also affects the flower bud initiation in the subsequent season by regulating biennial bearing (Fallahi and Greene, 2010). In commercial orchards, chemicals are widely used for blossom and fruit thinning. Since, 1989, new chemicals such as ammonium thiosulphate (ATS), hydrogen

cyanamide (Dormex^{TM}), endothalic acid (Endothal), pelargonic acid (Thinex^{TM}) and sulfcarbamide (Wilthin$^{®}$) have been tested as potential blossom thinners (Fallahi and Green, 2010). The mode of action of these chemicals is to reduce fruit set by damaging the anthers, stigma, style and pollen tubes which prevent fertilization. However, the application of the above mentioned chemicals in an organic apple orchard is prohibited. Organic fruit production is gaining impetus as the demand for high quality fruit from orchards that grow apple with minimal or no chemical input. Hand thinning of flowers and fruit in an orchard is laborious and would significantly increase cost of production. It has been suggested that fruitlets must be thinned up to a few weeks post-flowering to prevent biennial bearing, such a practice is not possible in commercial orchards by hand thinning (Tromp 2000; Greene, 2002). Therefore, hand thinning is an impractical approach for organic apple production particularly in a country like Australia, where labour costs are extremely high. Potential blossom thinning compounds that may be considered organic have been trialled in apples and resulted in varying success. These include lime sulphur (LS, a calcium polysulphide), vegetable oils such as corn oil, rape oil and soybean oil and other compounds such as acetic acid; sodium bicarbonate and starch (Meland, 1998; Bertschinger et al., 2000; Pendergrass et al. 2000; ZhiGuo and YouSheng, 2001). However, most studies concentrate on effects of organic thinning treatments on the fruit quality parameters following commercial harvest and some preliminary research work has been reported on their effects on cold storage life and quality of apple fruit. Guak et al., (2004) reported that 'Gala' and 'Fuji' apples treated with LS (1-4%) and stored for four months or three months respectively at 1ºC showed no significant effects for fruit firmness and soluble solids following storage. However, no research work has been reported on the effects of organic blossom thinning treatments on cold storage life and quality of apple fruit grown organically under Australian conditions. Some factors such as time and effectiveness of thinning, type and concentration of chemical used and apple cultivar have been reported to influence storage life and quality of conventionally grown apples (Basak, 1999). These properties are expected to remain optimum in the apple fruit during CA storage followed by ripening at 20ºC (Lopez et al., 2000). Apples such as 'Cripps Pink^{TM}' are available all year round; therefore, long term storability and quality maintenance of organically grown apples are extremely important to all organic growers.

In the previous chapter (6), LS alone or in combination with olive oil was tested as a potential blossom thinner on 'Gala' and 'Cripps Pink^{TM}' apples. The results showed that an application of 4% LS alone or in combination with olive oil (3%) at 75% bloom stage on 'Gala' apple and 'Cripps Pink^{TM}' apple trees reduced fruit set and increased fruit size. However, the cold storage life and quality parameters of apples of 'Gala' and 'Cripps Pink^{TM}' trees treated with organic blossom thinning applications of LS alone or in combination with olive oil following long-term cold storage are yet to be investigated. Therefore, the objectives of this study were to evaluate the effects of different concentrations of LS alone or in combination with organic olive oil on weight loss, fruit firmness, soluble solids concentration (SSC), titratable acidity (TA), SSC/TA ratio and levels of ascorbic acid following cold storage (0.5 ± 0.5 °C) for 60, 90 and 120 days plus 10 days of simulated shelf conditions (21 ± 1ºC).

7. 2. Materials and Methods

7. 2. 1. Plant materials

The experiment was conducted on organically grown, 15-year old 'Cripps Pink^{TM}' apple trees grafted on rootstock M26 and 'Gala' apple trees grafted on rootstock MM06 at the Newton Brothers Orchards in Manjimup (lat. 34°14´S, long.116°8´E),Western Australia. The trees were spaced 7.5 m between rows and 2.5 m within rows in the north-south direction. All the experimental trees received similar cultural practices including nutrition, irrigation and plant protection (McCoy, 2007) except varying experimental treatments.

7. 2. 2. Treatments

An aqueous emulsion containing different concentrations (1, 2, 3 or 4%) of lime sulphur (active constituent is 200g/L sulphur (s) as polysulfide) alone or in combination with organic olive oil (3%) and 0.5% synertrol oil as a surfactant was sprayed at 75% bloom stage onto whole trees until runoff. Unsprayed trees served as control. At commercial harvest in 2014, the fruit were harvested randomly from four quadrants of the tree and kept in cold (0.5 ± 0.5 °C) storage for 60, 90 and 120 days. At the end of each cold storage period the fruit were held for 10 days to simulate shelf conditions (21 ± 1ºC) prior to the fruit quality parameters such as weight loss, fruit firmness, soluble solids concentration (SSC), titratable acidity (TA), SSC/TA

ratio and levels of ascorbic acid. The experiment was laid out by following two-factor with two factors including thinning treatments and cultivars during each cold storage and simulated shelf conditions period. Ten fruit was treated as an experimental unit and was replicated three times.

7. 2. 3. Observations recorded

7. 2. 3. 1. Fruit weight loss (%)

The fruit weight loss was calculated by weighing ten fruit per replication before and after each storage period plus 10 days of simulated shelf conditions (21 ± 1ºC) by using a digital balance (A&D Limited, Tokyo, Japan). Fruit weight loss was calculated as follows and expressed as a percentage also detailed in Chapter 3, Section 3.8.4.

7. 2. 3. 2. Fruit firmness (N)

Fruit firmness was recorded individually from ten fruit following each storage period plus 10 days of simulated shelf conditions (21 ± 1ºC) using an electronic pressure tester (Model EPT-1 tensile tester, Lake City Technical Products Inc., Kelowna, BC, Canada) fitted with an 11-mm tip as outlined in chapter 3, Section 3.8.5. Fruit firmness was expressed as Newtons (N).

7. 2. 3. 3. SSC, TA and SSC/TA ratio

Juice was extracted from the fruit after each storage period plus 10 days of simulated shelf conditions (21 ± 1ºC). SSC of the fruit juice was determined using digital refractometer as described in Chapter 3, Section 3.8.7. TA was determined by titrating the juice with 0.1 N NaOH following the method as outlined in Chapter 3, Section 3.8.8. SSC/TA ratio was calculated by dividing SSC with the corresponding TA value also detailed in Chapter 3, Section 3.8.9.

7. 2. 3. 4. Ascorbic acid

The concentration of ascorbic acid from juice from the fruit after each storage period plus 10 days of simulated shelf conditions (21 ± 1ºC) was determined following the method of Malik and Singh, (2005) with some modifications as outlined in chapter 3, Section 3.8.10. The concentration of ascorbic acid was expressed as mg.100ml^{-1} fresh juice.

7. 3. Statistical analysis

The data were analysed employing two-way analysis of variance (ANOVA) using GenStat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK). The effects of various thinning treatments, cultivars and their interactions for different parameters were gauged within ANOVA for each storage period. The least significance differences (LSD) were calculated succeeding significant F-test ($P \leq 0.05$). To ensure validity of statistical analysis all the assumptions of ANOVA were examined.

7. 4. Results

7. 4. 1. Fruit weight loss (%)

Blossom thinning treatments with LS alone or in combination with olive oil had no significant ($P \leq 0.05$) effects on fruit weight loss following cold storage of 60 and 120 days plus 10 days of simulated shelf conditions (21 ± 1ºC) in both 'Gala' and 'Cripps Pink^{TM}' apples (Table 7. 1). As expected, the fruit weight loss (%) was higher in fruit stored for 120 days of cold storage (between 3.33- 3.85%) followed by fruit stored for 90 days (between 2.22 - 3.00%) in both cultivars (Fig. 7. 1 or table. 7. 1). Irrespective of the treatments, 'Gala' apples exhibited higher fruit weight loss compared to 'Cripps Pink^{TM}' apples, following the various storage periods plus 10 days of simulated shelf conditions (21 ± 1ºC). The mean fruit weight loss was highest (2.15, 2.79 and 3.89%) in 'Gala' apples compared to 'Cripps Pink^{TM}' (1.3, 2.44 and 3.30 %) following cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions (21 ± 1ºC) respectively. The interaction between the cultivars and the treatments were found to be significant for fruit weight loss following 90 and 120 days of cold storage plus 10 days of simulated shelf conditions only (Table 7. 1).

Table 7. 1. Effects of blossom thinning treatments of different concentrations of lime sulphur (LS) alone or in combination with organic olive oil and low temperature (0.5 ± 0.5 °C) storage period plus 10 days of simulated shelf conditions (21 ± 1ºC) on fruit weight loss (%) in 'Gala' and 'Cripps Pink™' apple trees.

	Fruit weight loss (%)								
	60 days			90 days			120 days		
Treatments	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	1.91	1.43	1.67	3.60	2.17	2.88 ab	4.13	2.79	3.46
LS 1%	1.83	1.46	1.65	3.05	2.94	2.99 a	3.80	3.40	3.60
LS 1% + Olive oil 3%	3.61	1.47	2.54	2.85	2.80	2.84 ab	4.33	3.37	3.85
LS 2 %	1.86	0.99	1.42	2.36	2.57	2.46 bc	3.66	3.40	3.53
LS 2 % + Olive oil 3%	2.26	1.25	1.75	2.94	2.48	2.71 ab	4.34	3.19	3.76
LS 3 %	2.03	1.18	1.60	2.05	2.40	2.22 c	3.85	3.49	3.67
LS 3 % + Olive oil 3%	1.42	1.18	1.30	2.54	1.95	2.24 c	3.13	3.55	3.34
LS 4 %	2.67	1.40	2.04	2.84	2.29	2.57 abc	4.18	3.38	3.78
LS 4 % + Olive oil 3%	1.74	1.31	1.53	2.90	2.38	2.64 abc	3.57	3.09	3.33
Mean (cultivars)	2.15 A	1.3 B		2.79 A	2.44 B		3.89 A	3.30B	
LSD ($P \leq 0.05$)				LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)		
Treatments	NS			0.41			NS		
Cultivars	0.38			0.19			0.21		
Treatments × cultivars	NS			0.58			0.62		

Any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different (LSD at $P < 0.05$). NS = not significant, n = four replicates (10 fruit per replicate).

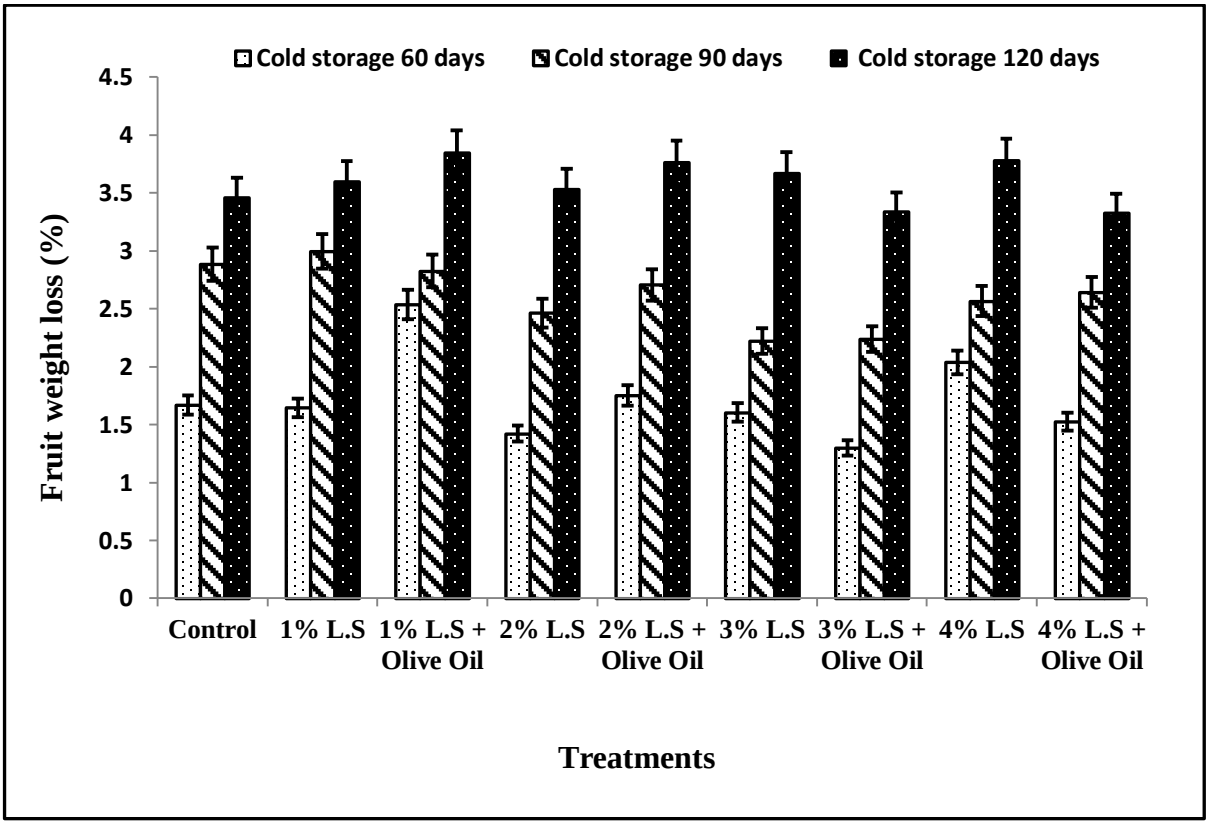

Figure 7. 1. Changes in mean apple fruit weight loss (%) influenced with the spray application of different concentrations of lime sulphur (LS) alone and in combination with organic olive oil applied at 75% bloom stage following cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions (21 ± 1ºC). Vertical bars represent LSD ($P \leq 0.05$).

7. 4. 2. Fruit firmness

Blossom thinning treatments with LS alone or in combination with olive oil, significantly reduced fruit firmness compared to control in 'Gala' and 'Cripps Pink^{TM}' apples following 60 and 120 days of cold storage plus 10 days of simulated shelf conditions (21 ± 1ºC) (Fig 7. 2). Fruit from trees that received LS combined with olive oil were significantly softer following 60 days cold storage plus 10 days of simulated shelf conditions (21 ± 1ºC) compared to control and LS treatments alone (Table 7. 2). However, this trend was not evident after 90 and 120 days of storage plus 10 days of simulated shelf conditions (21 ± 1ºC). LS treatments alone or in combination with olive oil showed significant differences only when compared with control after 120 days of storage plus 10 days of simulated shelf conditions (Fig. 7. 2). Irrespective of the treatment, there were significant differences between the two cultivars for fruit firmness following cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions (21 ± 1ºC). Mean fruit firmness of 'Gala' apples showed higher (59.04, 51.02 and 50.52 N) compared to 'Cripps Pink^{TM}' (72.32, 63.83 and 57.52 N) following cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions (21 ± 1ºC) respectively. The interaction between the treatments and cultivars for fruit firmness were found to be significant following cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions (Table 7. 2).

Table 7.2. Effects of blossom thinning treatments of different concentrations of lime sulphur (LS) alone or in combination with organic olive oil and low temperature (0.5 ± 0.5 °C) storage period plus 10 days of simulated shelf conditions (21 ± 1ºC) on fruit firmness (N) in 'Gala' and 'Cripps Pink™' apple.

	Fruit firmness (N)								
	60 days			90 days			120 days		
Treatments (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	83.3	67.74	75.52 a	63.69	61.31	62.50	64.33	60.35	62.34 a
LS 1%	70.05	58.42	64.24 cde	61.6	49.45	55.52	58.71	51.66	55.18 b
LS 1% + Olive oil 3%	74.09	59.64	66.86 bcd	65.62	49.72	57.67	55.18	47.59	51.38 b
LS 2 %	76.36	59.25	67.81 bc	57.29	53.81	55.55	50.12	51.99	51.06 b
LS 2 % + Olive oil 3%	64.65	56.28	60.47 e	67.24	50.7	58.97	57.6	51.56	54.58 b
LS 3 %	71.62	56.23	63.93 de	65.88	48.8	57.34	58.12	51.33	54.72 b
LS 3 % + Olive oil 3%	66.2	56.88	61.54 e	64.39	47.63	56.01	60.52	45.65	53.09 b
LS 4 %	80.26	59.93	70.09 b	63.78	51.96	57.87	51.34	49.01	50.17 b
LS 4 % + Olive oil 3%	64.32	59.04	61.68 e	65.01	45.78	55.39	61.8	45.51	53.65 b
Mean (cultivars)	72.32 A	59.27 B		63.83 A	51.02 B		57.52 A	50.52 B	
	LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)		
Treatments	3.439			NS			5.391		
Cultivars	1.621			2.250			2.541		
Treatments × cultivars	4.864			6.749			7.624		

Any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different (LSD at $P < 0.05$). NS = not significant, n = three replicates (10 fruit per replicate).

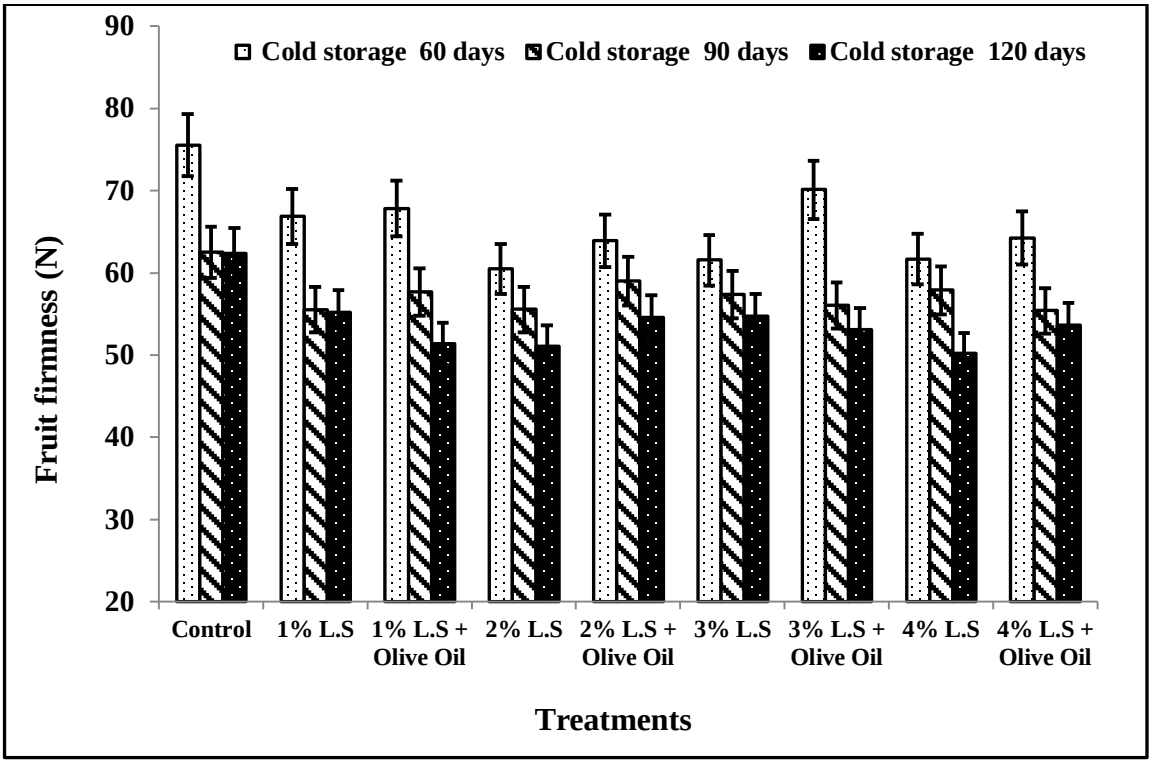

Figure 7. 2. Changes in mean fruit firmness (N) in 'Gala' and 'Cripps Pink^{TM}' apples as influenced by different concentrations of lime sulphur (LS) alone and in combination with organic olive oil applied at 75% bloom stage following 60, 90 and 120 days cold storage (0.5 ± 0.5 °C) plus 10 days of simulated shelf conditions (21 ± 1ºC) period. Vertical bars represent LSD ($P \leq 0.05$).

7. 4. 3. SSC, TA, SSC/TA ratio and ascorbic acid

Blossom thinning treatments with lime sulphur alone or in combination with olive oil sprayed on 'Gala' and 'Cripps Pink^{TM}' apple trees significantly ($P \leq 0.05$) affected mean SSC, TA and SSC/TA ratio in apple juice as compared to control following 60, 90 and 120 days of cold storage plus 10 days of simulated shelf conditions (21 ± 1ºC) (Table 7. 3, 7. 4 and 7. 5). However, the concentration of ascorbic acid did not differ significantly between the treatments and control (Fig. 7.4b). Mean SSC was significantly ($P \leq 0.05$) highest (12.55, 12.85 and 12.33% following cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions (21 ± 1ºC) in the treatment of lime sulphur (3%) only, control and lime sulphur (4%) respectively as compared to others treatments (Table 7. 3).

LS treatments alone or in combination with olive oil appeared to have lowered mean TA compared to control after 60, 90 and 120 days of cold storage Table (7. 4). Conversely, mean SSC/TA ratio was significantly higher in blossom-thinned fruit compared to control following 60, 90 and 120 days cold storage plus 10 days of simulated shelf conditions (Table 7. 5). When averaged over the cultivars, SSC and TA differed significantly with cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions among cultivars, except SSC with cold storage for 90 days and levels of ascorbic acid with cold storage for 90 and 120 days did not differ significantly (Figure 7. 3 a, b, 7. 4 a, b). SSC was higher in 'Cripps Pink^{TM}' apple (11.80 and 12.60%) than 'Gala' (11.54 and 11.10%) following cold storage for 90 and 120 days plus 10 days of simulated shelf conditions respectively. Similarly TA was higher in the juice of 'Cripps Pink^{TM}' (1.23, 1.02 and 0.99%) compared to 'Gala' (1.04, 0.87 and 0.74%) post-cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions respectively. The concentration of ascorbic acid was higher in 'Cripps Pink^{TM}' apple compared to 'Gala' following cold storage for 60 days plus 10 days of simulated shelf conditions. The interaction between the cultivars and different treatments were significant for SSC, TA and ascorbic acid, except SSC after 60 days and ascorbic acid after 120 days which were not significant (Tables 7. 3, 7. 4, 7. 5 and 7. 6).

Table 7. 3. Effects of blossom thinning treatments of different concentrations of lime sulphur (LS) alone or in combination with organic olive oil and low temperature (0.5 ± 0.5 °C) storage period plus 10 days of simulated shelf conditions (21 ± 1ºC) on SSC (%) in 'Gala' and 'Cripps Pink™' apples.

	SSC (%)								
	60 days			90 days			120 days		
Treatments	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	11.17	11.7	11.44c	12.2	13.5	12.85 a	10.6	12.45	11.53 c
L. S 1%	11.32	11.32	11.32 cd	11.45	11.5	12.98 a	10.95	12.33	11.64 bc
L. S 1% + Olive oil 3%	11.37	11.72	11.55 c	11.8	11.45	11.48 e	11.25	12.5	11.88 b
L. S 2 %	12.17	12.22	12.20 ab	13.33	12.63	11.63 de	11.75	12.68	12.21 a
L. S 2 % + Olive oil 3%	11.42	11.85	11.64 c	11.62	12.23	11.93 cd	10.9	12.65	11.78 bc
L. S 3 %	12.42	12.67	12.55 a	12.6	12.23	12.41 b	11.2	13.2	12.20 a
L. S 3 % + Olive oil 3%	11.7	11.77	11.74 bc	12.6	11.63	12.11 bc	11.1	12.6	11.85 b
L. S 4 %	11.87	11.6	11.74 bc	12.32	12.43	12.38 b	12.05	12.6	12.33 a
L. S 4 % + Olive oil 3%	10.35	11.3	10.82 d	11.95	12.22	12.09 bc	10.13	12.43	11.28 d
Mean (cultivars)	11.54 B	11.80 A		12.21	12.20		11.10 B	12.60 A	
LSD ($P \leq 0.05$)				LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)		
Treatments (T)	0.503			0.317			0.242		
Cultivars (C)	0.237			NS			0.114		
T × C	NS			0.448			0.342		

Any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different (LSD at P < 0.05). NS = not significant, n = four replicates (10 fruit per replicate).

Table 7. 4. Effects of blossom thinning treatments of different concentrations of lime sulphur (LS) alone or in combination with organic olive oil and low temperature (0.5 ± 0.5 °C) storage period plus 10 days of simulated shelf conditions (21 ± 1ºC) on TA (%) in 'Gala' and 'Cripps Pink™' apples.

	TA (malic acid %)								
	60 days			90 days			120 days		
Treatments	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	1.15	1.77	1.46 a	0.9	1.2	1.05 a	0.8	1.18	0.99 a
L. S 1%	1	1	1.0 e	0.85	1.12	0.99 ab	0.72	0.95	0.84 c
L. S 1% + Olive oil 3%	1.15	1.05	1.10 cde	0.75	0.97	0.86 cde	0.8	1	0.90 bc
L. S 2 %	1.1	1.17	1.14 bcd	1	1.08	1.038 a	0.73	1	0.863 c
L. S 2 % + Olive oil 3%	0.9	1.15	1.03 e	0.85	1.03	0.94 bc	0.6	1.05	0.83 c
L. S 3 %	1.1	1.35	1.23 b	0.83	0.9	0.86 ce	0.65	1.05	0.85 c
L. S 3 % + Olive oil 3%	1	1.05	1.03 e	0.83	0.8	0.81 e	0.68	0.8	0.74 d
L. S 4 %	1.02	1.3	1.16 bc	0.95	1.05	1.00 ab	0.95	1	0.98 ab
L. S 4 % + Olive oil 3%	0.9	1.2	1.05 de	0.88	1	0.94 bcd	0.75	0.9	0.83 c
Mean (cultivars)	1.04 B	1.23 A		0.87 B	1.02 A		0.74 B	0.99 A	
LSD ($P \leq 0.05$)				LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)		
Treatments (T)	0.092			0.073			0.076		
Cultivars (C)	0.044			0.034			0.036		
T × C	0.131			0.103			0.108		

Any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different (LSD at P < 0.05). NS = not significant, n = four replicates (10 fruit per replicate).

Table 7. 5. Effects of blossom thinning treatments of different concentrations of lime sulphur (LS) alone or in combination with organic olive oil and low temperature (0.5 ± 0.5 °C) storage period plus 10 days of simulated shelf conditions (21 ± 1ºC) on SSC/TA ratio in 'Gala' and 'Cripps Pink™' apple trees.

	SSC/TA ratio								
	60 days			90 days			120 days		
Treatments	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	9.76	6.6	8.18 d	13.6	11.29	12.45 d	13.39	10.65	12.02 e
L. S 1%	11.33	11.33	11.33 abc	13.52	10.29	11.90 d	15.15	13.01	16.19 a
L. S 1% + Olive oil 3%	9.9	11.3	10.60 abc	15.8	11.77	13.79 bc	14.15	12.5	15.24 ab
L. S 2 %	11.15	10.42	10.79 abc	13.32	11.81	12.57 d	16.27	12.74	15.03 ab
L. S 2 % + Olive oil 3%	12.77	10.42	11.60 a	13.72	11.95	12.83 cd	18.4	12.08	14.51 bc
L. S 3 %	11.33	9.46	10.39 c	15.31	13.8	14.56 ab	17.33	12.72	14.08 bcd
L. S 3 % + Olive oil 3%	11.7	11.24	11.47 ab	15.31	14.67	14.99 a	16.52	15.87	13.84 bcd
L. S 4 %	11.63	8.94	10.29 c	12.98	11.86	12.42 d	12.89	12.6	13.32 cde
L. S 4 % + Olive oil 3%	11.56	9.5	10.53 bc	13.7	12.28	12.99 cd	13.78	13.89	12.75 de
Mean (cultivars)	11.24	9.91		14.14 B	12.19 A		15.32 A	12.90 B	
LSD ($P \leq 0.05$)				LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)		
Treatments (T)	0.933			1.039			1.39		
Cultivars (C)	0.440			0.490			0.65		
T × C	1.320			1.469			1.96		

Any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different (LSD at P < 0.05). NS = not significant, n = four replicates (10 fruit per replicate).

Table 7. 6. Effects of blossom thinning treatments of different concentrations of lime sulphur (LS) alone or in combination with organic olive oil and low temperature (0.5 ± 0.5 °C) storage period plus 10 days of simulated shelf conditions (21 ± 1ºC) on concentration of ascorbic acid in 'Gala' and 'Cripps Pink^{TM}' apples.

	Ascorbic acid (mg.100 ml-1)								
	60 days			90 days			120 days		
Treatments	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	53.46	63.78	58.62	48.54	63.09	55.81	36.15	56.27	46.21
L. S 1%	53.51	71.76	62.63	62.31	67.01	64.66	49.66	50.83	50.25
L. S 1% + Olive oil 3%	57.22	58.99	58.10	58.94	61.36	60.15	58.3	47.59	52.94
L. S 2 %	55.19	61.06	58.12	65.12	67.49	66.30	51.86	51.3	51.58
L. S 2 % + Olive oil 3%	53.59	53.33	53.46	59.07	50.7	54.89	40.73	47.46	44.10
L. S 3 %	51.65	54.37	53.01	63.3	50.1	56.70	57.22	53.85	55.53
L. S 3 % + Olive oil 3%	57.48	56.05	56.76	71.76	52.56	62.16	44.31	47.42	45.87
L. S 4 %	53.29	61.96	57.63	52.25	58.77	55.51	65.2	50.44	57.82
L. S 4 % + Olive oil 3%	44.4	69.69	57.04	72.71	58.25	65.48	48.84	60.89	54.86
Mean (cultivars)	53.31 B	61.22 A		61.56	58.81		50.25	51.78	
LSD ($P \leq 0.05$)				LSD ($P \leq 0.05$)			LSD ($P \leq 0.05$)		
Treatments (T)	NS			NS			NS		
Cultivars (C)	3.73			NS			NS		
T × C	11.18			14.53			NS		

Any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different (LSD at P < 0.05). NS = not significant, n = four replicates (10 fruit per replicate).

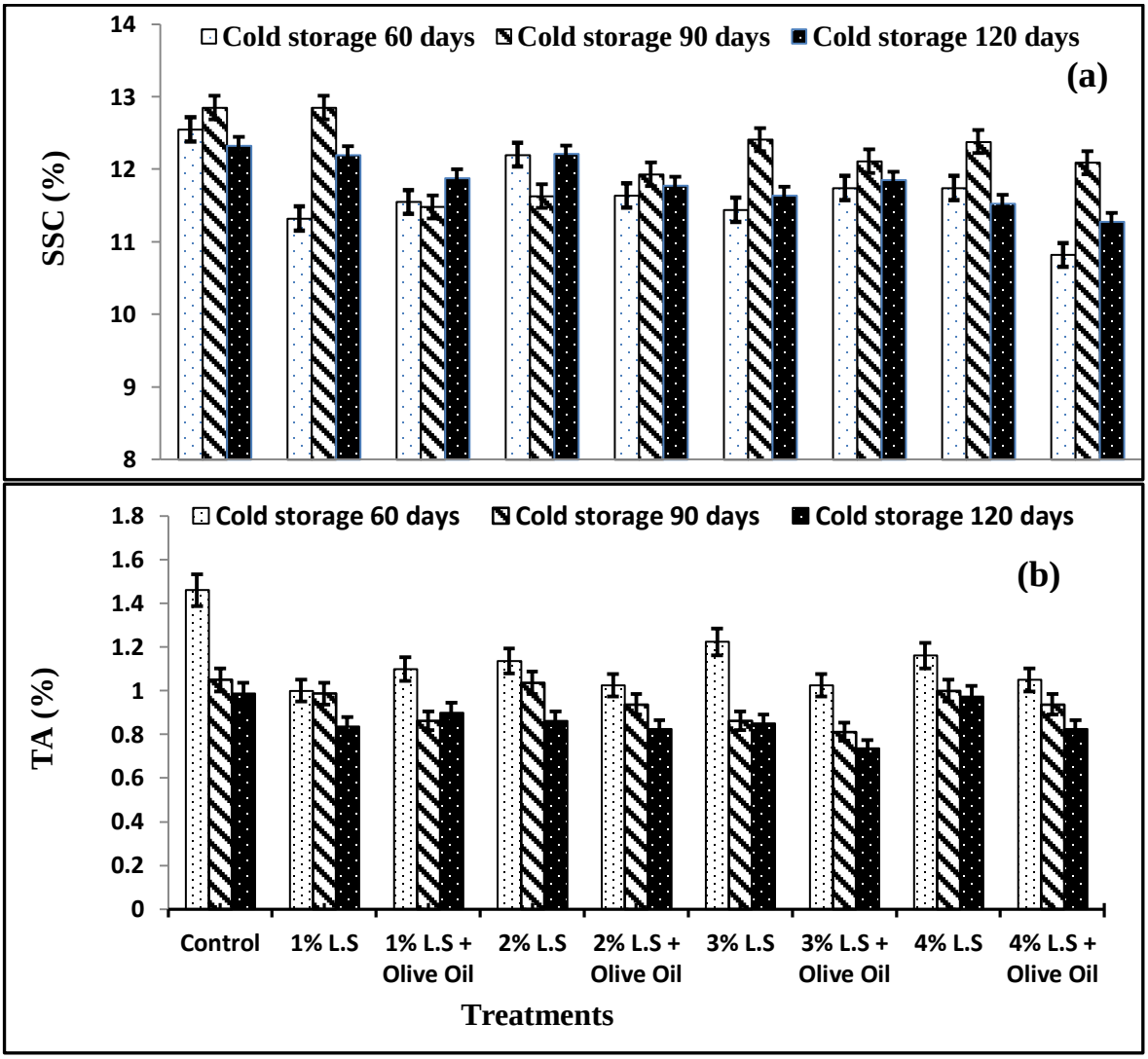

Figure 7. 3. Changes in mean SSC (%) (a) and TA (%) (b) influenced with the spray of different concentrations of lime sulphur (LS) alone and in combination with organic olive oil applied at 75% bloom stage following cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions (21 ± 1ºC). Vertical bars represent LSD ($P \leq 0.05$).

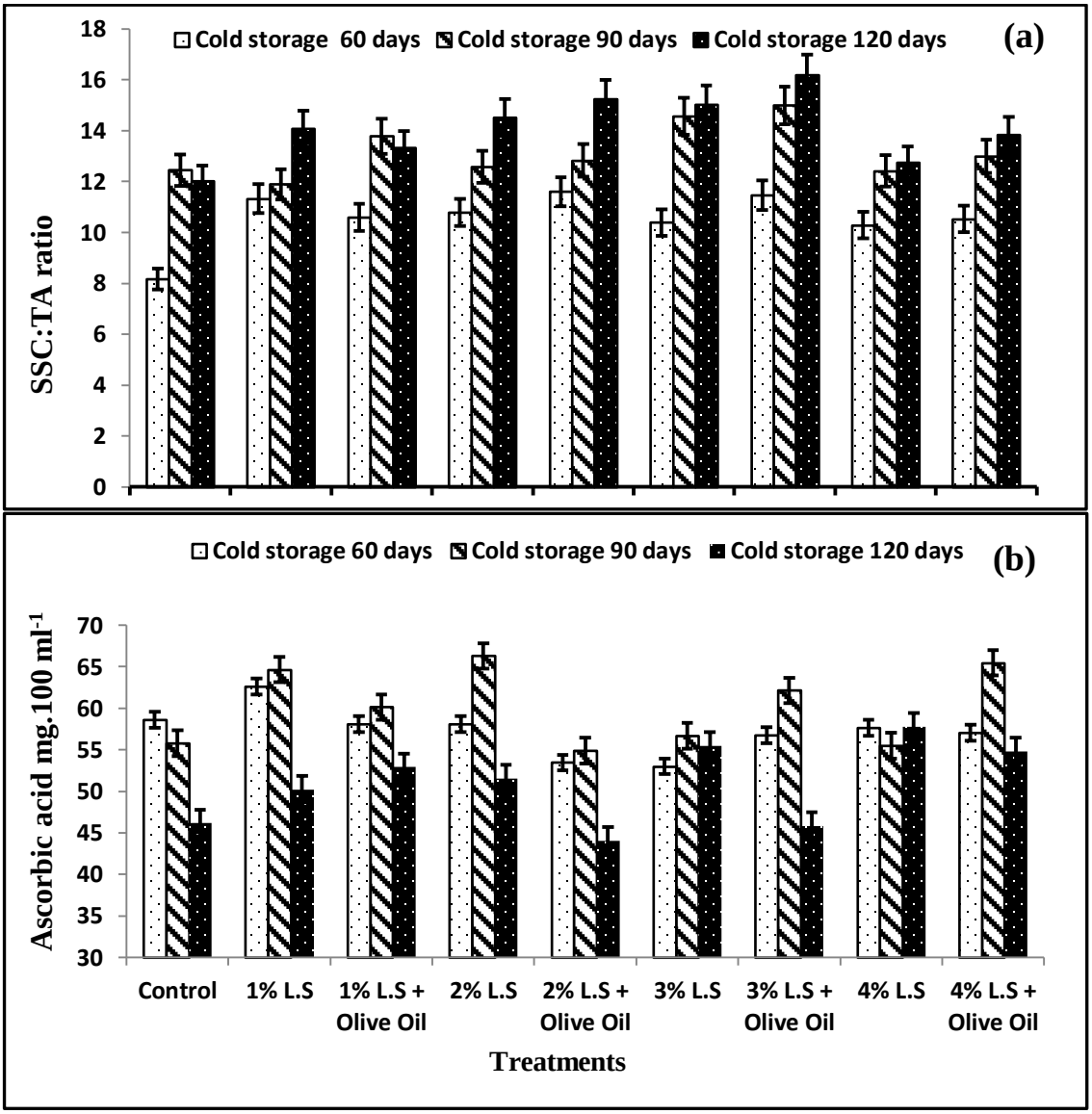

Figure 7. 4. Changes in mean SSC:TA ratio (a) and ascorbic acid (b) influenced with the spray of different concentrations of lime sulphur (LS) alone and in combination with organic olive oil applied at 75% bloom stage following cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions (21 ± 1ºC). Vertical bars represent LSD ($P \leq 0.05$).

7. 5. Discussion

Blossom thinning treatments with lime sulphur alone or in combination with olive oil significantly affected various fruit quality parameters in 'Gala' and 'Cripps Pink^{TM}' apples following cold storage plus 10 days of simulated shelf conditions. Fruit from trees that received LS treatments were significantly softer following 60, 90 and 120 days of cold storage plus 10 days of simulated shelf conditions compared to control in both 'Gala' and 'Pink Lady' apples (Fig 7. 1). Higher concentrations of LS (3 and 4%) with or without olive oil appeared to increase softening of fruit following cold storage plus 10 days of simulated shelf conditions irrespective of duration and cultivar. 'Cripps Pink^{TM}' apples were significantly softer than 'Gala' apples following storage plus 10 days of simulated shelf conditions (Table 7. 1). Therefore, cultivar response to LS treatments is an important factor to be considered during storage. Similarly, Basak, (1999) reported that the quality of stored apples is influenced by cultivar, the time and effectiveness of thinning, type and concentration of chemical used. Pre-harvest factors that also influence apple quality before and after storage include: climatic factors such as light intensity, temperature, and rainfall; cultural factors such as mineral nutrition, timing, and extent of thinning that affects crop load, orchard floor management, irrigation, tree management, and use of growth regulators; and genetic factors that involve choice of cultivar or clone, rootstocks, and inter stocks (Bramlage, 1993; Harker et al., 1997; Sams, 1999).

Apples were harvested at an acceptable maturity for commercial use. This also indicates that there might be a relationship between storage time, fruit firmness and fruit size, where increased fruit size results in reduced fruit firmness during long-term storage (Table 7. 1). Maturity at commercial harvest and fruit size influence post-harvest softening in apples (Johnston et al., 2002). The LS concentrations of 3-4% alone or with 3% of olive oil resulted in greater fruit size in both 'Gala' and 'Cripps Pink' apple (Chapter 6, Table 6. 2) and the enlargement of fruit size generally includes more and bigger air vacuoles which ultimately make the fruit softer than smaller fruit (Harker et al., 1997). Smaller fruit are considered to have stronger tissues as they have greater cell wall material per unit volume compared to larger fruit (Johnston et al., 2010). Larger apples have been reported to be softer than smaller fruit both at harvest and after storage (Blanpied et al., 1978; Marmo et al., 1985; Siddiqui and Bangerth, 1995) and therefore fruit firmness is negatively

correlated to fruit size both at harvest and after storage (Harker et al., 1997). Fruit firmness is also correlated with internal ethylene production. Increased internal ethylene concentration may have contributed to enhanced fruit softening in fruit that had received blossom thinning treatments with LS. The role of ethylene in enhancing softening in apple is well documented (Costa el at., 2010; Tacken el at., 2010). As expected, the percentage fruit weight loss increased with extended storage durations (Fig. 7. 2). The highest weight loss (3.85%) was recorded in the fruit stored for 120 days plus 10 days of simulated shelf conditions followed by fruit stored for 90 days plus 10 days of simulated shelf conditions (2.99%) in both cultivars. Jan et al., (2012) reported that the percentage fruit weight loss increases significantly with the extended storage duration. The loss of fruit weight is attributed to respiration and loss of moisture in the fruit with increasing storage duration, a fact confirmed by Erturk, (2003), Gavlheiro et al., (2003) and Ghafir et al., (2009). In this study, however, fruit respiration rate following storage was not recorded, which may be an important factor to be considered in future studies.

SCC of apple and other fruits is a major quality parameter along with the texture and composition of fruit (Weibel et al., 2004; Peck et al., 2006). SSC of apple fruit increased with extended storage duration up to 90 days (Fig 7. 3a). The maximum SSC (12.33%) was recorded in fruit with treatment of 4% of LS following storage for 120 days plus 10 days of simulated shelf conditions as compared to the same treatment fruit following storage for 60 days. SSC was higher in control fruit following cold storage plus 10 days of simulated shelf conditions compared to all LS treatments. The TA decreased with increasing storage durations. The treatment of LS alone (4%) significantly reduced TA in fruit stored for 120 days plus 10 days of simulated shelf conditions (Table 7. 4). This may be attributed to advancement of maturity of fruit as evidenced by lowered fruit firmness in this treatment. SSC and TA in 'Cripps Pink^{TM}' fruit was higher than 'Gala' fruit which is ascribed to varietal differences and the manner in which each cultivar responds to a treatment and furthermore its performance during storage. Ali et al., (2004) reported significant variations in SSC, TA and other physico-chemical characteristics of apples harvested from different varieties. Watkins et al., (2000) observed higher SSC in 'Empire' and 'Delicious' apple fruit stored in air at 0.5ºC for up to 7 months. Similarly the storage duration had a significant effect on SSC:TA ratio of apple juice. The SSC:TA ratio

increased with the extension of storage durations (Table 7. 5). The ascorbic acid of apple fruit decreased with extended storage durations (Table 7. 6). However, the blossom thinning treatments with LS did not significantly affect ascorbic acid concentration in fruit juice in both cultivars.

In conclusion, percentage of fruit weight loss was higher in cold-stored fruit 120 days plus 10 days of simulated shelf conditions between (3.33- 3.85%) followed by cold-stored fruit 90 days plus 10 days of simulated shelf conditions between (2.22 - 3.00%) in both 'Gala' and 'Cripps Pink^{TM}' apples. Spray application of higher concentrations of LS (3-4%) alone or with 3% of olive oil at blossom thinning showed a decreasing trend in firmness, SSC and TA following cold storage for 60, 90 and 120 days plus 10 days of simulated shelf conditions in 'Cripps Pink^{TM}' and 'Gala' apples. The fruit firmness reduced due to slightly advanced maturation of fruit from these treated trees. In future the effects of these treatments on respiration rate and ethylene production or internal ethylene concentration of the fruit at harvest and during cold storage may be monitored.

Chapter 8

Effects of pre-harvest spray applications of organic calcium and boron on concentrations of Ca and B in leaf and fruit, incidence of bitter pit, superficial scald, cold storage life and quality of 'Cripps Pink™' and 'Gala' apples

Abstract

Calcium (Ca) plays an important role in development of various physiological disorders, storage life and quality of apple fruit. Supplying sufficient levels of Ca into apple fruit is a major challenge particularly in organic apple production. Effects of various spray applications of different concentrations of organic calcium (Ca) or boron (B) alone and their combination during fruit growth and developments on regulation of uptake and supply of calcium into the apple fruit and leaf tissues at harvest, incidence of bitter pit and superficial scald, cold storage life and fruit quality in organically grown 'Gala' and 'Cripps Pink™' apples were investigated. Four pre-harvest sprays of an aqueous emulsion containing Biomin® calcium 1, 2 or 3 kg/ha alone or Biomin® Boron 1kg and 2 kg/ha alone and in combinations and synertrol oil (0.05%) as a surfactant was sprayed onto whole trees four times during fruit growth commencing from 30 days after full bloom stage at 25 day intervals. The effects of various Ca and B treatments on the concentrations of Ca and B in fruit and leaf tissues at harvest as well as incidence of bitter pit, superficial scald, soluble solids concentration (SSC), titratable acidity (TA), SSC:TA ratio and ascorbic acid in fruit juice following 0, 90 and 120 days of cold storage (0.5 ±0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days were recorded. All the pre-harvest sprays of calcium and boron have significantly increased calcium concentration in leaves and fruit in comparison to the control in 'Gala' and 'Cripps Pink™' apples. Pre-harvest spray applications of Ca alone were more effective in raising the concentration of Ca in leaf and fruit tissues than the spray applications of Ca in combination with boron sprays. Spray applications of Ca (3kg/ha) alone was most effective in increasing calcium in leaves and fruit in comparison to the control and other treatments. In conclusion, four pre-harvest sprays of emulsion containing Biomin® calcium (3 kg/ha) and synertrol oil (0.05%) as a surfactant, commencing from 30 days

after full bloom stage at 25 day intervals was most effective in curtailing bitter pit, superficial scald, extending cold storage life and maintaining fruit quality of organically grown 'Gala' and 'Cripps Pink^{TM}' apples.

8. 1. Introduction

Bitter pit in apples is related to calcium deficiency and is one of the main problems in the apple industry around the world. Environmental factors such as drought, low soil pH, crop load, and tree vigour are also associated with bitter pit in apples (Rosenberg et al., 2004). Although the incidence of bitter pit is triggered during the pre-harvest period in association with calcium deficiency, these symptoms normally develop progressively during storage. Bitter pit is a physiological disorder and the symptoms include brown, corky, roundish lesions predominantly under the epidermis, mainly at the calyx end (Ferguson and Watkins, 1989; Lotz, 1996). Bitter pit can also develop just below the skin and in the cortex, but is not externally visible (Ferguson and Watkins, 1989; Little and Holmes, 1999). Several studies have noted the relationship between the application of fertilizers and bitter pit within a growing season (Raese, 1994a; Raese, 1994b; Raese, 1994c; Raese, 1998; Ferguson and Watkins, 1989, Ferguson and Triggs, 1990; Bramlage, 1993). The incidence of bitter pit in apple fruit is commonly associated with low calcium concentrations (Bangerth, 1979; Moore et al., 2005), whilst higher nitrogen increases the susceptibility to bitter pit (Waller, 1990). Ferguson and Watkins, (1983) also confirmed that apple tissues low in calcium concentrations are sensitive to bitter pit. Four to five spray applications of calcium (0.7%) following post-bloom stage, significantly increased calcium concentration in the apple fruit, thereby reducing the incidence of bitter pit (Neilsen and Neilsen, 2002). Foliar Ca applications are highly successful in improving Ca concentration in the fruit and reducing bitter pit (Carbo et al., 1988; Hewitt and Watkins, 1991; Le Grange et al., 1998) and rapid uptake and penetration of Ca into fruit noticed during the first four weeks after full bloom or between six and 14 weeks after full bloom (Quinlan, 1969; Ferguson et al., 1987; Cline et al., 1991; Casero et al., 2002; Schlegel and Schönherr, 2002).

On the other hand, nutrient partitioning during various stages of apple fruit growth and development may be of importance to superficial scald which develops during storage. Superficial scald also called storage scald, is a common physiological disorder that develops during storage of apple fruit and tends to be genotype dependent (Emongor et al., 1994). Wills and Scott, (1981) reported that the concentration of nitrogen, potassium, magnesium and calcium in apples at pre- and post-harvest can affect fruit size and storage life. Calcium plays a very important role in plant growth and development as well as maintaining and modulating various cell functions (Wills and Scott, 1981). Sharples, (1980) reported that calcium affects cell metabolism and structure, not only conferring greater resistance to changes preceding softening, fungal invasion and the development of disorder, but it also delays the general rate of senescence of the tissues. Calcium also influences fruit quality (Raese and Drake, 2000). Loughlin and Jotic, (1978) reported a significant reduction in the incidence of internal breakdown and superficial scald with six spray applications of calcium (different concentrations) with the first two applications at 0.5 kg/100 litres, third application at 0.8 kg/100 litres and last three applications at 1 kg/100 litres. Calcium spray improved the appearance of the apple fruit, reduced cork spot and rot, showed less superficial scald, greener fruit skin colour, whiter flesh and increased juiciness (Raese and Drake, 2000). Fruit quality is affected and most often correlated with pre-harvest fruit mineral nutrients (Fallahi et al., 2010). Calcium spray application improves fruit quality parameters such as fruit finish, juiciness, red skin colour, texture and fruit firmness as well as reduces incidence of scald and bitter pit in apple fruit (Thomas and Stephen, 2000).

Boron application improves apple fruit quality by reducing development of external and internal cork formation and cracking on fruits (Jackson, 2003; Ganie et al., 2013, Wojcik et al., 1999). Boron is a component of plant cell wall and implicated in developing complexes with rhamnogalacturonan II. Consequently, boron crosslinks two chains of pectin polysaccharides and formulates a pectin polysaccharides system in the cell wall leading to improved cell wall integrity (Matoh, 1997; Goldbach and Wimmer, 2007; Dong et al., 2009 and Pham, 2009). Both boron and calcium formulates bridges within the pectin polysaccharide chains in the cell wall leading to stabilisation of cell

wall and membrane integrity (Easterwood, 2002; Dong et al., 2009 and Pham, 2009). Many research reports suggested the role of calcium and boron in improving apple fruit quality and cell wall stability and membrane integrity of the cell (Mason et al., 1975; Rossignol et al., 1977; Lidster and Porritt, 1978; Fallahi et al., 1987; Poovaiah, 1988).

Ca absorption into tree, leaf and fruit influenced by boron supply (Smith and Reuther, 1950; Pham, 2009) and an interaction between calcium and boron in apple seedlings has also been reported by Woodbridge et al. (1973). Conversely, Papadakis et al., (2003) also reported inconsistent effect of boron supply on calcium absorption into citrus trees. It was surmised that calcium spray application in combination with boron application will regulate uptake and supply of calcium into the apple fruit. However, in organic apple orchards, the use of these chemicals is prohibited. No research work has been reported on the efficacy of organic sources of calcium and boron alone and in combination in reducing the incidence of bitter pit, superficial scald and extending cold storage life and maintaining fruit quality under Australian conditions. Therefore, the effects of various spray applications of different concentrations of organic calcium or boron alone and their combination during fruit growth and developments on regulation of uptake and supply of calcium into the apple fruit and leaf tissues at harvest, incidence of bitter pit and superficial scald, cold storage life and fruit quality in 'Gala' and 'Cripps Pink^{TM}' apples were investigated.

8. 2. Materials and Methods

8. 2. 1. Plant materials

The experiment was conducted on two commercially important cultivars of apples 'Cripps Pink^{TM}' grafted on rootstock M26 and 'Gala' on rootstock MM06 apple trees of 15 years age at the Newton Brothers Orchards in Manjimup (latitude 34°14´ South, longitude 116°8´ East) Western Australia. The trees were spaced 7.5 m between rows and 2.5 m within rows in the North-South orientation. All the experimental apple trees were organically grown received similar cultural practices including nutrition, irrigation and plant protection except varying experimental treatments.

8. 2. 2. Organic Chemicals

Biomin® Calcium (15% calcium) and Biomin® Boron (15% boron) was purchased from J H Biotech, Inc, Australia. Synertrol oil, an organic surfactant was purchased from Organic Crop Production, Pty Ltd, Lilyfield, NSW, Australia.

8. 2. 3. Treatments and experimental design

An aqueous emulsion containing Biomin® calcium 1, 2 or 3 kg /ha alone or Biomin® Boron 1kg and 2 kg/ha alone and in combinations (Table 8.1) and synertrol oil (0.05%) as a surfactant was sprayed onto whole trees four times during fruit growth commencing from 30 days after full bloom stage at 25 day intervals. 'Cripps Pink™' and 'Gala' trees were sprayed using a sprayer (The Selecta Trolleypak Mk II, Acacia Ridge, Australia). Control trees were kept unsprayed. Calcium spray treatment was applied in the morning, whilst boron treatment was applied in the evening on the same day. The experiment was laid out by following two-factor (treatments and cultivars) factorial randomised block design with four replicates. Single trees were treated as one experimental unit. The concentrations of Ca and B in fruit and leaf tissues were determined at harvest. The fruit were harvested at commercial maturity from the experimental trees. Fruit free from visual symptoms of diseases and blemishes were packed in cardboard boxes and kept in cold storage (0.5 ±0.5°C) for 0 (at commercial harvest), 90 and 120 days. Twenty fruit were included in each replication and replicated three times. Following the cold storage period, the fruit were kept at simulated shelf conditions (21 ± 1ºC) for 10 days. Incidence of bitter pit, scald, fruit colour, soluble solids concentration (SSC), titratable acidity (TA), SSC:TA ratio and ascorbic acid were determined from the apple fruit juice.

8. 2. 4. Observations recorded

8. 2. 4. 1. Determination of calcium and boron concentrations from leaf and fruit

Leaves (25 per tree) and ten fruit per tree were collected for nutrient analysis at commercial harvest during 2012. The leaves and fruit from each tree were collected from unshaded positions at about 1.5 m height from the north, east, south and west

quadrants of the trees. All leaves and fruit collected were free from disease or insect damage. Fruit leaves were washed, dried and grinded to powder prior to digestion. Calcium and boron concentrations in leaf and fruit tissue were analysed by using Radial Inductively Coupled Plasma Atomic Emission Spectrometry (VISTA – PRO, CCD Simultaneous ICP-OES, VARIAN, Australia) which operated in simultaneous mode. The detailed procedure has also been described in Chapter 3, Section 3.7.2.

8. 2. 4. 2. Bitter pit and superficial scald index

Bitter pit and superficial scald was recorded using a 10-point visual peel damage scale on the fruit. The severity of incidence was recorded using a rating scale from 0 - 10. 0 = no visible injury, 1 = slight injury, 5 = moderate injury, 10 = severe injury. The index was calculated by following the formula as reported by Pesis et al., (2010).

$$\text{Bitter pit or Scald index} = \sum_{0}^{10} \frac{(\text{index level}) \times (\text{fruits at this level})}{\text{total no of fruits}}$$

8. 2. 4. 3. Fruit weight loss (%)

The fruit weight loss was estimated by weighing ten fruit per replication before and after storage and simulated shelf conditions (21 ± 1ºC) for 10 days by using a digital balance (A&D Limited, Tokyo, Japan). Fruit weight loss was calculated as detailed in Chapter 3, Section 3. 9. 4. Fruit weight loss was expressed as a percentage.

8. 2. 4. 4. Fruit firmness

Fruit firmness from ten randomly selected fruit was determined using an electronic pressure tester (Model EPT-1 pressure tester, Lake City Technical Products Inc., Kelowna, BC, Canada) as outlined in Chapter 3, Section 3. 9. 5. Fruit firmness was expressed as Newtons (N).

8. 2. 4. 5. SSC, TA and SSC:TA ratio

SSC was determined from freshly extracted juice using digital refractometer as detailed in Chapter 3, Section 3. 9. 7. TA was determined by titrating the juice with 0.1 N NaOH following the method outlined in Chapter 3, Section 3. 9. 8. SSC/TA ratio was calculated dividing SSC with the corresponding TA value as detailed in Chapter 3, Section 3. 9. 9.

8. 2. 4. 6. Ascorbic acid

The concentration of ascorbic acid from freshly extracted juice was determined following the method of Jagota and Dani, (1982) and Malik and Singh, (2005) with some modifications. The concentration of ascorbic acid was expressed as mg ascorbic acid per 100 ml fresh juice as outlined in Chapter 3, Section 3. 9. 10.

8. 3. Statistical analysis

The experimental data were subjected to two-way analysis of variance (ANOVA) using GenStat 14th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK). The effects of various treatments and their interactions for different parameters were assessed within ANOVA and least significance differences (LSD) were calculated following significant F-test ($P \leq 0.05$). All the assumptions of analysis were checked to ensure validity of statistical analysis.

8. 4. Results

8. 4. 1. Concentration of Calcium in Leaves and Fruit

Spray applications of organic calcium alone or in combination with boron have significantly ($P \leq 0.05$) increased calcium concentration in leaves in comparison to the control in 'Gala' and 'Cripps Pink^{TM}' apples (Table 8. 1). When averaged over the two cultivars, the spray applications of calcium (3kg/ha) alone significantly ($P \leq 0.05$) increased concentration of calcium in leaves and fruit in comparison to the control (Fig. 8. 1a & b). This treatment also resulted in the highest accumulation of calcium in the fruit compared to all other treatments (Table 8.1). The interaction between the treatments and the cultivars was found to be significant for concentrations of calcium in leaves and fruit. When averaged over treatments, mean concentrations of calcium in leaves and fruit differ significantly among the cultivars. 'Cripps Pink^{TM}' exhibited significantly higher calcium in leaves (1.56%) than 'Gala (1.13%), whilst 'Gala' showed significantly higher calcium in fruit (0.033%) than 'Cripps Pink (0.031%) Table (8. 1). The application of boron with calcium did not significantly contribute to the accumulation of calcium in the leaves or fruit when compared to application of calcium alone (Table 8. 1).

Table 8.1. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on the concentrations of calcium in leaf and fruit of 'Gala' and 'Cripps Pink™' apples.

	Leaf Ca (%)			Fruit Ca (%)		
Treatment	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	1.07	1.40	1.24 c	0.026	0.024	0.025 e
1kg (Ca)	1.20	1.56	1.38 ab	0.038	0.031	0.035 b
2kg (Ca)	1.18	1.64	1.42 ab	0.039	0.035	0.037 b
3kg (Ca)	1.38	1.64	1.51 a	0.040	0.045	0.043 a
1kg (B)	0.95	1.60	1.27 b	0.025	0.027	0.026 e
1kg (Ca)+ 1kg (B)	1.04	1.64	1.34 b	0.031	0.027	0.029 d
2kg (Ca)+1kg (B)	1.18	1.64	1.41 ab	0.032	0.025	0.029 d
3kg (Ca)+1kg (B)	1.22	1.51	1.37 ab	0.027	0.032	0.030 cd
2kg (B)	1.0	1.53	1.26 bc	0.025	0.028	0.027 e
1kg (Ca)+2kg (B)	1.04	1.60	1.32 b	0.037	0.030	0.034 bcd
2kg (Ca)+2kg (B)	1.20	1.46	1.32 b	0.031	0.030	0.031 cd
3kg (Ca)+2kg (B)	1.26	1.36	1.31 b	0.028	0.030	0.029 d
Mean (cultivars)	1.13 B	1.56 A		0.033 A	0.031 B	
LSD ($P \leq 0.05$)						
Treatments (T)	0.145			0.004		
Cultivars (C)	0.059			0.002		
T × C	0.204			0.006		

+ = followed by, any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different using LSD at ($P \leq 0.05$). NS = not significant, n = four replicates (20 fruit per replicate) and (100-150 leaves per replicate).

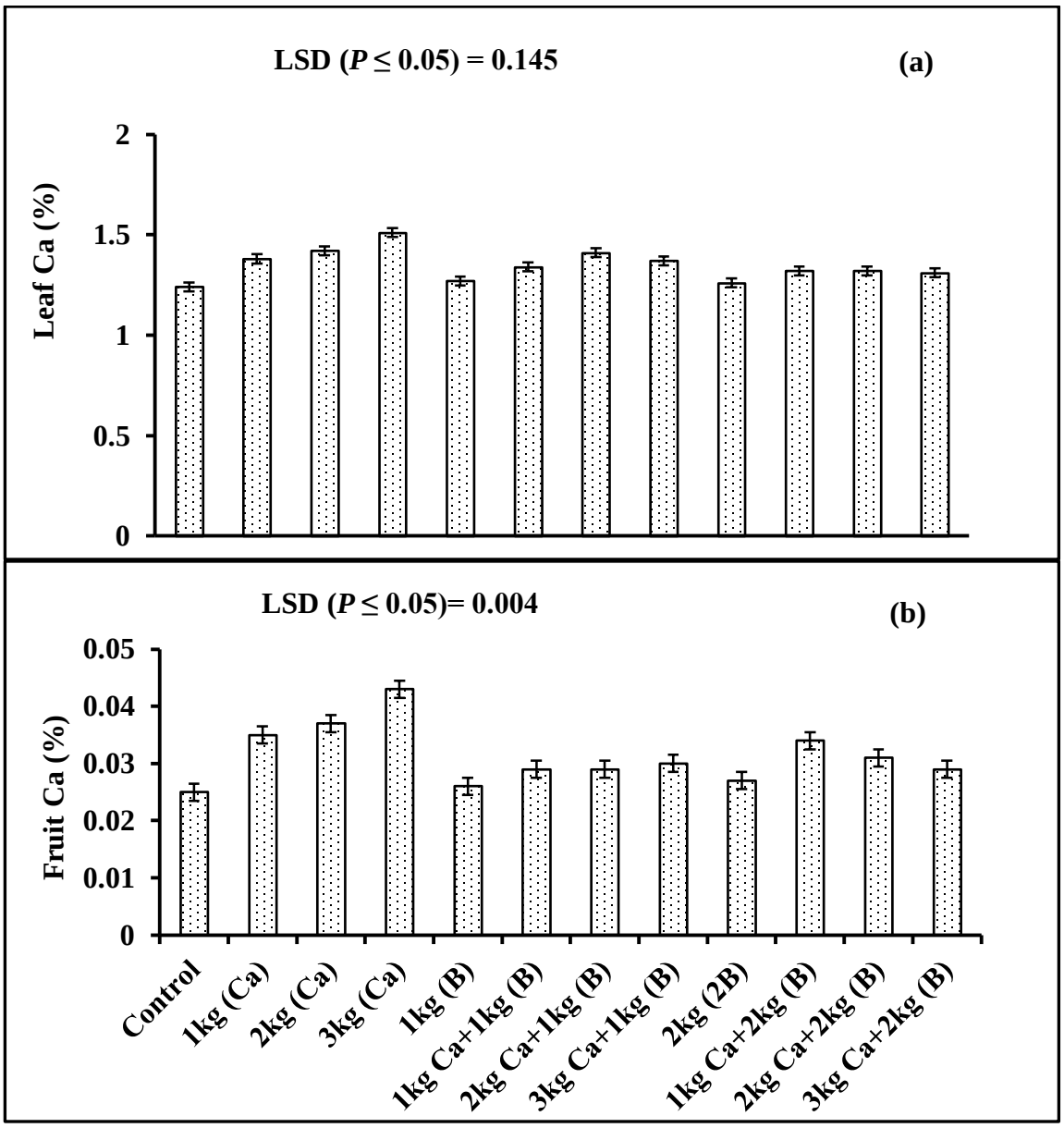

Figure 8.1. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on mean concentrations of calcium in leaf (a) and fruit (b). Vertical bars represent standard error of means.

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8. 4. 2. Concentration of Boron in Leaves and Fruit

All spray treatments of boron alone or in combination with Ca on 'Gala' and 'Cripps Pink^{TM}' apple trees showed significantly ($P \leq 0.05$) increased concentration of boron in leaves and fruit in comparison to the leaves and fruit of untreated trees at commercial harvest (Table 8. 2). A single spray application of boron (2 kg/ha) significantly increased concentration of boron in the leaves of 'Gala' and 'Cripps Pink^{TM}' apples compared to all other treatments (Table 8. 2). This treatment also resulted in the highest concentrations of boron in the fruit as compared to all other treatments except trees treated with 1 kg/ha boron alone. As expected, calcium treatments alone did not significantly increase boron concentration in the leaves. The applications of boron (2 kg/ha) along with calcium during the season, appeared to increase boron concentration in the leaves and fruit compared to 1 kg/ha application of boron with calcium and this was more evident in the leaves (Table 8. 2 and Figs 8. 2 a and b). Irrespective of the treatments, the mean concentrations of boron in leaves and fruit were higher (31.23-35.62 mg kg^{-1} respectively) in 'Cripps Pink^{TM}' than 'Gala' (29.25-32.55 mg/Kg^{-1} respectively). The interaction between the cultivars and the treatments differed significantly for concentration of boron in leaves and fruit (Table. 8. 2).

Table 8. 2. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on the concentrations of boron in leaf and fruit of 'Gala' and 'Cripps Pink^{TM}' apples.

	Leaf B ($mgKg^{-1}$)			Fruit B ($mgKg^{-1}$)		
	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	28.38	28.65	28.51 d	16.27	12.63	14.45 f
1kg (Ca)	27.92	30.56	29.24 cd	23.30	21.75	22.53 ef
2kg (Ca)	26.86	30.89	28.88 d	29.83	20.90	25.36 de
3kg (Ca)	27.44	29.89	28.67 d	28.75	24.66	26.71 de
1kg (B)	30.28	32.02	31.17 b	39.41	57.08	48.24 ab
1kg (Ca)+ 1kg (B)	27.85	33.48	30.67 bc	36.74	29.67	33.21 cd
2kg (Ca)+1kg (B)	28.94	30.75	29.85 bcd	37.05	38.70	37.87 bc
3kg (Ca)+1kg (B)	27.55	31.32	29.43 cd	24.29	43.18	33.73 cd
2kg (B)	33.58	33.48	33.53 a	46.84	63.88	55.36 a
1kg (Ca)+2kg (B)	30.57	31.52	31.05 b	43.41	46.11	44.76 b
2kg (Ca)+2kg (B)	30.26	31.97	31.12 b	42.76	31.29	37.02 cb
3kg (Ca)+2kg (B)	31.32	30.13	30.73 bc	25.91	41.63	33.77 cd
Mean (cultivars)	29.25 B	31.23 A		32.55 B	35.62 A	
LSD ($P \leq 0.05$)						
Treatments (T)	1.39			5.03		
Cultivars (C)	0.567			2.054		
T × C	1.963			7.116		

+ = followed by, any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different using LSD at ($P \leq 0.05$). NS = not significant, n = four replicates (20 fruit per replicate) and (100-150 leaves per replicate).

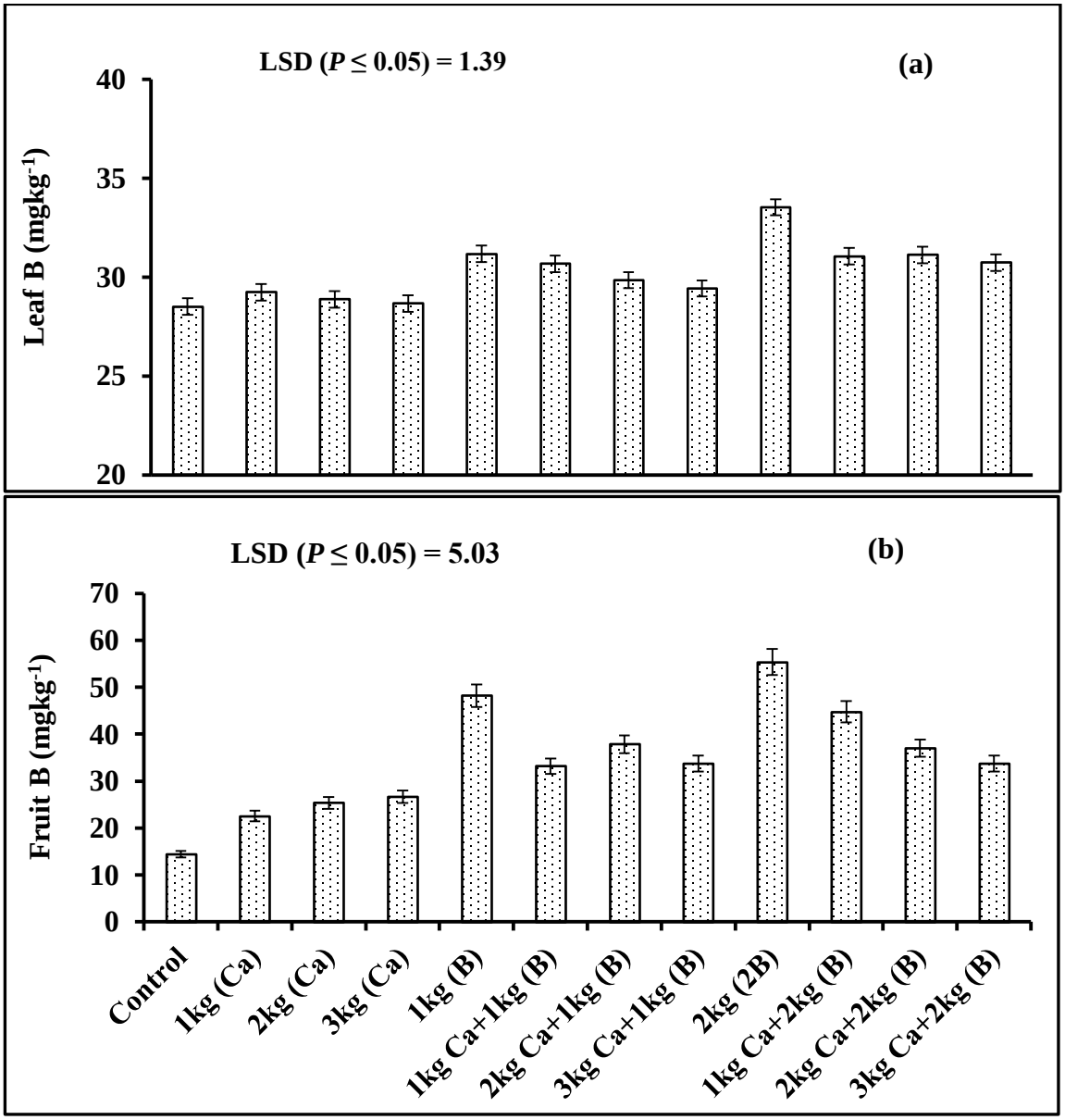

Figure 8. 2. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on mean concentrations of boron in leaf (a) and fruit (b). Vertical bars represent standard error of means.

8. 4. 3. Bitter pit

At commercial harvest, 'Cripps Pink^{TM}' apples showed significantly higher incidences of bitter pit compared to 'Gala' apples, where bitter pit was not detected (Table 8. 3). Calcium treatments alone reduced incidence of bitter pit in 'Cripps Pink^{TM}' apples, whilst application of 2 kg/ha boron alone significantly increased percentage of bitter pit compared to calcium treatments alone. Following 90 days of cold storage and simulated shelf conditions for 10 days, bitter pit was evident in most treatments across both cultivars. However, 'Gala' apples treated with 3 kg/ha calcium alone or two or 3 kg/ha calcium followed by 1 kg/ha boron were still free of bitter pit. The incidence of bitter pit was greater in trees treated with 2 kg/ha boron alone and this trend was evident even after 120 days of cold storage and simulated shelf conditions for 10 days. Calcium and boron sprays on 'Gala' and 'Cripps Pink^{TM}' apple trees significantly ($P \leq 0.05$) affected bitter pit incidence in 'Gala' and 'Cripps Pink^{TM}' apple fruit at commercial harvest and during cold storage for 90 and 120 days and simulated shelf conditions for 10 days (Table 8. 3). However, when averaged over the cultivars, the spray application of 2 kg/ha boron resulted in the highest bitter pit incidence at commercial harvest and during cold storage 90 and 120 days followed by simulated shelf conditions for 10 days (1.28, 1.28, and 1.36% respectively). Meanwhile, the spray applications of 1kg/ha, 2kg/ha or 3kg/ha calcium alone resulted in reduction of percentage of bitter pit incidence at commercial harvest and during 90 and 120 days followed by simulated shelf conditions for 10 days as compared to control and others treatments (Figs 8. 3 a, b and c). On the other hand, irrespective of the treatments, there were significant differences between cultivars for bitter pit in fruit. 'Gala' apple fruit did not show detectable incidence of bitter pit at commercial harvest and the bitter pit was slightly increased following 90 and 120 days cold storage and simulated shelf conditions for 10 days (Table (8. 3). Nevertheless, the bitter pit incidence was higher (1.24, 0.58, and 0.60) in 'Cripps Pink^{TM}' apple fruit (1.24, 0.58, and 0.60 respectively) at commercial harvest and during cold storage for 90 and 120 and simulated shelf conditions for 10 days respectively. The interactions between different treatments and the cultivars were noticed to be significantly ($P \leq 0.05$) different for bitter pit incidence in 'Gala' and 'Cripps Pink^{TM}' apple fruit only at commercial harvest (Table 8. 3).

Table 8. 3. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on incidence of bitter pit on 'Gala' and 'Cripps Pink™' apple fruit following 0, 90 and 120 days of cold storage (0.5 ± 0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days.

Bitter pit index (0 -10)									
Cold storage period									
	0 day			90 days			120 days		
	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	0.00	1.50	1.20 ab	0.50	0.55	0.53 abc	0.31	0.00	0.16 b
1kg (Ca)	0.00	0.20	0.10 fg	0.15	0.15	0.15 b	0.00	0.10	0.05 c
2kg (Ca)	0.00	0.10	0.05 g	0.08	0.05	0.06 c	0.00	0.05	0.03 c
3kg (Ca)	0.00	0.13	0.06 g	0.00	0.05	0.03 c	0.000	0.10	0.05 c
1kg (B)	0.00	0.28	0.14 f	0.60	0.60	0.60 abc	0.000	0.30	0.15 b
1kg (Ca)+ 1kg (B)	0.00	1.00	0.50 def	0.05	0.50	0.28 bc	0.000	0.10	0.15 b
2kg (Ca)+1kg (B)	0.00	1.93	0.96 abc	0.00	0.40	0.20 bc	0.250	1.80	1.03 ab
3kg (Ca)+1kg (B)	0.00	2.40	1.20 ab	0.00	0.35	0.18 bc	0.050	1.80	0.93 ab
2kg (B)	0.00	2.55	1.28 a	0.10	2.45	1.28 a	0.32	2.40	1.36 a
1kg (Ca)+2kg (B)	0.00	2.38	1.19 ab	0.60	1.25	0.93 ab	0.625	1.15	0.80 ab
2kg (Ca)+2kg (B)	0.00	1.68	0.96 abc	0.35	0.65	0.50 abc	0.625	1.70	1.13 ab
3kg (Ca)+2kg (B)	0.00	0.75	0.38 efg	0.30	0.00	0.15 b	0.312	1.20	0.76 ab
Mean (cultivars)	0.00 B	1.24 A		0.23 B	0.58 A		0.18 B	0.60 A	
LSD ($P \leq 0.05$)									
Treatments (T)	0.37			0.73			0.741		
Cultivars (C)	0.15			0.30			0.3027		
T × C	0.53			NS			NS		

+ = followed by, any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different using LSD at ($P \leq 0.05$). NS = not significant, n = four replicates (20 fruit per replicate).

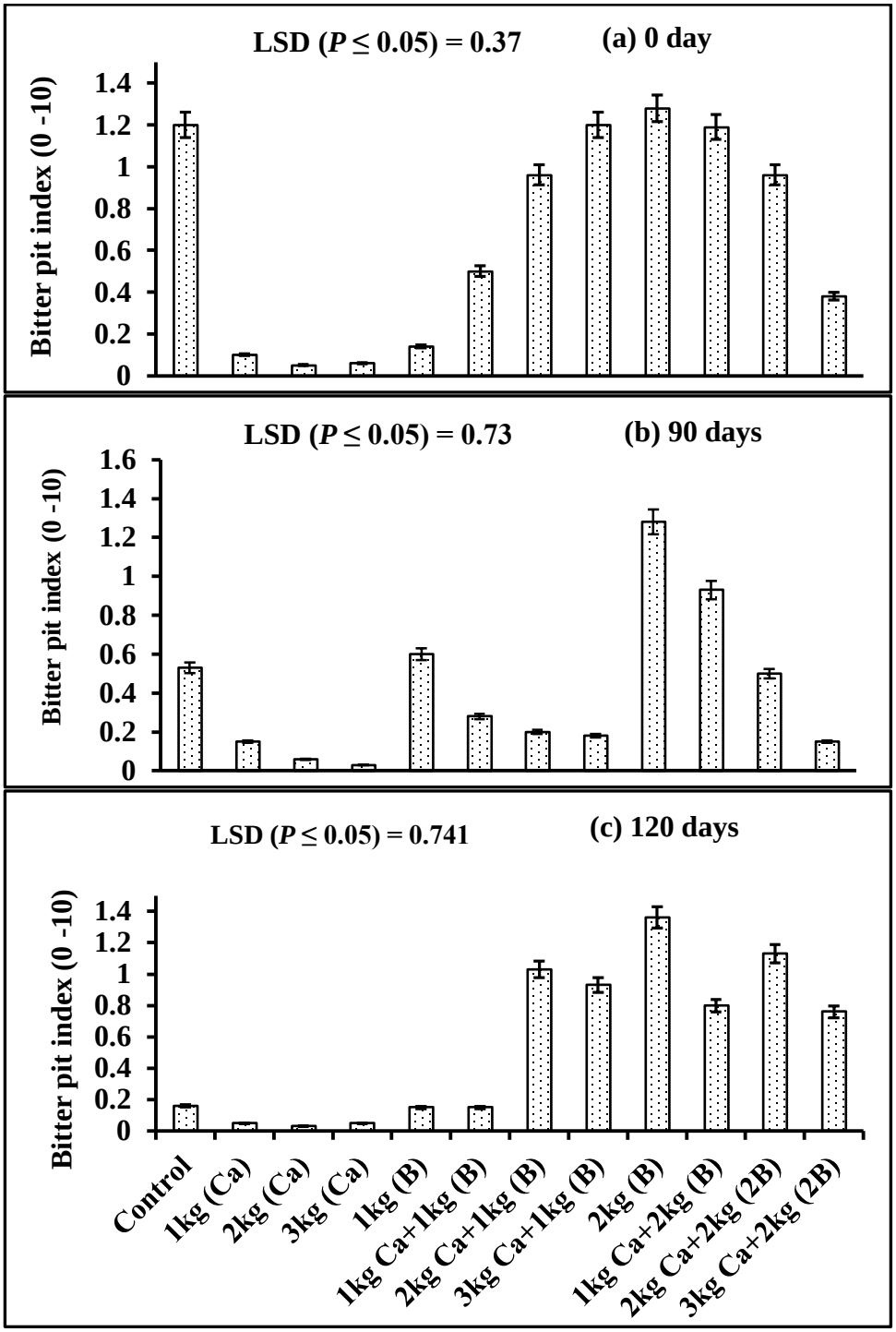

Figure 8. 3. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on mean incidence of bitter pit on 'Gala' and 'Cripps Pink^{TM}' apple fruit following 0, 90 and 120 days of cold storage (0.5 ±0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days. Vertical bars represent standard error of means.

8. 4. 4. Superficial scald

The various calcium and boron treatments alone or in combination had varying effects on incidence of superficial scald in both 'Gala' and 'Cripps Pink^{TM}' apples. At commercial harvest, 'Gala' apples were free from superficial scald (Fig 8. 4). However, at harvest 'Cripps Pink^{TM}' apples treated with boron (2 kg) exhibited significantly higher incidence of superficial scald compared to the fruit from all other treatments. Calcium treatments alone or in combination with boron had no significant effects on reducing superficial scald at commercial harvest compared to control. Spray applications of boron (2 kg/ha) significantly increased incidence of superficial scald following 90 and 120 days cold storage and simulated shelf conditions (21 ± 1ºC) for 10 days, compared to control and all other treatments. Calcium treatments alone appeared to have reduced the incidence of superficial scald compared to control following 90 and 120 days of cold storage and simulated shelf conditions (21 ± 1ºC) for 10 days, with the spray applications of 2 and 3 kg/ha of calcium being most effective. In general, 'Cripps Pink^{TM}' apples were more susceptible to superficial scald than 'Gala' apples (Table 8. 4). All treatments of calcium and boron significantly ($P \leq 0.05$) affected superficial scald incidence in 'Gala' and 'Cripps Pink^{TM}' apple fruit at commercial harvest and 90 and 120 days of cold storage and simulated shelf conditions (21 ± 1ºC) for 10 days (Table 8. 4). The fruit sprayed with 1 kg/ha, 2 kg/ha and 3 kg/ha calcium alone resulted in lowest incidence of superficial scald at commercial harvest, 90 and 120 days of cold storage and simulated shelf conditions (21 ± 1ºC) for 10 days. Irrespective of the treatments, there were significant differences between cultivars for superficial scald incidence on apple fruit. 'Gala' apple fruit did not show detectable incidence of superficial scald at commercial harvest, 90 and 120 days of cold storage and simulated shelf conditions (21 ± 1ºC) for 10 days (Table 8. 4). However, the superficial scald incidence was higher on 'Cripps Pink^{TM}' apple fruit (0.50, 0.19, and 2.27% respectively) at commercial harvest, 90 and 120 days of cold storage and simulated shelf conditions (21 ± 1ºC) for 10 days. The interactions between different treatments and the cultivars were noticed to be significant ($P \leq 0.05$) for superficial scald incidence in 'Gala' and 'Cripps Pink^{TM}' apple fruit at commercial harvest, 90 and 120 days of cold storage and simulated shelf conditions (21 ± 1ºC) for 10 days (Table 8. 4).

Table 8. 4. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on incidence of superficial scald on 'Gala' and 'Cripps Pink^{TM}' apple fruit following 0, 90 and 120 days of cold storage (0.5 ± 0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days.

Superficial scald index (0 -10)									
Cold storage period									
	0 day			90 days			120 days		
Treatments	Gala	Gala	Cripps Pink	Mean (T)	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	0.00	0.09	0.20	0.14 cd	0.075	0.038 b	0.00	2.81	1.41 abc
1kg (Ca)	0.00	0.03	0.10	0.07 de	0.10	0.050 b	0.12	1.66	0.89 bcd
2kg (Ca)	0.00	0.02	0.03	0.02e	0.00	0.00 b	0.00	0.69	0.34 cd
3kg (Ca)	0.00	0.00	0.04	0.02e	0.03	0.013 b	0.25	0.00	0.13 d
1kg (B)	0.00	0.28	0.28	0.28 bc	0.05	0.025 b	0.73	2.56	1.64 bcd
1kg (Ca)+ 1kg (B)	0.00	0.01	0.35	0.18 cd	0.03	0.013 b	0.19	1.12	0.66 cd
2kg (Ca)+1kg (B)	0.00	0.00	0.64	0.32 bc	0.00	0.00 b	0.31	2.69	1.50 bcd
3kg (Ca)+1kg (B)	0.00	0.00	0.72	0.36 bc	0.13	0.063 b	0.30	3.12	1.71 abc
2kg (B)	0.00	0.12	1.48	0.80 a	1.85	0.93 a	0.40	5.12	2.76 a
1kg (Ca)+2kg (B)	0.00	0.12	0.86	0.49 b	0.00	0.00 b	0.62	3.91	2.27 ab
2kg (Ca)+2kg (B)	0.00	0.07	0.61	0.34 bc	0.05	0.03 b	0.00	2.75	1.38 bcd
3kg (Ca)+2kg (B)	0.00	0.10	0.71	0.40 bc	0.03	0.01 b	0.50	0.75	0.64 cd
Mean (cultivars)	0.00 B	0.07 B	0.50 A		0.194 A		0.29 B	2.27 A	
LSD ($P \leq 0.05$)									
Treatments (T)	0.34			0.24			1.31		
Cultivars (C)	0.14			0.10			0.53		
T × C	0.48			0.34			1.85		

+ = followed by, any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different using LSD at ($P \leq 0.05$). NS = not significant, n = four replicates (20 fruit per replicates).

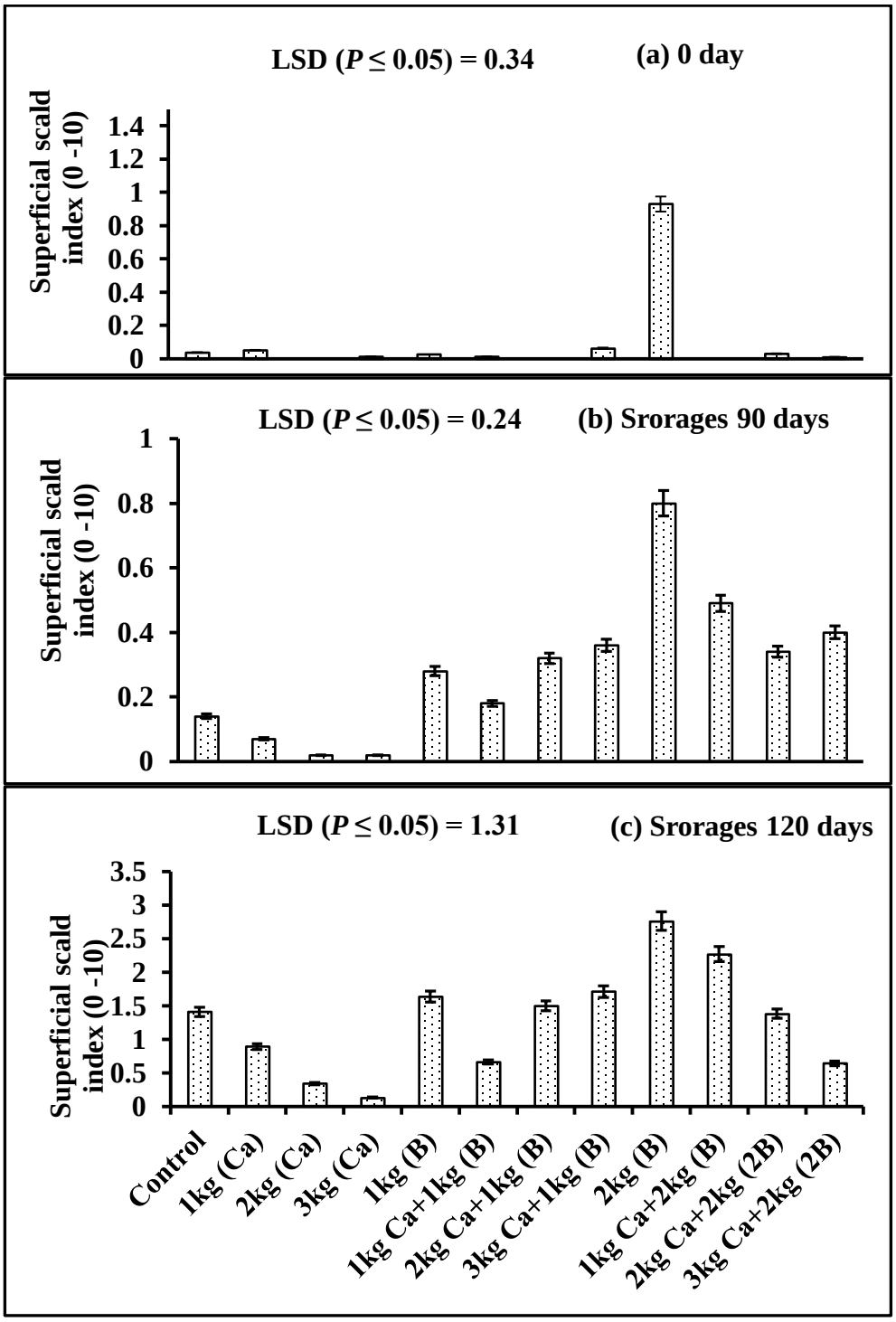

Figure 8. 4. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on mean incidence of superficial scald on 'Gala' and 'Cripps Pink^{TM}' apple fruit following 0, 90 and 120 days of cold storage (0.5 ±0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days. Vertical bars represent standard error of means.

8. 4. 5. Fruit firmness

All calcium and boron spray treatments resulted in significant ($P \leq 0.05$) increase in fruit firmness in comparison to the control fruit at commercial harvest, following 90 and 120 days of cold storage (0.5 ±0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days in both 'Gala' and 'Cripps Pink^{TM}' apple (Fig 8. 5). Mean fruit firmness was significantly higher when trees were sprayed with 3kg/ha calcium alone (98.0, 83.34 and 75.93 N) or with 3 kg/ha of calcium followed by 1kg/ha of boron (94.95, 78.34 and 75.90 N) compared to all other treatments at commercial harvest, following 90 and 120 days of cold storage and simulated shelf conditions for 10 days (Figs 8. 5a, b, c). Irrespective of the treatments, there were significant differences between cultivars for fruit firmness. Fruit firmness was higher (92.2 N) in 'Gala' at commercial harvest compared to 'Cripps Pink'. Whilst 'Cripps Pink^{TM}' apples exhibited higher in firmness following 90 and 120 days of cold storage and simulated shelf conditions for 10 days (78.6 N and 73.3 N respectively) compared to 'Gala'. The interaction between the cultivars and the treatments did not differ significantly for fruit firmness at commercial harvest and following cold storage for 90 and 120 days and simulated shelf conditions for 10 days (Table 8. 5).

Table 8. 5. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on firmness (N) of 'Gala' and 'Cripps Pink^{TM}' apple fruit following 0, 90 and 120 days of cold storage (0.5 ± 0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days.

Firmness (N)									
Storage period									
	0 day			90 days			120 days		
Treatments	Gala	Cripps P	Mean (T)	Gala	Cripps P	Mean (T)	Gala	Cripps P	Mean (T)
Control	86.6	80.8	83.75 e	68.6	71.6	70.06 d	67.2	66.7	66.95 c
1kg (Ca)	90.8	88.3	89.55 bcd	68.7	76.6	72.66 c	67.9	72.7	70.28 ab
2kg (Ca)	93.5	86.2	89.89 bcd	75.8	74.8	75.33 bc	69.3	67.4	68.35 b
3kg (Ca)	97.2	98.8	98.00 a	81.2	85.5	83.34 a	73.6	78.2	75.90 a
1kg (B)	97.9	89.1	93.54 abc	73.0	83.7	78.34 abc	73.3	76.7	75.00 a
1kg (Ca) + 1kg (B)	89.8	87.4	88.59 cd	68.6	78.0	73.33 bc	66.8	73.0	69.85 ab
2kg (Ca) +1kg (B)	95.4	93.5	94.42 abc	74.7	84.7	79.71 ab	68.7	78.4	73.51 ab
3kg (Ca) +1kg (B)	97.6	92.3	94.95 ab	75.9	79.9	77.88 abc	74.9	77.0	75.93 a
2kg (B)	89.1	87.7	88.41 cd	75.9	79.3	77.60 abc	67.4	73.8	70.56 ab
1kg (Ca) +2kg (B)	85.5	86.2	85.84 d	71.6	78.3	74.91 bc	68.9	73.6	71.25 ab
2kg (Ca) +2kg (B)	87.9	86.8	87.34 d	71.2	73.8	72.51 c	70.3	71.0	70.65 ab
3kg (Ca) +2kg (B)	95.6	86.4	91.02 bc	72.0	77.4	74.70 bc	71.8	71.8	71.76 ab
Mean (cultivars)	92.2 A	88.6 B		73.1 B	78.6 A		70.2 B	73.3 A	
LSD ($P \leq 0.05$)									
Treatments (T)	5.24			5.92			5.17		
Cultivars (C)	2.14			2.42			2.11		
T × C	NS			NS			NS		

+ = followed by, any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different using LSD at ($P \leq 0.05$). NS = not significant, n = four replicates (20 fruit per replicate).

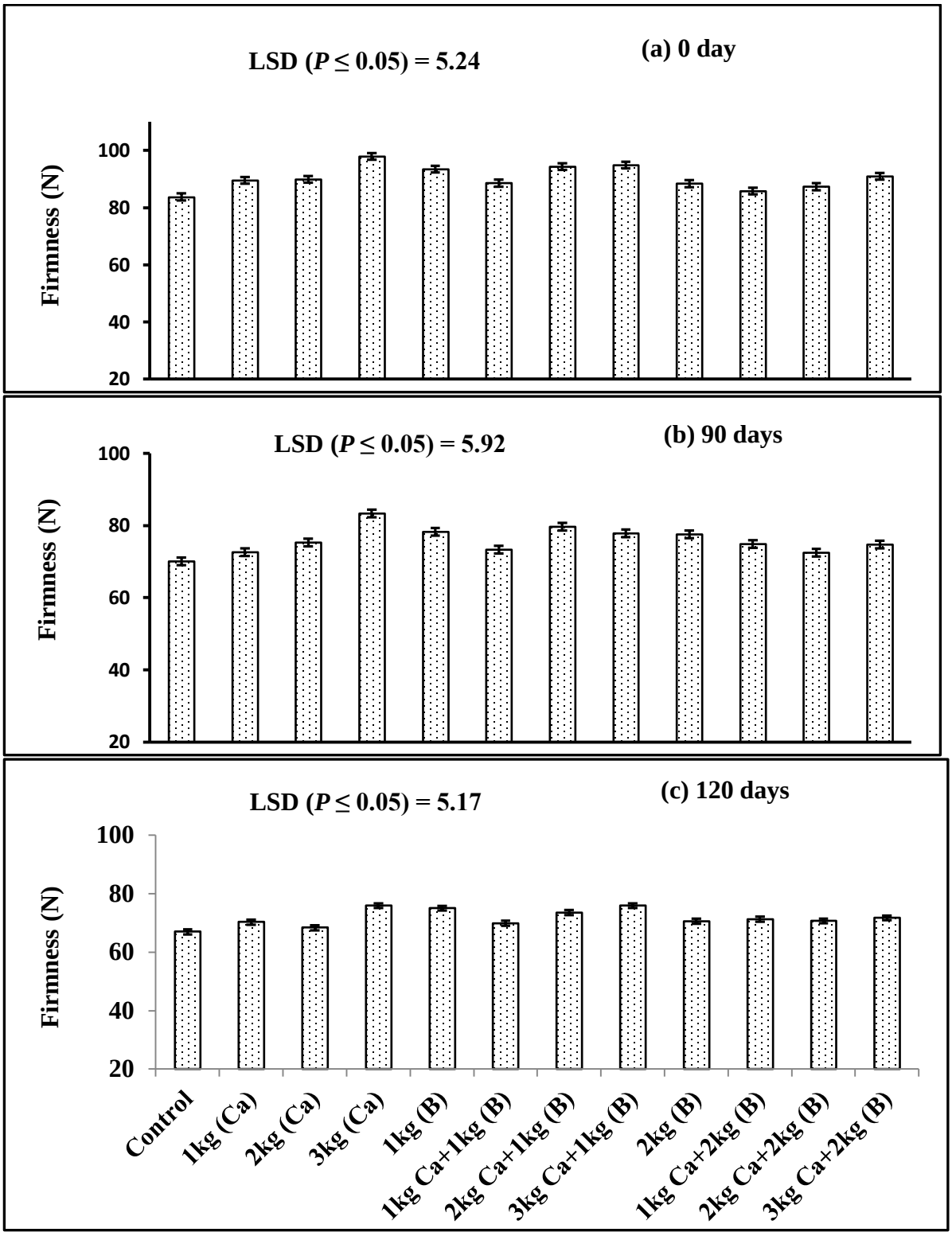

Figure 8. 5. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on mean firmness (N) 'Gala' and 'Cripps Pink^{TM}' apple fruit following 0, 90 and 120 days of cold storage (0.5 ±0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days. Vertical bars represent standard error of means.

8. 4. 6. SSC, TA, SSC: TA ratio and ascorbic acid

All the spray treatments of calcium and boron applied to 'Gala' and 'Cripps Pink^{TM}' apple trees have significantly ($P \leq 0.05$) increased mean SSC, TA and ascorbic acid in apple juice as compared to control at commercial harvest, following 90 and 120 days of cold storage (0.5 ±0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days. Meanwhile, most of the spray treatments resulted in lower mean SSC:TA ratio compared to control at commercial harvest, following 90 and 120 days of cold storage and simulated shelf conditions for 10 days (Figs 8. 6, 8. 7, 8. 8 and 8. 9). Mean SSC was significantly ($P \leq 0.05$) highest when the trees were sprayed with 3 kg of calcium followed by 1kg of boron (14.33, 15.85 and 15.77%) at commercial harvest, following 90 and 120 days of cold storage and simulated shelf conditions for 10 days respectively as compared to untreated trees (Fig 8. 6 a, b and c). Similarly, mean TA was significantly highest in the fruit harvested from the trees sprayed with 3 kg/ha of calcium followed by 1kg/ha of boron at commercial harvest, following 90 and 120 days of cold storage and simulated shelf conditions for 10 days respectively as compared to untreated trees (Fig 8.7 a, b and c). Mean SSC:TA ratio was significantly lowest when the trees were sprayed with 3 kg/ha calcium followed by 1 kg/ha of boron at commercial harvest, following 90 and 120 days of cold storage and simulated shelf conditions for 10 days (Figs 8. 8 a, b and c). Irrespective of the treatment, mean SSC was higher (13.79%) in 'Cripps Pink^{TM}' at commercial harvest than 'Gala' (Table 8. 6). Meanwhile, SSC was higher in 'Gala' apple fruit following cold storage for 90 and 120 days and simulated shelf conditions for 10 days (16.88 and 14.64% respectively) than 'Cripps Pink^{TM}' (14.50 and 13.98% respectively) (Table 8. 11). Mean TA was higher in the juice of 'Cripps Pink^{TM}' apple (2.54%, 2.39% and 1.74% respectively) at commercial harvest and following cold storage for 90 and 120 days and simulated shelf conditions for 10 days compared to 'Gala' (1.91%, 1.44 and 1.38% respectively) Table (8. 7). SSC:TA ratio was significantly higher in 'Gala' at commercial harvest and following cold storage for 90 and 120 days and simulated shelf conditions for 10 days respectively compared to the SSC:TA ratio of 'Cripps Pink^{TM}' apple (Table 8. 8). The concentrations of ascorbic acid were higher in fruit at commercial harvest, also after 90 and 120 days of storage and simulated shelf conditions for 10 days from trees that had received spray applications of

3 kg/ha calcium as compared to all other treatments (Fig 8. 9a, b and c). Irrespective of the treatment, ascorbic acid was higher (60.86 mg·100ml^{-1} FJ) in 'Cripps Pink^{TM}' at commercial harvest than 'Gala' (51.35 mg·100ml^{-1} FJ) (Table 8. 9). Meanwhile, the ascorbic acid was higher in 'Gala' apple fruit following cold storage for 90 days and simulated shelf conditions for 10 days compared to 'Cripps Pink'. There were no significant differences between the cultivars following 120 days of storage (Table 8. 9). The interaction between treatments and cultivars was significant for levels of ascorbic acid at commercial harvest and also after 90 days of storage.

Table 8. 6. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on soluble solids concentration (SSC) in the juice of 'Gala' and 'Cripps Pink^{TM}' apple fruit following 0, 90 and 120 days of cold storage (0.5 ± 0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days.

SSC (%)									
Storage period									
	0 day			90 days			120 days		
Treatments	Gala	Cripps P	Mean (T)	Gala	Cripps P	Mean (T)	Gala	Cripps P	Mean (T)
Control	10.70	12.73	11.71 c	12.55	13.08	12.81 h	12.62	13.40	13.01 e
1kg (Ca)	11.90	12.35	12.13 c	12.98	13.35	13.16 gh	13.55	13.27	13.41 de
2kg (Ca)	11.55	12.90	12.23 c	13.58	13.45	13.51 fg	13.85	13.15	13.50 cde
3kg (Ca)	12.08	13.15	12.61 bc	13.93	14.18	14.05 ef	11.55	14.17	12.86 e
1kg (B)	14.20	14.13	14.16 a	15.35	15.13	15.24 abc	15.65	15.05	15.35 abc
1kg (Ca)+ 1kg (B)	13.58	14.20	13.89 a	15.03	15.08	15.05 bc	15.32	15.12	15.22 abc
2kg (Ca)+1kg (B)	13.38	14.95	14.16 a	16.40	15.10	15.75 a	15.95	14.40	15.17 abc
3kg (Ca)+1kg (B)	13.93	14.73	14.33 a	16.28	15.43	15.85 a	16.92	14.05	15.77 a
2kg (B)	13.35	14.28	13.81 a	14.00	14.50	14.25 de	14.72	13.52	14.12 abcd
1kg (Ca)+2kg (B)	12.40	14.13	13.26 ab	14.18	14.45	14.31 de	14.12	13.20	13.66 bcd
2kg (Ca)+2kg (B)	12.83	14.03	13.43 ab	15.08	14.40	14.74 cd	15.95	14.40	14.11 abc
3kg (Ca)+2kg (B)	14.75	13.90	14.33 a	16.88	14.50	15.69 ab	16.67	14.87	15.49 ab
Mean (cultivars)	12.89 B	13.79 A		16.88 A	14.50 B		14.64 A	13.98 B	
LSD ($P \leq 0.05$)									
Treatments (T)	0.953			0.618			1.635		
Cultivars (C)	0.389			0.252			0.668		
T × C	NS			0.874			NS		

+ = followed by, any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different using LSD at ($P \leq 0.05$). NS = not significant, n = four replicates (20 fruit per replicate). FJ = fruit juice.

Table 8. 7. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on titratable acidity (TA) in the juice of 'Gala' and 'Cripps Pink™' apple fruit following 0, 90 and 120 days of cold storage (0.5 ± 0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days.

TA (malic acid %)									
	0 day			90 days			120 days		
	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	1.58	1.65	1.61 f	1.150	1.975	1.56 d	1.125	1.650	1.39 c
1kg (Ca)	1.75	1.88	1.81 ef	1.200	2.150	1.68 cd	1.175	1.600	1.39 c
2kg (Ca)	1.73	2.05	1.89 def	1.350	2.325	1.84 bc	1.425	1.575	1.50 bc
3kg (Ca)	1.70	2.08	1.89 def	1.250	2.400	1.83 bc	1.325	1.900	1.61 ab
1kg (B)	2.18	2.73	2.45 bc	1.550	2.475	2.01 b	1.450	1.800	1.63 ab
1kg (Ca)+ 1kg (B)	2.05	2.93	2.49 bc	1.375	2.450	1.91 b	1.550	1.675	1.61 ab
2kg (Ca)+1kg (B)	2.05	2.90	2.48 bc	1.650	2.800	2.23 a	1.425	1.925	1.68 ab
3kg (Ca)+1kg (B)	2.28	3.40	2.84 a	1.675	2.825	2.25 a	1.600	2.000	1.80 a
2kg (B)	1.88	2.50	2.19 c	1.475	2.500	1.99 b	1.225	1.800	1.51 bc
1kg (Ca)+2kg (B)	1.70	2.58	2.14 c	1.425	2.475	1.95 b	1.250	1.850	1.55 bc
2kg (Ca)+2kg (B)	2.05	2.90	2.28 c	1.525	2.175	1.85 bc	1.425	1.575	1.50 bc
3kg (Ca)+2kg (B)	2.20	3.13	2.66 b	1.625	2.100	1.86 bc	1.625	1.575	1.60 ab
Mean (cultivars)	1.91 B	2.54 A		1.437 B	2.387 A		1.383 B	1.744 A	
LSD ($P \leq 0.05$)									
Treatments (T)	0.311			0.197			0.182		
Cultivars (C)	0.127			0.080			0.075		
T × C	0.440			0.278			0.258		

+ = followed by, any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different using LSD at ($P \leq 0.05$). NS = not significant, n = four replicates (20 fruit per replicate). FJ = fruit juice.

Table 8. 8. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on SSC/TA ratio in the juice of 'Gala' and 'Cripps Pink^{TM}' apple fruit following 0, 90 and 120 days of cold storage (0.5 ± 0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days.

SSC/TA ratio									
	Storage period								
	0 day			90 days			120 days		
Treatments	Gala	Cripps Pink	Mean (s)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	6.80	7.94	7.37 a	10.93	6.62	8.77 a	11.29	8.14	9.72 a
1kg (Ca)	6.85	6.62	6.73 ab	10.86	6.25	8.55 abc	11.54	8.31	9.93 a
2kg (Ca)	6.75	6.40	6.57 bc	10.10	5.84	7.97 abc	9.83	8.38	9.10 ab
3kg (Ca)	7.18	6.41	6.80 ab	11.17	5.95	8.56 abc	8.65	7.50	8.07 b
1kg (1B)	6.54	5.20	5.87 def	9.94	6.18	8.06 abc	10.84	8.42	9.63 ab
1kg Ca+1kg (1B)	6.68	4.90	5.79 def	11.04	6.17	8.60 ab	9.98	9.04	9.51 ab
2kg Ca+1kg (1B)	6.52	5.18	5.85 def	10.15	5.54	7.84 bcd	11.36	7.53	9.44 ab
3kg Ca+1kg (1B)	6.14	4.36	5.25 f	9.82	5.36	7.59 d	10.44	8.97	9.71 a
2kg (2B)	7.13	5.77	6.45 bcd	9.58	5.81	7.70 cd	12.15	7.53	9.834 a
1kg Ca+1kg (2B)	7.34	5.54	6.44 bcd	10.19	5.87	8.03 abcd	11.41	7.18	9.30 ab
2kg Ca+1kg (2B)	6.89	5.48	6.18 bcde	9.97	6.62	8.30 abcd	10.43	8.75	9.59 ab
3kg Ca+1kg (2B)	6.72	4.48	5.60 ef	10.40	6.91	8.65 ab	10.47	7.47	8.97 ab
Mean (cultivars)	6.80 A	5.69 B		10.34 A	6.09 B		10.70 A	8.10 B	
LSD ($P \leq 0.05$)									
Treatments (T)	0.655			0.760			NS		
Cultivars (C)	0.267			0.310			0.553		
T × C	0.926			NS			NS		

+ = followed by, any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different using LSD at ($P \leq 0.05$). NS = not significant, n = four replicates (20 fruit per replicate). FJ = fruit juice.

Table 8. 9. Effects of pre-harvest spray application of organic calcium and organic boron on ascorbic acid in in the juice of 'Gala' and 'Cripps Pink™' apple fruit at commercial harvest and after storage periods.

Ascorbic acid (mg·100ml^{-1} FJ)									
	0 day			90 days			120 days		
Treatments	Gala	Cripps Pink	Mean (s)	Gala	Cripps Pink	Mean (T)	Gala	Cripps Pink	Mean (T)
Control	47.33	60.50	53.92 c	48.54	55.09	51.82 c	36.50	51.95	44.23 c
1kg (Ca)	50.31	58.60	54.45 c	62.31	67.01	64.66 ab	50.10	51.30	50.70 b
2kg (Ca)	53.94	63.82	58.88 b	71.76	50.10	60.93 ab	51.86	51.78	51.82 b
3kg (Ca)	58.68	67.10	62.89 a	72.71	67.49	70.10 a	61.53	58.60	60.07 a
1kg (1B)	49.19	63.17	56.18 c	52.25	58.77	55.51 b	65.20	50.44	57.82 ab
1kg Ca+1kg (1B)	49.79	59.63	54.71 c	58.94	61.36	60.15 ab	58.30	47.59	52.94 b
2kg Ca+1kg (1B)	53.42	58.38	55.90 c	59.07	50.70	54.89 b	40.77	47.46	44.12 c
3kg Ca+1kg (1B)	51.95	59.25	55.60 c	65.12	52.56	58.84 b	44.31	47.42	45.87 c
2kg (2B)	48.80	60.45	54.63 c	63.30	58.25	60.78 ab	48.84	49.71	49.28 bc
1kg Ca+1kg (2B)	48.07	60.32	54.20 c	58.51	55.23	56.87 b	58.30	49.75	54.02 b
2kg Ca+1kg (2B)	52.56	59.25	55.90 c	59.07	51.39	55.23 b	40.77	49.19	44.98 bc
3kg Ca+1kg (2B)	52.21	59.89	56.05 c	65.07	52.56	58.81 b	48.63	47.42	48.02 bc
Mean (cultivars)	51.35 A	60.86 B		61.39 A	57.38 B		50.43	50.22	
LSD ($P \leq 0.05$)									
Treatments (T)	2.393			9.52			11.56		
Cultivars (C)	0.977			3.89			NS		
T × C	3.385			13.47			NS		

+ = followed by, any two means within a column followed by different lowercase letters and within a row followed by different uppercase letters are significantly different using LSD at ($P \leq 0.05$). NS = not significant, n = four replicates (20 fruit per replicate). FJ = fruit juice.

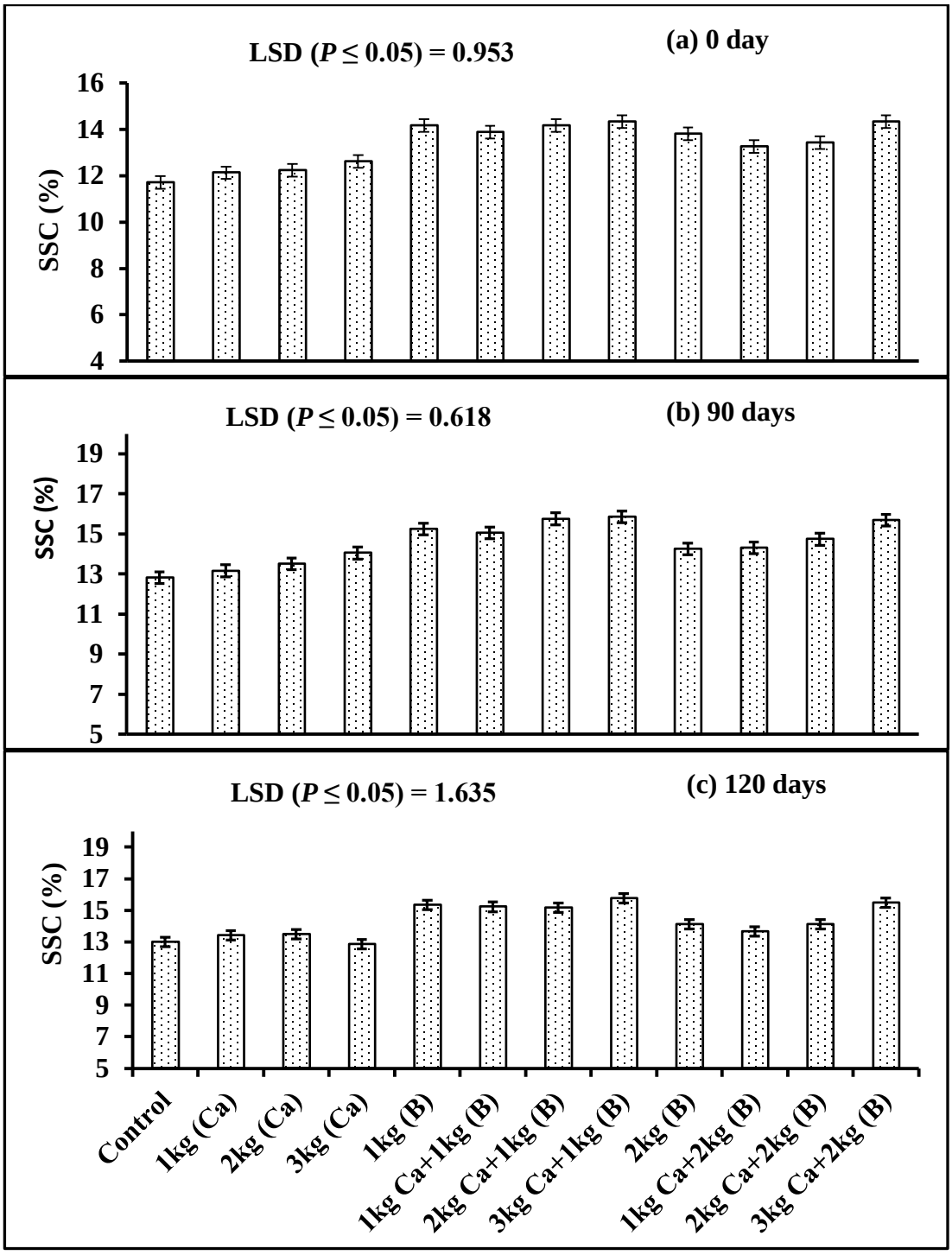

Figure 8. 6. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on SSC of 'Gala' and 'Cripps PinkTM' apple fruit following 0, 90 and 120 days of cold storage (0.5 ±0.5°C) and simulated shelf conditions (21 ± 1°C) for 10 days. Vertical bars represent standard error of means.

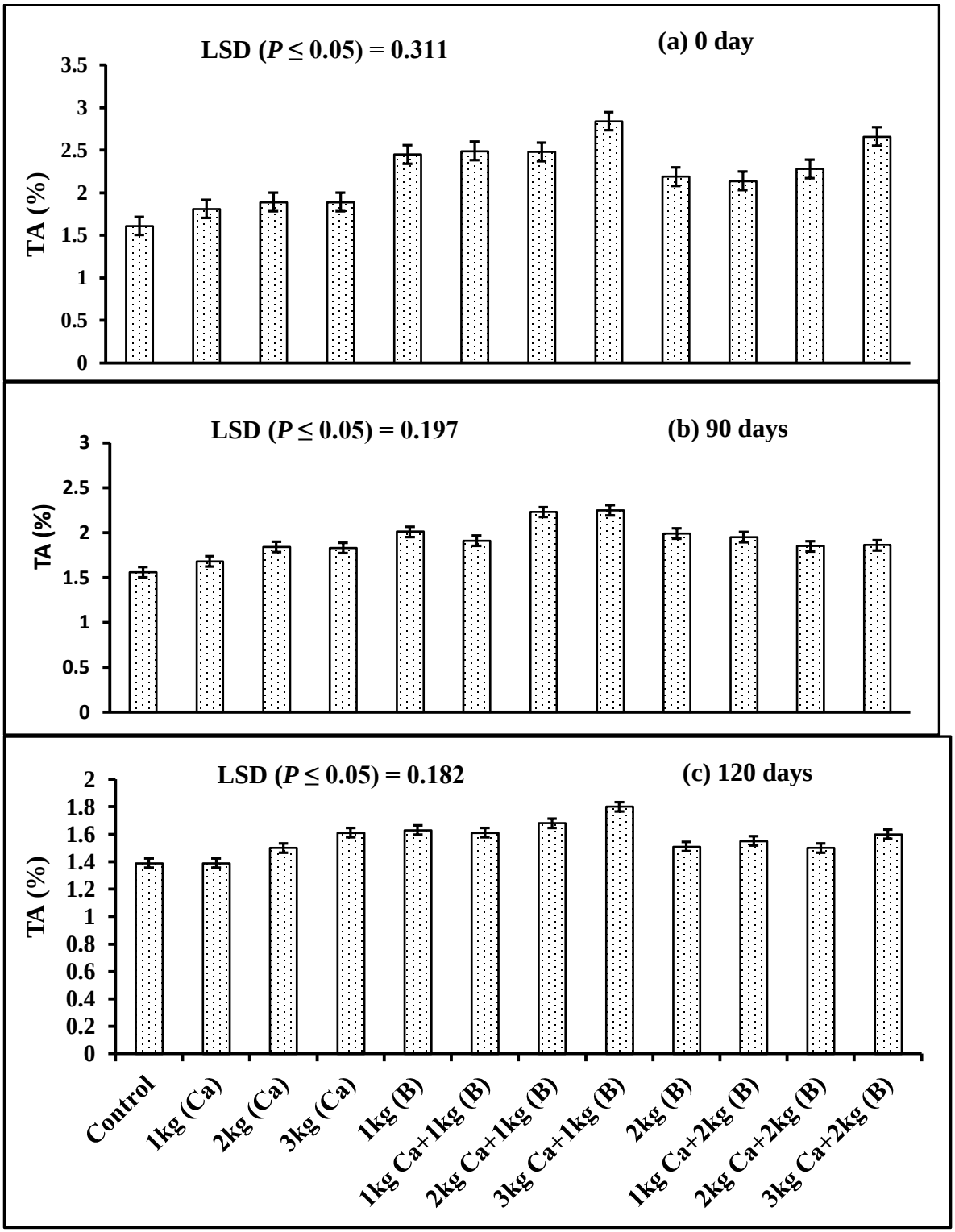

Figure 8. 7. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on TA of 'Gala' and 'Cripps Pink^{TM}, apple fruit following 0, 90 and 120 days of cold storage (0.5 ±0.5°C) and simulated shelf conditions (21 ± 1°C) for 10 days. Vertical bars represent standard error of means.

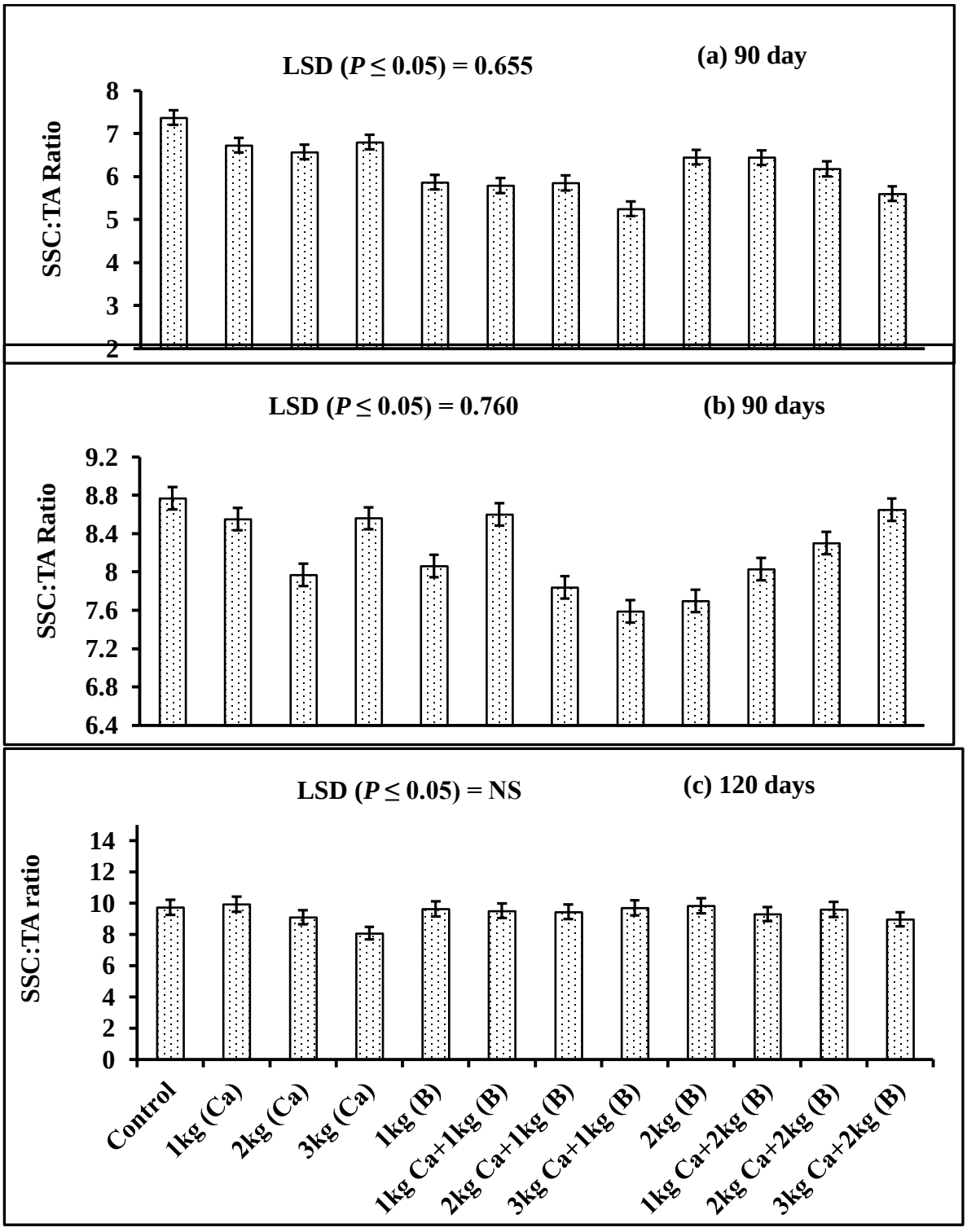

Figure 8. 8. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on SSC/TA ratio of 'Gala' and 'Cripps Pink^{TM}' apple fruit following 0, 90 and 120 days of cold storage (0.5 ±0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days. Vertical bars represent standard error of means.

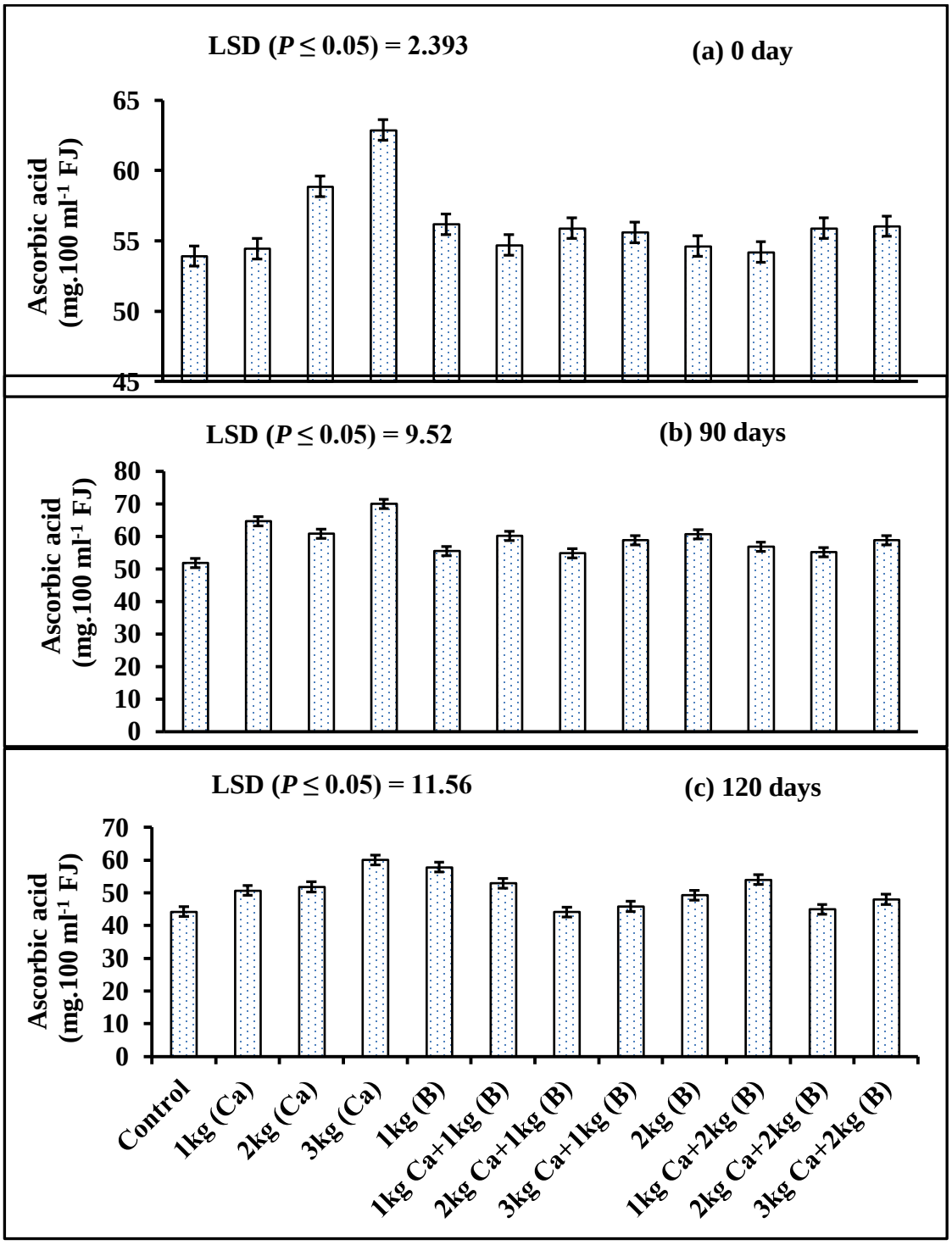

Figure 8. 9. Effects of pre-harvest spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone and in combination on levels of ascorbic acid of 'Gala' and 'Cripps Pink^{TM}' apple fruit following 0, 90 and 120 days of cold storage (0.5 ±0.5°C) and simulated shelf conditions (21 ± 1ºC) for 10 days. Vertical bars represent standard error of means.

8. 5. Discussion

Four exogenous spray applications of Biomin® calcium (Ca) and Biomin® boron (B) alone or calcium followed by boron significantly increased concentration of calcium and boron in the leaves and fruit of both 'Gala' and 'Cripps Pink™' apples compared to control (Table 8. 1). Pre-harvest spray applications of Ca alone were more effective in uplifting the concentration of Ca in leaf and fruit tissues than the spray applications of Ca in combination with boron sprays. Similarly, reduced absorption of Ca into sweet orange trees has also been reported when boron supply was at higher levels (Smith and Reuther, 1950). Calcium sprays applied at the rate of 3 kg/ha alone resulted in the highest concentration of calcium in the fruit compared to all other treatments. Similar results have also been reported by Raese and Drake (1993) in 'Delicious' apples, where calcium concentrations in the peel and fruit pulp were increased with pre-harvest sprays of calcium chloride. However, Casero et al., (2004) reported that when calcium is applied in the early stages of the fruit growth period in 'Golden Delicious' apples, there was little accumulation of calcium in the fruit, while a greater increase was recorded when calcium was applied at later fruit growth stages. They ascribed this to the fact that in the initial stages, calcium is provided to the fruitlets through root absorption while in the later stages, when fruit calcium absorption reduces, exogenous calcium applications increased fruit calcium levels.

Bitter pit is a serious physiological disorder in apple production, despite extensive research on the numerous factors involved in the development of this disorder. Bitter pit in apples is closely associated with the Ca concentration of the fruit (Ferguson et al., 2012; Perring and Pearson, 1987). It has been suggested that high Ca concentration in fruit at harvest considerably reduces the incidence of this disorder (Shear, 1975). Except for applying Ca to the soil and employing optimal orchard practices, pre-harvest foliar Ca applications to increase the Ca concentration in fruit and reduce bitter pit are widely used (Beyers, 1963; Terblanche et al., 1970; Hewett and Watkins, 1991; Yuri et al., 2002). Although bitter pit is one of the ten physiological disorders that are associated with low fruit calcium concentrations, its correlation with Ca is least understood (Perring, 1986). Four pre-harvest spray applications of Ca (1, 2 and 3 kg/ha) alone on apple trees has significantly reduced bitter pit index at commercial harvest and following cold storage for 90 and 120 days

and simulated shelf conditions (21 ± 1ºC) for 10 days as compared to control and all others treatments. Possibly, this may be partly attributed to increased Ca concentration in fruit with the spray applications of 1, 2 and 3 kg/ha Ca as previously reported. Increased Ca concentration in the fruit and leaves on trees treated with 1, 2 and 3 kg/ha Ca alone has increased uptake and penetration of Ca into fruit, during fruit growth (Table 8. 3) and (Figs 8. 3 a, b and c). Bramlage, (1995) noted that the reduction of the incidence of bitter pit could be explained in part by the total supply of Ca provided by the different sources. Earlier, Bramlage et al., (1985) also noted that the Ca applications shortly after bloom, as the first three sprays were greater influence in raising Ca levels in the fruit consequently reduced bitter pit in apple fruit. Wojcik, (1999) reported that 'Jonagold' apples sprayed three times with Ca recorded higher Ca concentration, which delayed the fruit ripening process during storage. However, Conway et al., (1994) noted that Ca sprays in orchards have consistently less effect on the incidence of apple bitter pit. Apple fruit with low calcium levels have been reported to develop more superficial scald than those with high levels. Calcium applications of 2 or 3kg/ha alone reduced the superficial scald index at commercial harvest in both 'Gala' and 'Cripps Pink^{TM}' apples. This reduction was also observed in both apple cultivars following 90 and 120 days of cold storage and simulated shelf conditions (21 ± 1ºC) for 10 days (Fig 8. 4). The highest superficial scald index was recorded when the trees were sprayed with 2 kg/ha boron at commercial harvest and following different periods of cold storage (Fig 8. 4). Also the superficial scald index increased during storage period from 0, 80 after 90 days of storage to 2.27 after 120 days of storage with application of 2kg/ha boron (Table 8. 4). 'Cripps Pink^{TM}' apple fruit exhibited higher incidence of superficial scald than 'Gala' apple fruit at commercial harvest and also following 90-120 days cold storage and simulated shelf conditions (21 ± 1ºC) for 10 days (Table 8. 4). Various reports claimed that scald susceptibility in apple is affected by many factors including cultivar, stage of maturity, growing area and storage conditions (D'Souza, 1989; Lau, 1990; Sfakiotakis et al., 1993; Barden and Bramlage, 1994). Previously, Bramlage, (1993) noted higher boron concentrations in apple may adversely affect post-harvest quality of apples. Apple fruit with high concentration of boron tend to ripen early and are more susceptible to incidence of superficial scald before and after harvest (Bramlage, 1993). Fruit and leaves of trees that received four pre-harvest sprays of boron (2 kg/ha) exhibited higher levels of boron compared to

all other treatments (Table 8. 2). This may have subsequently resulted in highest incidence of superficial scald as compared to other treatments. Similarly, Yogaratnam and Johnson, (1982) reported that higher boron concentration in apples enhances the incidence of internal disorders, particularly water core and internal breakdown. Higher boron concentration in apples could result in increased decay and decreased fruit firmness (Bramlage and Thompson, 1963; Bramlage and Weis, 1991). Bramlage et al., (1974) showed a negative correlation between fruit calcium level and scald development, and found scald to be more prevalent when peel calcium was less. Likewise, Sharples et al., (1979) reported that apples with low calcium levels often develop more scald than those with high levels.

At commercial harvest, fruit firmness increased significantly with four pre-harvest spray applications of 1, 2 or 3 kg/ha Ca alone (Table (8. 5) and (Figs 8. 6). Meanwhile, the highest firmness was observed in fruit trees treated with 3kg/ha calcium alone (Table 8. 8). Fruit firmness decreased following cold storage of 90 and 120 days and simulated shelf conditions (21 ± 1ºC) for 10 days, however the fruit firmness was still higher which were harvested from trees treated with 1, 2 and 3kg/ha Ca alone as compared to control and other treatments. Similarly, Wojcik, (1999) also reported that ‘’Jonagold’’ apples sprayed three times with Ca increased Ca concentration in the fruit consequently retarding the apple fruit ripening process during cold storage and maintained higher fruit firmness. Improved fruit firmness with four spray applications of Ca may be ascribed to increased availability of Ca in fruit and leaves of ‘Gala’ and ‘Cripps Pink^{TM}’ apples. The increased fruit firmness with spray applications of Ca may be ascribed to enhanced stabilisation of cell wall and membrane integrity (Easterwood, 2002; Dong et al., 2009; Pham, 2009). Similarly, there are many earlier reports outlining the relationship between calcium concentration and apple fruit firmness. Bramlage et al., (1979) noted ‘McIntosh’ fruit firmness at harvest to increase slightly with increased flesh Ca concentration. Foliar applications of calcium resulted in firmer ‘Golden Delicious’ and ‘Cox’s Orange Pippin’ apples compared to non-treated apples (Raese and Drake, 1993; Watkins et al., 1989). (Peryea, 1991; Weis et al., 1980) also reported that pre-harvest calcium sprays seem to be effective in increasing fruit firmness in ‘Granny Smith’ and ‘Gala’ apple fruit. On the other hand, results of many studies clearly showed that the apples from the trees sprayed with boron after bloom were more mature than from control

trees that had less firmness (Wojcik, 2002). This is consistent with the results of this experiment, where firmness in 'Gala' and 'Cripps Pink^{TM}' apples was lower in trees sprayed with 1 or 2 kg/ha of boron.

The mean SSC and TA in apple juice at commercial harvest and following cold storage for 90 and 120 days and simulated shelf conditions (21 ± 1ºC) for 10 days respectively decreased when trees were applied with four sprays of 1, 2 or 3kg/ha Ca and increased in fruit when trees were treated with boron (B) (Figs 8. 6 and 8. 7). As confirmed byAgar et al (1999), Rosen and Kader (1989), Kader (1986) the less increase in SSC with increasing Ca concentrations may be due to the delay in natural physiological processes like ripening and senescence by higher concentrations Ca due to the inhibition or retardation of conversion of complex polysaccharides with simple sugars. Similarity effect of Ca was also observed on concentration of ascorbic acid in apple juice. Higher concentration of ascorbic acid was observed in fruit of trees treated with 2 and 3 kg/ha Ca (Fig 8. 9).

In conclusion, four pre-harvest applications of Ca alone were more effective in raising concentration of Ca in leaf and fruit tissues, reducing bitter pit index and superficial scald index and improving apple fruit quality and extending cold storage life in both cultivars as compared to the spray applications of Ca in combination with boron spray applications. Four pre-harvest spray applications of emulsion containing Biomin® calcium (3 kg/ha) and synertrol oil (0.05%) as a surfactant, commencing from 30 days after full bloom stage at 25 day intervals was most effective in minimising bitter pit, superficial scald, extending cold storage life and maintaining fruit quality of organically grown 'Gala' and 'Cripps Pink^{TM}' apples.

Chapter 9

General discussion, conclusion and future research

9.1. Introduction

Apple is one of the most commercially important temperate fruit worldwide including Europe, North America, South America, Africa and Australia (FAO, 2014). Organic production of fruit is becoming a commercial reality and occupies 116,000 ha in the world and organic apple is grown on 35,268 ha, followed by apricots' production area (10,683 ha) (Kirby and Granatstein, 2010). Organic apple is grown in Australia with over 60 certified growers (McCoy, 2007). In Western Australia, organic apple production is gaining momentum with annual production of 300 tonnes only (McCoy, 2007). As a prelude, apple fruit thinning is essential in the production of high quality fruit and the regularity of bearing (Link, 1973, 2000). Hand-thinning is expensive and time consuming. Chemical-free flower and fruit thinning is applicable in organic apple production as well as cost effective and minimises alternate bearing (Bertschinger et al., 1998). The efficacy of organic bloom thinners on reducing crop load, maintaining storage life and fruit quality of organically grown apples is yet to be investigated under Australian conditions. Among different physiological disorders in apple fruit, bitter pit and superficial scald are major ones (Witney et al., 1991; Emongor et al., 1994). Ca application controls the bitter pit and superficial scald in apple (Ferguson et al., 1979; Loughlin and Jotic, 1978). Calcium plays an important role in reducing incidence of bitter pit in apple (Moor et al., 2005). Calcium chloride application is not permitted in organic apple production. However, no research work has been reported on the effects of application of organic calcium or boron on reducing the incidence of bitter pit in organically grown apple fruit in Australia. Therefore, crop regulation using organic chemicals such as lime sulphur alone and in combination with different types of oils and effects of exogenously applied calcium and boron alone and in combination on cold storage life and apple fruit quality was investigated.

9. 1. 1. Bloom thinning

Fruit thinning is one of the most expensive components of apple production and is practiced to reduce fruit crop load. Crop load has a major impact on apple fruit quality and the regularity of bearing (Link, 2000). Therefore, there continues to be continued interest and research in perfecting suitable chemical thinning techniques to reduce crop load in apples (Beulah and Looney, 2004) and (Bound, 2010). Early thinning of apple fruit is important because of its impact on fruit size and flower bud initiation in the following season (Fallahi and Greene, 2010). Chemical thinning continues to be the most important practice in modern apple production (Looney, 1986). Various chemicals such as 6-benzyladenine at 100mg.L^{-1}(Yuan and Greene 2000), carbaryl (1000 mg L^{-1}) , ethephon (474 mg L^{-1}), naphthalene acetic acid (58 mg L^{-1}) (Ebert and Bangerth, 1982) and Tergitol TMN-6 (0.75% to 1.25%) (Fallahi and Greene, 2010) have been tested for bloom thinning on different apple cultivars. Fallahi and Willemsen, (2001) reported that chemical bloom thinners are caustic and reduce fruit set by damaging different flower parts, including anthers, stigma, style and pollen tubes, and thus prevent fertilization. In organic orchards, chemical-free flower and fruit thinning eliminates the use of chemicals as well as reducing production costs and reduces alternate bearing (Bertschinger et al., 1998). Guak et al., (2004) reported that a spray application of lime sulphur (LS) (up to 4%) at 85% full bloom reduced fruit set in 'Fuji' and 'Gala' apple trees. Stopar, (2004) reported a severe thinning when 3% lime sulphur was applied to 'Golden Delicious' apple trees which led to reduced crop load and increased fruit weight.

Organic apple growers have long used lime sulphur (LS) during bloom to reduce the number of viable flowers (Edwards, 1998). While the Pacific Northwest apple industry in the US has widely used LS to control diseases and improve fruit finish, its potential for regulating cropping in apples has not been fully explored or exploited in Australia. LS is permitted under current guidelines for organic fruit production. It acts by preventing fruit-set by hindering fertilisation process. This response to LS is cultivar and location specific and was found to be effective for pome and stone fruits (Meland, 1998a; Bertschinger et al., 2000; Webster and Spencer, 2000; Lenahan and Whiting, 2006; Chun et al., (2012). Fallahi (2006) used

lime sulphur and fish oil and a combination of these chemicals and found them to be effective organic blossom thinners for apples and peaches.

9. 1. 2. Bitter pit and superficial scald

Bitter pit is mainly related to lower levels of calcium in apple fruit and remains one of the main problems in the apple industry around the world. Several studies have noted the relationship between the application of fertilizers, and bitter pit within a growing season (Witney et al., 1991). Bangerth (1979) found that the incidence of bitter pit in apple is commonly associated with low calcium concentrations which were also confirmed by Moore et al (2005). Ferguson and Watkins (1983) also reported that apple tissues are sensitive to bitter pit when calcium concentrations in the fruit are low and they correlated reduced levels of calcium in fruit flesh with a higher risk of bitter pit development. Neilsen and Neilsen (2002) found post-bloom applications of calcium (0.7%) 4-5 times significantly increased calcium concentration in apple fruit thus reducing bitter pit. More recently, the emphasis is on the important relationship between the fruit's element Ca levels and quality retention.

Superficial scald like bitter pit is a common physiological disorder that affects various fruit such as apples and pears (Emongor et al., 1994). It is characterized by irregular brown patches of damaged cells developing under the cuticle, thus adversely affecting the quality and value of the apple fruit (Paliyath et al., 1997). Apple scald appears during marketing or after storage (Soria et al., 1999) and is influenced by several factors including cultivar, stage of maturity, growing and storage conditions (Diamantidis et al., 2002). Recently, Pesis et al., (2010) reported that ethylene which regulates climacteric fruit ripening may play a role in the development of superficial scald in apple fruit in cold storage. Wills and Scott (1981) have reported that the concentration of nitrogen, potassium, magnesium and calcium in apples at pre- and post-harvest phase can affect fruit size and storage life. Calcium also plays a very important role in plant growth and development and in maintaining and modulating various cell functions (Conway et al., 1991). Sharples, (1980) reported that calcium affects cell metabolism and structure, and offers greater resistance to changes preceding softening, fungal invasion and the development of

disorders. Ca is also known to delay the general rate of senescence of the tissues (Sharples 1980). It is thought to be the most important mineral element determining fruit quality (Raese and Drake, 2000). O'Loughlin and Jotic, (1978) found that six pre-harvest spray applications of calcium at different concentrations significantly reduced the incidence of internal breakdown and superficial scald in 'Red Delicious' apple fruit. Calcium spray on the apple fruit is known to improve the fruit's finish, reduce cork spot, rot, superficial scald and produces greener fruit skin colour, whiter flesh and more juiciness in 'Anjou' pear fruit (Raese and Drake, 2000). Emongor et al., (1994) reported that apples with low calcium levels often develop more scald than those with higher levels. William, (1993) also found that low calcium adversely affects post-harvest fruit quality due to development of internal breakdown, and appearance of scald on apples and pears. Calcium sprays exerted a far greater influence on the level of internal breakdown than did the range of rootstocks (O'Loughlin and Jotic, 1978).

9.2. Efficacy of lime sulphur alone or in combination with different types of oils on blossom thinning and fruit quality of organically grown 'Gala', 'Granny Smith' and 'Cripps Pink' apples (2011-2012 growing season)

Lime sulphur (LS) and oil products have been used by organic growers in some countries and the reports suggest partly satisfying thinning results (Weibe et al., 2008). Byers, (2003) reported that chemical products may act as blossom thinners by decreasing pollen viability or fertility (i.e., act as pollenicides). They may also decrease stigmatic surface receptivity, or interfere with pollen-pistil interactions at the stigmatic surface or impede pollen tube growth in the pistil (Weibe et al., 2008). In this experiment, spray of lime sulphur (5%) alone or in combination with different types of oils (canola oil, fish oil and olive oil each at 3% concentration) were effective as blossom thinners when applied at 75% bloom in 'Gala', 'Granny Smith' and 'Cripps Pink' apples as compared to control (Fig 4. 1). All the LS spray treatments alone and in combination with different types of oils significantly ($P \leq 0.05$) reduced fruit set, fruit retention and leaf scorch in comparison to the control in all three cultivars (Table 4. 1). The application of lime sulphur possibly resulted in scalding of petals and other flower parts which may have subsequently hampered effective fertilisation and consequently reduced fruit set in apple. However, these

observations were not recorded in this study. The reduction in fruit set in trees treated with LS alone or LS in combination with oils (Table 4. 1) can possibly be partially attributed to the reduction in pollen viability and germination. Similarly, Myraa et al., (2011) has reported that application of LS at blossom time reduces percentage pollen germination which ultimately reduces the fruit set in apple. Likewise, application of LS (4%) at 85% bloom has been reported to reduce fruit set in 'Gala' and 'Fuji' apple and reduced the crop load. Application of LS (5%) in combination with 3% olive oil resulted in the lowest percentage fruit set compared to control and all other treatments, while the control trees showed the highest fruit set (Fig 4. 1). LS alone or in combination with olive oil significantly ($P \leq 0.05$) increased leaf scorch in 'Gala', 'Granny Smith' and 'Cripps Pink' apples compared to control (Table 4.1). LS in combination with canola oil and fish oil significantly increased leaf scorch as compared to control and LS alone or LS in combination with olive oil. This finding signifies the potential role of LS and certain oils such as canola and fish oil in burning of leaves. The exact mechanism of how olive oil in combination with LS reduces leaf burning caused by lime sulphur is yet to be investigated in detail. LS applications significantly ($P \leq 0.05$) influenced fruit size, fruit weight and fruit firmness compared to the control (Table 4. 2). The improved fruit size and fruit weight may be attributed to increased availability of photosynthates from the leaves to the fruit due to reduced number of fruit with the thinning treatments. A direct linear negative relationship ($r = - 0.26$, $y = -0.1006x + 66.238$) between percentage fruit set and fruit size was observed with the spray applications of LS alone and in combination with different types of oils (Fig. 4. 3a). Similarly, a direct negative relationship between percentage fruit set and fruit weight ($r = -0.35$, $y = 0.7217x +145.7$) (Fig. 4. 3b) also supports the experimental findings. Fruit size is the result of the combination of number of cells and cell size (Smith, 1950; Bain and Robertson, 1951; Martin et al., 1964; Sugiura et al., 1995). Reducing fruit numbers at, or soon after flowering has an effect on reducing competition for photosynthates among fruit, which may allow remaining individual fruit to develop greater cell numbers. Fruit size and fruit weight are closely correlated, both being inversely related to fruit retention. LS (5%) in combination with olive oil reduced fruit set and fruit retention leading to largest fruit size with highest weight and higher fruit firmness. This observation is also supported by earlier reports by Stanley et al., (2000), Matej S. et

al., (2002), Guak et al., (2004), Osborne et al., (2005) and Fallahi, and Fallahi, (2006) in various apple cultivars, who explained that lower fruit load also gives individual fruit a greater share of resources by allowing cells to increase to their maximum size. Mean fruit firmness decreased in treatments where percentage fruit retention was higher LS (5%) in combination with oils treatments (Fig 4. 1 and 4. 2). These results agree with those of Garriz et al., (2000) who found that fruit flesh firmness was significantly lower in 'Braeburn' trees with high crop loads than in trees with moderate or low crop loads. Link, (2000) suggested that in trees with high crop loads, the supply of carbohydrates for cell wall synthesis is lowered which may result in reduced fruit firmness. Though the trends relating to firmness and fruit retention were investigated in the current study, low bloom thinning produced softer fruit than trees thinned at high bloom thinning. Jones et al., (1997b) also reported increased firmness with reduced crop load following chemical thinning of 'Pink Lady' and 'Jonagold' with ethephon and benzyladenine. LS applied alone or in combination with different types of oils at bloom-time influenced SSC, TA and ascorbic acid in apples fruit juice at harvest compared to control (Table 4. 4). In all cultivars, SSC, TA and ascorbic acid increased with increasing bloom thinning i.e reduced fruit set and fruit retention. Similarly, Guak et al., (2004) also reported higher soluble solids and titratable acidity in 'Gala' and 'Fuji' apples with high bloom thinning. The current results are also in agreement with the findings of Stopar et al., (2002) who reported that fruit from low-cropping trees exhibited significantly higher percentage of soluble solids and better flesh firmness compared with the high cropping trees. A possible explanation for this result is that high bloom thinning causes fruit to mature earlier than low thinning, particularly when combined with the increased soluble solids observed in high bloom thinning. Also bloom thinning treatments significantly ($P \leq 0.05$) improved the skin colour of 'Gala' and 'Cripps Pink' apple fruit. This is represented by the mean values of L* and (h) being lower in fruit treated with lime sulphur (5%) in combination with olive oil compared to all other treatments (Table 4.4). The mean a*, b* and Chroma (*C) were highest on the fruit treated with lime sulphur (5%) in combination with 3% fish oil (fig 4 .5a and b).

9. 3. Effects of number and time of application of lime sulphur on blossom thinning and fruit quality in 'Gala', 'Granny Smith' and 'Cripps Pink' apples (2011-2012 growing season)

Time of application of various blossom thinners is a very important factor influencing the effectiveness of blossom thinning in apples. In apples, the best time to spray for blossom thinning, is when the king flower (central dominant blossom in the cluster) is open and fertilized, and only one side bloom is open but not fertilized (Fallahi and Willemsen, 2002). Knight, (1986) regarded the timing of chemical application as critical in order to achieve effective thinning for many apple cultivars. Spray application of carbaryl at petal fall gave the best improvement in apple fruit size (Knight, 1986). Guak et al., (2004) found that LS (up to 4%) applied at 85% full bloom reduced fruit set in 'Fuji' and 'Gala' apple trees. All LS spray applications significantly reduced fruit set and fruit retention in 'Gala', 'Granny Smith' and 'Cripps Pink' apples as compared to control (Table 5.1). These results conform with findings of (Guak et al., 2004; Osborne, 2006) who reported that 4% LS applied at 85% full bloom reduced fruit set and fruit retention in 'Gala' and 'Fuji' apple. The reduction in fruit set and fruit retention in this study were more pronounced when LS was applied twice (first at 25% bloom and second at 75% bloom stage) and also with a single application of LS when applied at a later bloom stage (75%) (Table 5. 1). Reduced fruit set may be attributed to higher blossom thinning. Similarly, Fallahi et al., (2004) reported that double applications of blossom thinners such as ammonium thiosulfate caused higher blossom thinning in 'Fuji' apples. Therefore, the reduction in fruit set and fruit retention observed with LS sprayed at 75% bloom (either as a single or as a double spray) could be ascribed to LS impeding fertilisation in the flowers. Similarly, Myraa et al., (2011) reported that spray application of LS at blossom time reduced pollen germination, which ultimately reduces the fruit set.

Post-bloom thinning must be implemented during a critical period of fruit development. Timing of post-bloom sprays on apple has been based on fruit size and/or days after bloom (Williams, 1979). Chemical thinning is applied during the post-bloom time period because it is the best time for carbohydrate demand by the actively growing fruits. Paull, (1994) noted that BA is an effective thinner in several apple cultivars, at a range of concentrations and timings. Fruit set in 'Delicious' was

reduced with a 25 mgL^{-1} BA spray applied before full bloom (Masabni, 1988). LS is most effective as a blossom thinner when applied at 80% bloom (Knight 1986). All LS treatments had a significant ($P \leq 0.05$) effect on leaf scorching in 'Gala', 'Granny Smith' and 'Cripps Pink' apples compared to control (Fig 5. 1). Higher percentage of leaf scorching was observed in the trees of all cultivars treated with double application of LS at 25% bloom and at 75% bloom. This finding indicates that LS is responsible for the burning of leaves on the apple tree, whilst reducing fruit set and retention as also confirmed in the previous experiment. Stephen et al., (1996) reported that application of Armothin (5%) reduced fruit set in peach, accompanied by some phytotoxicity symptoms (slight leaf yellowing and burning and young shoot dieback in the interior of the canopy) observed without affecting the yield or fruit quality. The adverse effects of LS however, had no long lasting effects on the health of the trees that received the treatments during the season the experiment was conducted. The potential phytotoxic effects of LS needs to be explored and trees receiving LS treatments need to be monitored in future seasons to determine any long term effects of LS on the health of the trees.

All the blossom thinning treatments improved fruit size compared to the control (Table 5. 2). Double application LS at 25% bloom and at 75% bloom and single application of LS at 75% bloom reduced fruit set and fruit retention leading to larger fruit size with higher weight compared to single spray of LS at 25% bloom and untreated trees (Table 5. 2). Guak et al., (2004); Osborne et al., (2006); Fallahi and Green, 2010; Link, (2000) suggested that reduction of crop load with blossom thinning treatments resulted in improved fruit weight and size in apple. Stanley et al., (2000) also reported that lower fruit load allows individual fruit a greater share of resources thereby allowing cells to increase to the maximum size. There is a negative relationship between fruit set and fruit number (Fig 5. 3a) and similarly between fruit set and fruit weight (Fig 5. 3b). This has been confirmed by many researchers and is one of the main reasons for adopting fruit thinning strategies in apple orchards (Wertheim, 1997) and (Neilsen et al., 2001). Increasing the crop load increased the number of small fruit on the tree and decreases the average fruit size and weight (Stopar et al., 2002). Fruit size is affected by cell division and differences in fruit size are mainly due to differences in the number and individual size of cells within the

fruit cortex and pith (Smith, 1950; Martin et al., 1964; Sugiura et al., 1995; Webster, 1997). Webster, (1997) reported that cell numbers are determined within the first few weeks of fruit development. The characteristic size for each cultivar is determined predominantly by the degree of cell multiplication occurring after pollination (Smith, 1950). Therefore, the timing of the application of blossom thinners such as LS is critical and this study suggests that application at 75% bloom is beneficial. Factors affecting cell number and cell size of apples are economically important because they will determine final fruit size (Westwood et al., 1967). All LS treatments at different blossom stages significantly influenced fruit firmness (Table 5. 2), TA and SSC/TA ratio (Table 5.3) in 'Gala', 'Granny Smith' and 'Cripps Pink' apple. SSC and concentration of ascorbic acid in apple juice at harvest did not differ significantly ($P \leq 0.05$) in all cultivars. Fruit firmness, SSC/TA ratio and titratable acidity increased with double application of LS, first at 25% bloom and second at 75% bloom and with single application of LS at 75% bloom. These results are similar to that reported by Guak et al., (2004) where they found increased fruit firmness, juice soluble solids and titratable acidity in 'Gala' and 'Fuji' apples treated with high LS concentrations. Furthermore, Garriz et al., (2000) also found that fruit firmness was significantly lower in 'Braeburn' trees carrying high crop loads than in trees with moderate or low crop loads. Therefore, improved fruit firmness observed in the current experiment with LS treatments at 75% bloom may be attributed to reduced crop load. Jones et al., (1997b) also reported increased firmness with reduced crop load following chemical thinning in 'Pink Lady' and 'Jonagold' apples with ethephon and benzylaminopurine (BA). However, Link, (2000) suggested that the reduced firmness often observed in heavily cropped trees could be due to limited carbohydrate supply for cell wall synthesis. In the current study, bloom thinning produced softer fruit in trees that were less thinned and therefore carried a higher crop load. It is possible that high bloom thinning resulted in earlier fruit maturity and increased soluble solids than those treated with lower bloom thinning.

On the other hand, bloom thinning treatments with LS significantly improved ($P \leq 0.05$) the skin colour of 'Gala' and 'Cripps Pink' apple fruit. Mean values L*, a* and hue angle (h°) were lower in fruit treated with double application of LS at 25% bloom and at 75% bloom in both cultivars compared to all other treatments

indicating that fruit were more red. Meanwhile, the mean b* and chroma were highest on the fruit treated with a single application of LS at 75% bloom in both cultivars compared to other treatments. Sparks, (2007) reported that apple trees that bear larger fruit develop colour earlier and reach maturity earlier. Trees sprayed with LS at 75% bloom (either once or twice) resulted in larger size fruit (Table 5. 2) which could indicate that these fruit matured earlier and hence the improvement in colour.

9.4. Efficacy of different concentrations of lime sulphur alone or in combination with olive oil on blossom thinning and fruit quality in organically grown 'Cripps Pink' and 'Gala' apples (2013-2014 growing season)

LS applications alone or in combination with olive oil at 75% full bloom significantly ($P \leq 0.05$) reduced fruit set in both 'Gala' and 'Cripps Pink' apple cultivars compared to control trees (Table 6. 1). This showed that blossom thinning was achieved in 'Gala' and 'Cripps Pink' apple trees with LS, an organic compound approved by the EU legislation for organically grown fruit. The results of the LS experiments from the previous growing seasons are further confirmed by these results. Guak et al., (2004) and Osborne, (2006) also reported similar results whereby 4% LS applied at 85% full bloom reduced fruit set in 'Gala' and 'Fuji' apple. Trees treated with LS concentrations of 3% or 4% alone recorded the lowest fruit set whilst trees that received LS at 1% with or without olive oil and control trees recorded higher fruit set (Fig. 6.1). According to Myraa et al., (2011) LS applications at blossom time reduce pollen germination, which ultimately reduces the fruit set. Therefore, it may be assumed that higher concentrations of LS were more effective in potentially inhibiting pollen germination or pollen tube growth, leading to reduced fruit set in 'Gala' and 'Cripps Pink' apple trees compared to lower concentrations of LS. Similar suggestions have been made by Bertshinger et al., (1998) and Embree and Foster, (1999) with regards to chemicals such as Safer-Soap, PEG-1000, Anti-Stress, Nutri-Safe and Biofilm.

Treatments of 4% LS alone or with olive oil resulted in the lowest fruit retention compared to control in both 'Gala' and 'Cripps Pink' apples (Table 6. 1). Earlier findings by Guak et al., (2004) and Osborne et al., (2006) also support the findings in the current study. Application of 4% LS at 85% bloom on 'Fuji' and

'Gala' apple trees resulted in a higher proportion of fruiting sites with one fruit and a lower proportion of fruiting sites with more than two fruits (Guak et al., 2004).

The treatments of LS alone or in combination with olive oil had a significant ($P \leq 0.05$) effect on leaf scorch in 'Gala' and 'Cripps Pink' apples compared to control (Table 6. 1 and Fig 6. 1). Higher percentage of scorched leaves was observed in the trees of both cultivars treated with the higher concentrations (3-4%) of LS alone. However, when LS was used in conjunction with olive oil, percentage of scorched leaves reduced significantly (Table 6. 1). This finding indicates that LS at higher concentrations is probably phytotoxic and is responsible for the burning of leaves on the trees. Stopar, (2004) similarly reported stunting of leaves in 'Golden Delicious' apples treated with 3% LS, which did not recover during the growing season. In contrast, Guak e al., (2004) reported no damage to 'Fuji' and 'Gala' apples treated with 4% LS. The results from this study indicate that when LS is combined with olive oil, some of the phytotoxic effects could be overcome.

Size differences in fruit are primarily due to differences in the number and individual size of cells within the fruit cortex and pith (Smith 1950; Martin et al., 1964; Sugiura et al., 1995; Webster, 1997). According to Smith, (1950), the extent of cell multiplication occurring after pollination, determines the fruit size for each cultivar. However, for each cultivar, the relationship between increase in fruit weight and increase in cell number is different (Smith, 1950). Webster, (1997) reported that cell numbers are determined within the first few weeks of fruit development. The cell division in the flesh (pith and cortex) of the fruit stops about 4-6 weeks after blossom (Smith, 1950; Bain and Robertson, 1951). Fruit weight and size are closely correlated, both being inversely related to fruit set (Figs. 6. 3a and 6. 3b). In this experiment, blossom thinning treatments with LS improved fruit size compared to the control (Table 6. 2). LS (4%) alone or in combination with olive oil produced larger fruit with higher fruit weights (Table 6. 2) which can be attributed to reduced fruit set and fruit retention in trees receiving the same treatment. Earlier reports also suggested that reduction of crop load with blossom thinning treatments resulted in improved fruit weight and size in apple (Guak et al., 2004; Osborne et al., 2006; Fallahi and Green., 2010; Link, 2000). Stanley et al., (2000) has also reported that lower fruit load also gives individual fruit a greater share of resources allowing cells

to increase to the maximum size. Increasing the crop load increased the number of small fruit on the tree and decreased the average fruit size and fruit weight (Stopar et al., 2002). This inverse relationship between fruit size and fruit number on the crown has been confirmed by many researchers and is one of the main reasons for adopting fruit thinning strategies in apple orchards (Wertheim, 1997) and (Neilsen et al., 2001). Webster, (1997) stated that competition for photosynthates among growing fruit can be reduced by reducing the number of fruit set at, or soon after flowering. This allows the fruit remaining on the tree to increase their cell numbers and also size of the individual cells.

Spray applications of LS at bloom-time significantly influenced fruit firmness, SSC, TA and concentration of ascorbic acid in apple juice at harvest (Table 6. 3). In both 'Gala' and 'Cripps Pink' apples, fruit firmness, SSC and TA decreased with increasing concentrations of LS, while concentration of ascorbic acid increased marginally compared to control. Similar results have been reported in 'Gala' and 'Fuji' apples treated with high LS concentrations (4%) earlier by Guak et al., (2004), where they found reduced fruit firmness, SSC and TA in treated fruit compared to control. However, Chun et al., (2012) reported no significant effects of various LS concentrations on fruit firmness, SSC and TA in 'Fuji' and 'Hongro' apples. These differences in observations could be attributed to differences in growing areas, climate and differences in soil conditions. Garriz et al., (2000) reported that in 'Braeburn' apple trees, fruit firmness was significantly lower in trees with high crop loads compared to trees with moderate or low crop loads. However, Jones et al. (1997b) reported increased fruit firmness with reduced crop load following chemical thinning of 'Pink Lady' and 'Jonagold' with ethephon and BA. Link, (2000) suggested that the fruit firmness in trees that are heavily cropped may be reduced because these fruit receive limited carbohydrates for cell wall synthesis. In the current study, bloom thinning produced softer fruit than trees with less thinning and control trees. It is possible that reducing crop load with high concentrations of LS resulted in fruit maturing earlier compared to trees with a higher crop load such as control. This is particularly evident in fruit with increased SSC values observed in trees that recorded fruit retention (Table 6. 3).

Different concentrations of LS alone or in combination with olive oil significantly ($P \leq 0.05$) influenced the skin colour of both 'Gala' and 'Cripps Pink' apple fruit (Table 6. 4, 6. 5). Lightness and hue angle were lower on fruit of trees treated with higher concentration lime sulphur alone or in combination with olive oil indicating redder fruit compared to control and all other treatments. Mean a*, b* and chroma or red colour saturation were highest in the fruit of trees treated with higher concentration 3-4 of lime sulphur alone or in combination with olive oil compared to control and all others treatments (Figs. 6.5b, 6.5c and 6.5d). This improvement in colour in part could be ascribed to advanced maturity of fruit in trees treated with high concentrations of LS. These findings are in contrast to Guak et al., (2004) who reported no significant effects of LS treatments on fruit colour in 'Fuji' or 'Gala' apples. Colour development in apples is dependent on a number of factors, environment and geographic location playing an important part. Therefore these differences in results could be linked to the different geographic locations and climate at these locations.

9. 5. Experiment. Effects of organic blossom thinning with lime sulphur alone or in combination with olive oil on cold storage life and quality of 'Gala' and 'Cripps Pink' apples

Pre-harvest factors that influence apple quality at harvest and after storage include: climatic factors including light intensity, temperature, and rainfall; cultural factors such as mineral nutrition, timing and extent of blossom thinning that affects crop load, orchard floor management, irrigation, tree management, and use of growth regulators; and genetic factors that involve choice of cultivar or clone, rootstocks, and inter stocks (Bramlage, 1993; Harker et al., 1997; Sams, 1999). In this experiment, applications of different concentrations of LS alone or in combination with 3% olive oil significantly affected various fruit quality parameters in 'Gala' and 'Cripps Pink' apples following long-term cold storage (0.5 ± 0.5 °C) for 60, 90 and 120 days. Fruit from trees that received LS alone or in combination with olive oil treatments were significantly softer following 60, 90 and 120 days of cold storage compared to control in both 'Gala' and 'Cripps Pink' apples (Fig 7. 2). Higher concentrations of LS (3 and 4%) with or without olive oil appeared to increase softening of fruit following storage irrespective of duration and cultivar. 'Cripps

Pink' apples were significantly softer than 'Gala' apples post-storage (Table 7.2). Therefore cultivar response to LS treatments is an important factor to be considered during storage. Basak, (1999) reported the storage quality of apples is influenced by cultivar, the time and effectiveness of thinning, type and concentration of chemical used.

The experimental apples were harvested at an acceptable maturity for commercial use (Table 7. 2) before long-term cold storage. This also indicates that there might be a relationship between storage time, fruit firmness and fruit size, where, increased fruit size results in reduced fruit firmness during long-term. Maturity at commercial harvest and fruit size influence post-harvest softening in apples (Johnston et al., 2010). In chapter six, the LS concentrations of 3-4% alone or with 3 % of olive oil resulted in greater fruit size in both 'Gala' and 'Cripps Pink' apples (Chapter 6 Table 6. 2). This is in agreement with many studies, which have found that larger fruit are generally softer than smaller fruit (Harker et al., 1997). Smaller fruit are considered to have stronger tissues as they have greater cell wall material per unit volume compared to larger fruit (Johnston et al., 2010). Larger apples have been reported to be softer than smaller fruit both at harvest and after storage (Blanpied et al., 1978; Marmo et al., 1985; Siddiqui and Bangerth, 1995) and therefore fruit firmness is negatively correlated to fruit size both at harvest and after storage (Harker et al., 1997). Fruit firmness is also correlated with internal ethylene concentration and the role of ethylene in enhancing softening in apple is well documented (Fabrizio et al., 2010) and (Tacken et al., 2010). Therefore, an increased internal ethylene concentration may have contributed to enhanced fruit softening in fruit that had received blossom thinning treatments with LS following various storage periods. The percentage fruit weight loss increased with increasing the storage durations (Fig. 7. 2). The highest weight loss (3.85%) was recorded in fruit stored for 120 days followed by fruit stored for 90 days (2.99%) in both cultivars. Sarhad, (2012) reported that the percentage fruit weight loss increased significantly with incremental increase in storage duration so that it increased to 4.05 and 4.53% with 120 and 150 days respectively. The loss of fruit weight is attributed to respiration and loss of moisture in the fruit with increasing storage durations, facts confirmed by Erturk, (2003); Gavlheiro et al., (2003); and Ghafir et al., (2009). In

this study, however, fruit respiration rate following storage was not recorded, which will be an important factor to be considered in future studies. SSC of apple and other fruits is a major quality parameter which is correlated with the texture and composition of fruit (Weibel et al., 2004; Peck et al., 2006). SSC of apple fruit increased with extending storage durations (Fig 7. 3). The highest SSC (12.33%) was recorded in fruit that had received high concentration of LS (4%) and stored for 120 days as compared to fruit that received LS treatment of 4% and stored for 60 days. SSC measured in fruit from all storage durations was higher in control fruit compared to LS treatments alone or in combination with olive oil. Likewise, TA decreased with increasing storage durations (Table 7. 4). LS alone at 4% concentration significantly reduced TA in fruit stored for 120 days compared to control. This can be attributed to hastened maturity of fruit as evidenced by lowered fruit firmness in this treatment. SSC and TA in 'Cripps Pink' fruit was higher than 'Gala' fruit which is ascribed to varietal differences and the manner in which each cultivar responds to a treatment and furthermore its performance during storage. Ali et al., (2004) reported significant variations in SSC, acidity and other physico-chemical characteristics of apples harvested from different varieties. Watkins et al., (2000) observed higher SSC in 'Empire' and 'Delicious' apple fruit stored at 0.5ºC for up to 7 months. Similarly the storage duration had a significant effect on SSC:TA ratio of apple juice. The SSC:TA ratio increased with the increase in storage durations (Table 7. 5). The ascorbic acid of apples fruit decreased with increase in the storage durations (Table 7. 6). However, the blossom thinning treatments with LS did not significantly affect ascorbic acid concentration in fruit juice in both cultivars.

9. 6. Effects of pre-harvest spray applications of organic calcium and organic boron on incidence of bitter pit, scald, cold storage life and quality

Exogenous spray applications of organic calcium alone or calcium followed by boron, significantly increased concentration of calcium in the leaves and fruit of both 'Gala' and 'Cripps Pink' apples compared to control (Table 8. 1). Four sprays of calcium (3 kg/h) alone resulted in the highest concentration of calcium in the fruit compared to all other treatments. Similar results have been reported by Raese and Drake, (1993) in 'Delicious' apples, where calcium concentrations in the peel and pulp were increased with pre-harvest sprays of calcium chloride. Casero et al., (2004)

however, reported that when calcium is applied in the early part of the fruit growth period in 'Golden Delicious' apples, there was little accumulation of calcium in the fruit, while a greater increase was recorded when calcium was applied at later fruit growth stages. They ascribed this to the fact that in the initial stages, calcium is provided to the fruitlets through root absorption while in the later stages, when fruit calcium absorption reduces, exogenous calcium applications increased fruit calcium levels.

During the 2011/2012 trial, the bitter pit incidence was reduced significantly with four pre-harvest spray applications of 1, 2 or 3kg/ha Ca alone at commercial harvest and following cold storage of 90 and 120 days an additional 10 days at room temperature as compared to control and others treatments (Table 8. 3). This may be partly attributed to increased Ca concentration in fruit with the application of 1, 2 or 3 kg Ca. Increased Ca concentration in fruit and leaves in trees treated with 1, 2 or 3kg Ca alone may be related to the increased uptake and penetration of Ca into fruit, during fruit growth (Table 8. 3) and (Figs 8. 3 a, b, and c). Bramlage, (1995) noted that the reduction of the incidence of bitter pit could be explained in part by the total supply of Ca provided by the different sources. Bramlage et al., (1985) noted that Ca applications shortly after bloom, as the first three sprays were greater influence in raising Ca levels in fruit, thrus reduced bitter pit in apple fruit. Wojcik, (1999) reported that 'Jonagold' apples sprayed three times with Ca recorded higher Ca concentration, which delayed the fruit ripening process during storage. Conway et al. (1994) also noted that Ca sprays have consistently less effect on the incidence of apple bitter pit. Four pre-harvest spray applications of Ca (2 or 3kg/ha) alone reduced the incidence of superficial scald at commercial harvest in both 'Gala' and 'Cripps Pink' apples (Table 8. 4). This reduction was also observed in both apple cultivars following 90 and 120 days of cold storage and an additional period of 10 days at room temperature (Fig 8. 4). The highest incidence of superficial scald was recorded when the trees were sprayed with 2kg boron at commercial harvest and following different periods of cold storage (Fig 8. 4). Also the percentage of superficial scald increased during the storage period from 0.80% after 90 days of storage to 2.27% after 120 days of storage with application of 2kg/ha boron (Table 8. 4). 'Cripps Pink' apple fruit exhibited higher incidence of superficial scald than 'Gala' apple fruit at

commercial harvest and also following long term cold storage followed by 10 days of storage at room temperature (Table 8. 4).

Research by lngle and D'Souza, (1989), Lau, (1990), Sfakiotakis et al., (1993) and Barden and Bramlage, (1994) have all suggested that scald susceptibility in apple is affected by many factors including cultivar, stage of maturity, growing area and storage conditions. Bramlage, (1993) noted high boron concentrations in apple may adversely affect post-harvest quality of apples. Apple fruit with high concentration of boron tend to ripen early and are more susceptible to superficial scald before and after harvest (Bramlage, 1993). Fruit and leaves of trees that received 2 kg/ha boron had higher levels of boron compared to all other treatments (Table 8. 2). This may have subsequently resulted in higher incidence of superficial scald as compared to other treatments. These results are in agreement with those reported by Yogaratnam and Johnson, (1982) who noted that high boron concentration in apples enhances the incidence of internal disorders, particularly water core and internal breakdown. High boron concentration in apples could result in increased decay and decreased fruit firmness (Bramlage and Thompson, 1963; Bramlage and Weis, 1991). Bramlage et al., (1974) showed a negative correlation between fruit calcium level and scald development, and found scald to be more prevalent when peel calcium was less. Likewise, Sharples et al., (1979) reported that apples with low calcium levels often develop more scald than those with high levels.

At commercial harvest fruit firmness increased significantly with four pre-harvest spray applications of 1, 2 or 3kg Ca/ha alone (Table 8. 5) and (Figs 8. 5). While fruit firmness decreased following cold storage of 90 and 120 days. Fruit firmness was still higher in fruit harvested from trees treated with 1, 2 or 3kg/ha Ca alone as compared to control and other treatments. The improved fruit firmness may be ascribed to increased availability of Ca in fruit and leaves of 'Gala' and 'Cripps Pink' apples treated with 1, 2 or 3 kg/ha Ca alone. Similarly, Sorkel, (2007) stated there are many reports outlining the relationship between calcium concentration and apple fruit firmness. Bramlage et al., (1979) noted 'McIntosh' fruit firmness at harvest to increase slightly with increased flesh Ca concentration. Foliar applications of calcium resulted in firmer 'Golden Delicious' and 'Cox's Orange Pippin' apples compared to non-treated apples (Raese and Drake, 1993; Watkins et al., 1989).

Peryea, (1991) and Weis et al., (1980) also reported that pre-harvest calcium sprays seem to be effective in increasing fruit firmness in 'Granny Smith' and 'Gala' apple fruit. On the other hand, results of many studies clearly showed that the apples from trees sprayed with boron after bloom were more mature than from control trees and were less firm compared to control (Wojcik, 2002). This agrees with the results from experiments on Ca and boron where firmness measurements in all apple cultivars were lower in trees sprayed with 1 or 2 kg/ha of boron. Meanwhile, the highest firmness was observed in fruit from the trees treated with 3kg/ha calcium alone (Table 8. 5). This is further substantiated by Wojcik, (1999) who reported that 'Jonagold' apples sprayed three times with Ca had increased Ca concentration in the fruit, which delayed and lowered the fruit ripening process during storage. Calcium and boron sprayed on 'Gala' and 'Cripps Pink' apple trees significantly ($P \leq 0.05$) affected SSC, TA and ascorbic acid in apple juice. Mean SSC and TA in apple juice at commercial harvest and following cold storage for 90 and 120 days respectively decreased when trees were sprayed with 1, 2 and 3kg/ha Ca and increased in fruit trees treated with boron (Figs 8. 6 and 8. 7). Concentration of ascorbic acid in apple juice was higher in the fruit of trees treated with 2 or 3kg/ha Ca (Fig 8. 9).

In conclusion, the results of this experiment showed that pre-harvest boron applications were not successful in decreasing occurrence of bitter pit and improving fruit quality of 'Gala' and 'Cripps Pink' apple fruit at commercial harvest or after long term cold storage. Pre-harvest applications of Ca alone reduced incidence of bitter pit and superficial scald and improved fruit quality in 'Gala' and 'Cripps Pink' apple fruit. Four pre-harvest spray applications of Ca (3kg/ha) seems to be promising in organic apple production to minimize incidence of bitter pit, superficial scald and to maintain post-harvest fruit quality.

9. 7. Conclusions

1. Pre-harvest spray application of an aqueous emulsion containing lime sulphur (5%) in combination with olive oil (3%) at 75% bloom stage was more effective in reducing fruit set and crop load, increasing fruit size, reduced loss of firmness, maintained SSC, TA and fruit colour at commercial harvest and lowest leaf scorch as compared to the spray application of LS in combination with canola oil or fish oil and control on 'Gala', 'Granny Smith' and 'Cripps Pink' apple trees.

2. Two spray applications of 5% LS first at 25% bloom and second at 75% bloom did not prove better than a single spray application of LS at 75% bloom stage in reducing fruit set, crop load, improving fruit size, colour and quality as well as minimising leaf scorch on 'Gala', 'Granny Smith' and 'Cripps Pink' apple trees.

3. A spray application of reduced concentration (4%) of LS alone as compared to the previous year or in combination with olive oil (3%) at 75% bloom on 'Gala' apple and 'Cripps Pink' apple trees were equally effective in reducing fruit set, and increasing the fruit size and improving fruit skin colour. Meanwhile, the percentage leaf scorch was higher in both treatments as compared to the previous year suggesting that some other agro-climatic factors not included in the current experiment also play an important role in regulating LS induced leaf scorch in apple which warrants to be investigated.

4. Fruit from trees which were sprayed with 4% LS alone or in combination with olive oil (3%) at 75% bloom stage on 'Gala' apple and 'Cripps Pink' apple resulted in greater fruit size (chapter six). The same treatment resulted in reduced fruit weight loss and firmness following 60, 90 and 120 days cold storage (0.5 ± 0.5 °C) plus 10 days of shelf conditions (21 ± 1ºC) as compared to other treatment fruit. Fruit received this treatment maintained fruit quality for 90 days in cold storage (0.5 ± 0.5 °C) plus 10 days of shelf conditions (21 ± 1ºC).

5. Four pre-harvest sprays of emulsion containing Biomin® calcium (3 kg/ha) alone and synertrol oil (0.05%) as a surfactant, commencing from 30 days after full bloom stage at 25 day intervals proved more effective than Ca applications in combination with Biomin® boron and other treatments in reducing bitter pit, superficial scald, and extending cold storage life as well as maintaining fruit quality of organically grown 'Gala' and 'Cripps Pink' apples.

9. 8. Recommendations to the organic apple industry

1. Bloom thinning treatment with exogenous spray application of 4% LS in combination with 3% olive oil and synertrol oil (0.05%) as a surfactant at 75% bloom stage on 'Gala', 'Granny Smith' and 'Cripps Pink' apple trees may be recommended to reduce fruit set and increase the fruit size and improve fruit colour.

2. Four pre-harvest sprays of emulsion containing Biomin® calcium (3 kg/ha) and synertrol oil (0.05%) as a surfactant, commencing from 30 days after full bloom stage at 25 day intervals may be used to curtail bitter pit, superficial scald and extend cold storage life as well as maintaining fruit quality of organically grown 'Gala' and 'Cripps Pink' apples.

9. 9. Future research

This research has explored the effects of organic sprays to reduce crop loads in an organic apple orchard. The importance of organic calcium in reducing the incidence of bitter pit and superficial scald at commercial harvest and after long term cold storage has also been highlighted. Future work therefore should be focussed on the following areas:

1. Fruit respiration rate, ethylene production and internal ethylene concentration (IEC) are important factors that affect and determine fruit maturity and quality. The effects of organic bloom thinning applications and pre-harvest applications of calcium and boron on fruit respiration rate, ethylene production and IEC need to be explored at commercial harvest and during the cold and controlled atmosphere (CA) storage period.

2. Response to pre-harvest applications of bloom thinning sprays and pre-harvest sprays of calcium and boron to control bitter pit, are also dependent on environmental factors such as location, climate and weather patterns and soil conditions. Therefore the experimental trials should be extended across multiple locations in the apple growing regions of Western Australia to better understand how these treatments would affect the cultivars selected in this study under varied environmental conditions.

3. The effects of organic spray treatments on bloom thinning and calcium and boron sprays on fruit respiration rate and ethylene production or internal ethylene concentration of fruit at harvest, during long-term cold/CA storage periods may be investigated.

9. 10. Disclaimer

These recommendations to the organic apple industry are purely based on experiments conducted at only one location - Manjimup, Western Australia. Similar trials conducted at different locations may result in varied results that may not conform to results from this PhD study. The project investigator, Mubarak Alrashedi, and Curtin University accept no liability for whatever reason due to negligence or otherwise arising from the reliance or use of these recommendations.

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