Effect of Nutrition on Postharvest Quality and Grey Mould Development in Strawberries

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Abstract

Strawberries are an extremely perishable fruit mainly due to their soft texture and sensitivity to fungal infection. The fungal pathogen *Botrytis cinerea* is responsible for grey mould on strawberries and is the main causal agent of postharvest decay and subsequent economic loss. As an alternative to fungicides, manipulation of plant nutrition, such as calcium and boron, has been suggested as a means of disease management. This project investigated the effects of calcium and boron application on fruit quality and grey mould development in strawberry.

The effect of calcium on fruit quality, grey mould development and leaf blight in strawberry cultivars 'Aromas' and 'Selva' was investigated through preharvest and postharvest applications. To determine the effect of preharvest application, calcium sulphate in 0.25X strength Hoagland's solution was applied at 0, 100, 300 and 500 ppm Ca through fertigation. Fully-ripened fruit were harvested and evaluated for postharvest quality at harvest and then after storage at 10^{o}C, 90±5% RH for 2 to 10 days. Although fruit firmness of both cultivars declined slightly during storage, this was not affected by preharvest calcium application. Similarly, preharvest calcium treatment had no effect on the external appearance, pH, soluble solids content (SSC) or titratable acidity (TA).

No grey mould development was observed on fruit at harvest when flowers were inoculated with a conidia suspension of *B. cinerea* (10^4 conidia per mL). However, fruit harvested from plants that received calcium at any concentration had less incidence of grey mould during storage at 10^{o}C, 90±5% RH for 14 days than fruit harvested from plants that received no calcium for both cultivars. For 'Aromas', 79% and 51% of fruit, and for 'Selva', 69% and 43% of fruit, showed rot when treated with 0 and 500 ppm Ca, respectively. The shelf life of 'Aromas' and 'Selva' increased by about 8% when plants received 500 ppm Ca in comparison with plants that received 0 ppm Ca.

After 7 days of incubation at 22 to 24^{o}C, there was no difference between blight lesions on wound-inoculated detached leaves from different calcium treatments for either cultivar. However, the lesions on 'Selva' were smaller than on 'Aromas'. The calcium levels in leaves from plants that received calcium at any concentration were adequate for strawberry growing and significantly higher ($P < 0.05$) than in leaves from plants that received 0 ppm Ca. However, calcium treatment did not ensure transfer of calcium to fruit tissues.

Calcium lactate and calcium chloride were used as postharvest calcium treatments at 1500, 3000 and 4500 ppm Ca. Fruit of 'Selva' were dipped in calcium solution for 5 min and wound-inoculated with *B. cinerea* (10^6 conidia per mL). Calcium lactate and calcium chloride at 3000 and 4500 ppm Ca, respectively, were most effective in delaying Botrytis rot development on 'Selva' after 7 days of storage at 10^{o}C, 90±5% RH. Storage for least 24 h after calcium dips prior to inoculation was required to delay the development of fruit rot. Fruit harvested early in the season seemed to be less susceptible to grey mould than those harvested later. However, calcium treatment tended to be more effective when applied to late-season fruit.

Preharvest boron treatment, applied as for calcium but at 0, 0.25, 0.5 and 1.0 ppm B, had no effect on fruit firmness of either cultivar. However, firmness of 'Aromas' fruit was slightly greater than 'Selva' fruit for all treatments. The amount of boron applied had no effect on the external appearance, pH, SSC or TA for either cultivar after storage of fruit for up to 10 days.

Application of boron had no effect on fruit grey mould development in either cultivar. Furthermore, boron had minimal effect on the incidence of blight on wound-inoculated detached leaves of 'Aromas' 7 days after inoculation. However, blight lesion diameters on 'Selva' leaves in the 1.0 ppm B treatment (8.0 mm) were significantly smaller ($P < 0.001$) than in the 0 ppm B treatment (13.0 mm).

Phytotoxicity was observed in boron treatments even at the level considered optimum for strawberry growing. Severity increased with increasing boron concentration but no consistent effect on flower death or flower abortion was observed.

In conclusion, strawberry is sensitive to boron toxicity. Calcium may enhance fruit firmness and, consequently, delay grey mould development if calcium penetrates the fruit. Postharvest calcium treatment tended to be more effective in delaying development of grey mould when applied to late-season fruit. Calcium lactate is a potential alternative to calcium chloride for reducing decay caused by *B. cinerea* in strawberry without providing undesirable bitterness. This finding may provide a basis for application in industry.

Declaration

I declare that the work presented in this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

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Abbreviations

µL	microlitre
ANOVA	Analysis of Variance
ARC	Australian Research Council
B	boron
Ca	calcium
cm	centimetre
DN	day-neutral
e.g.	for example
et al.	and others
FAO	Food and Agricultural Organisation of the United Nations
g	gram
GA	General Appearance
h	hour
i.e.	that is
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometer (ARL model 3580 B)
kg	kilogram
kgf	kilogram-force
L	litre
LD	long-day
LSD	Least Significant Difference
mg	milligram
min	minute
mL	millilitre
mm	millimetre
mm^2	square millimetre
^oC	degree celsius

pH	potential of hydrogen
ppm	parts per million
RH	relative humidity
RO	reverse osmosis
SARDI	South Australia Research and Development Institute
SD	short-day
SE	standard error
SNW	sterile nanopure water
SSC	soluble solids content
TA	titratable acidity
TSS	total soluble solids
UC	University of California
v/v	volume by volume

Chapter One
General Literature Review

1.1 Introduction

Strawberries are an extremely perishable fruit, with a short postharvest life mainly due to their soft texture and high sensitivity to fungal infection (Bitencourt De Souza *et al.*, 1999). The fungal pathogen *Botrytis cinerea* is responsible for grey mould on strawberries and is the main causal agent of postharvest decay and subsequent economic loss. Control of grey mould in strawberry has been attempted via several means. Although fungicide application has been the conventional method, attention is now turning to alternatives such as maintaining plant nutrition not only to control plant growth and fruit quality but to also control grey mould. Calcium and boron application have been reported to enhance plant growth and development as well as fruit quality in a number of other crops. Based on these previous findings, the research presented in this thesis investigated the effects of calcium and boron applications on grey mould development and fruit quality in strawberries.

1.2 Strawberry

The strawberry (*Fragaria* X *ananassa* Duchesne), which belongs to the family Rosaceae (Maas, 1998), is widely grown from the low-latitude tropics and subtropics to the colder high-latitude areas (Darnell *et al.*, 2003). The cultivation area is over 270,000 ha worldwide and estimated world production in 2006 was around 4 million tonnes [Food and Agricultural Organisation of the United Nations (FAO), 2007]. About 31% of the total production is in the United States, with significant production also in Spain (8%), Russia (6%), Turkey and Korea (5% each). The production in Australia was about 23,737 tonnes or approximately 0.6% of the world production in 2006 (FAO, 2007).

Strawberry is a perennial plant which is usually described as herbaceous, because the plant has no persistent woody system (Tasmanian Department of Agriculture, 1978). However, it is a true woody plant, as indicated by the presence of secondary xylem in roots and crowns (Darrow, 1966; Esau, 1977 *in* Darnell *et al.*, 2003). The plant comprises a shortened stem, or crown, from which arises leaves, runners or stolons, roots, axillary crowns and inflorescences (Maas, 1998; Darnell *et al.*, 2003). Flowering in strawberry is greatly influenced by environmental conditions, particularly temperature and day length (Maas, 1998). According to their photoperiodic flowering response, strawberries are classified into three types: short-day (SD) or June-bearing plants (in the northern hemisphere) which initiate flower buds under short days; long-day (LD) or ever-bearing plants which initiate flowers under long days; and day-neutral (DN) plants that are relatively insensitive to day length for flowering (Darnell *et al.*, 2003). However, cold temperature may also induce dormancy in day-neutral cultivars (Maas, 1998). The various climates across Australia allow different regions to grow strawberry at different times of the year. In South Australia, the strawberries are produced and released to the market from summer (November) until late autumn (the middle of May). Given market requirements and the suitability of a DN cultivar to the South Australian climate, 'Selva' is the major cultivar grown.

Strawberry is an aggregate fruit and not a true fruit, in that the edible portion is the enlarged receptacle, which has many one-seeded fruit or achenes (a combination of seed and ovary tissue) located on the outer surface (Figure 1.1) (Perkins-Veazie, 1995). Strawberry fruit is non-climacteric and ripens rapidly (Perkins-Veazie, 1995; Wills *et al.*, 1998). Fruit develop into a fully red (ripe) stage within 30 to 40 days after anthesis, depending on cultivar and environment (Perkins-Veazie, 1995). Many physiochemical changes occur in the ripening fruit that determine consumer perception of fruit quality (Wills *et al.*, 1998). During ripening, fruit continue to increase in size, accumulate soluble solids content (SSC), decline in titratable acidity (TA) (Perkins-Veazie, 1995) and show distinct changes in pigmentation and

softening (Woodward, 1972; Spayd and Morris, 1981). Fruit soften as colour development progresses (Perkins-Veazie, 1995). The changes in fruit texture are not completely understood. However, it seems likely that much of the softening results from degradation of the middle lamella of cell walls due to pectins dissolving (Wills *et al.*, 1998).

The SSC of fruit is composed of sugars, acids and other substances dissolved in the cell sap. Sugar is a major component which can be measured directly by chemical means (Wills *et al.*, 1998). SSC in strawberry fruit increases steadily during development. However, SSC in ripe berries can vary from 4 to 11%, depending on cultivar and environment (Perkins-Veazie, 1995). TA of strawberry is generally expressed as percent citric acid, the predominant organic acid in strawberry. TA ranges from 0.45 to 1.81% and is dependent upon the nutritional and environmental conditions during production, fruit maturity and cultivar (Perkins-Veazie, 1995). The ratio of SSC to TA often determines consumer perception of fruit flavour, rather than SSC or TA alone (Perkins-Veazie, 1995; Wills *et al.*, 1998). For acceptable flavour, a minimum of 7% SSC and/or a maximum of 0.8% TA are recommended (Mitcham *et al.*, 2007).

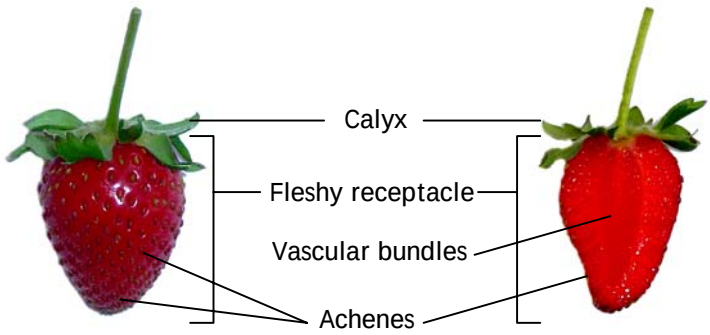


Figure 1.1 A strawberry fruit, illustrating calyx, enlarged receptacle (edible portion) and achenes.

Strawberries are grown on a wide variety of soil types and grow best on slightly acid or acid soil, the ideal soil pH being between 6.0 and 6.5 (Matlock, 1954). A sandy or gravely loam which is in good physical condition, well drained and high in organic matter is recommended (Peacock, 1939 *in* Matlock, 1954). In addition, an adequate or balanced supply of nutrients is important for the growing strawberry. Nutrient deficiencies cause poor growth, yield reduction, fruit disorders and disease susceptibility.

In addition to the fact that strawberry fruit develop and ripen rapidly resulting in a short postharvest life, fruit become increasingly susceptible to fungal infection with increased ripeness. Ripe fruit are especially susceptible to grey mould.

1.3 Grey mould

Grey mould, also called Botrytis fruit rot or ash mould, is an important airborne disease of strawberry worldwide and seriously reduces yields and postharvest quality (Paulus, 1990; Maas, 1998). The disease, caused by the fungus *Botrytis cinerea* Pers. ex Fr., results mainly from latent infection of flower parts, which usually develops into rot once fruit begin to ripen (Maas, 1998). The grey mould fungus also attacks fruit at the green, white or pink stage (Maas, 1998) and can infect flowers (causing blossom blight), petioles, leaves, crowns and roots (Plakidas, 1964; Paulus, 1990).

1.3.1 Symptoms of grey mould

Blossom blight is characterised by petals and pedicels turning brown and drying (Maas, 1998). Entire blossoms and inflorescences die if the infection is aggressive (Jarvis and Borecka, 1968).

Fruit rot may occur on any part of the fruit. However, it frequently begins on the stem end next to the calyx or on the side that touches another rotten fruit, the soil or a dead leaf (Plakidas, 1964). The disease may develop on young green and white fruit (Figure 1.2A) but the fruit become more susceptible as they mature. Infected tissue is

first seen as light brown but remains firm (Figure 1.2B). The whole fruit soon becomes rotten and covered with a greyish, fluffy mycelium (Figure 1.2C) before becoming dried (not leaky as for other rots), tough, mummified and covered with a grey, dusty powder of the conidia of *B. cinerea* (Figure 1.2D), which gives the disease its name "grey mould" (Maas, 1998; Plakidas, 1964).

1.3.2 Causal agent of grey mould

B. cinerea is the anamorph of *Botryotinia fuckeliana* (de Bary) Whetzel (Maas, 1998). The pathogen produces branched and septate hyphae. Conidiophores, produced directly from hyphae, are stout (16-30 μm in diameter), tall (2-5 mm long), dark brown and irregularly branched, chiefly near the apex and have rounded apical cells bearing clusters of colourless or grey, one-celled, ovoid conidia (8-14 x 6-9 μm) (Maas, 1998). The conidiophores and clusters of conidia resemble a grape-like cluster. Conidia are released rapidly in humid weather and are carried by air currents. The fungus produces black, hard, flat, irregular sclerotia which are infrequent on strawberry plants in the field but appear on dead petioles, residues of preceding crops, dead weeds and straw mulch (Braun and Sutton, 1987; Maas, 1998).

The grey mould fungus is a necrotroph and infects hundreds of host plant species, chiefly in temperate climates (Maas, 1998; Holz *et al.*, 2004). In some host organs, the pathogen produces lesions, while in others it remains latent or quiescent until the tissues senesce and die, when it grows as a weak pathogen or saprotroph (Maas, 1998). As strawberries have a worldwide distribution and are mostly grown in temperate regions, they are more likely to have issues with *B. cinerea*.

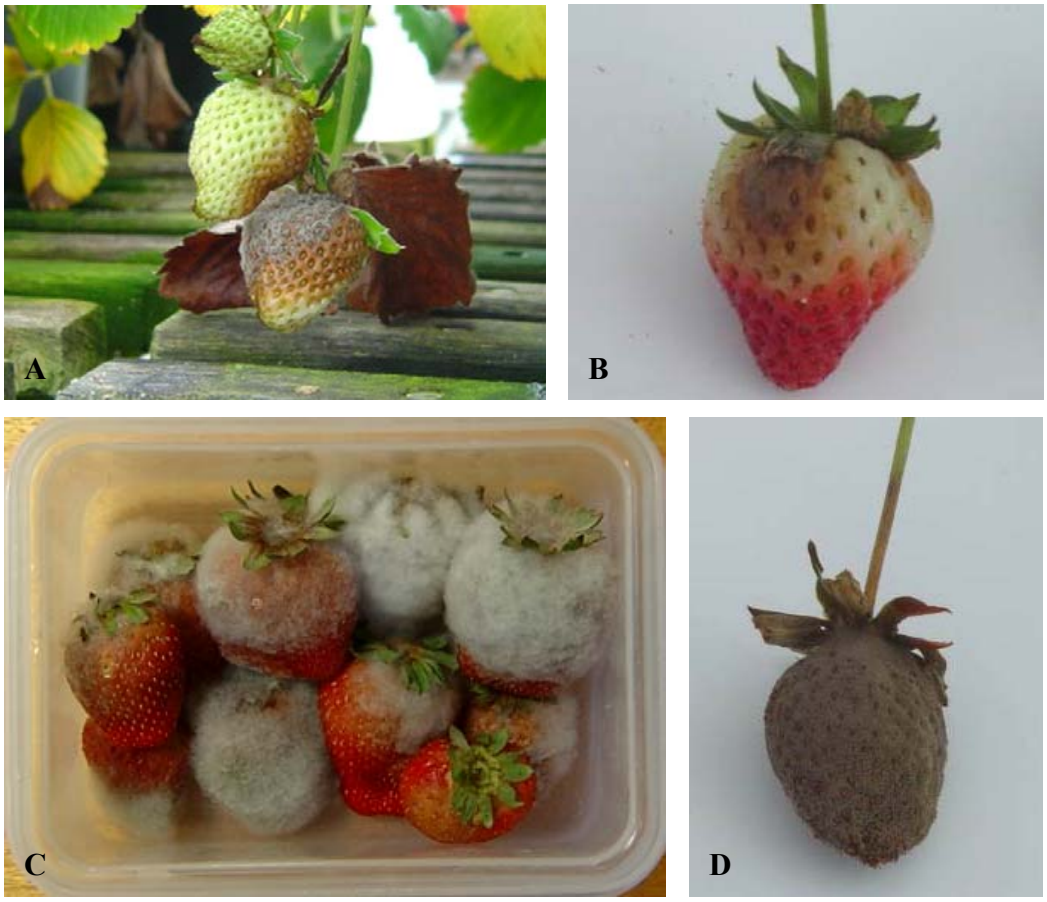


Figure 1.2 Grey mould on strawberry. (A) Infected young green fruit. (B) Brown lesion near the stem end where calyx and stamens are attached. (C) A greyish mass of mycelium on the surface of stored fruit. (D) Mummified fruit covered with the grey, dusty powder of *Botrytis cinerea* conidia.

1.3.3 Infection pathways of *B. cinerea*

Latent infection of *B. cinerea* in flowers has been described as the main pathway by which the fungus infects the fruit. However, not all fruit infections are the result of flower and latent infections. Generally, there are three main pathways of fungal infection of strawberry: flowers, ripening fruit and leaf residues.

1.3.3.1 Infection of flowers

Young blossoms are very susceptible to infection by *B. cinerea*, resulting in blossom blight or a symptomless latent infection of flowers (Jarvis and Borecka, 1968). The fungus readily infects the petals, stamens and pistils (Figure 1.3). Conidia germinate on stigmas, then the hyphae grow through the styles and, in some cases, can grow into and colonise the carpel (Bristow *et al.*, 1986), but the growth is very slow (Maas, 1998). In infected stamens, however, hyphae of *B. cinerea* grow rapidly down the filaments into the base of the calyx and then to the stem-end or neck portion of the receptacle where a high proportion of mould is initiated (Bristow *et al.*, 1986; Powelson, 1960), as indicated by the frequent initiation of rot at the proximal end of receptacles of ripened strawberry (Droby and Lichter, 2004). Hence, it seems likely that stamens may be more important than styles as a site of latent infection. Because infected stamens commonly provide a direct pathway to the receptacle whereas the carpels mature into achenes, hyphae do not grow beyond the achene and into the ripening receptacle.

In addition, the infection of senescent floral parts by *B. cinerea* provides a source of mycelium that is capable of invading the tissues of the healthy strawberry fruit. Removal of petals, stamens and calyxes shortly after fertilisation markedly reduced the incidence of fruit rot, demonstrating the importance of those parts as pathways by which *B. cinerea* gains entry to the fruit (Powelson, 1960).

1.3.3.2 Infection of ripening fruit

Although it is generally recognised that infection of strawberry flower parts by *B. cinerea* is common, field observations indicate that the major losses are caused by direct infection of the ripening fruit (Powelson, 1960; Barkai-Golan, 2001). Fruit with wounds or bruises occurring during picking, storage and transportation are likely to provide opportunities for *B. cinerea* to infect via air-borne conidia. In addition, healthy fruit can be invaded by mycelium in petals or diseased fruit adhering to it (Figure 1.4) (Maas, 1998).

1.3.3.3 Infection and extensive colonisation of leaf residues

The fungus infects apparently healthy strawberry leaves at any time during the growing season but symptoms do not usually develop until the infected leaves senesce and die. The fungus then becomes active, colonises dead tissue and eventually sporulates to produce the inoculum for subsequent infections (Braun and Sutton, 1988).

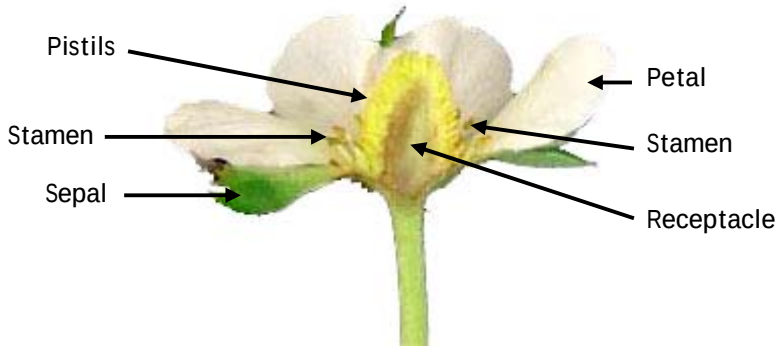


Figure 1.3 Longitudinal section of strawberry flower, illustrating pathways of flower infection: petals, pistils and stamens. Infected stamens are commonly a direct pathway to the receptacle whereas carpels/pistils mature into achenes where hyphae do not grow beyond the achene and into the ripening receptacle.

Figure 1.4 Infection of ripening fruit. *Botrytis cinerea* spreads by fruit-to-fruit contact such as this.

1.3.4 Disease cycle

The disease cycle of grey mould on strawberry is shown in Figure 1.5. The fungus overwinters as sclerotia or as mycelium in plant debris, straw mulch, weed residues, mummified fruit and strawberry leaf residues (Sosa-Alvarez *et al.*, 1995). However, nearly all primary inoculum of *B. cinerea* for infection of flower originates from mycelium on dead strawberry leaf residues in the field (Braun and Sutton, 1987). In early spring, the mycelium becomes active and sporulates on the surface of the plant debris. Conidia are spread by wind, splashing rains, irrigation water (Maas, 1998) and the hands of the fruit pickers (Plakidas, 1964) to infect floral parts, including stamens and petals. In the presence of moisture, the germination of conidia and the infection can occur within a few hours (Plakidas, 1964). Infected flowers may develop blossom blight but usually remain symptomless and the fungus establishes within the receptacle of the young fruit as a "latent" or "quiescent" infection. Mycelium of *B. cinerea* then invades the adjacent receptacle as the fruit ripen and causes fruit rot. The

pathogen sporulates on diseased flowers and fruit, which become important sources of secondary inoculum in annual production systems where there are multiple flowering and harvest cycles over several months (Maas, 1998).

Grey mould is generally favoured by moderate temperatures (15 to 25^{o}C) and long periods of high relative humidity or surface wetness during the flowering period (Maas, 1998). Frequent rains, especially during the harvest period, induce maximum disease incidence (Paulus, 1990; Powelson, 1960) and disease severity (Plakidas, 1964). The incidence of infection of flowers by *B. cinerea* at 15 to 20^{o}C increases from near zero to more than 90% as the duration wetness increases from 6 to 24 h (Bulger *et al.*, 1987) namely due to the need for free water for conidia to germinate (Peng *et al.*, 1992).

NOTE: This figure is included on page 10 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.5 Disease cycle of grey mould on strawberry (modified from http://ohioline.osu.edu/hyg-fact/3000/3017.html).

1.3.5 Control of grey mould

The major methods of grey mould control in strawberry can be grouped into three categories of physical (e.g. cold storage, heat treatments, controlled or modified atmospheres, ionising radiation and ultraviolet illumination), biological and chemical control. Application of chemical fungicides is the primary method used to control grey mould in commercial strawberry crops, but public concern over food safety and the development of fungicide resistance by pathogens have encouraged the development of alternative means (Ippolito and Nigro, 2000). Enhancing natural mechanisms of resistance to postharvest diseases is important, especially with fruit (Conway *et al.*, 1994). Adjustment of nutritional status of plants has been reported as a means for disease suppression (Yermiyahu *et al.*, 2006). Given the relevance of nutrient elements, such as calcium and boron, to disease resistance through enhancing skin strength or hardness, increasing the content of these elements in plants should enhance natural resistance (Conway *et al.*, 1994). Hence, additional calcium and boron in strawberry fruit may increase fruit firmness, which may, in turn limit infection of fruit by *B. cinerea*.

1.4 Effect of calcium on fruit quality and postharvest diseases

Calcium (Ca) is the plant nutrient most closely related to fruit quality, and firmness in particular (Sams, 1999). Calcium appears to be involved in maintaining firmness due to its role as a major component of pectins and in strengthening cell wall and membrane structure (Maas, 1998; Sams, 1999). Calcium application usually leads to an increase in calcium concentration that may affect the structure and functions of cell walls and membranes, and cell metabolism events (Chéour *et al.*, 1991). However, additional calcium may have little effect if tissues contain adequate amounts of calcium for maintaining cell integrity (Conway and Sams, 1987). A correlation between strawberry fruit firmness and its response to calcium treatment has been observed by Chéour *et al.* (1991), who noted that calcium treatment increased calcium more in the softer ‘Glooscap’ fruit, which contained less calcium than the firm ‘Kent’ fruit at the time of treatment.

The role of calcium in maintaining firmness has been associated with plant disease resistance (Maas, 1998). It is suggested that addition of calcium to fruit can either enhance resistance of fruit to postharvest pathogens or reduce susceptibility to postharvest diseases and disorders. Moline (1994) explained that the additional calcium may cause a reduction of pathogen conidia germination and germ tube elongation by limiting nutrients available to pathogens on the fruit surface. Under normal conditions, as fruit mature, there is increased availability of nutrients for pathogens on the surface of the fruit. However, calcium may enhance resistance of fruit to pathogens by interacting with cell wall components. Postharvest pathogens produce pectolytic enzymes, which cause softening of host tissues (Conway *et al.*, 1994). Calcium ions bind tightly to the pectins in the cell walls and produce cationic bridges between pectic acids, or between pectic acids and other acidic polysaccharides (Figure 1.6). These bridges make the cell walls less accessible to the action of pectolytic enzymes (Conway *et al.*, 1994; Moline, 1994).

Calcium deficiency is common but the deficiency is rarely due to insufficient calcium in the soil (Maas, 1998). In South Australia, calcium deficiency is a state wide problem where strawberries are grown even though additional calcium has been applied (Paul Mason, personal communication, 2 May 2005), suggesting calcium uptake by plants and calcium mobility in plants are relatively low. Conway *et al.* (1994) suggested that disorders that result from inadequate calcium in fruit may be due to poor calcium distribution rather than low calcium uptake, because, in the same plant, the calcium content of leaves is often higher than that of fruit. Dunn (2003) reported that following soil application of calcium, leaf tissues had significantly more calcium than the petiole, which had significantly more than the fruit tissue. Environmental factors such as low soil moisture and cool, cloudy, humid weather, can limit calcium uptake and calcium distribution within the plant (Conway *et al.*, 1994; Maas, 1998). However, the distribution of calcium in the plant is not well understood. Various methods of increasing the calcium content of fruit have, therefore, been investigated through both preharvest and postharvest applications.

NOTE: This figure is included on page 13 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.6 Schematic representation of cell walls, illustrating the Ca^{+} bridges between pectin molecules in the cell walls (inset) (www.2mcdaniel.edu/Biology/botf99/cellstructure/cell%20wall.html).

1.4.1 Preharvest calcium application

Preharvest calcium application may be considered as a cultural practice for maintaining adequate calcium concentration in fruit. Calcium-containing compounds have been applied as supplemental fertilisers in soil amendments or foliar sprays. Calcium chloride ($CaCl_2$) and calcium nitrate [$Ca(NO_3)_2$] are commonly used for foliar sprays (Bramlage *et al.*, 1985). For example, application of calcium nitrate at the rate of 15% calcium in the nutrient irrigation reduced severity of leaf grey mould of tomato plants grown in perlite by 70% (Elad and Volpin, 1993).

Application of calcium chloride has been reported to control fruit disorders and improve fruit quality of apples and pears. Raese and Drake (1993) demonstrated that 'Delicious' and 'Golden Delicious' apples from trees sprayed with calcium chloride three to four times per year at the rates of 3.60 and 4.76 g per L of water had fruit calcium concentrations of 10% or more greater than the unsprayed control trees. Similar results were obtained for 'Anjou' pears when the trees were sprayed with calcium chloride at the rate of 2.38 g per L of water. Raese and Drake (1993) also reported that calcium chloride sprays reduced the incidence of bitter pit, scald and internal breakdown in apples and the incidence of cork spot in pears. However, the calcium chloride spray treatments improved fruit firmness, TA and juiciness ratings only in apples. The reasons for this were not discussed.

Chéour *et al.* (1990) treated strawberry plants by foliar application of calcium chloride at rates of 0 to 20 kg per ha between 3 and 9 days before harvest. This led to increased calcium content of strawberry fruit, delayed fruit ripening and reduced grey mould (*B. cinerea*) development. Additionally, the delay in ripening increased with increasing calcium concentration. Similarly, Wójcik and Lewandowski (2003) observed that fruit at harvest were firmer and more resistant to Botrytis fruit rot than those in the control when the fruit were sprayed with calcium chloride at a rate of 1.5 kg per ha per spray every 5 days from the petal fall stage. Although the sprays had no

effect on SSC and TA of fruit at harvest, the SSC and TA of sprayed fruit were higher than the untreated control after 3 days of storage at 18^{o}C.

However, the application of calcium chloride and calcium nitrate can cause phytotoxicity. Relatively low concentrations of calcium chloride can produce serious foliar injury, while calcium nitrate is more likely to produce fruit injury (Bramlage *et al.*, 1985). Thus calcium chelates have been applied as an alternative source of calcium for fruit crops.

Bramlage *et al.* (1985) compared foliar sprays of calcium chloride, calcium phosphate and a polyphenolic acid chelate of calcium with the aim of increasing calcium uptake into 'McIntosh' apple fruits without causing phytotoxicity. Both calcium chloride and calcium chelate sprays increased fruit calcium concentrations and reduced senescent breakdown. Calcium phosphate sprays did not increase fruit calcium concentration. In contrast, they increased fruit phosphorus concentration, which was not associated with reduced breakdown. Foliar damage sometimes occurred in all calcium treatments, but it was not severe and the calcium treatment did not damage apple fruit. As strawberry is delicate, foliar application of calcium may cause phytotoxicity.

Naphun *et al.* (1997) reported that sprays of 200 ppm Ca^{2+} chelated with carboxylic acids (CALHARD®) once a week at the rate of 200 L per 100 m^2 markedly increased strawberry fruit firmness. They found that the force required to puncture the skin of calcium-treated strawberries was greater than that of controls, as was the content of calcium and ascorbic acid. Calcium treatment increased ascorbic acid and calcium content and maintained fruit firmness by delaying decay caused by grey mould, however, the level of grey mould control was not reported. The paper made no mention of whether or not phytotoxicity occurred after applying calcium chelate.

Since strawberry is particularly sensitive to damage by chloride (Maas, 1998), alternative calcium-containing compounds have been explored. Calcium sulphate ($CaSO_4$) has been reported to improve soil structure without having an effect on the soil pH and has been associated with improved root health (Maas, 1998). As it is less expensive than other calcium sources, calcium sulphate was considered to be a potential alternative to calcium nitrate for decrease of grey mould in tomato plants. Elad and Volpin (1993) reported that application of calcium sulphate to the soil at the rates of 1 and 3 g per kg before planting tomato seedlings reduced the disease severity by 30 and 40%, respectively. However, studies conducted for strawberry have yet to establish whether or not calcium sulphate application can improve fruit quality and control grey mould development. This is particularly true for the cultivars 'Aromas' and 'Selva' and research is required to test this possibility.

Applying supplementary calcium into soil has been suggested as the most efficient way of increasing calcium in plant organs (Conway *et al.*, 1994). However, previous research on strawberry indicated that calcium is unlikely to be translocated from roots to fruit. Increasing calcium concentration in soil through application of calcium sulphate increased leaf calcium content but not that of fruit (Dunn and Able, 2006). To increase calcium content in fruit, García *et al.* (1996) recommended that calcium should be applied directly to the fruit surface.

1.4.2 Postharvest calcium application

Postharvest calcium treatment is a means of applying calcium directly to a fruit, and may be the best method of increasing flesh calcium content (Conway *et al.*, 1994). Calcium is applied directly onto fruit by dipping, vacuum infiltration or pressure infiltration. Postharvest calcium application by dipping fruit in solutions of calcium salts has been shown to increase fruit calcium content and to increase fruit firmness in storage. Dipping apples in calcium chloride solutions can increase flesh calcium, while adding food thickeners such as Keltrol® to calcium dip solutions can further increase calcium uptake in apples (Conway *et al.*, 1994). The calcium chloride plus

Keltrol® dip was also effective in reducing the occurrence of pitting and surface marking in 'Van' cherry (Lidster *et al.*, 1979).

Vacuum or pressure infiltration of calcium salt solutions has been shown to be an effective method of getting calcium into apple. Scott and Wills (1977) compared the use of vacuum infiltration to increase the uptake of calcium with normal dipping procedures. The vacuum infiltrated apples had a greater reduction in the incidence of bitter pit than the conventionally dipped apples. The senescence of vacuum infiltrated fruit was also retarded to a greater extent. Vacuum infiltration, therefore, was the most effective method of adding calcium to the apple in that study.

The optimum method of treating fruit with calcium solutions was determined for apple by Conway (1982). 'Delicious' apples were treated with 0, 2, 4, 6 or 8% solutions of calcium chloride by dipping (2 min), vacuum infiltration (2 min; 33.32 kPa) or pressure infiltration (2 min; 103 kPa). The fruit were then inoculated with a conidial suspension of *Penicillium expansum*. There was no decrease in decay caused by *P. expansum* nor increase in calcium concentration in the flesh of apples dipped in the various concentrations of calcium chloride. The least decay and the highest concentration of calcium in flesh were found in those apples pressure infiltrated with 8% calcium chloride. Decay was reduced as the concentration of calcium chloride in the pressure infiltrated fruit increased. In vacuum infiltrated fruit, calcium concentrations increased as the concentration of the calcium solution increased, but not enough to reduce decay by more than 10%, even with the 8% calcium chloride solution. However, the paper did not provide data on the amount of calcium in fruit, information which could be used to predict the calcium content in fruit that would reduce decay caused by the fungus. Although increasing calcium concentration in apple fruit was achieved by vacuum and pressure infiltrations, the soft texture of strawberry may limit its use.

As reported above for apple, postharvest calcium chloride applied by dipping had little effect on strawberry fruit quality and fruit calcium content. Bitencourt De Souza *et al.* (1999) reported that 'Sequoia' strawberry fruit treated with 0.5 and 1.0% calcium chloride solution by immersion did not significantly differ from the untreated control in pH, total soluble solids (TSS), total titratable acidity (TA), the ratio of TSS to TA, the soluble and total pectin content and the ratio between soluble and total pectin. The calcium treatment did not significantly increase the levels of calcium in the fruit; however it increased postharvest life of fruit from 3 (control) to 21 days. The fruit showed no symptoms of fungal infection, which was assumed to be due to the incorporation of calcium into the cell wall of fruit, conferring resistance to infection. However, given that calcium content in fruit tissues did not increase with calcium treatment, it is possible that calcium chloride might affect the fungal growth directly. There is no evidence in the literature to support this idea.

The use of calcium chloride may impart bitterness or flavour differences which result from residual calcium chloride on the surface of the fruit (Luna-Guzmán and Barrett, 2000). Postharvest treatment with calcium lactate was suggested as a potential alternative to calcium chloride for shelf life extension of fresh-cut cantaloupe and treated fruit were approximately 25 to 33% firmer than cut and untreated cantaloupe samples, without providing undesirable bitterness (Luna-Guzmán and Barrett, 2000). Dipping of strawberries of cultivars 'Cardinal' and 'Sunrise' in calcium lactate improved berry firmness and character (Morris *et al.*, 1985); however, the effect on grey mould development was not mentioned in that study. There is evidence in rose that calcium decreased the ability of *B. cinerea* to utilise pectate and to produce polygalacturonase by up to 100% (Volpin and Elad, 1991). Given the potential benefit of calcium lactate in increasing fruit firmness, further research on its effect on grey mould development is warranted.

1.5 Effect of boron on fruit quality and postharvest diseases

Boron (B) is a micronutrient element required at very low concentration, but it is essential for vegetative growth. The growth of strawberry plants and the quality of fruit were found to be directly related to boron concentrations in soil and in leaves (Bhargava and Chadha, 1988).

Boron has been reported to influence quality in prune, macadamia, cherry and apple (reviewed in Silva *et al.*, 2003). Moreover, an increase in fruit set and yield following foliar boron applications has been reported in almonds (Nyomora *et al.*, 1999), olives (Perica *et al.*, 2001), sunflower (Asad *et al.*, 2003), grapevine (Treeby *et al.*, 2004) and alfalfa (Dordas, 2006).

Although the exact role of boron in plant structure and function is not well understood, a close relationship between the primary cell walls and boron nutrition has been observed. Up to 90% of the cellular boron has been localised in the cell wall (Blevins and Lukaszewski, 1998) and evidence suggests that boron may play a role in maintaining the structural integrity of plant membranes (Pilbeam and Kirkby, 1983). Treatment of cells with borate buffer prior to treatment of the cells with the chelating agent EDTA (which chelates calcium) prevented the cells from separating from one another, which normally occurs when the cells are treated only with the chelating agent EDTA (Ginzburg, 1961 *in* Pilbeam and Kirkby, 1983). The authors suggested that boron may complex with hydroxyl groups on the membranes near to the site of attack and block the access of the EDTA to the calcium that is required to cause the cells to adhere to each other. Hence, boron provides stability to that membrane in conjunction with calcium. This idea was supported by a report that boron-deficient cell walls in tomato contained less calcium than boron-sufficient cell walls (Blevins and Lukaszewski, 1998).

Boron deficiency causes both leaf and fruit symptoms. Boron deficient plants first exhibit abnormal or retarded growth of the apical growing points (Pilbeam and

Kirkby, 1983). Interveinal chlorosis usually occurs on older leaves of grapevines with boron deficiency and the yellowed tissue later becomes necrotic (Treeby *et al.*, 2004; Dordas, 2006). Generally, boron deficiency reduces pollen germination and growth of pollen tubes, which consequently results in the development of malformed fruit, which reduces crop yield and quality (Wójcik and Lewandowski, 2003).

In strawberry, boron deficiency causes asymmetrical leaves, deformed fruit and stubby roots. Boron deficiency, in addition, is associated with poor root growth, which results in deficiencies of other nutrients and the plants become stunted (Maas, 1998). However, there is a narrow range between boron deficiency and toxicity for strawberry (Gupta, 1979). As such strawberry is very sensitive to boron (Latimer, 1943) and boron can easily be toxic to strawberries. Hoagland and Snyder (1933) reported that 0.1 ppm B in the culture solution completely prevented boron deficiency symptoms in cultivars 'Banner', 'Nich Ohmer' and 'Klondike'. Eaton (1944, *in* Bhargava and Chadha, 1988) reported that 1 ppm B in the nutrient solution produced the largest plants in sand culture. Plants grown in a nutrient solution containing 5 ppm B were severely stunted, whilst 25 ppm B killed the plants before the end of the growing season. A soil boron range between 0.05 and 0.25 ppm B is considered adequate for plant growth and yield of 'Sparkle' strawberry (Gilbert and Robbins, 1950). Boron requirement has not been determined for the cultivars grown in South Australia, which indicates the need for further research.

In addition to the effect on plant growth and development, boron deficiency tends to enhance susceptibility to disease due to changes in membrane permeability in deficient plants. Boron has been reported to reduce the development of disease caused by *Verticillium albo-atrum* in tomato and cotton, *Fusarium solani* in bean, *Rhizoctonia solani* in mungbean, pea and cowpea, tobacco mosaic virus in bean (reviewed by Graham and Webb, 1991), *Sphaerotheca fuliginea* causing powdery mildew in bottle gourd and *Sclerospora graminicola* causing downy mildew in pearl millet (reviewed by Stangoulis and Graham, 2007).

Blevins *et al.* (1996) suggested that there was a trend for a decrease in tip dieback on blueberry following foliar boron treatments for 4 years. The annual total boron applied per plant was 45.4, 68.0, 90.7 and 226.8 mg for the first, second, third and fourth year of application, respectively. The foliar application of boron increased blueberry weight per plant by 10% over the 4-year period and increased the number of berries per plant by 12%. However, the mode of action of boron in reducing tip dieback and increasing blueberry yield has not been explained and is still unclear.

Although boron has been shown to reduce disease development of several plant species, there have been no reports of the effects of boron on grey mould caused by *B. cinerea* in strawberries. Application of adequate boron to strawberry plants may, therefore, enhance plant growth, fruit quality and fruit firmness as well as reduce susceptibility to grey mould.

As for calcium, boron is relatively immobile within plants and is not readily translocated from older to younger plant tissue (Gupta, 1979). Frequent applications of boron are required in most situations and applications at low rates can minimise the risk of toxicity (Incitec Pivot Limited, 2004). The principal methods of applying boron are by foliar spraying or by adding it directly to the soil (Gupta, 1979). Soil applications of boron are commonly made alone or in mixed fertilisers. Boron-containing fertilisers are applied to soil by broadcast or band applications. For foliar sprays, Solubor® and boric acid are most commonly used to supply boron (Gupta, 1979). Boron foliar spray is more effective than soil application (Wójcik and Lewandowski, 2003; Singh *et al.*, 2007) and is most frequently recommended in fruit production (Wójcik and Lewandowski, 2003). Given the sensitivity of strawberry to boron, foliar application of boron may damage the leaves. Additionally, the lack of knowledge on the effect of boron application to soil on fruit quality and grey mould development indicates the need for further study.

1.6 Justification and aims

Strawberry is extremely perishable and has a short shelf life due to its soft texture, rapid ripening and high susceptibility to fungal infection, particularly to *B. cinerea* which causes grey mould (also widely known as Botrytis fruit rot). The disease results from either latent infection of flowers, which develops into rot when fruit begins to ripen, or from infection via wounds during storage. Control of grey mould is usually achieved through fungicide applications, which have a role early in the season. However, the risk of improper use of chemicals for controlling the disease is a concern and consumers are now looking for products where alternative natural means have been used. Maintaining adequate plant nutrition, such as calcium and boron which are responsible for membrane integrity and cell wall stabilisation, may increase the strength of the cell walls and limit penetration by fungal pathogens.

Preharvest applications of calcium chloride and calcium nitrate have been reported to increase fruit calcium content, improve fruit quality and delay disease development. However, calcium chloride and calcium nitrate can cause phytotoxicity. Due to the sensitivity of strawberry to damage by chloride, an alternative source of calcium, such as calcium sulphate, which is less expensive, has been considered to replace the use of calcium chloride for strawberry. However, calcium may not be translocated readily in the plants, especially from roots to fruit, thus applying calcium directly to fruit after harvest should also be considered as a method of increasing flesh calcium content.

Postharvest calcium application has been shown to increase fruit calcium content and to increase fruit firmness. Vacuum infiltration is an effective method of adding calcium to fruit. However, dipping fruit in calcium solutions may be more suitable for applying calcium to strawberry due to its delicate texture. Although postharvest application of calcium chloride has been reported to increase fruit calcium content and prevent decay caused by fungi, the use of calcium chloride may impart bitterness or flavour differences. Calcium lactate has been used as an alternative calcium source

to increase the calcium content of strawberry fruit without reported undesirable bitterness.

Strawberry is sensitive to boron, in that the range between deficient and toxic levels of boron is very narrow. However, boron deficiency affects plant growth and fruit quality in South Australian conditions. Although boron has been reported to have a major influence on quality and to have beneficial effects in reducing disease in several plants; investigation of its effect on fruit quality and grey mould development in strawberry is limited.

B. cinerea can infect strawberry leaves and cause leaf blight (or grey mould) or symptomless latent infection until senescence and death, and the fungus then sporulates to produce the inoculum for subsequent infections. Increased calcium and boron content in leaves may strengthen the cell wall and inhibit development of *B. cinerea* on leaves. Control of grey mould by means of reducing initial inoculum may be a potentially beneficial approach to control of grey mould on fruit. This may lead to the reduction of inoculum in the field and hence contribute to the management of grey mould.

The success of calcium and boron application in enhancing fruit quality, fruit firmness and reducing the incidence of disease in various plants suggests that application of these elements might increase the firmness of strawberry, thereby conferring reduced susceptibility to fungal infection. In particular, the effect of calcium and boron on grey mould development and postharvest quality of the two SA-grown strawberry cultivars ‘Aromas’ and ‘Selva’ is still not known. The aims of this research therefore were to investigate the effect of preharvest and postharvest application of calcium and preharvest application of boron to strawberry in terms of grey mould development and fruit quality.

Chapter Two
General Materials and Methods

2.1 Introduction

This chapter describes the materials and methods that were common to the majority of the experiments reported in this thesis. Materials and methods that were specific to a particular experiment are described in the relevant chapters.

2.2 Plant materials

Strawberry (*Fragaria* X *ananassa* Duchesne) cultivars 'Aromas' and 'Selva' were chosen for this study.

'Selva' is widely grown and produced in South Australia but this cultivar has low tolerance of two-spotted mite and red spider mite and is susceptible to disease, especially to powdery mildew (Shasta Nursery, 2006).

'Aromas' is a new cultivar, which is superior to 'Selva' for productivity and fruit quality (Shasta Nursery, 2006). 'Aromas' is also relatively resistant to a number of diseases, including powdery mildew, anthracnose and crown rot (University of California, 2001a), and is relatively tolerant to several soil-borne diseases (University of California, 2001b).

The plant materials were provided by the Berry Growers of South Australia through an Australian Research Council (ARC) linkage grant (LPO347787). Fruit were provided by Mason Fresh Berries Pty Ltd. (Woodside, South Australia) and A.F. Parker & Sons, Woodside, South Australia. The certified virus-free runners for glasshouse experimentation were provided by Toolangi Strawberry Runner Growers Co-operative Ltd, Victoria. Where the runners were not used immediately in

experimentation, they were maintained in the University of California, Davis (UC Davis) soil.

The runners were grown in 15-cm diameter plastic pots containing approximately 2.5 kg of sand. Different kinds of sand were used in different experiments, which are indicated in the relevant chapters.

All experiments were conducted in laboratories and in glasshouses at the Waite Campus of the University of Adelaide (N-34^{o} 58' 13" W-138^{o} 37' 51") between May 2003 and August 2007.

2.3 Standard nutrient solutions

The standard nutrient solutions; with calcium (+Ca) (Table 2.1), without calcium (-Ca) (Table 2.2) or without boron (-B) (Table 2.3); used for the relevant preharvest experiments were a modification of Hoagland's solution (Hoagland and Arnon, 1938). The recipes presented in Tables 2.1 were to make 20 L of 10X full strength Hoagland's solution with the appropriate modification. A 0.25X full strength Hoagland's solution was then made by dilution with reverse osmosis (RO) water and was used for irrigation. Irrigation (approximately 100 mL of nutrient solution) was applied twice per week and RO water was applied as necessary to maintain sand moisture.

The micronutrient solution was prepared by dissolving the following chemicals (in grams) in 1 L of nanopure water: H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.85; $ZnSO_4.7H_2O$, 0.22; $CuSO_4.5H_2O$, 0.08; MoO_3, 0.016; $CoSO_4.5H_2O$, 0.42; $NiSO_4.5H_2O$, 0.125. In -B experiments, H_3BO_3 was not added to micronutrient solution.

Table 2.1 Composition of 10X full strength Hoagland's solution [modified from Hoagland and Arnon (1938)]. The volume was made up to 20 L with reverse osmosis water.

Chemical	mL in 20L of nutrient solution
(+Ca)	
1M $Ca(NO_3)_2$	1000
1M KNO_3	1000
1M $MgSO_4$	400
1M KH_2PO_4	200
0.5% $FeSO_4$	200
Micronutrient solution	200
(-Ca)	
1M $NaNO_3$	2000
1M KNO_3	1000
1M $MgSO_4$	400
1M KH_2PO_4	200
0.5% $FeSO_4$	200
Micronutrient solution	200
(-B)	
1M $Ca(NO_3)_2$	1000
1M KNO_3	1000
1M $MgSO_4$	400
1M KH_2PO_4	200
0.5% $FeSO_4$	200
Micronutrient solution (-B)	200

2.4 Assessment of postharvest fruit quality

2.4.1 Shelf life

Quality was assessed using a 9-point general appearance (GA) scale (9 = the best condition and 1 = the worst condition) as described in Able *et al.* (2002). When fruit reached a GA of 5.5, it was considered to have reached the end of its storage life.

2.4.2 External appearance

The presence of external defects, such as shrivel, loss of shine and calyx browning, was evaluated using a scoring system of 0 to 4 (0 = no defects, 1 = slight, 2 = moderate, 3 = severe and 4 = extreme defects).

2.4.3 Fruit firmness

Fruit firmness was determined by measuring penetration force in kilogram-force (kgf) using a Fruit Pressure Tester (Penetrometer; FT 110, Facchini, Alfonsine, Italy) equipped with a 6-mm diameter plunger tip. A puncture test was performed on the fruit cheek, approximately midway between the calyx and blossom end, by holding fruit against a hard surface before forcing the plunger tip into the fruit at a uniform speed so that the depth of penetration was consistently to the scribed line on the tip.

2.4.4 pH

The pH of the juice was determined by using a combination electrode and pH meter (Adelab Scientific, Model 209, South Australia). Juice samples were collected by wrapping fruit halves with cheesecloth and squeezing with a hand press. Juice was then filtered through cheesecloth to make a clear juice for measurement.

2.4.5 Soluble solids content (SSC)

Soluble Solids Content was determined by using a hand refractometer (Bellingham + Stanley Limited, Switzerland). A drop of juice sample (prepared as per 2.4.4) was placed on a hand refractometer, the result was read and demonstrated as percent sugar.

2.4.6 Titratable acidity (TA)

Titratable Acidity was measured as described by El-Kazzaz *et al.* (1983). Six grams of the juice sample were diluted to 50 mL with nanopure water and titrated to pH 8.1, using 0.1N NaOH. Three independent samples per treatment were analysed and each sample was titrated in triplicate. Results were expressed as percent citric acid using the formula below.

$$TA = \frac{\text{mL (NaOH) x N(NaOH) x acid milliequivalent factor x 100}}{\text{mL juice titrated}}$$

where acid milliequivalent factor for citric acid which is the predominant organic acid in strawberry = 0.64 (Garner *et al.*, 2003).

2.5 Nutrient analysis

Plant material required preparation before proceeding to analysis for nutrient concentrations. Three replicates of leaf samples per treatment were collected, washed and rinsed in deionized water to remove soil and dust particles and pesticide residues that may influence analytical results. The excess water was drained from the leaf samples which were then placed in a paper bag and dried in an oven at 70^{o}C for 48 h or until a constant dry weight was obtained. The oven-dried samples were then ground to a fine powder using a high-speed grinder before providing to Waite Analytical Services, Waite Campus.

To prepare fruit samples, after harvest, the calyx of each fruit was removed and the fruit was then cut into small pieces which were randomly placed into small tubes (approximately 6 g for each sample per tube). Three replicates of fruit samples per treatment were prepared. Fruit samples were then dried at 70^{o}C for 48 h. The prepared samples were then provided to Waite Analytical Services for nutrient analysis.

For nutrient analysis, samples were digested with 69% v/v nitric acid and 30% v/v hydrogen peroxide prior to analysis. The digests were analysed by an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (model ARL 3580B, Analytical Research Laboratories, Switzerland), to obtain iron (Fe), manganese (Mn), boron (B), copper (Cu), molybdenum (Mo), cobalt (Co), nickel (Ni), zinc (Zn), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), phosphorus (P), sulphur (S), aluminium (Al) and cadmium (Cd), with the results reported in parts per million (ppm).

To ensure nutrient load was not too high. All sand used in different experiments were analysed for nutrient content (Appendix 4). Sand was dried and ground before extraction and analysis using ICP-OES.

2.6 *B. cinerea* isolation, maintenance and inoculation

2.6.1 Isolation and maintenance

An isolate of *B. cinerea* was obtained from an infected strawberry fruit and was cultured on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) amended with 100 mg/L streptomycin sulphate (Sigma, St Louis, MO). Pathogenicity was verified by inoculating the fungus into fruit before use in this study. Stock cultures were maintained by transferring mycelial plugs from the edge of actively growing cultures onto fresh PDA every two weeks. Virulence was maintained every three weeks by reinoculating as described by Sosa-Alvarez *et al.* (1995). Discs (5.0 mm in diameter) were cut from immature strawberry fruit and placed on PDA prior to plugs (4.5 mm in diameter) cut from the 2-week-old fungus grown on PDA being placed on the fruit disk. The pathogen was reisolated after it had grown through the fruit disk.

2.6.2 Preparation of conidia suspension

Conidia were produced by placing a disc (4.5 mm in diameter) cut from the edge of cultures of the fungus onto V-8 juice agar (prepared as per Appendix 2) (El-Kazzaz *et al.*, 1983). A two-week-old sporulating culture was flooded with 20 mL of Tween-20 solution [three drops of Tween 20 (Amresco®, Ohio) in 1 L sterile nanopure water (SNW)]. Conidia were dislodged with a sterile glass rod and the conidia suspension was then filtered through two layers of sterile cheesecloth. Conidia were washed three times by centrifugation at 1000 rpm for 5 min (Volpin and Elad, 1991), resuspended in fresh Tween-20 solution. The concentration of conidia was counted by using haemacytometer and adjusted to the concentrations employed in each experiment, which ranged from 10^4 to 10^6 conidia per mL (as indicated in the relevant sections).

2.6.3 Inoculation of flowers

In a preliminary study, plants were inoculated by spraying at 50% bloom with 10 mL of *B. cinerea* conidia suspension (4 x 10^4 conidia per mL). In subsequent experiments, individually tagged newly opened flowers were sprayed with a conidia suspension of *B. cinerea* (10^4 conidia per mL) to run-off (approximately 1 mL per flower). Inoculated flowers were then covered with a plastic bag for 8 h to maintain moisture (Figure 2.1). Controls were treated with sterile water in the same manner.

2.7 Assessment of grey mould development

Fruit from each treatment were surface-sterilised by dipping in 2% sodium hypochlorite for 1 min. Sterilised fruit were then placed on a sterile, moist cloth in plastic containers and monitored daily during storage at 10^{o}C and 90±5% relative humidity (RH) for 20 days. Number of fruit exhibiting rot was recorded and presented as a percentage of rotten fruit.

2.8 Statistical analysis

Data were subjected to analysis of variance using GenStat (6th Edition, Rothamsted Experimental Station, UK) and the differences between the means of the treatments were compared by the LSD test at a significance level of 0.05. General regression equation (normally polynomial) of GA during storage were used to calculate the shelf life of fruit (GA = 5.5). Means and standard errors were calculated using Microsoft Excel.

Figure 2.1 Inoculated plants, showing how flowers were covered with a plastic bag to maintain moisture after inoculating with *Botrytis cinerea* or sterile water (as a control).

Chapter Three
Effect of Preharvest Calcium Application

3.1 Introduction

The preharvest application of calcium-containing compounds as supplemental fertilisers in soil amendments or foliar sprays could be considered as a potential cultural practice to maintain adequate calcium concentration in fruit. Calcium chloride ($CaCl_2$) and calcium nitrate [$Ca(NO_3)_2$] are commonly used for foliar sprays (Bramlage *et al.*, 1985). Foliar sprays of calcium chloride and a polyphenolic acid chelate of calcium increased fruit calcium concentrations and reduced senescent breakdown in 'McIntosh' apple fruits (Bramlage *et al.*, 1985). Similarly, foliar application of calcium chloride to strawberry plants 3 to 9 days before harvest led to increased calcium content of strawberry fruit, delayed fruit ripening and reduced grey mould development (Chéour *et al.*, 1990). Incidence of grey mould was significantly lower in fruit from plants that received five sprays of calcium chloride at rates of 2 kg of calcium per ha per spray than those that received water spray as a control (Singh *et al.*, 2007). However, foliar sprays of calcium can sometimes cause phytotoxicity (see Section 1.4.1). Application of supplementary calcium into soil would therefore seem to be an efficient way to potentially increase calcium in fruits (Conway and Sams, 1984).

Because strawberry is particularly sensitive to damage by chloride salt (Maas, 1998), alternative calcium-containing compounds have been explored to replace the use of calcium chloride. Calcium sulphate ($CaSO_4$) has been reported as improving soil structure without having an effect on the soil pH and has been associated with improved root health (Maas, 1998). Knowledge of how calcium sulphate amendments influence strawberry fruit quality and incidence of grey mould is scant. Thus, the aim of the research presented in this chapter was to investigate the effect of preharvest

application of calcium sulphate on the incidence of grey mould caused by *B. cinerea* and postharvest quality in the strawberry cultivars 'Aromas' and 'Selva'.

3.2 Materials and methods

3.2.1 Plant materials and growth conditions

Strawberry plants of two cultivars, 'Aromas' and 'Selva', were used in this study. All experiments were conducted in the glasshouse as previously described in Section 2.2.

3.2.2 Treatments

3.2.2.1 Using a closed pot system

In an initial experiment, a closed pot system was set up and amounts of all nutrients were mixed into the potting mix prior to planting (Figure 3.1) so that all nutrients were available for plant growth from the onset. Three calcium concentrations (400, 1200 and 2000 ppm Ca) were trialled and Mount Compass sand was used as potting soil. The sand was air-dried, passed through a 2-mm stainless steel sieve to remove organic matter and other debris and again air-dried in the glasshouse. The 2.5 kg of sieved Mount Compass sand was weighed into individual plastic bags.

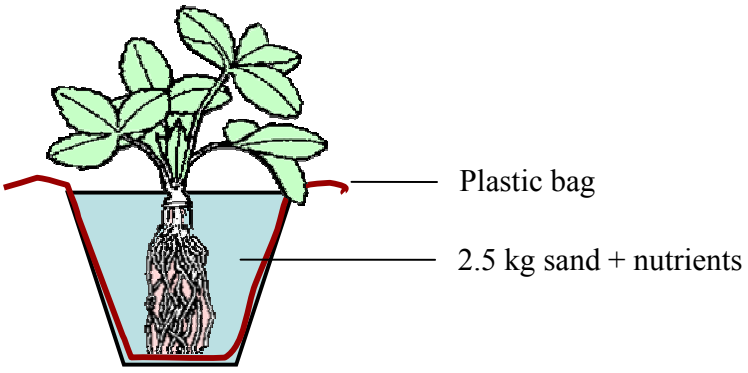


Figure 3.1 The closed pot system used to apply calcium. Strawberry plants were planted in 2.5 kg of Mount Compass sand amended with nutrients in a 15-cm diameter plastic pot lined with a plastic bag.

Nutrient solutions were added into each of the planting bags in the order and volumes presented in Table 3.1. Potassium hydroxide (KOH) was the first solution added in order to adjust the pH of the Mount Compass sand to 6.5 which is the ideal pH for growing strawberry. Once all of the nutrient solutions were added, calcium sulphate ($CaSO_4$, Labchem, Ajax Finechem, Seven Hills, New South Wales) was added at the concentration required (Table 3.2) and mixed thoroughly into the sand.

For planting, a 15-cm diameter plastic pot was lined with a plastic bag and filled with one-third of the prepared Mount Compass sand from a selected planting bag. A four-week-old strawberry plant was removed from the UC Davis soil, placed into the planting pot and filled with the remaining amended Mount Compass sand from the planting bag. This process was used for each of the 144 plants. The plants were watered with nanopure water twice a week to 10% field capacity of Mount Compass sand by weighing.

3.2.2.2 Using an automatic fertigation system

The second experiment was designed to use a free-draining system to avoid the problems encountered in the closed pot system. Nutrients were dissolved in irrigation water and continuously applied to the soil through irrigation lines (Figure 3.2). Because of immobility of calcium in plants, especially from old to young tissue, and also the poor solubility of calcium sulphate in water (3 g per L), such applications needed to be made often and the experimental calcium concentrations in the nutrient solutions were changed to 100, 300 and 500 ppm Ca. This trial was carried out over a 3-year period (January 2004 to December 2006). The planting method was similar to the closed pot system (Section 3.2.2.1) except that runners were planted directly into 15-cm diameter plastic pots containing 2.5 kg of Golden Grove sand and pots were not lined with plastic bags. Reverse osmosis (RO) water was applied to run through before applying nutrient solution. There were three plants per replicates and three replicates per calcium treatment with complete randomisation. Each calcium treatment consisted of two groups of plants; one as a control and one for inoculation with *B. cinerea*.

Table 3.1 The composition of standard nutrient solutions applied to Mount Compass sand prior to planting in the closed pot system [modified from Hoagland and Arnon (1938)].

Chemical name	**Formula**	**Stock solution (g/L)**	**Volume of nutrient solution in soil (mL/kg soil)**	**Final nutrient content in soil (mg/kg soil)**
Potassium hydroxide	KOH	40	2.0	80.0
Magnesium sulphate	$MgSO_4.7H_2O$	18.0	5.0	90.0
Manganese sulphate	$MnSO_4.H_2O$	1.5	2.0	3.0
Molybdic acid	MoO_3	0.005	1.0	0.005
Nickel sulphate	$NiSO_4.5H_2O$	0.075	2.0	0.15
Cobalt sulphate	$CoSO_4.7H_2O$	0.5	2.0	1.0
Copper sulphate	$CuSO_4.5H_2O$	2.5	2.0	5.0
Boric acid	H_3BO_3	0.05	2.0	0.1
Potassium nitrate	KNO_3	75	5.0	375.0
Calcium nitrate	$Ca(NO_3)_2.4H_2O$	143	2.9	409.4
Potassium dihydrogen orthophosphate	KH_2PO_4	75	1.2	90
Ferrous sulphate	$FeSO_4.7H_2O$	0.7	2.0	1.4
Zinc sulphate	$ZnSO_4.7H_2O$	13.2	0.5	6.6

Table 3.2 Experimental calcium treatments applied to Mount Compass sand immediately prior to planting in the closed pot system.

Calcium treatment (ppm Ca)	**$Ca(NO_3)_2$ (mg/kg soil)**	**$CaSO_4$ (mg/kg soil)**	**Final calcium content (mg/kg soil)**
400	409.4	1019.0	1428.4
1200	409.4	3736.4	4145.8
2000	409.4	6453.7	6863.1

Figure 3.2 The automatic fertigation system in the glasshouse. The nutrient solutions are held in the 20-L tanks (in the background) and allowed to flow by gravity into potting mix for 1 min each time (approximately 100 mL of nutrient solution run through the potting mix).

The stock solutions of each chemical for making a standard nutrient solution were prepared and stored in individual 1-L polythene bottles to prevent reactions with glass and Si contamination. 10X full strength Hoagland's solution (20 L) was prepared as per Section 2.3, Table 2.1 and stored in 20-L polythene containers. A 0.25 full strength Hoagland's solution with appropriate levels of calcium was used for fertigation. To prepare fertigation solution (Table 3.3), 500 mL of the 10X full strength Hoagland's solution and calcium sulphate at the concentration needed in the selected tank were added before 35 mL of 0.1 M KOH was added to adjust pH to 6.5 and then the volume was made up to 20 L with RO water. The nutrient solution was applied into potting mix until run off (100 mL per plant) every 2 days.

Table 3.3 Calcium content in 20 L fertigation tank used in the automatic fertigation system. The appropriate amount of calcium was added for each treatment to make up to 20 L of 0.25 Hoagland's solution and 100 mL of nutrient solution applied to plants every 2 days.

Calcium treatment (ppm Ca)	$Ca(NO_3)_2$ [a] (mg)	$CaSO_4$ (mg)	Final calcium content (mg)
100	4102.25	3389.9	7492.2
300	4102.25	16976.7	21079.0
500	4102.25	30563.6	34666.0

[a] From 500 mL of 10X full strength Hoagland's solution.

3.2.2.3 Using a manual fertigation system

This trial was conducted to repeat the treatments in the automatic fertigation system (Section 3.2.2.2) except that a minus-calcium (-Ca) treatment was included and the nutrient solution was applied manually to plants by pouring into soil. The trial was carried out over a 3-year period (May 2004 to May 2007). Strawberry plants were planted in a manner similar to the automatic fertigation system (see Section 3.2.2.2). 10X full strength Hoagland's solution (20 L) with no calcium (0 ppm Ca), where nitrate was supplied as sodium nitrate, was made according to the recipe in Section

2.3, Table 2.1. A 0.25 full strength Hoagland's solution for fertigation was prepared as described in Section 3.2.2.2. The amount of calcium sulphate was incorporated into the nutrient solution in the fertigation tank at the concentration needed (Table 3.4). The nutrient solution (100 mL) was applied to each plant by hand every 2 days.

Table 3.4 The amount of calcium sulphate added into a 20 L fertigation tank for use in manual fertigation. The appropriate amount of calcium sulphate was added for each treatment to make up to 20 L of 0.25 Hoagland's solution and 100 mL of nutrient solution applied by hand to plants every 2 days.

Calcium treatment (ppm Ca)	**$CaSO_4$ (mg)**
0	-
100	6793.4
300	20380.2
500	33967.1

3.2.3 Inoculation of flowers with *B. cinerea*

A conidia suspension of *B. cinerea* was prepared as previously described in Section 2.6.2. Because *B. cinerea*- inoculated flowers die if infection is severe, the method of inoculation and the concentration of conidia suspension that allow the fungus to remain quiescent in the flowers but do not cause the inoculated flowers to die were examined. To examine the method of inoculation, newly opened flowers were inoculated by one of three methods: (1) by spraying a whole plant at 50% bloom with 10 mL of conidia suspension (4 X 10^4 conidia per mL), (2) by placing 100 µL of conidia suspension (10^6 conidia per mL) onto each flower and (3) by dusting the flowers lightly with unwashed dry conidia. To inoculate, subjected plants were moved and inoculated with *B. cinerea* in a separate bench.

To examine the optimum concentration of conidia suspension, 50 µL of conidia suspension at one of four concentrations: 10^3, 10^4, 10^5 and 10^6 conidia per mL were

dropped onto each newly opened flower. The method of inoculation and the concentration of conidia suspension that caused the least number of flowers to die were then used in subsequent trials as described in Section 2.6.3.

3.2.4 Storage conditions and postharvest assessment

The harvested fruit were kept in a plastic container lined with heavy-duty paper towel and stored at 10^{o}C, 90±5% relative humidity (RH) until assessment.

To evaluate postharvest fruit quality, fruit from uninoculated plants were evaluated initially and then after storage at 10^{o}C and 90±5% RH for 2, 4, 6, 8 and 10 days. Ten fruit were used for each replicate and for each storage duration. Fruit were assessed for postharvest quality as described in Section 2.4.

Plant samples were collected and prepared for nutrient analysis (ICP-OES) as described in Section 2.5.

3.2.5 Assessment of grey mould development

To assess grey mould development, fruit from inoculated flowers or from water-treated flowers (as a control) were harvested when fully ripened and were investigated for grey mould development as described in Section 2.7.

3.2.6 Statistical analysis

Data were statistically analysed as described in Section 2.8. The percentage of rotten fruit was subjected to arcsine square root transformation before conducting analysis of variance (ANOVA). A two-way ANOVA was made to determine the effect of treatment and storage period on postharvest quality. The differences between the means of the treatments determined according to least significant difference (LSD) at $P < 0.05$. The correlation between nutrient concentration in the fertigation solution, leaf nutrient status and fruit nutrient status was determined using GenStat (6th Edition, Rothamsted Experimental Station, UK).

3.3 Results

3.3.1 Using a closed pot system

Three months after transplanting, plants which were growing in pots lined with plastic bags appeared unhealthy, with marginal leaf necrosis, stunting and consequent production of few fruit. As there were not enough fruit to continue the experiment, new trials were set up where the runners were planted directly into soil in pots and the method of nutrient application was changed to the fertigation system (Figure 3.2, Section 3.2.2.2).

3.3.2 Inoculation of flowers with *B. cinerea*

There was no significant difference between the methods of inoculation in causing inoculated flowers to die (Table 3.5). However, no flowers aborted when 10 mL of *B. cinerea* conidia suspension (4 x 10^4 conidia per mL) was sprayed onto the whole plant at 50% bloom. A lower percentage of flowers died when inoculated with a conidia suspension at 10^4 or 10^6 conidia per mL than at 10^3 and 10^5 conidia per mL regardless of cultivar (Table 3.6). Based on these preliminary experiments, spraying conidia suspension at 10^4 conidia per mL onto flowers was chosen as the inoculation method for subsequent trials.

3.3.3 Calcium treatments through automatic fertigation system

3.3.3.1 Effect on grey mould development

Grey mould was not observed on fruit of either 'Aromas' (Figure 3.3) or 'Selva' (Figure 3.4) at harvest regardless of whether artificially inoculated or not. Harvested fruit were then investigated for the incidence of grey mould fruit rot during storage at 10^{o}C, 90±5% RH for 20 days. In general, the percentage of fruit with Botrytis rot increased with time of storage; however, the incidence of fruit rot was lower when flowers were artificially inoculated. By the end of the storage period, for 'Aromas', all calcium treatments showed 100% of fruit with rot except for the 300 ppm Ca treatment inoculated with *B. cinerea* (88.9%) (Figure 3.3). Also, the incidence of fruit rot was less for controls that received the 300 ppm Ca treatment than for the other

calcium treatments. However, when flowers were artificially inoculated, the incidence of fruit rot was significantly higher ($P < 0.05$) in the 500 ppm Ca treatment than in the 100 and 300 ppm Ca treatments. On the other hand, calcium application had an effect on delaying fruit rot development in 'Selva' in both the control and inoculation treatments. The percentage of fruit rot incidence was less in the higher calcium treatments than in the 100 ppm Ca treatment.

Table 3.5 The percentage of flowers that died resulting from different methods of inoculation with *Botrytis cinerea* conidia suspension. Data shown are means ± SE.

Method of inoculation	% Flowers dead[a]	% Aborted flower[a]
Spraying[b]	68.00 ± 4.58	0.00
Droplet[c]	62.53 ± 10.24	4.17 ± 4.17
Dusting[d]	60.40 ± 10.76	5.57 ± 5.57

[a] Means of all treatments.
[b] Spraying a whole plant at 50% bloom with 10 mL of conidia suspension (4 X 10^4 conidia per mL).
[c] Placing 100 µL of conidia suspension (10^6 conidia per mL) onto each flower.
[d] Dusting the flowers lightly with unwashed dry conidia.

Table 3.6 The percentage of flower death resulting from inoculation with *Botrytis cinerea* conidia suspension at different concentrations. Data shown are means ± SE.

Concentration of conidia suspension[a] (conidia per mL)	% Flower dead[b]	
	'Aromas'	'Selva'
10^3	23.33 ± 8.82	20.00 ± 5.77
10^4	10.00 ± 5.77	10.00 ± 0.00
10^5	13.33 ± 6.67	23.33 ± 13.33
10^6	n/a	8.89 ± 8.89

[a] Placing 50 µL of conidia suspension onto each newly opened flower.
[b] Means of all treatments.
n/a = data not available.

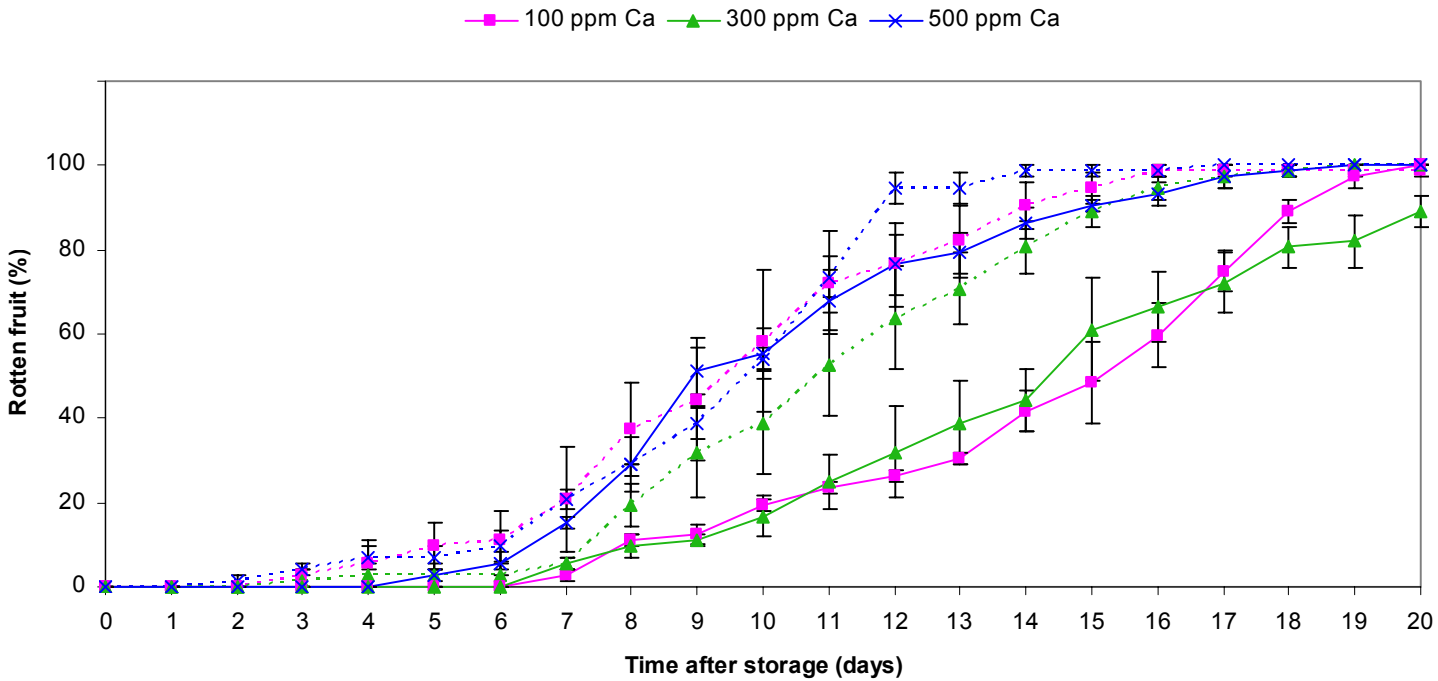


Figure 3.3 Effect of preharvest calcium application through an automatic fertigation system on the percentage of fruit displaying rot for 'Aromas' fruit from *Botrytis cinerea*-inoculated flowers (solid line) and water-treated flowers as control (dashed line). Data shown are non-transformed means ± SE from $n = 72$.

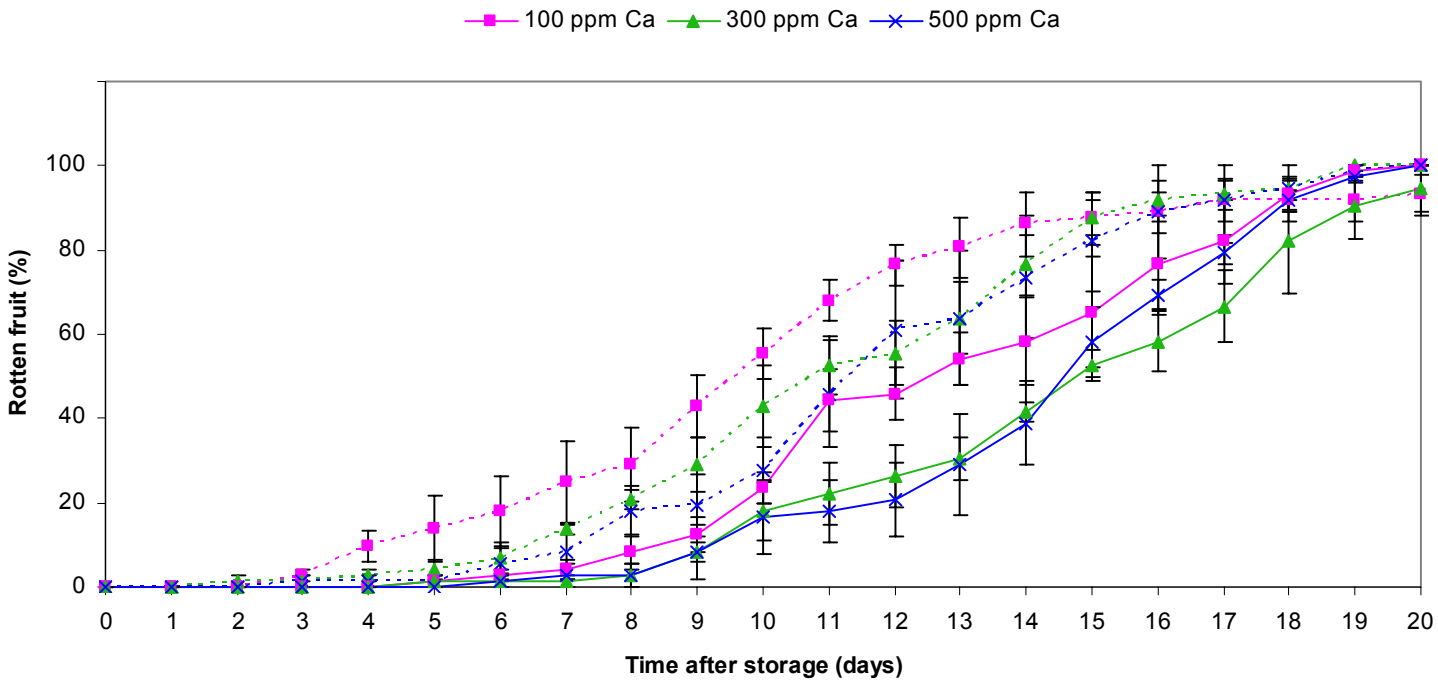


Figure 3.4 Effect of preharvest calcium application through an automatic fertigation system on the percentage of fruit displaying rot for 'Selva' fruit developed from *Botrytis cinerea*-inoculated flowers (solid line) and water-treated flowers as control (dashed line). Data shown are non-transformed means ± SE from $n = 72$.

3.3.3.2 Effect on shelf life

Preharvest application of calcium through an automatic fertigation system had an effect on the shelf life of fruit of both cultivars except for the inoculated 'Aromas' (Figure 3.5). In the control, where fruit developed from flowers inoculated with sterile water, 'Aromas' fruit in the 300 ppm Ca treatments had a significantly longer ($P < 0.05$) shelf life than those in the 100 or 500 ppm Ca. Similarly, the shelf life of 'Selva' fruit in either the 300 or 500 ppm Ca treatments was significantly longer ($P < 0.05$) than that in the 100 ppm Ca.

When the flowers were artificially inoculated, the shelf life of 'Aromas' did not significantly increase with increasing calcium concentration in the nutrient solution, whereas, the shelf life of 'Selva' in the 300 ppm Ca was significantly greater ($P < 0.05$) than in the 100 ppm Ca treatment.

3.3.3.3 Effect on fruit firmness

Fruit firmness at harvest of fruit from plants that received 500 ppm Ca in the nutrient solution was significantly greater ($P < 0.05$) than that in 100 and 300 ppm Ca treatments in both cultivars. However, fruit firmness fluctuated during storage (Figure 3.6). In 'Aromas', the fruit firmness in all treatments significantly decreased ($P < 0.05$) on the second day of storage and significantly increased ($P < 0.05$) on day 4 except for the fruit in the 300 ppm Ca treatment where the firmness slightly decreased on day 4 but then increased progressively until the end of storage. After 10 days of storage, only firmness for fruit from plants that received 500 ppm Ca significantly decreased ($P < 0.05$) and fell below the initial values.

In 'Selva', the fruit firmness in the 100 and 300 ppm Ca treatments gradually increased during storage and was significantly higher at day 10 than at harvest. Fruit firmness in 300 and 500 ppm Ca treatments slightly decreased on the second day of storage. However, the firmness of fruit in the 500 ppm Ca remained constant from day 2 until the end of storage.

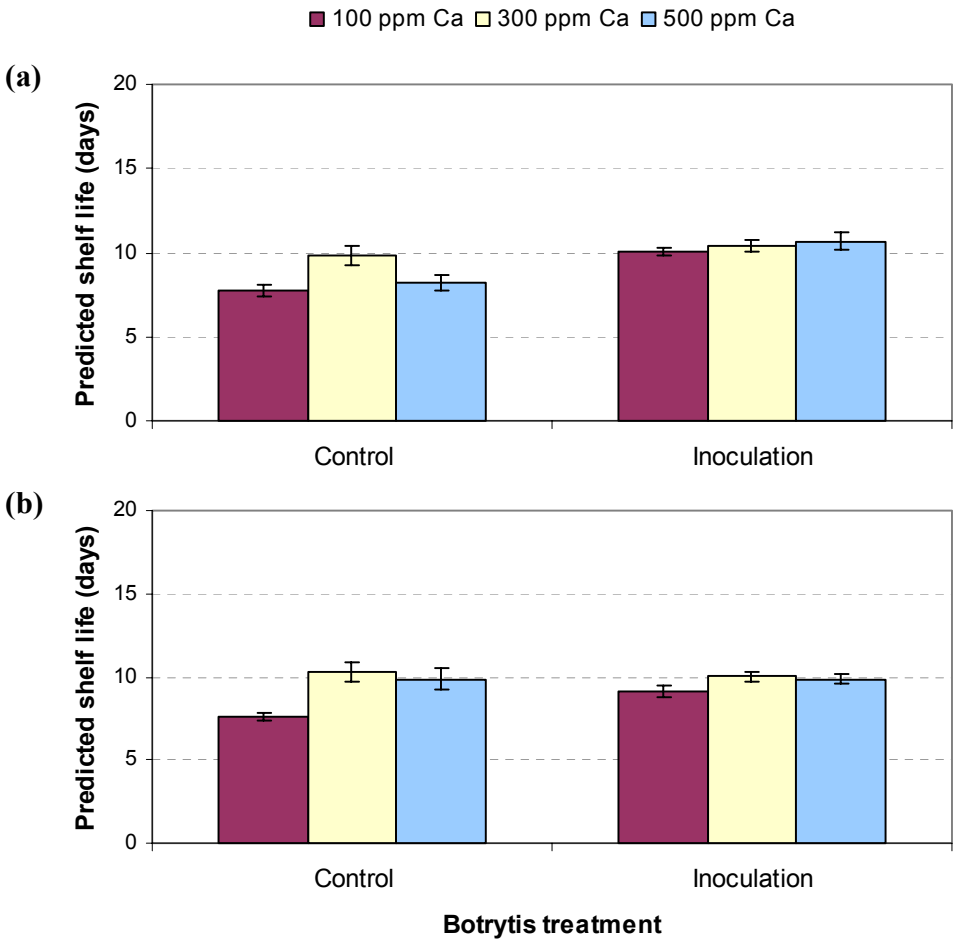


Figure 3.5 Effect of preharvest calcium application through the automatic fertigation system on shelf life of 'Aromas' (a) and 'Selva' (b) during storage at 10°C, 90±5% RH for 10 days. Shelf life was predicted in fruit from *Botrytis cinerea*-inoculated plants and from uninoculated control plants which were treated with sterile distilled water. Data shown are means ± SE from $n = 72$.

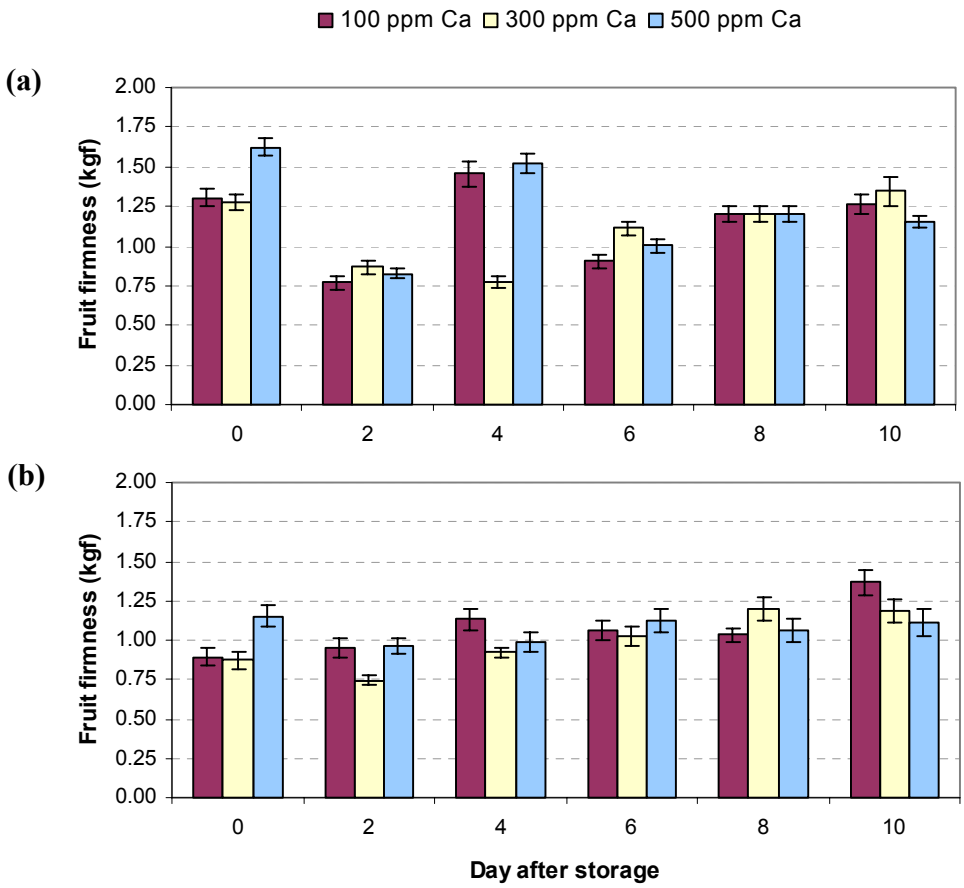


Figure 3.6 Effect of preharvest calcium application through the automatic fertigation system on fruit firmness of 'Aromas' (a) and 'Selva' (b) during storage at 10^{o}C, 90±5% RH for 10 days. Data shown are means ± SE from n = 30.

3.3.3.4 Effect on postharvest quality

3.3.3.4.1 External appearance

The degree of shrivel, loss of shine and calyx browning of both 'Aromas' (Table 3.7) and 'Selva' (Table 3.8) generally increased with time of storage at 10^{o}C and 90±5% RH. The degree of calyx browning in 'Aromas' sharply increased after 6 days of storage, whereas in 'Selva' calyx browning gradually increased such that at day 10 of storage it was significantly greater ($P < 0.05$) than at harvest. However, increasing calcium application did not delay calyx browning. Likewise, calcium did not affect shine retention in either cultivar during storage for up to 10 days. The degree of shrivel at harvest in 'Selva' tended to decrease as the amount of calcium increased in the nutrient solution, however, by the end of storage, the degree of shrivel was the greatest in the highest calcium treatment (500 ppm Ca). Although, shrivel of 'Aromas' fruit was significantly increased ($P < 0.05$) by the end of storage of 10 days, calcium application did not influence its changes.

3.3.3.4.2 Soluble solids content

The effect of preharvest calcium application on SSC is shown in Figures 3.7a and 3.8a. Initial SSC of 'Aromas' and 'Selva' in the 300 ppm Ca treatment was higher than those in the other treatments, especially in 'Aromas'. The SSC of both cultivars decreased with time of storage but the calcium level did not significantly affect those changes and the extent of change over time during storage was more in 'Selva' than in 'Aromas', particularly during the first 6 days of storage.

3.3.3.4.3 pH

The pH was between 3.30 and 3.50 regardless of cultivar or calcium treatment. In 'Aromas', the pH at harvest in the 300 ppm Ca treatment was significantly higher ($P < 0.05$) than that in the other two treatments but by the end of storage at 10^{o}C and 90±5% RH, the pH did not differ between calcium treatments (Figure 3.7b). Whereas calcium treatment did not affect the pH at harvest of 'Selva', by the end of storage,

the pH in the 300 ppm Ca was significantly lower ($P < 0.05$) than in the 100 or 500 ppm Ca treatments.

3.3.3.4.4 Titratable acidity

Preharvest calcium treatment through the automatic fertigation system did not affect the TA at harvest of 'Aromas'. However, after day 8 of storage at 10^{o}C and 90±5% RH, the TA was significantly higher ($P < 0.05$) in the 500 ppm Ca than in the 100 or 300 ppm Ca (Figure 3.7c). Although, the initial TA of 'Selva' in 500 ppm Ca was significantly higher ($P < 0.05$) than in the 100 and 300 ppm Ca treatments, the TA in all treatments slowly declined to day 10 after an initial TA slight increase at day 2 (Figure 3.8c).

3.3.3.4.5 Effect on calcium content in fruit and leaf tissues

The replicated raw data obtained from the nutrient analysis were used to calculate the mean and standard error values for 18 nutrients present in leaf and fruit tissues obtained from the cultivars 'Aromas' (Table 3.9 and 3.10) and 'Selva' (Table 3.11 and 3.12) receiving three different calcium treatments through the automatic fertigation system. Nutrient content determined in this study was compared with the nutrient levels for strawberry leaf tissues recommended by Reuter and Robinson (1997) (see Appendix 3).

Calcium concentrations either in leaves or in fruit were not significantly affected by increasing calcium application. Leaf tissues of both 'Aromas' and 'Selva' in any of the three calcium treatments had calcium concentrations in the marginal level (3000 – 15000 mg/kg) according to recommended nutrient levels for strawberry leaf tissues by Reuter and Robinson (1997). Calcium concentrations in fruit were lower than those in leaves in both cultivars. However, 'Aromas' fruit had a slightly higher calcium concentration than 'Selva' fruit. Likewise, magnesium, phosphorus and sulphur concentrations were less in fruit in comparison with in leaves in both 'Aromas' and 'Selva' regardless of calcium treatment.

Table 3.7 Effect of preharvest calcium application through the automatic fertigation system on external appearance of 'Aromas' during storage at 10°C, 90±5% RH for 0 to 10 days. Defect score: 0, no defects; 1, slight; 2, moderate; 3, severe and 4, extreme defects.

Days	Calcium treatment (ppm Ca)	Shrivel	Loss of shine	Calyx browning
0	100	0.3	0.5	0.0
	300	0.1	0.4	0.3
	500	0.2	0.2	0.1
2	100	0.5	0.4	0.3
	300	0.5	0.5	0.1
	500	0.5	0.5	0.5
4	100	0.7	0.7	0.4
	300	0.5	0.4	0.1
	500	0.4	0.4	0.8
6	100	1.2	0.9	0.8
	300	1.0	0.9	0.6
	500	1.0	0.7	0.9
8	100	0.9	0.9	1.1
	300	0.7	0.7	1.8
	500	1.0	0.9	1.4
10	100	1.5	1.2	2.4
	300	1.6	1.6	2.2
	500	1.4	1.3	2.4
	LSD (5%)	0.4	0.4	0.3

Data shown are means from $n = 30$.

Table 3.8 Effect of preharvest calcium application through the automatic fertigation system on external appearance of 'Selva' during storage at 10°C, 90±5% RH for 0 to 10 days. Defect score: 0, no defects; 1, slight; 2, moderate; 3, severe and 4, extreme defects.

Days	Calcium treatment (ppm Ca)	Shrivel	Loss of shine	Calyx browning
0	100	0.4	0.6	0.3
	300	0.4	0.3	0.7
	500	0.1	0.2	0.3
2	100	0.4	0.4	0.6
	300	0.4	0.4	0.7
	500	0.5	0.3	1.2
4	100	0.6	0.5	0.4
	300	0.7	0.8	1.3
	500	0.6	0.6	1.0
6	100	1.1	1.2	1.0
	300	0.7	1.0	1.3
	500	0.9	1.0	2.2
8	100	1.2	1.1	1.1
	300	1.3	1.2	1.6
	500	1.0	1.0	1.6
10	100	1.0	1.2	2.3
	300	1.0	1.0	1.7
	500	1.2	1.4	2.3
	LSD (5%)	0.4	0.4	0.5

Data shown are means from $n = 30$.

While sulphur concentrations in leaf tissues increased with increased calcium treatment, phosphorus and magnesium concentrations decreased in both cultivars. According to recommended nutrient levels for strawberry leaf tissues by Reuter and Robinson (1997), however, the concentrations of phosphorus and magnesium were more than adequate and in the marginal range, respectively.

With increased calcium application, iron concentration increased in leaf tissues but decreased in fruit tissues in both cultivars. There were significantly lower ($P < 0.05$) manganese concentrations in the 500 ppm Ca treatment than in the 100 ppm Ca treatment in both cultivars.

Calcium application did not affect boron, copper, cobalt, zinc, sodium or potassium in either leaves or fruit in both cultivars. In all treatments, the concentrations of boron, copper, sodium and potassium in leaf tissues were in the adequate range whereas zinc concentrations were below the deficient level (Reuter and Robinson, 1997) with no deficiency symptoms observed. The concentrations of potassium in leaves and in fruit were similar.

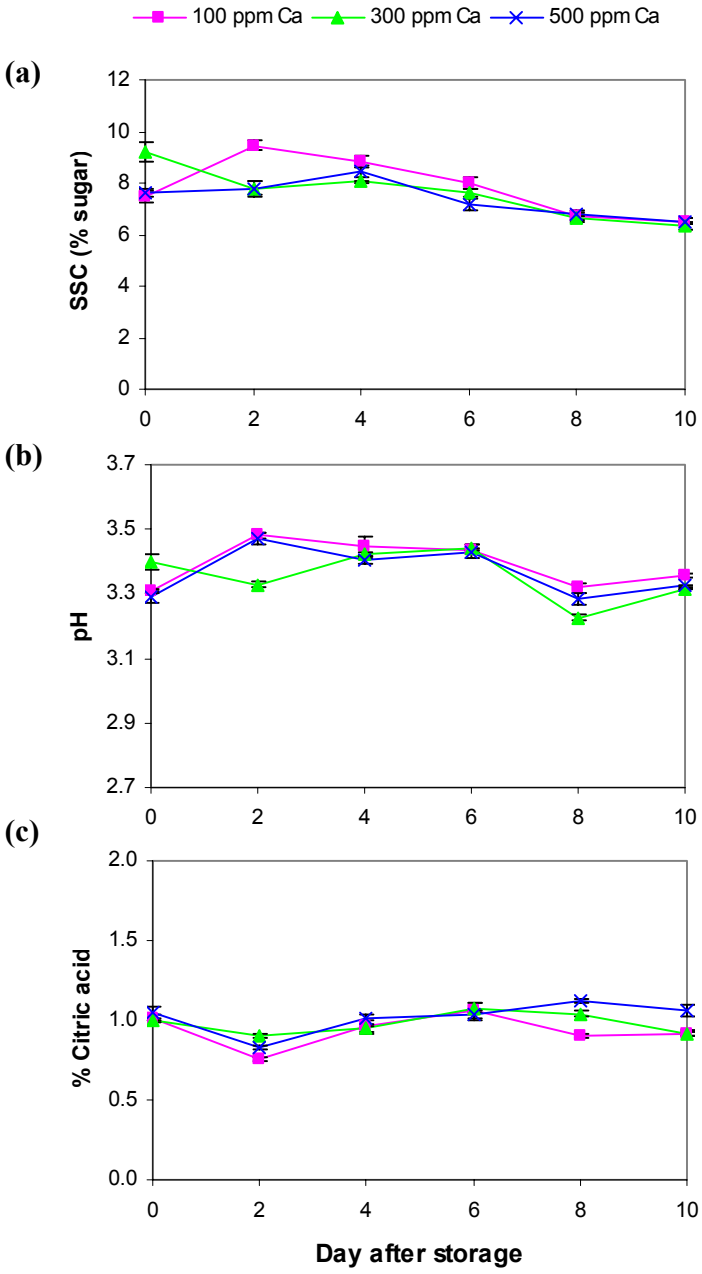


Figure 3.7 Effect of preharvest calcium application through the automatic fertigation system on soluble solids content (a), pH (b) and titratable acidity (c) of 'Aromas' during storage at 10°C, 90±5% RH for 0 to 10 days. Data shown are means ± SE from $n = 3$.

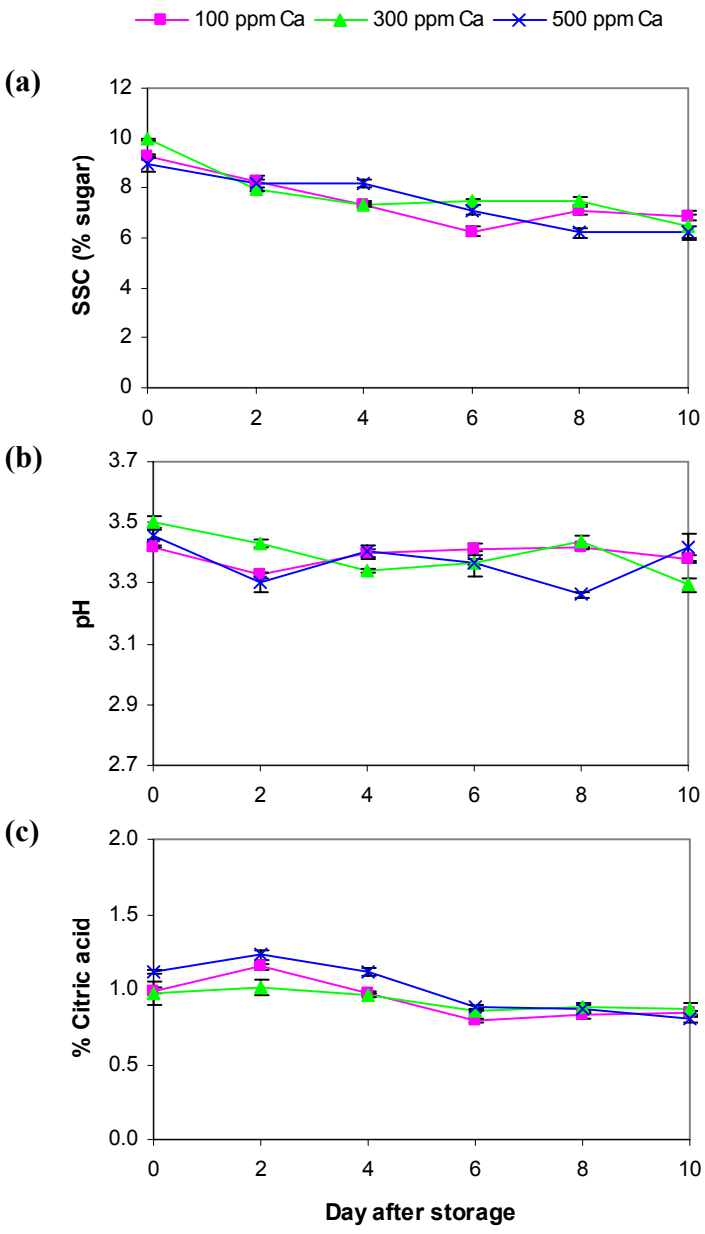


Figure 3.8 Effect of preharvest calcium application through the automatic fertigation system on soluble solids content (a), pH (b) and titratable acidity (c) of 'Selva' during storage at 10°C, 90±5% RH for 0 to 10 days. Data shown are means ± SE from $n = 3$.

Table 3.9 Effect of calcium treatment through the automatic fertigation on foliar concentration of nutrients in 'Aromas'. The nutrient concentrations were determined using ICP-OES analysis. Leaf tissues were collected from plants that received 100, 300 and 500 ppm Ca in 0.25 Hoagland's solution (400 mL per week) for 24 months. Data are presented as means ± SE from $n = 3$.

Nutrient (mg/kg)	'Aromas' leaf tissues		
	100 ppm Ca	300 ppm Ca	500 ppm Ca
Iron	34.01 ± 0.98	34.59 ± 1.99	35.10 ± 0.80
Manganese	73.14 ± 2.11	75.21 ± 2.44	78.24 ± 5.24
Boron	55.81 ± 2.41	54.18 ± 3.69	56.63 ± 3.14
Copper	3.16 ± 0.50	3.13 ± 0.11	2.94 ± 0.17
Molybdenum	< 1.00	< 1.00	< 1.00
Cobalt	1.57 ± 0.16	1.47 ± 0.08	< 0.90
Nickel	1.20 ± 0.12	1.61 ± 0.32	< 1.00
Zinc	12.26 ± 1.69	10.07 ± 0.57	10.10 ± 0.97
Calcium	10866.67 ± 744.61	12166.67 ± 733.33	12566.67 ± 648.93
Magnesium	3733.33 ± 202.76	3266.67 ± 133.33	2866.67 ± 240.37
Sodium	6.47 ± 1.40	6.05 ± 1.64	6.08 ± 1.04
Potassium	22666.67 ± 333.33	21766.67 ± 1233.33	23333.33 ± 1452.97
Phosphorus	6633.33 ± 384.42	5800.00 ± 461.88	5366.67 ± 375.65
Sulphur	1803.33 ± 6.67	2300.00 ± 57.74	2500.00 ± 208.17
Aluminium	9.04 ± 1.25	4.17 ± 0.80	3.53 ± 0.37
Cadmium	< 0.20	< 0.20	< 0.20
Lead	< 3.00	< 3.00	< 3.00
Selenium	< 9.00	< 9.00	< 9.00

= deficient range according to Reuter and Robinson (1997).
= marginal range according to Reuter and Robinson (1997).

Table 3.10 Effect of calcium treatment through the automatic fertigation on nutrient concentrations in 'Aromas' fruit. The nutrient concentrations were determined using ICP-OES analysis. Fruit tissues were collected from plants that received 100, 300 and 500 ppm Ca in 0.25 Hoagland's solution (400 mL per week) for 24 months. Data are presented as means ± SE from $n = 3$.

Nutrient (mg/kg)	'Aromas' fruit tissues		
	100 ppm Ca	300 ppm Ca	500 ppm Ca
Iron	426.67 ± 21.86	446.67 ± 92.62	340.00 ± 52.92
Manganese	32.88 ± 1.70	32.06 ± 0.49	24.90 ± 3.41
Boron	22.97 ± 1.46	21.60 ± 0.68	22.99 ± 2.66
Copper	1.48 ± 0.14	1.79 ± 0.19	1.69 ± 0.30
Molybdenum	< 1.00	< 1.00	< 1.00
Cobalt	3.08 ± 0.18	2.79 ± 0.17	1.72 ± 0.16
Nickel	1.39 ± 0.04	2.12 ± 0.20	1.10 ± 0.00
Zinc	4.90 ± 0.17	7.02 ± 0.42	4.60 ± 0.71
Calcium	3166.67 ± 284.80	3300.00 ± 360.56	3300.00 ± 635.09
Magnesium	1776.67 ± 122.38	1550.00 ± 51.32	1443.33 ± 191.95
Sodium	10.09 ± 1.38	10.31 ± 4.86	22.97 ± 8.20
Potassium	22666.67 ± 666.67	22333.33 ± 333.33	20566.67 ± 976.96
Phosphorus	3933.33 ± 392.99	3733.33 ± 88.19	3900.00 ± 808.29
Sulphur	1253.33 ± 81.72	1376.67 ± 12.02	1223.33 ± 62.27
Aluminium	2.32 ± 0.11	2.11 ± 0.42	2.88 ± 0.49
Cadmium	< 0.30	< 0.20	< 0.20
Lead	< 3.00	< 3.00	< 3.00
Selenium	< 10.00	< 10.00	< 9.00

Table 3.11 Effect of calcium treatment through the automatic fertigation on foliar concentration of nutrients in 'Selva'. The nutrient concentrations were determined using ICP-OES analysis. Leaf tissues were collected from plants that received 100, 300 and 500 ppm Ca in 0.25 Hoagland's solution (400 mL per week) for 24 months. Data are presented as means ± SE from $n = 3$.

Nutrient (mg/kg)	'Selva' leaf tissues		
	100 ppm Ca	300 ppm Ca	500 ppm Ca
Iron	40.34 ± 0.53	39.61 ± 1.46	35.68 ± 1.85
Manganese	79.21 ± 1.89	64.66 ± 3.77	60.53 ± 7.17
Boron	64.54 ± 5.77	64.45 ± 4.90	64.22 ± 4.65
Copper	3.84 ± 0.26	3.02 ± 0.36	3.20 ± 0.21
Molybdenum	< 1.00	< 1.00	< 1.00
Cobalt	1.80 ± 0.46	1.73 ± 0.35	1.11 ± 0.03
Nickel	1.44 ± 0.05	1.31 ± 0.05	< 1.00
Zinc	11.99 ± 1.51	10.25 ± 0.25	11.26 ± 0.59
Calcium	12733.33 ± 837.32	12966.67 ± 1281.06	12333.33 ± 1185.09
Magnesium	3100.00 ± 100.00	2633.33 ± 202.76	2266.67 ± 66.67
Sodium	6.70 ± 0.59	7.32 ± 1.70	9.86 ± 3.06
Potassium	24333.33 ± 881.92	21666.67 ± 881.92	21966.67 ± 1183.69
Phosphorus	6133.33 ± 218.58	4700.00 ± 360.56	4400.00 ± 57.74
Sulphur	2300.00 ± 57.74	2500.00 ± 264.58	2733.33 ± 133.33
Aluminium	2.96 ± 0.54	1.95 ± 0.32	2.48 ± 0.32
Cadmium	< 0.20	< 0.20	< 0.20
Lead	< 2.00	< 3.00	< 2.00
Selenium	< 9.00	< 9.00	< 9.00

= deficient range according to Reuter and Robinson (1997).
= marginal range according to Reuter and Robinson (1997).

Table 3.12 Effect of calcium treatment through the automatic fertigation on nutrient concentrations in 'Selva' fruit. The nutrient concentrations were determined using ICP-OES analysis. Fruit tissues were collected from plants that received 100, 300 and 500 ppm Ca in 0.25 Hoagland's solution (400 mL per week) for 24 months. Data are presented as means ± SE from $n = 3$.

Nutrient (mg/kg)	'Selva' fruit tissues		
	100 ppm Ca	300 ppm Ca	500 ppm Ca
Iron	353.33 ± 68.88	220.41 ± 35.78	248.57 ± 35.89
Manganese	23.29 ± 1.19	21.64 ± 1.61	18.31 ± 0.59
Boron	23.91 ± 1.19	24.31 ± 1.98	22.32 ± 0.45
Copper	1.66 ± 0.17	1.54 ± 0.23	1.71 ± 0.11
Molybdenum	< 1.00	< 1.00	< 1.00
Cobalt	3.23 ± 0.50	3.13 ± 0.50	1.75 ± 0.09
Nickel	1.95 ± 0.13	1.63 ± 0.39	1.36 ± 0.07
Zinc	6.42 ± 1.12	7.13 ± 0.73	6.05 ± 0.58
Calcium	2633.33 ± 317.98	2833.33 ± 240.37	2600.00 ± 57.74
Magnesium	1303.33 ± 69.60	1196.67 ± 122.52	1050.00 ± 49.33
Sodium	6.26 ± 2.51	9.55 ± 0.19	10.61 ± 1.01
Potassium	21000.00 ± 577.35	20466.67 ± 1826.05	18666.67 ± 1855.92
Phosphorus	3633.33 ± 218.58	3300.00 ± 200.00	2733.33 ± 133.33
Sulphur	1183.33 ± 86.47	1426.67 ± 41.77	1350.00 ± 83.86
Aluminium	1.15 ± 0.02	1.67 ± 0.24	1.22 ± 0.43
Cadmium	< 0.30	< 0.20	< 0.30
Lead	< 3.00	< 3.00	< 3.00
Selenium	< 10.00	< 9.00	< 9.00

3.3.4 Calcium treatments through a manual fertigation system

3.3.4.1 Effect on grey mould development

Similar to the results obtained previously in the automatic fertigation system, there was no grey mould observed on fruit in both 'Aromas' and 'Selva' at harvest regardless of whether flowers were artificially inoculated or not. However, the percentage of fruit with rot increased with time of storage and was greater on uninoculated (control) plants than those from inoculated plants for both cultivars.

The results in Figure 3.9 indicate that calcium treatment had an effect on the incidence of Botrytis fruit rot in 'Aromas' during storage at 10^{o}C, 90±5% RH for 20 days, particularly in the uninoculated control plants where the incidence of Botrytis fruit rot was significantly lower ($P < 0.05$) in fruit from calcium-treated plants than in those from no calcium-treated plants. After 14 days of storage, the percentage of fruit with rot in the uninoculated control plants significantly decreased ($P < 0.05$) to 24%, 24% and 51% when plants received 100, 300 and 500 ppm Ca, respectively, in comparison with plants that received no calcium (79%). Similar results obtained in fruit from *B. cinerea*-inoculated plants, the percentage of fruit that developed rot in the 0 ppm Ca treatment was significantly greater ($P < 0.05$) than that in the 100 and 300 ppm Ca treatments after 14 days of storage.

Similarly, calcium treatment influenced the incidence of fruit rot in 'Selva' but only in the uninoculated plants where the numbers of fruit with rot in the calcium treatments was generally lower than that in the 0 ppm Ca treatment from day 8 until the end of the storage period (Figure 3.10). However, when plants were artificially inoculated, the percentage of fruit with rot was only significantly lower ($P < 0.05$) in the 500 ppm Ca than the 0 ppm Ca after 12 days of storage.

3.3.4.2 Effect on shelf life

The effect of calcium application through the manual fertigation system on shelf life of fruit is shown in Figure 3.11. The shelf life of 'Aromas' fruit obtained from plants

that received calcium was significantly longer ($P < 0.05$) than that of fruit obtained from plants that received no calcium regardless of whether inoculated with *B. cinerea* or not, except the uninoculated control in the 100 ppm Ca treatment. In contrast to 'Aromas' control fruit, 'Selva' control fruit had a shorter shelf life when plants were supplemented with calcium. The shelf life of 'Selva' fruit increased with the increasing calcium concentrations only when fruit were obtained from inoculated flowers such that there was a significant difference ($P < 0.05$) between the 0 ppm Ca and the calcium treatments at 300 and 500 ppm Ca. In both cultivars, the shelf life of fruit from inoculated flowers was significantly longer ($P < 0.05$) than that of the control regardless of calcium treatments.

3.3.4.3 Effect on fruit firmness

Preharvest application of calcium using the manual fertigation system had no effect on fruit firmness at harvest of fruit from both cultivars (Figure 3.12). In 'Aromas', fruit firmness in the 0 ppm Ca treatment slightly increased during the first 6 days of storage but significantly declined ($P < 0.05$) on day 8 before increasing to the initial values on day 10. Regardless of calcium treatment, there was no significant loss of firmness by the end of storage period of 10 days. During storage, however, the firmness of fruit in the 500 ppm Ca significantly decreased ($P < 0.05$) and appeared significantly lower ($P < 0.05$) than the other treatments on day 6 before increasing to the original values observed on day 8 and 10.

Unlike 'Aromas', 'Selva' fruit firmness significantly decreased ($P < 0.05$) by the end of storage in comparison to the "at harvest" values except in the 100 ppm Ca treatment. After storage for 4 days, the fruit firmness in all treatments except in the 100 ppm Ca significantly declined ($P < 0.05$) and the firmness in the 300 ppm Ca was significantly lower ($P < 0.05$) than the 0 and 100 ppm Ca. On day 6 of storage, the firmness of 'Selva' fruit increased with increasing calcium concentration whilst that of 'Aromas' fruit tended to decrease.

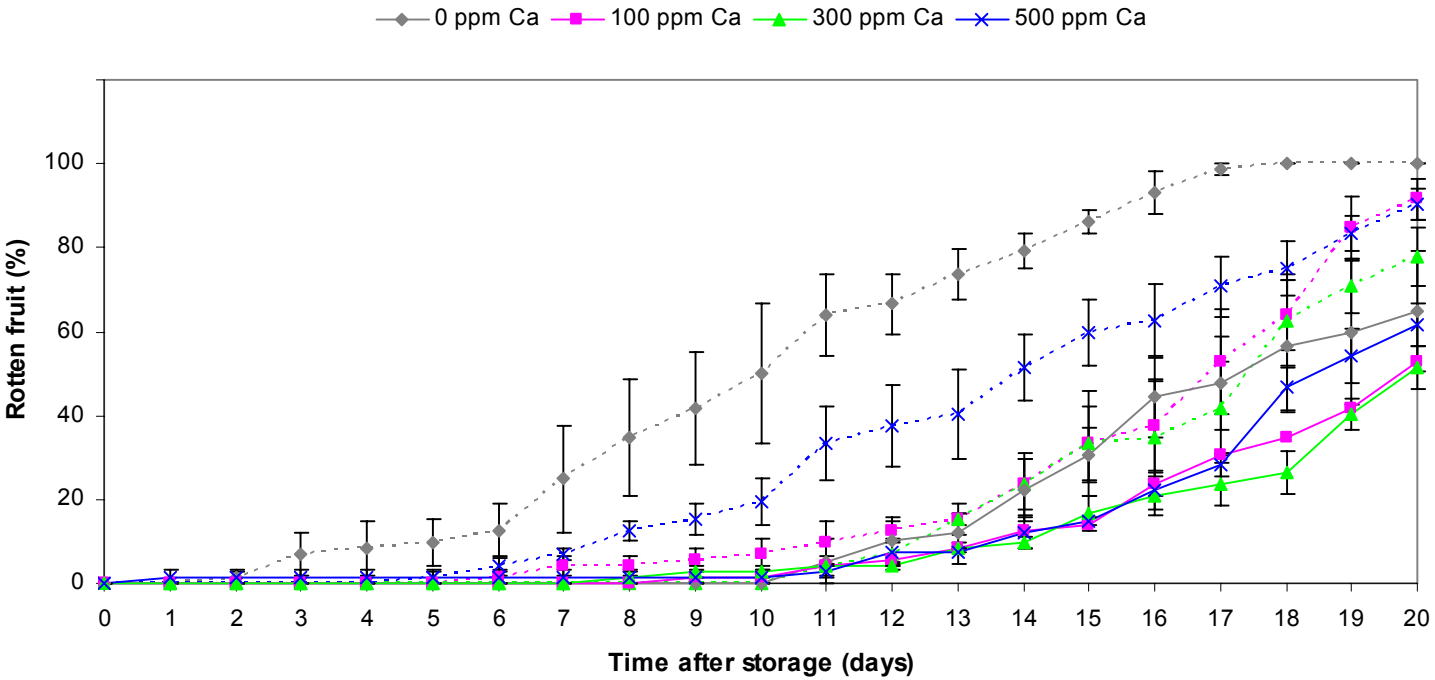


Figure 3.9 Effect of preharvest calcium application through the manual fertigation system on the percentage of fruit displaying rot for 'Aromas' fruit developed from *Botrytis cinerea*-inoculated flowers (solid line) and water-treated flowers as control (dashed line). Data shown are non-transformed means ± SE from $n = 72$.

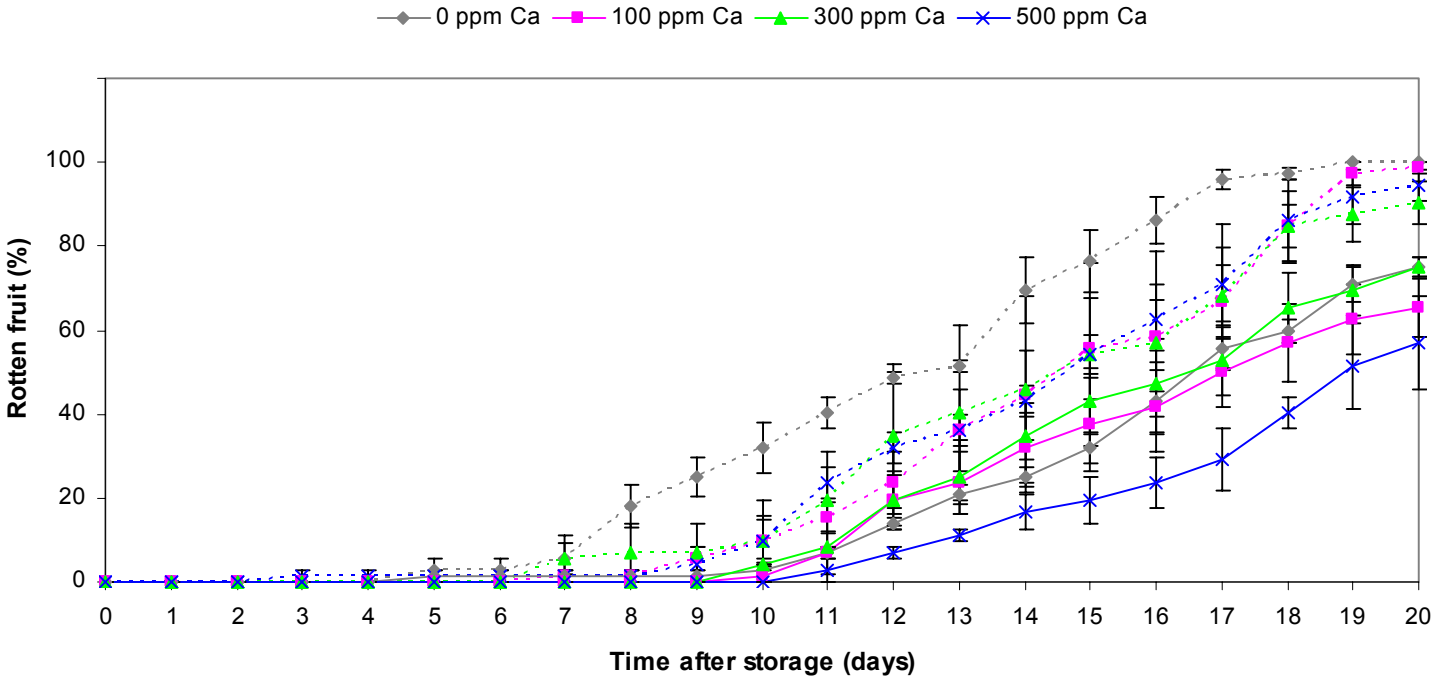


Figure 3.10 Effect of preharvest calcium application through the manual fertigation system on the percentage of fruit displaying rot for 'Selva' fruit developed from *Botrytis cinerea*-inoculated flowers (solid line) and water-treated flowers as control (dashed line). Data shown are non-transformed means ± SE from $n = 72$.

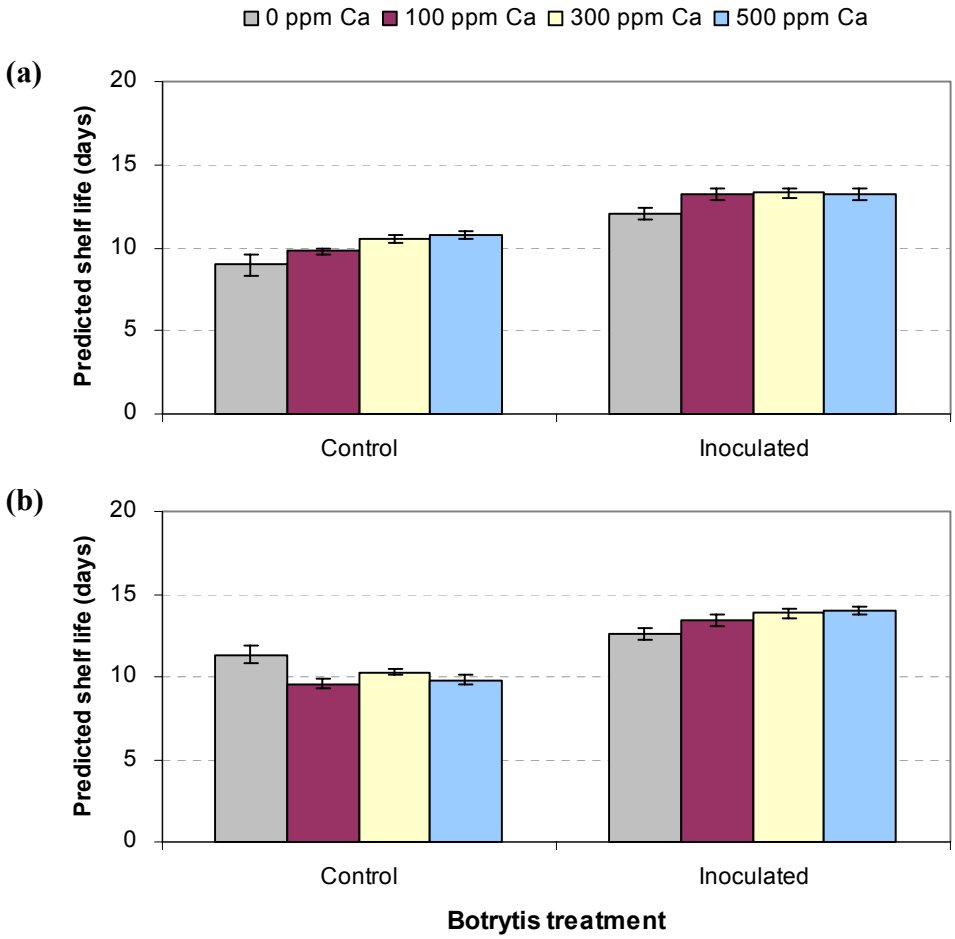


Figure 3.11 Effect of preharvest calcium application through the manual fertigation system on shelf life of 'Aromas' (a) and 'Selva' (b) during storage at 10°C, 90±5% RH for 10 days. Shelf life was predicted in fruit from *Botrytis cinerea*-inoculated plants and from uninoculated control plants which treated with sterile distilled water. Data shown are means ± SE from n = 72.

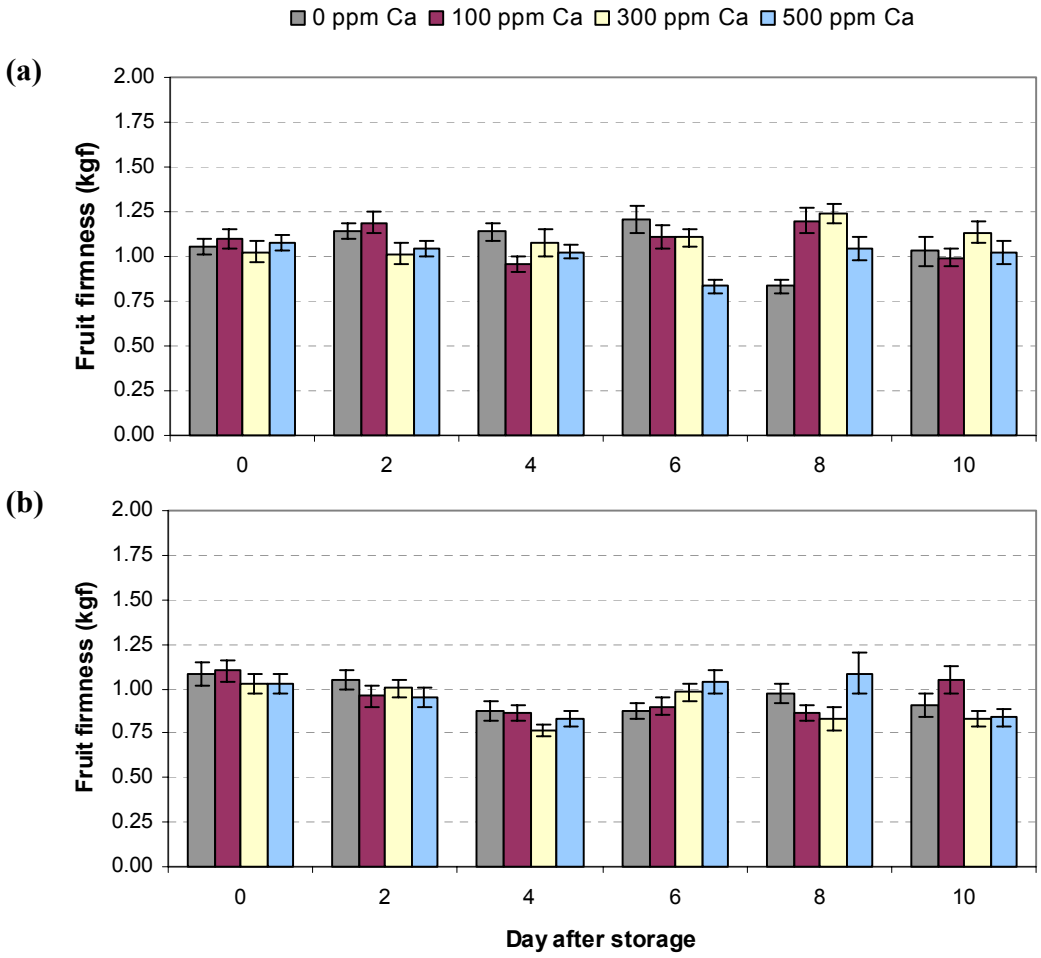


Figure 3.12 Effect of preharvest calcium application through the manual fertigation system on fruit firmness of 'Aromas' (a) and 'Selva' (b) during storage at 10°C, 90±5% RH for 10 days. Data shown are means ± SE from n = 30.

3.3.4.4 Effect on postharvest quality

3.3.4.4.1 External appearance

Preharvest calcium application through the manual fertigation system did not affect external appearance at harvest as measured by shrivel, loss of shine and calyx browning, of both cultivars (Tables 3.13 and 3.14). The degree of shrivel of both cultivars generally increased over 10 days of storage at 10^{o}C, 90±5% RH. However, there was no difference among treatments except on day 10 in 'Aromas' when the degree of shrivel was significantly lower ($P < 0.05$) in calcium treatments than in the no calcium treatment. Likewise, on day 10 of storage, loss of shine and calyx browning in 'Aromas' fruit were significantly lower ($P < 0.05$) when calcium was added to plants. In contrast, calcium treatment did not affect loss of shine in 'Selva' fruit and the degree of loss of shine increased during storage. Similarly, the degree of calyx browning in 'Selva' fruit increased with increasing time of storage with no effect of calcium treatment on its changes except on day 10, where the calyx browning on fruit from plants that received calcium was significantly lower ($P < 0.05$) than that on fruit from plants that received no calcium.

3.3.4.4.2 Soluble solids content

Changes in the SSC with storage time are shown in Figure 3.13a and 3.14a. In general, SSC of 'Selva' was slightly higher than that of 'Aromas'. For both cultivars, the SSC at the end of storage of 10 days at 10^{o}C, 90±5% RH was the same as at harvest. During storage, however, the SSC of 'Aromas' in the 500 ppm Ca slightly declined after 4 days and increased to the original level at the end of storage. Conversely, those in the 0, 100 and 300 ppm Ca treatments slightly increased during the first 4 days and decreased at day 6 before increasing to the initial values in day 10. In contrast, the SSC of 'Selva' in the 500 ppm Ca slightly increased during the first 4 days of storage and then gradually decreased to the initial value by the end of storage whereas the SSC for the other calcium treatments fluctuated during 8 days of storage before increasing to the original level at day 10.

3.3.4.4.3 pH

The pH at harvest was between 3.20 and 3.50 regardless of cultivar or calcium treatment. In 'Aromas', the pH in all treatments was the same at the end of storage as at the beginning, after fluctuating during storage, especially at day 6 when the pH in all treatments except in the 500 ppm Ca treatment significantly decreased ($P < 0.05$) in comparison to the "at harvest" pH (Figure 3.13b). In contrast, the pH of 'Selva' fruit increased from harvest to day 4 and then decreased to the original pH by the end of storage (Figure 3.14b).

3.3.4.4.4 Titratable acidity

In general, TA decreased with time of storage at 10^{o}C, 90±5% RH for 10 days in both cultivars (Figure 3.13c and 3.14c). Preharvest calcium application at the concentration of 500 ppm Ca had an effect on TA at harvest in the cultivar 'Aromas' only. TA of 'Aromas' fruit from all treatments was significantly lower ($P < 0.05$) after storage for 10 days than at harvest, except in the 500 ppm Ca treatment, where the TA remained constant until the end of storage period. TA of 'Selva' fruit for all treatments significantly decreased ($P < 0.05$) during the first 4 days of storage at 10^{o}C, 90±5% RH and appeared to increase slightly from day 4 to 10.

3.3.4.4.5 Effect on calcium content in fruit and leaf tissues

At 12 months after planting, both 'Aromas' and 'Selva' plants treated in the manual fertigation system were stunted regardless of whether calcium was applied or not. However, plants in the 0 ppm Ca presented the symptom of leaf chlorosis whereas those in the calcium treatments showed curly leaves (Figure 3.15). The nutrient concentrations in leaf and fruit tissues of both cultivars after applying nutrient solutions for 24 months are shown in Table 3.15, 3.16, 3.17 and 3.18. Calcium concentrations either in leaves or in fruit were significantly higher ($P < 0.05$) in the calcium treatment than in the 0 ppm Ca treatment. However, calcium concentrations did not significantly increase with increased calcium application. The level of

calcium in leaf tissues was present in the marginal range (Reuter and Robinson, 1997) across the four calcium treatments.

Iron was found to be deficient in leaf tissues obtained from all treatments. Mean iron concentration in leaf tissues of both cultivars increased with increased calcium treatments from 0 to 500 ppm Ca, but not significantly. In contrast, manganese, magnesium, phosphorus and potassium generally decreased with increasing calcium treatment and the concentrations of phosphorus and potassium (only in 'Aromas') in leaves were in the adequate level in the 0 ppm Ca treatment and were in the marginal range in the calcium treatments. However, manganese was below the deficient level, especially in 'Aromas', but plants did not show symptoms of deficiency.

In both cultivars, potassium and phosphorus concentrations in leaves and fruit were similar whereas manganese and magnesium concentrations were significantly lower ($P < 0.05$) in fruit than in leaves.

Calcium treatment did not influence copper or zinc concentrations. Leaf tissues of both cultivars had adequate copper but deficient zinc. However, there was no zinc deficiency symptom apparent in the plants.

Boron concentrations were at more than adequate levels in leaf tissues of both cultivars. While boron and sulphur concentrations were higher in leaves than in fruit, cobalt and sodium concentrations were higher in fruit than in leaves. Sodium decreased with increased calcium application and was significantly higher ($P < 0.05$) in both leaves and fruit in the 0 ppm Ca treatment than in the other calcium treatments.

Table 3.13 Effect of preharvest calcium application through the manual fertigation system on external appearance of 'Aromas' during storage at 10°C, 90±5% RH for 0 to 10 days. Defect score: 0, no defects; 1, slight; 2, moderate; 3, severe and 4, extreme defects.

Days	Calcium treatment (ppm Ca)	Shrivel	Loss of shine	Calyx browning
0	0	0.1	0.1	0.2
	100	0.1	0.1	0.3
	300	0.1	0.1	0.3
	500	0.2	0.3	0.4
2	0	0.5	0.5	0.8
	100	0.4	0.3	0.3
	300	0.5	0.4	0.1
	500	0.3	0.5	0.6
4	0	1.1	0.9	0.5
	100	0.9	0.7	0.1
	300	0.6	0.4	0.3
	500	0.7	0.5	0.4
6	0	1.2	0.7	0.3
	100	1.0	0.5	0.1
	300	1.2	1.1	0.4
	500	1.5	1.1	0.3
8	0	1.1	0.9	0.7
	100	1.3	1.1	0.5
	300	1.0	1.0	0.5
	500	1.1	1.2	0.4
10	0	2.0	1.6	3.4
	100	1.4	0.6	1.4
	300	1.0	0.7	1.2
	500	1.5	1.1	1.4
	LSD (5%)	0.4	0.4	0.3

Data shown are means from $n = 30$.

Table 3.14 Effect of preharvest calcium application through the manual fertigation system on external appearance of 'Selva' during storage at 10°C, 90±5% RH for 0 to 10 days. Defect score: 0, no defects; 1, slight; 2, moderate; 3, severe and 4, extreme defects.

Days	Calcium treatment (ppm Ca)	Shrivel	Loss of shine	Calyx browning
0	0	0.0	0.3	0.0
	100	0.1	0.3	0.1
	300	0.0	0.0	0.0
	500	0.0	0.0	0.0
2	0	0.2	0.3	0.2
	100	0.4	0.3	0.1
	300	0.4	0.4	0.2
	500	0.3	0.3	0.1
4	0	0.5	0.7	0.2
	100	0.3	0.5	0.3
	300	0.3	0.5	0.3
	500	0.5	0.5	0.5
6	0	0.6	0.5	0.5
	100	1.0	0.8	0.7
	300	0.6	0.2	0.5
	500	0.7	0.6	0.7
8	0	0.5	0.5	0.9
	100	0.7	0.8	1.2
	300	0.8	0.7	1.0
	500	0.8	0.8	0.8
10	0	1.0	1.1	2.3
	100	1.5	1.2	1.8
	300	1.2	1.1	1.7
	500	1.2	1.0	1.9
	LSD (5%)	0.3	0.4	0.3

Data shown are means from $n = 30$.

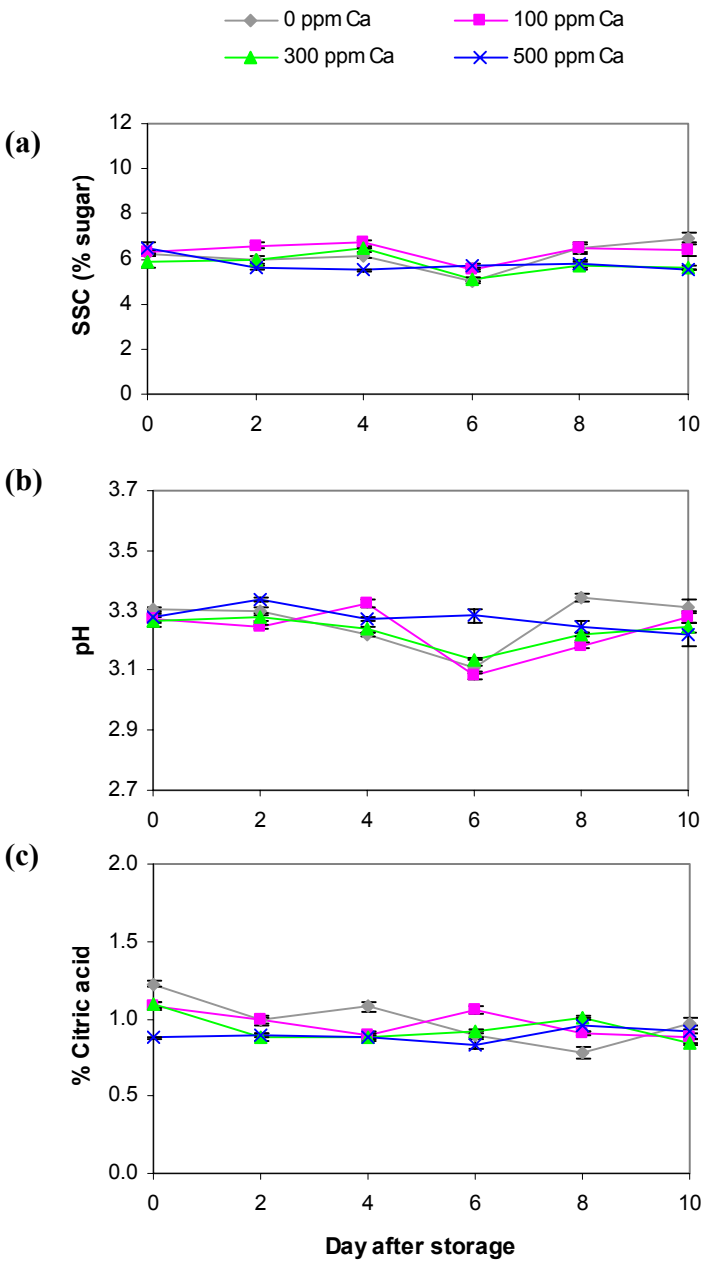


Figure 3.13 Effect of preharvest calcium application through the manual fertigation system on soluble solids content (a), pH (b) and titratable acidity (c) of 'Aromas' during storage at 10°C, 90±5% RH for 0 to 10 days. Data shown are means ± SE from $n = 3$.

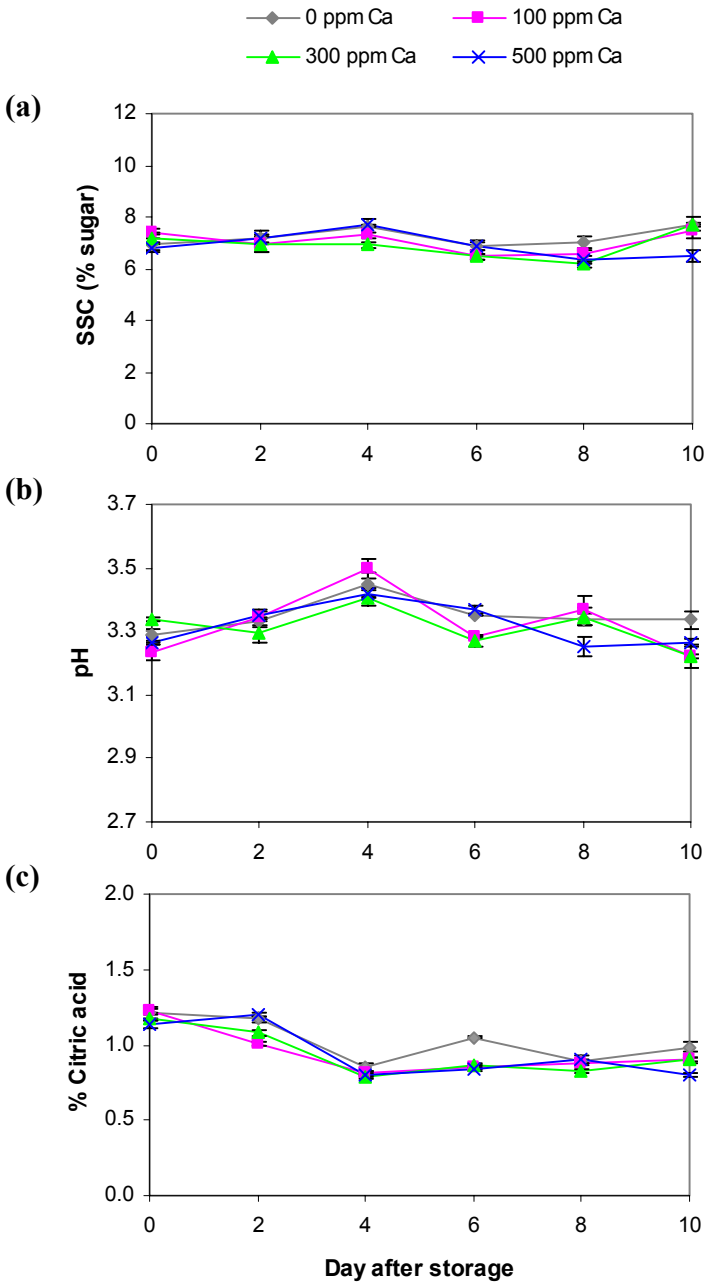


Figure 3.14 Effect of preharvest calcium application through the manual fertigation system on soluble solids content (a), pH (b) and titratable acidity (c) of 'Selva' during storage at 10°C, 90±5% RH for 0 to 10 days. Data shown are means ± SE from n = 3.

(a)

(b) **(c)**

Figure 3.15 Appearance of plants grown in soil and manually fertigated with various concentrations of calcium: (a) chlorosis of leaves on plants that received 0 ppm Ca in comparison with healthy leaves, from left to right, plants received 500, 300, 100 and 0 ppm Ca; (b) interveinal chlorosis on leaves of 'Aromas' and (c) of 'Selva'.

Table 3.15 Effect of calcium treatment through the manual fertigation on foliar concentration of nutrients in 'Aromas'. The nutrient concentrations were determined using ICP-OES analysis. Leaf tissues were collected from plants that received 0, 100, 300 and 500 ppm Ca in 0.25 Hoagland's solution (400 mL per week) for 24 months. Data are presented as means ± SE from $n = 3$.

Nutrient (mg/kg)	'Aromas' leaf tissues			
	0 ppm Ca	100 ppm Ca	300 ppm Ca	500 ppm Ca
Iron	19.97 ± 1.49	29.38 ± 1.13	30.18 ± 0.35	32.49 ± 0.93
Manganese	26.05 ± 3.82	13.33 ± 0.67	17.88 ± 1.65	16.59 ± 2.86
Boron	73.98 ± 8.52	56.57 ± 1.23	59.40 ± 2.32	69.39 ± 4.18
Copper	7.40 ± 0.50	5.50 ± 0.53	5.44 ± 0.10	5.29 ± 0.08
Molybdenum	< 1.00	< 1.00	< 1.00	< 1.00
Cobalt	0.85 ± 0.1	< 0.60	< 0.60	< 0.60
Nickel	< 1.00	< 1.00	< 1.00	< 1.00
Zinc	12.30 ± 1.33	11.60 ± 0.67	12.99 ± 0.98	10.66 ± 0.89
Calcium	3933.33 ± 284.80	11100.00 ± 888.82	11266.67 ± 317.98	12466.67 ± 933.33
Magnesium	7100.00 ± 208.17	5100.00 ± 208.17	4333.33 ± 88.19	4266.67 ± 145.30
Sodium	381.41 ± 204.30	8.65 ± 4.24	25.11 ± 9.67	12.86 ± 2.68
Potassium	24000.00 ± 1527.53	12100.00 ± 850.49	14666.67 ± 545.69	16100.00 ± 1473.09
Phosphorus	4233.33 ± 384.42	2133.33 ± 33.33	1830.00 ± 15.28	1616.67 ± 32.83
Sulphur	1546.67 ± 69.84	2220.00 ± 156.20	2533.33 ± 66.67	2833.33 ± 240.37
Aluminium	6.15 ± 0.87	5.24 ± 1.63	5.53 ± 0.57	8.39 ± 1.68
Cadmium	< 0.30	< 0.20	< 0.20	< 0.20
Lead	< 3.00	< 3.00	< 3.00	< 3.00
Selenium	< 10.00	< 9.00	< 9.00	< 9.00

= deficient range according to Reuter and Robinson (1997).
= marginal range according to Reuter and Robinson (1997).

Table 3.16 Effect of calcium treatment through the manual fertigation on nutrients concentration in 'Aromas' fruit. The nutrient concentrations were determined using ICP-OES analysis. Fruit tissues were collected from plants that received 0, 100, 300 and 500 ppm Ca in 0.25 Hoagland's solution (400 mL per week) for 24 months. Data are presented as means ± SE from $n = 3$.

Nutrient (mg/kg)	'Aromas' fruit tissues			
	0 ppm Ca	100 ppm Ca	300 ppm Ca	500 ppm Ca
Iron	238.30 ± 46.95	224.80 ± 39.83	256.67 ± 9.07	181.46 ± 3.46
Manganese	10.79 ± 1.07	7.48 ± 0.41	9.07 ± 0.60	10.69 ± 0.72
Boron	21.19 ± 1.72	19.46 ± 0.89	21.56 ± 0.72	21.82 ± 0.23
Copper	5.44 ± 0.38	6.77 ± 0.58	6.90 ± 0.38	7.15 ± 0.64
Molybdenum	< 1.00	< 1.00	< 1.00	< 1.00
Cobalt	1.34 ± 0.14	1.18 ± 0.09	1.22 ± 0.02	1.23 ± 0.07
Nickel	< 1.00	< 1.00	< 1.00	< 1.00
Zinc	7.43 ± 0.56	10.92 ± 1.22	12.70 ± 0.59	11.78 ± 0.38
Calcium	890.00 ± 105.99	1823.33 ± 63.33	2143.33 ± 179.47	2300.00 ± 0.00
Magnesium	2600.00 ± 115.47	1606.67 ± 40.96	1566.67 ± 56.08	1590.00 ± 47.26
Sodium	2110.00 ± 146.40	370.00 ± 60.28	365.46 ± 105.74	277.86 ± 78.48
Potassium	19033.33 ± 348.01	16300.00 ± 305.51	17533.33 ± 463.08	18166.67 ± 233.33
Phosphorus	2933.33 ± 133.33	1950.00 ± 45.09	1876.67 ± 78.81	1973.33 ± 14.53
Sulphur	1160.00 ± 72.34	1016.67 ± 54.87	1210.00 ± 60.28	1180.00 ± 32.15
Aluminium	8.09 ± 2.34	5.11 ± 0.70	9.49 ± 1.04	14.87 ± 5.30
Cadmium	< 0.20	< 0.20	< 0.30	< 0.20
Lead	< 3.00	< 3.00	< 3.00	< 3.00
Selenium	< 9.00	< 10.00	< 10.00	< 9.00

Table 3.17 Effect of calcium treatment through the manual fertigation on foliar concentration of nutrients in 'Selva'. The nutrient concentrations were determined using ICP-OES analysis. Leaf tissues were collected from plants that received 0, 100, 300 and 500 ppm Ca in 0.25 Hoagland's solution (400 mL per week) for 24 months. Data are presented as means ± SE from $n = 3$.

Nutrient (mg/kg)	'Selva' leaf tissues			
	0 ppm Ca	100 ppm Ca	300 ppm Ca	500 ppm Ca
Iron	14.95 ± 1.04	31.65 ± 0.71	30.87 ± 0.69	33.30 ± 0.62
Manganese	22.87 ± 3.33	20.82 ± 2.22	26.41 ± 1.37	25.38 ± 2.03
Boron	57.28 ± 3.34	55.65 ± 1.48	61.49 ± 0.63	64.55 ± 4.28
Copper	6.63 ± 0.52	6.85 ± 0.20	5.94 ± 0.10	6.14 ± 0.14
Molybdenum	< 1.00	< 1.00	< 1.00	< 1.00
Cobalt	1.24 ± 0.26	0.65 ± 0.03	< 0.60	0.73 ± 0.08
Nickel	< 1.00	< 1.00	< 1.00	< 1.00
Zinc	11.28 ± 0.39	12.51 ± 0.56	11.37 ± 0.25	11.72 ± 0.24
Calcium	3700.00 ± 208.17	11300.00 ± 832.67	12833.33 ± 317.98	13733.33 ± 1490.34
Magnesium	6633.33 ± 33.33	4266.67 ± 202.76	3500.00 ± 152.75	3433.33 ± 352.77
Sodium	134.02 ± 6.39	37.15 ± 6.25	27.45 ± 4.54	18.11 ± 5.56
Potassium	19866.67 ± 1658.65	14533.33 ± 762.31	16933.33 ± 560.75	17866.67 ± 1102.02
Phosphorus	3366.67 ± 233.33	2266.67 ± 33.33	1820.00 ± 45.09	1800.00 ± 56.86
Sulphur	1503.33 ± 35.28	2006.67 ± 109.75	2700.00 ± 208.17	2500.00 ± 264.58
Aluminium	10.47 ± 4.48	16.41 ± 4.37	14.46 ± 1.58	7.09 ± 0.85
Cadmium	< 0.20	< 0.20	< 0.30	< 0.20
Lead	< 3.00	< 3.00	< 3.00	< 3.00
Selenium	< 9.00	< 10.00	< 10.00	< 10.00

= deficient range according to Reuter and Robinson (1997).
= marginal range according to Reuter and Robinson (1997).

Table 3.18 Effect of calcium treatment through the manual fertigation on nutrient concentrations in 'Selva' fruit. The nutrient concentrations were determined using ICP-OES analysis. Fruit tissues were collected from plants that received 0, 100, 300 and 500 ppm Ca in 0.25 Hoagland's solution (400 mL per week) for 24 months. Data are presented as means ± SE from $n = 3$.

Nutrient (mg/kg)	'Selva' fruit tissues			
	0 ppm Ca	100 ppm Ca	300 ppm Ca	500 ppm Ca
Iron	159.43 ± 18.46	219.47 ± 51.83	165.42 ± 28.44	180.50 ± 41.81
Manganese	11.64 ± 0.77	9.07 ± 0.93	10.33 ± 0.83	10.12 ± 0.21
Boron	20.34 ± 2.85	23.14 ± 0.37	26.32 ± 1.24	24.32 ± 0.61
Copper	6.33 ± 1.45	8.29 ± 0.67	7.11 ± 0.33	7.63 ± 0.50
Molybdenum	< 1.00	< 1.00	< 1.00	< 1.00
Cobalt	1.72 ± 0.27	1.21 ± 0.22	1.15 ± 0.09	1.24 ± 0.05
Nickel	< 1.00	< 1.00	< 1.00	< 1.00
Zinc	11.78 ± 2.41	13.00 ± 1.08	10.87 ± 0.24	12.41 ± 0.85
Calcium	786.67 ± 95.63	2186.67 ± 165.86	2296.67 ± 254.97	2300.00 ± 57.74
Magnesium	1956.67 ± 71.72	1503.33 ± 27.28	1213.33 ± 29.06	1146.67 ± 23.33
Sodium	1843.33 ± 137.15	326.16 ± 116.12	127.13 ± 18.07	180.78 ± 8.89
Potassium	18733.33 ± 2105.02	17333.33 ± 635.96	15566.67 ± 440.96	15933.33 ± 260.34
Phosphorus	2600.00 ± 264.58	2110.00 ± 106.93	1756.67 ± 78.81	1726.67 ± 23.33
Sulphur	1153.33 ± 147.23	1063.33 ± 62.27	1006.67 ± 66.42	1096.67 ± 65.66
Aluminium	3.97 ± 2.18	11.09 ± 3.78	4.40 ± 0.51	5.33 ± 1.25
Cadmium	< 0.20	< 0.20	< 0.30	< 0.20
Lead	< 3.00	< 3.00	< 3.00	< 3.00
Selenium	< 9.00	< 10.00	< 10.00	< 9.00

3.4 Discussion

During initial experimentation, it became obvious that there were a number of issues related to the type of system used to distribute nutrients and the inoculation of flowers. Preliminary results on inoculation of flowers in this present study showed that spraying plants at 50% bloom with 10 mL of *B. cinerea* conidia suspension (4 X 10^4 conidia per mL) caused 77, 65 and 62% of inoculated flowers on plants that received 400, 1200 and 2000 ppm Ca at the time of planting, respectively, to exhibit damage and to abort (data not shown). The method of inoculation and the concentration of conidia suspension were, therefore, optimised before conducting the later experiments.

Although the percentage of inoculated flowers that died did not differ between methods of inoculation, more than 50% of the inoculated flowers died after inoculation using either method (Table 3.5). Generally, young blossoms are highly susceptible to infection by *B. cinerea*, resulting in blossom blight (Maas, 1998) or flower death when the infection is aggressive (Jarvis and Borecka, 1968). This could happen in particular when the flowering period is wet or the flower has been damaged (Bristow *et al.*, 1986). In this present study, spraying a whole plant with conidia suspension (4 X 10^4 conidia per mL) might have provided excess conidia and moisture, resulting in severe damage to the flowers, whereas the droplet method tended to be less destructive. However, the concentration of conidia used in the droplet method (10^6 conidia per mL) was probably too high for artificial inoculation. Holz *et al.* (2004) recommended the use of conidia suspensions of 10^3 to 10^5 conidia per mL to achieve symptom expression during inoculation. In this present study, the concentration of 10^4 conidia per mL using a droplet method was less destructive than the other tested concentrations in causing inoculated flowers to die in both 'Aromas' and 'Selva' (Table 3.6) and, as such, was used in the subsequent trials. Spraying, the quickest and the most convenient inoculation method, was used in the later experiments.

Plants transplanted into a closed pot system became unhealthy and consequently produced few and small fruit. Likewise, Dunn (2003) reported yield reduction and a high percentage of small fruit after transplanting but the sensory quality of strawberry was not influenced. This problem could be an issue with planting in a closed pot system or an adverse reaction to transplanting. Because the runners were initially planted in UC Davis potting soil before transfer to an experimental soil, roots, in particular, could have been damaged during the transplanting procedure and as such affected plant growth. In addition, plants would have had to adjust to the new growth conditions such as a different potting soil. However, the closed pot system, which has been commonly used in nutrition studies in other plants, was perhaps the main factor causing unhealthy plants in this present study. Palmer (2007) also reported that most of strawberry plants died when grown using the closed pot system, possibly due to excess nutrients and poor drainage. Poor drainage results in waterlogging and as such affects root growth as well as the absorption of nutrients (Matlock, 1954). The closed pot system which contains all the nutrients at the time of planting may also have been excessive for normal strawberry growth (Palmer, 2007). Because calcium is relatively immobile in plants and is not readily relocated from old to young tissues, and strawberry requires a continuous supply of available calcium for normal growth (Chiu and Bould, 1976), such applications need to be made on a regular basis, i.e. weekly or possibly twice per week (Incitec Pivot Limited, 2004). Thus, an alternative experimental system of using free-draining pots and frequent application of nutrients dissolved in irrigation water was used in subsequent experiments. The application of nutrient solutions was made through two fertigation systems (automatic and manual) to compare the effectiveness of such calcium application systems on incidence of grey mould and postharvest quality of fruit.

Similar trend of results on the effect of calcium on postharvest grey mould development and fruit quality were obtained by using either automatic or manual fertigation systems, so the focus of this discussion will be on the manual system,

which contained a 0 ppm Ca control treatment and may also be easier to manipulate for industry purposes.

In this present study, preharvest calcium application reduced the incidence of Botrytis fruit rot and increased shelf life of both 'Aromas' and 'Selva', which is consistent with results reported for cultivars 'Kent', 'Tribute' (Chéour *et al.*, 1990), 'Nyoho' (Phun *et al*., 1999 *in* Wójcik and Lewandowski, 2003) and 'Elsanta' (Wójcik and Lewandowski, 2003), in that foliar spray of calcium enhanced resistance to Botrytis rot, delayed fruit ripening and prolonged storage life. However, there was no consistent effect of calcium treatment on postharvest quality, in terms of external appearance, firmness, SSC, pH and TA, in this present study. Likewise, preharvest foliar application of calcium had no effect on postharvest quality of 'Honeoye' strawberry fruit and calcium treatment did not influence fruit calcium status (Erincik *et al*., 1998 *in* Wójcik and Lewandowski, 2003). The difference in response to calcium application may result from a difference in the ability of the cultivars to accumulate calcium ions (Chéour *et al.*, 1990), amount of calcium taken into the plant and calcium mobility in the plant (Maas, 1998).

As described in Section 1.4, the ability of calcium to improve fruit quality and, perhaps, enhance plant resistance to diseases is probably related to poor calcium distribution in the plant rather than poor calcium uptake, because, in the same plant, leaves are often higher in calcium concentration than in fruit (Conway *et al.*, 1994). The results obtained in this present study, where calcium was applied to roots, support this concept as does the report by Dunn (2003) that leaves contained more calcium than fruit. However, there was a strong correlation between calcium content in leaves and in fruit in both 'Aromas' ($P < 0.05$, $r = 0.903$) and 'Selva' ($P < 0.05$, $r = 0.891$). A similar relationship was also obtained for strawberry cultivars 'Kent' and 'Tribute' (Chéour *et al.*, 1990) and 'Elsanta' (Wójcik and Lewandowski, 2003) when the plants were treated with calcium chloride by foliar application. Chéour *et al*.

(1990) stated that calcium determination in leaves may be used to predict levels in fruit at harvest and to diagnose the need for treatment.

Although calcium content in leaf and fruit tissues appeared to increase with increasing calcium concentration in the nutrient solution, there was no significant increase. However, the trend suggested that calcium concentration in the nutrient solutions was correlated with calcium content in leaves ($P < 0.05$, $r = 0.744$) and in fruit ($P < 0.05$, $r = 0.837$) of 'Aromas' and in leaves ($P < 0.05$, $r = 0.783$,) and in fruit ($P < 0.05$, $r = 0.679$,) of 'Selva'. However, this correlation did not exist when nutrient solutions were automatically applied to plants. The influence of the application method could not be directly compared due to the lack of a 0 ppm Ca treatment in the automatic fertigation system.

It is worth noting though that even after 24 months of no calcium being supplied, foliar calcium concentrations were just marginal and increasing calcium application did not increase the concentration to the sufficient range. In addition, no typical calcium deficiency symptoms (such as leaf tipburn) were seen. However, plants that received 0 ppm Ca exhibited a foliar chlorosis (Figure 3.15) which resembled a symptom of iron deficiency. Although iron contents in leaves from all calcium treatments were at deficiency level (<50 mg per kg) according to Reuter and Robinson (1997), iron deficiency symptom did not appear on plants that received calcium where foliar contents of iron were 29 to 34 mg per kg. This indicates that the critical level of deficiency may vary depending on the cultivar. Hence, the critical levels of iron deficiency and the symptoms for different cultivars should be determined in order to make a correct diagnosis of deficiency.

In general, iron content decreases with increasing calcium concentrations (Yusuf Genc, personal communication, 6 June 2007). In contrast, the results obtained in this present study indicated that iron content in the leaf tissues increased with increasing calcium concentration. In a similar experiment in wheat, the plant had chlorotic

leaves when it had deficient levels of foliar iron in a low calcium treatment, thus it could be assumed that calcium deficiency induces iron deficiency (Yusuf Genc, personal communication, 13 August 2007).

The results obtained in this present study indicated that the incidence of Botrytis fruit rot was less in fruit that developed from artificially inoculated flowers than for those from uninoculated (water-treated) controls, where rot is likely to have originated from natural inoculation. It is possible that artificial inoculation with *B. cinerea* may induce defence reactions, such that the plants attempt to defend themselves against invasion. One early defence reaction is the collapse of epidermal cells upon successful penetration (Tenberge, 2004). Host defence responses may be stimulated by the fungus itself. In onion, foliar spray with a biotic elicitor derived from *Botrytis allii* significantly reduced the incidence and extent of Botrytis rot (Perkovskaya and Dmitriev, 2000). It would be of interest to investigate whether inoculation with *B. cinerea* would induce defence reactions in plants.

3.5 Conclusions

Preharvest application of calcium sulphate reduced the incidence of postharvest Botrytis fruit rot but increasing calcium concentration did not significantly decrease the fruit rot incidence. Postharvest quality was not influenced by calcium application, except that fruit firmness at harvest of both 'Aromas' and 'Selva' was somewhat higher in the 500 ppm Ca treatment than in the lower calcium treatments in the automatic fertigation system. However, there was a strong positive correlation between calcium concentration in the nutrient solution, leaf calcium content and fruit calcium content. Thus, a field trial with the same method of application should be investigated. In addition, due to the immobility of calcium in plant tissues, spray application of calcium sulphate directly to flowers and developing fruit merits further investigation. Moreover, a further study involving inoculation of flowers with *B. cinerea* should be conducted to determine if artificial inoculation induces defence reactions in plants.

Chapter Four
Effect of Preharvest Boron Application

4.1 Introduction

Although boron (B) is only required at very low concentrations, it is one of the most commonly deficient micronutrients in plants, including strawberry (Maas, 1998). Under conditions of boron deficiency, pollen germination and growth of pollen tubes are reduced which consequently reduces crop yields and deteriorates fruit quality (Wójcik and Lewandowski, 2003). In addition, application of boron has been reported to reduce the incidence of plant diseases (Stangoulis and Graham, 2007). However, the influence of boron on fruit quality and reduction of disease development has been described more often in other plants than strawberry. Foliar sprays of boron have been reported to increase fruit set and yield in almonds (Nyomora *et al.*, 1999), olives (Perica *et al.*, 2001), sunflower (Asad *et al.*, 2003), grapevine (Treeby *et al.*, 2004) and alfalfa (Dordas, 2006). Nott *et al.* (1999) reported that a single spray application of boron at 3.5 kg per ha reduced root hair infection by *Plasmodiophora brassicae* in Brassica vegetables.

In strawberry, deficiency symptoms include deformed fruit, asymmetrical leaves and stunted rootlets (Matlock, 1954). Moreover, boron-deficient strawberry plants are susceptible to injury and inhibited in growth (Hoagland and Snyder, 1933).

Three sprays of boron as Borvit® at a rate of 160 g B per ha per spray to 'Elsanta' strawberry increased boron content in fruit and leaf tissues but did not influence soluble solids content (SSC) and titratable acidity (TA) of fruit at harvest (Wójcik and Lewandowski, 2003) suggesting no impact on strawberry fruit quality. In addition, fruit sprayed with boron were not more resistant to Botrytis rot than the untreated control. Although there is no evidence in the literature, given potential differences in susceptibility to Botrytis, abilities to take up boron and requirements

for boron, it is likely that cultivars also behave differently in their response to boron application.

The aim of the study presented in this chapter was, therefore, to examine the effect of preharvest boron application on grey mould development and postharvest quality in the strawberry cultivars 'Aromas' and 'Selva'.

4.2 Materials and methods

4.2.1 Plant materials and growth conditions

Strawberry plants of two cultivars, 'Aromas' and 'Selva' were used in this study. All experiments were conducted between August 2005 and August 2007 in the glasshouse as previously described in Section 2.2.

4.2.2 Treatments

A free-draining system was used in this study. Nutrients were dissolved in reverse osmosis (RO) irrigation water and applied manually to plants by pouring into the soil. Boron was applied as boric acid (H_3BO_3, BDH AnalaR® Merck Pty Ltd, Kilsyth, Victoria) at four concentrations: 0, 0.25, 0.5 and 1.0 ppm B. This boron range was chosen based on the boron requirement of 'Sparkle' strawberry for good growth and excellent fruit quality, which lies between 0.05 and 0.25 ppm B (Gilbert and Robbins, 1950). Strawberry plants were planted in Waikerie sand which contains boron less than 0.08 mg per kg (see Appendix 4) and planting was carried out in a similar manner to that described in Section 3.2.2.2. Treatments were arranged in completely randomised designs, with three plants per replicate and three replicates per boron treatment. Each boron treatment consisted of two groups of plants where one was "inoculated" with sterile water as a control and one was inoculated with *B. cinerea* when required.

The stock solutions of each chemical were prepared as described in Section 3.2.2.2. 10X full strength Hoagland's solution (20 L) without boron was made according to the recipe in Section 2.3, Table 2.1. To prepare a 0.25 full strength Hoagland's solution for fertigation, 500 mL of the 10X full strength Hoagland's solution plus boric acid at the concentration needed in the appropriate tank (Table 4.1) were added. KOH (0.1 M) was then added at the necessary amount to adjust the pH to 6.5 and then the volume was made up to 20 L with RO water. The nutrient solution was applied to the potting mix until it drained through (100 mL per plant), and initially applied every 2 days. After 4 weeks, the nutrient solution was applied twice weekly due to phytotoxicity symptoms and RO water was applied on every other day.

Table 4.1 The amount of boric acid and potassium hydroxide added into a 20 L fertigation tank. The appropriate amount of boric acid and potassium hydroxide were added for each treatment to make up to 20 L of 0.25 Hoagland's solution and 100 mL of nutrient solution applied by hand to plants every 2 days.

Boron treatment (ppm B)	**0.1 M H_3BO_3 (mL/20 L)**	**0.1 M KOH (mL/20 L)**
0	0	120
0.25	4.63	160
0.5	9.82	180
1.0	19.65	200

4.2.3 Inoculation of flowers with *B. cinerea*

A conidia suspension of *B. cinerea* was prepared as previously described in Section 2.6.2. Based on the experiment described in Section 3.3.2, plants were inoculated by spraying 0.5 mL of conidia suspension at a concentration of 10^4 conidia per mL onto each newly opened flower. Controls were "inoculated" with 0.5 mL of sterile water.

4.2.4 Storage conditions and postharvest assessment

The harvested fruit were kept in a plastic container lined with heavy-duty paper towel and stored at 10^{o}C and 90±5% relative humidity (RH) until assessment as detailed below.

To evaluate postharvest fruit quality, fruit from uninoculated plants were evaluated initially and then after storage at 10^{o}C and 90±5% RH for 4, 8 and 10 days. Ten fruit were used for each replicate and different fruit were used for each storage duration. Fruit were assessed for postharvest quality (including external appearance, SSC, TA and pH) as described in Section 2.4.

After applying nutrient solution for 12 months, plant samples were collected and prepared for nutrient analysis (ICP-OES) as described in Section 2.5.

4.2.5 Assessment of grey mould development

To determine the impact of inoculation with *B. cinerea* on flower development, inoculated flowers were assessed by counting the number that died, aborted and developed into fruit.

To assess grey mould development, fruit from *B. cinerea*-inoculated flowers or from water-inoculated flowers as a control were harvested when fully ripened and were examined for grey mould development as described in Section 2.7, unless indicated otherwise.

4.2.6 Statistical analysis

Data were statistically analysed as described in Section 2.8. The percentage data was subjected to arcsine square root transformation before conducting analysis of variance (ANOVA). A two-way ANOVA was made to determine the effect of treatment and storage period on postharvest quality. The differences between the means of the treatments determined according to least significant difference (LSD) at $P < 0.05$.

4.3 Results

For the duration of the experiment, fruit yield was poor in subsequent experiments. Both 'Aromas' and 'Selva' plants developed leaf tip burn (Figure 4.1a) and leaves of 'Aromas' were yellow with green veins (Figure 4.1b). Few flowers were produced, a number of which died and aborted. Due to these problems, the experimental design was slightly adjusted, particularly in the study of the effect of preharvest boron application on grey mould development on fruit.

4.3.1 Effect on grey mould development

In the first few rounds of inoculation, more than 50% of flowers which were inoculated with either *B. cinerea* conidia suspension or sterile water as a control died and so did not develop into fruit. This problem prevented analysis of grey mould development on fruit. The focus of the study was, therefore, changed to consider if *B. cinerea* caused inoculated flowers to die or abort and also to look at whether preharvest boron application influenced the death and abortion of *B. cinerea*-inoculated flowers. Data obtained in this experiment were pooled from all three replicates of each boron treatment (i.e. nine plants per treatment).

In 'Aromas', artificial inoculation with *B. cinerea* did not result in flower death, as indicated by no difference in the percentage of flowers that died between water-treated control and inoculated flowers, except in the 0.5 ppm B treatment where more inoculated flowers than control flowers died (Figure 4.2). Preharvest boron application did not reduce the number of dead flowers among the plants inoculated with *B. cinerea*. In the water-inoculated control, however, fewer flowers died on the plants that received 0.5 ppm B than the other boron treatments.

Whether flowers were inoculated with *B. cinerea* or not, plants that received 1.0 ppm B had a higher percentage of flowers that aborted than the other boron treatments, except the 0.25 ppm B treatment in the uninoculated control (Figure 4.2b). Apart from the 0.25 ppm B treatment where the number of aborted flowers decreased,

inoculation with *B. cinerea* did not influence the occurrence of flower abortion in comparison to the water-inoculated control.

In the water-treated control, as a consequence of less flower death and abortion, more fruit was obtained in the 0.5 ppm B treatment than in the other boron treatments (Figure 4.2c). In the *B. cinerea*-inoculated plants, fewer fruit were obtained from the 1.0 ppm B treatment than the other boron treatments reflecting the greater percentage of flower abortion.

As for 'Aromas', there was no difference in percentage of dead flowers between uninoculated controls and *B. cinerea*-inoculated plants of 'Selva' regardless of boron treatment (Figure 4.3a). However, fewer flowers died in the 1.0 ppm B treatment than in the other boron treatments, particularly, those in the uninoculated control plants. Preharvest boron application reduced the percentage of flowers that aborted among the water-inoculated control plants. Inoculation with *B. cinerea* caused more flowers to abort in the 0.25 and 1.0 ppm B treatments than in the water-inoculated control.

As a consequence of less flower abortion, greater fruit yield was obtained in the 0 and 0.5 ppm B treatments than in the 0.25 and 1.0 ppm B treatments in the inoculated plants (Figure 4.3c).

There was no grey mould on fruit at harvest or during storage at 10^{o}C and 90±5% RH after 7 days, for both 'Aromas' and 'Selva', whether the flowers had been inoculated with *B. cinerea* or not (data not shown).

(a)

(b)

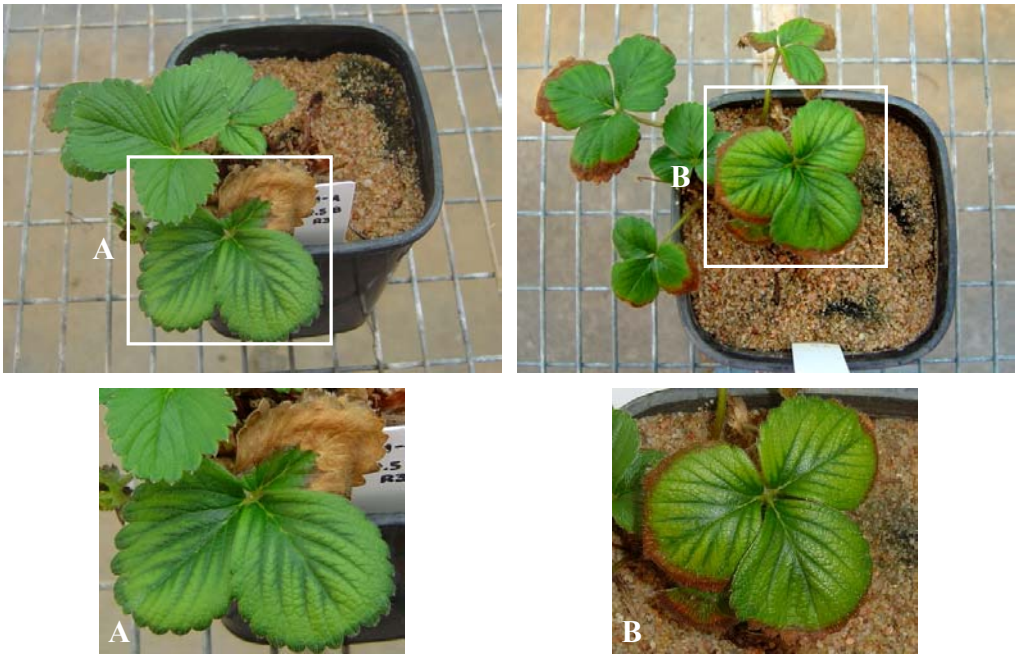


Figure 4.1 Appearance of plants grown in soil and fertigated with various concentrations of boron: (a) marginal scorch on 'Selva' leaves after applying nutrient solutions for 4 weeks; (b) yellowing with green-veining and marginal greening (inset A) and marginal burn (inset B) on 'Aromas' leaves after applying nutrient solutions for 6 months.

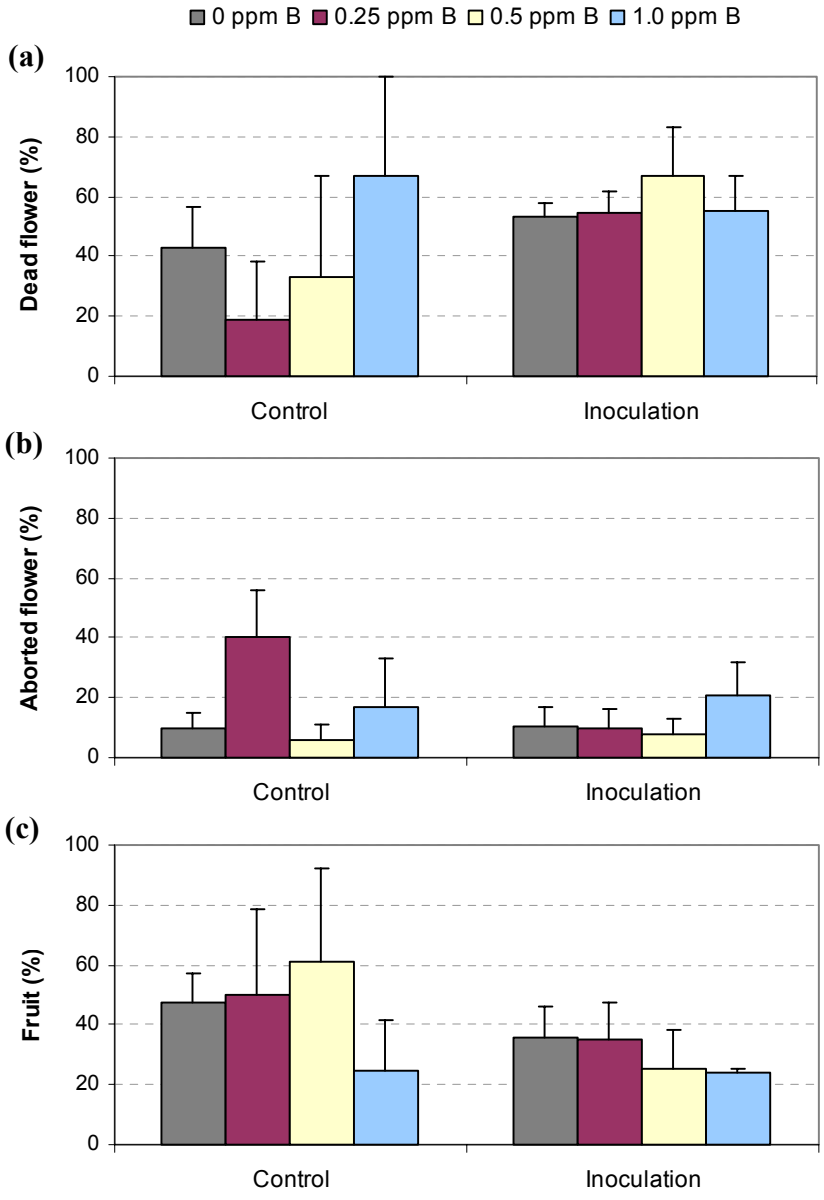


Figure 4.2 Effect of preharvest boron application and inoculation with *Botrytis cinerea* on the number of 'Aromas' flowers that died (a), aborted (b) and developed into fruit (c). Data shown are non-transformed means ± SE from n = 22, 10, 11 and 8 for 0, 0.25, 0.5 and 1.0 ppm B treatments in water-inoculated controls and n = 28, 30, 22 and 29 for 0, 0.25, 0.5 and 1.0 ppm B treatments in inoculation trials.

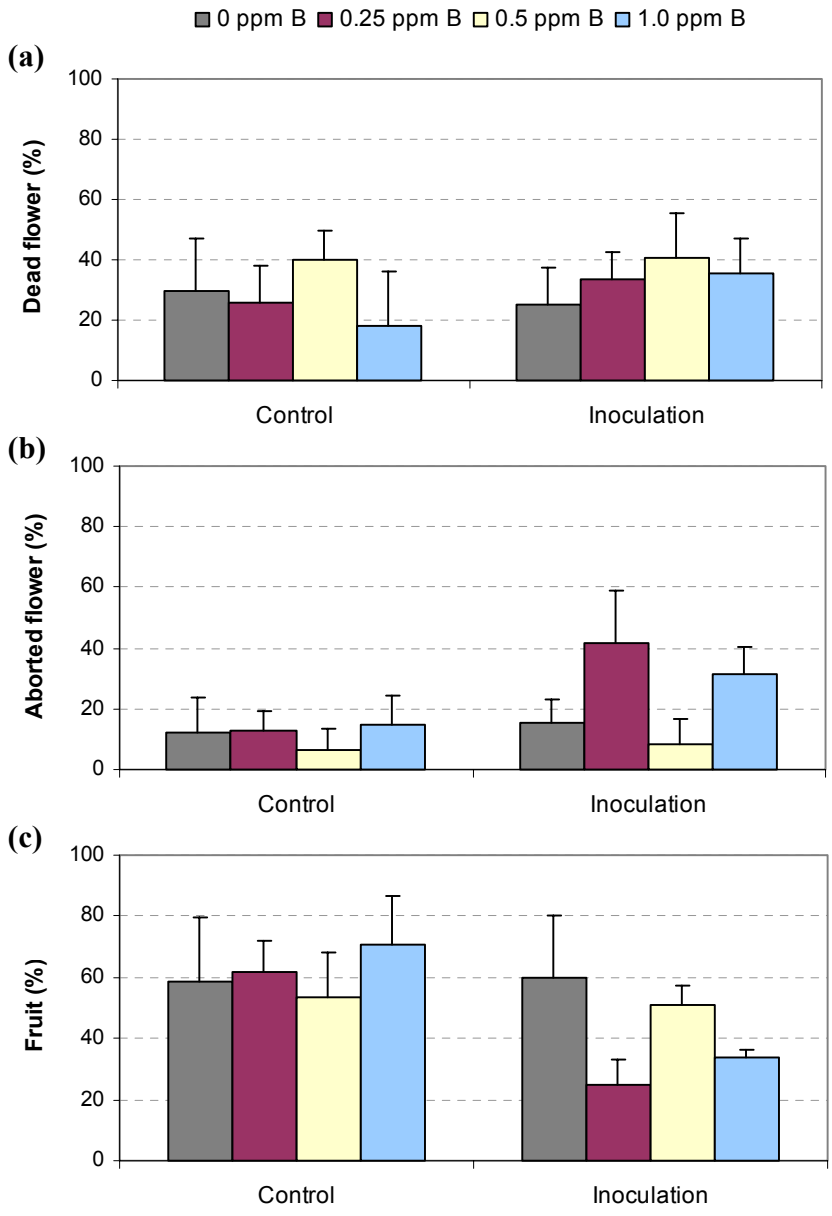


Figure 4.3 Effect of preharvest boron application and inoculation with *Botrytis cinerea* on the number of 'Selva' flowers that died (a), aborted (b) and developed into fruit (c). Data shown are non-transformed means ± SE from n = 20, 21, 19 and 28 for 0, 0.25, 0.5 and 1.0 ppm B treatments in water-inoculated controls and n = 38, 24, 23 and 29 for 0, 0.25, 0.5 and 1.0 ppm B treatments in inoculation trials.

4.3.2 Effect on fruit firmness

Preharvest boron application did not increase fruit firmness of either 'Aromas' or 'Selva' (Figure 4.4). In 'Aromas', fruit from untreated plants were generally firmer than those from boron-treated plants, especially at harvest where fruit firmness in the 0 ppm B was significantly greater ($P < 0.05$) than that in the 0.25, 0.5 and 1.0 ppm B treatments. There was no difference in fruit firmness between treatments where boron was added. However, fruit in the boron treatments was slightly firmer by the end of storage at 10 days than at harvest, whereas the firmness in the 0 ppm B treatment significantly decreased ($P < 0.05$) in this time.

Firmness of 'Selva' fruit did not differ between treatments and did not change over the first 8 days of storage. On day 10, however, fruit firmness in all treatments slightly increased, except in the 1.0 ppm B treatment in which the fruit was significantly less firm ($P < 0.05$) than at the earlier times.

4.3.3 Effect on postharvest quality

4.3.3.1 External appearance and shelf life

Preharvest application of boron did not influence the occurrence of defects in external appearance in terms of shrivel, loss of shine and calyx browning at harvest in either 'Aromas' (Table 4.2) or 'Selva' (Table 4.3). For both cultivars, the degree of all defects generally increased with time of storage. However, by the end of storage (10 days), 'Aromas' fruit from plants that received boron were less shrivelled and more shiny than those from plants that did not receive boron. In addition, the occurrence of calyx browning on day 10 in both cultivars was slightly greater in the 1.0 ppm B treatment than in the other boron treatments.

When considering the quality, as determined by rating general appearance (GA), fruit at harvest did not differ between the boron treatments and significantly decreased ($P < 0.05$) with time of storage for both cultivars (Figure 4.5). During storage for 10 days, the GA of 'Aromas' fruit was not different between the boron treatments,

except on day 4 in that the GA of fruit in the 0.5 ppm B treatment was significantly higher ($P < 0.05$) than that in the 1.0 ppm B treatment. However, by day 10 of storage, all fruit samples in any treatment were below the acceptable marketability point of GA = 5.5. For 'Selva', fruit harvested from plants that received boron rated higher in term of GA than those harvested from plants that received no boron, especially on day 8 and 10. In addition, after 10 days of storage, the GA of fruit in the 0.25 and 1.0 ppm B treatments (GA = 5.57 and 5.72, respectively) was still above the acceptable marketability point.

The data obtained from the GA ratings for fruit stored at 10^{o}C, 90±5% RH for 10 days were subsequently used to calculate the expected shelf life of fruit for each boron treatment (Figure 4.6). In both cultivars, fruit harvested from 0.25 and 1.0 ppm B treatments had a longer shelf life than fruit harvested from 0 and 0.5 ppm B treatments; however, there was a significant difference ($P < 0.05$) only in 'Selva'.

4.3.3.2 Soluble solids content

Initial SSC of 'Aromas' in the 0.25 ppm B treatment was significantly higher ($P < 0.05$) than that in the other treatments (Figure 4.7a). For fruit from plants treated with 0, 0.5 and 1.0 ppm B, the SSC tended to decline during the first 8 days of storage but then increased on day 10. However, the SSC of fruit from the 0.25 ppm B treatment fluctuated during storage such that SSC was low on day 4, high on day 8 and then low again on day 10.

For 'Selva' (Figure 4.8a), the SSC at harvest in the 0 ppm B treatment was significantly higher ($P < 0.05$) than that in the boron treatments and significantly decreased ($P < 0.05$) during storage for 8 days. In comparing boron treatments, the SSC in the 0.25 ppm B treatment was significantly higher ($P < 0.05$) than that in 0.5 and 1.0 ppm B treatments at harvest. However, the SSC in the 0.25 ppm B treatment was significantly lower ($P < 0.05$) at the end of storage for 10 days than the SSC at harvest.

There was no difference in SSC between the two cultivars, as all SSC values obtained for 'Aromas' and 'Selva' were in the same range.

4.3.3.3 pH

pH of 'Aromas' fruit increased with the time of storage and was significantly higher ($P < 0.05$) on day 10 than at harvest in all treatments (Figure 4.7b). The pH on day 10 did not differ between the treatments. However, during the first 8 days of storage, the pH in the 0 ppm B treatment was lower than that in other boron treatments although the difference was not always significant. For 'Selva', the pH at harvest differed slightly among treatments then sharply increased after 4 days of storage and decreased until day 10, except for the 0.25 and 1.0 ppm B treatments where the pH increased to the same value as on day 4 (Figure 4.8b). However, it appeared that fruit of 'Selva' (pH 3.15 to 3.35) was generally more alkaline than 'Aromas' (pH 3.05 to 3.20).

4.3.3.4 Titratable acidity

TA at harvest was significantly higher ($P < 0.05$) in the 0 ppm B treatments than the other boron treatments in both 'Aromas' (Figure 4.7c) and 'Selva' (Figure 4.8c). However, TA decreased slightly by the end of storage for 10 days regardless of boron treatment and cultivar for both cultivars. When comparing the boron treatments, the following trend of TA changes during storage was observed. In the 0 ppm B treatment, TA of 'Aromas' significantly decreased ($P < 0.05$) and of 'Selva' gradually decreased over 10 days of storage. However, TA of both cultivars in different boron concentration treatments fluctuated slightly, appearing to slightly decrease at day 4 then increase on day 8 to levels equal to or above TA seen at harvest and decrease on day 10. TA of 'Selva' was generally much less than of 'Aromas'.

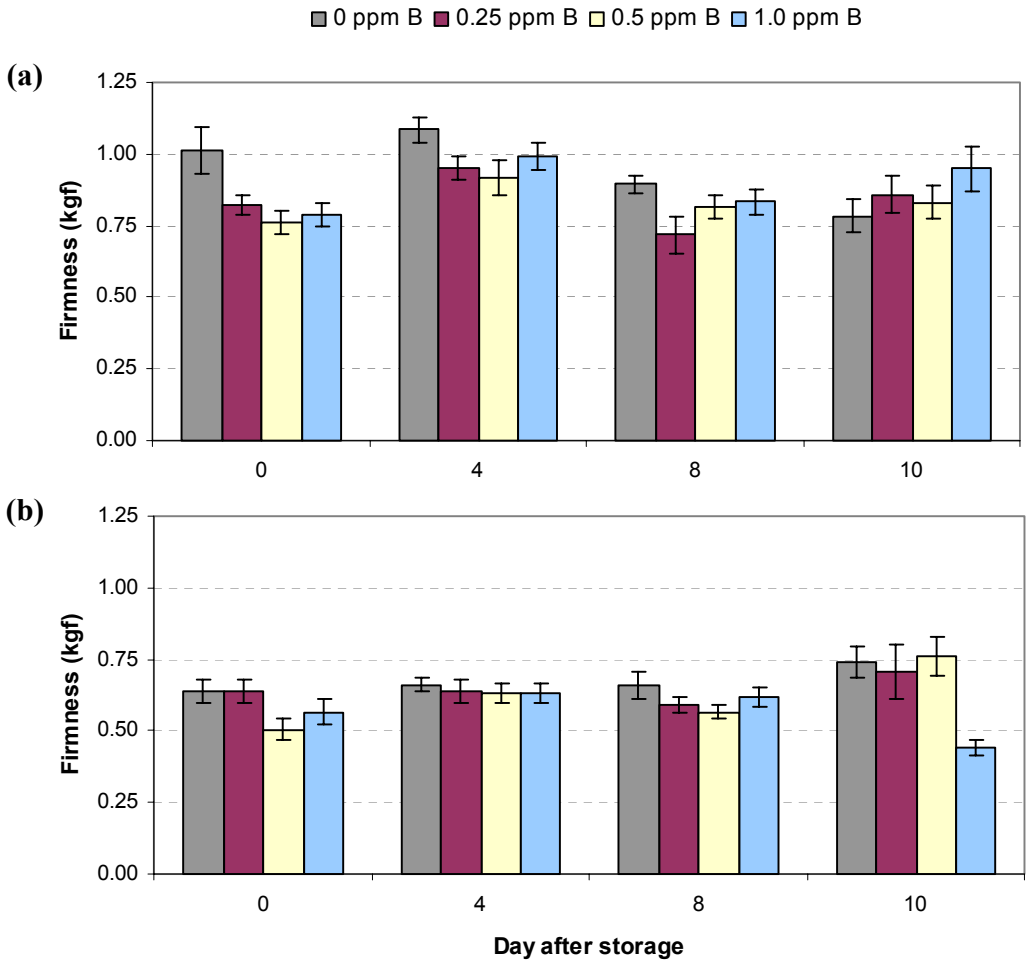


Figure 4.4 Effect of preharvest boron application on fruit firmness of 'Aromas' (a) and 'Selva' (b) during storage at 10°C, 90±5% RH for 10 days. Data shown are means ± SE from n = 30.

Table 4.2 Effect of preharvest boron application on external appearance of 'Aromas' during storage at 10°C, 90±5% RH for 0 to 10 days. Defect score: 0, no defects; 1, slight; 2, moderate; 3, severe and 4, extreme defects.

Days	Boron treatment (ppm B)	Shrivel	Loss of shine	Calyx browning
0	0	0.4	0.3	0.0
	0.25	0.1	0.1	0.0
	0.5	0.2	0.2	0.0
	1.0	0.2	0.1	0.0
4	0	0.9	0.8	0.7
	0.25	0.8	0.7	0.4
	0.5	1.1	1.0	0.4
	1.0	1.0	1.0	0.6
8	0	1.5	1.2	0.8
	0.25	1.8	1.9	1.7
	0.5	1.8	1.6	1.2
	1.0	1.5	1.5	1.1
10	0	1.6	1.7	1.7
	0.25	1.0	1.0	1.4
	0.5	1.3	1.2	1.2
	1.0	1.4	1.4	1.8
	LSD (5%)	0.4	0.4	0.3

Data shown are means from $n = 30$.

Table 4.3 Effect of preharvest boron application on external appearance of 'Selva' during storage at 10°C, 90±5% RH for 0 to 10 days. Defect score: 0, no defects; 1, slight; 2, moderate; 3, severe and 4, extreme defects.

Days	Boron treatment (ppm B)	Shrivel	Loss of shine	Calyx browning
0	0	0.2	0.3	0.0
	0.25	0.2	0.2	0.0
	0.5	0.3	0.2	0.0
	1.0	0.2	0.1	0.0
4	0	1.1	1.1	0.4
	0.25	1.1	1.0	0.4
	0.5	0.6	0.7	0.9
	1.0	1.0	0.9	0.6
8	0	1.1	1.3	1.3
	0.25	1.1	0.9	0.8
	0.5	1.1	1.1	0.8
	1.0	1.1	0.9	0.7
10	0	1.5	1.4	1.7
	0.25	1.3	1.4	1.6
	0.5	1.7	1.6	1.9
	1.0	1.7	1.6	2.1
	LSD (5%)	0.4	0.4	0.3

Data shown are means from $n = 30$.

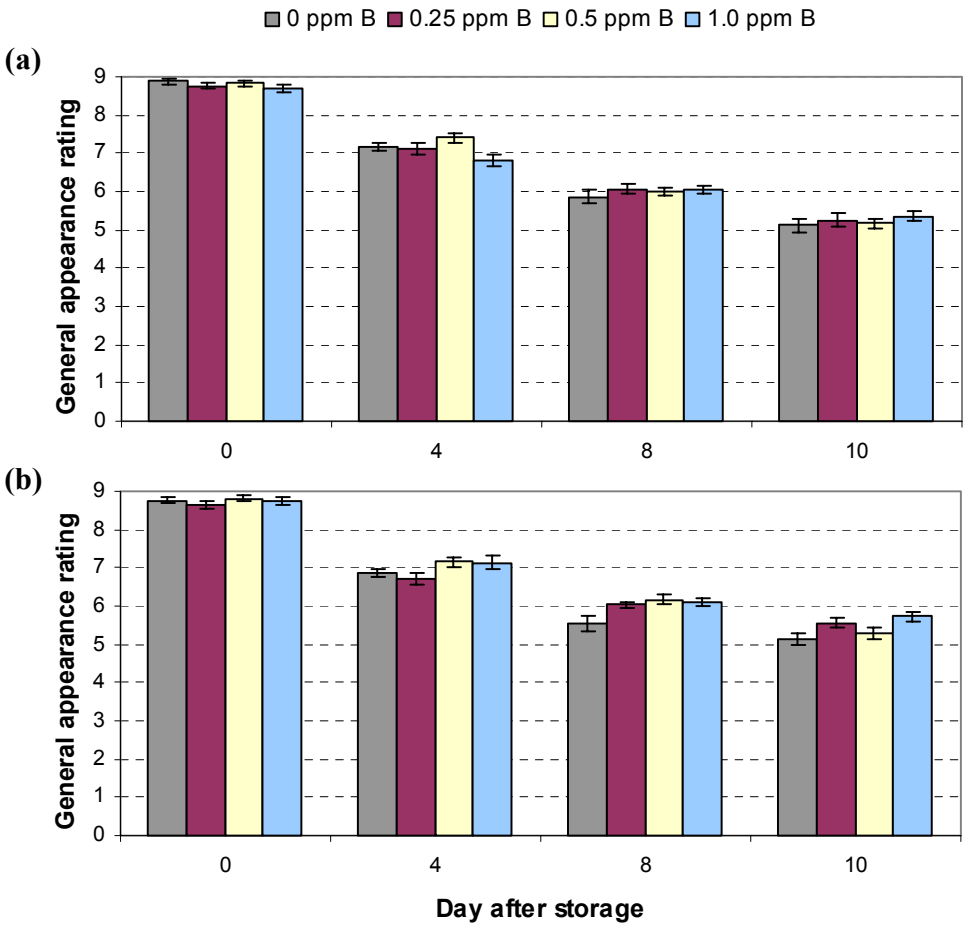


Figure 4.5 Effect of preharvest boron application on general appearance of fruit from 'Aromas' (a) and 'Selva' (b) at harvest then after 4, 8 and 10 days of storage at 10^{o}C, 90±5% RH. Data shown are means ± SE from n = 30.

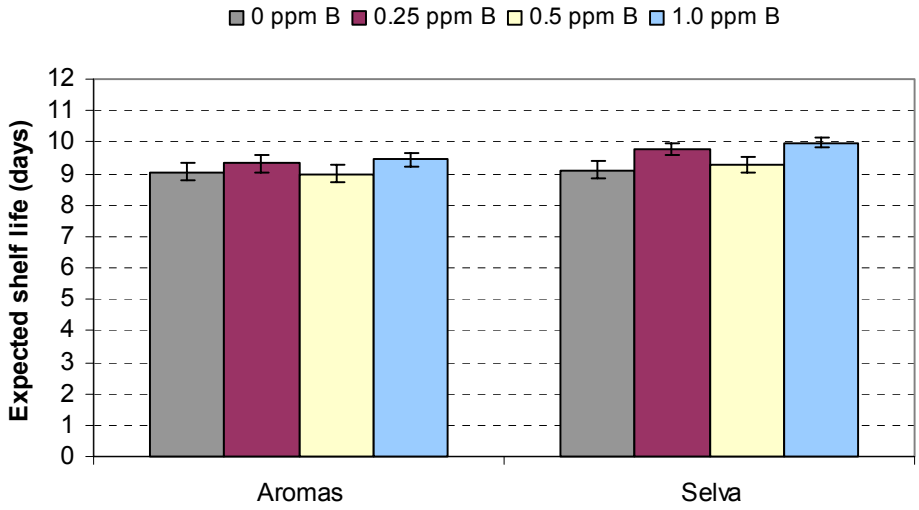


Figure 4.6 Effect of preharvest boron application on the expected duration of shelf life for 'Aromas' and 'Selva'. Shelf life was predicted from general appearance rating for fruit stored at 10°C, 90±5% RH for 10 days. Data shown are means ± SE from *n* = 30.

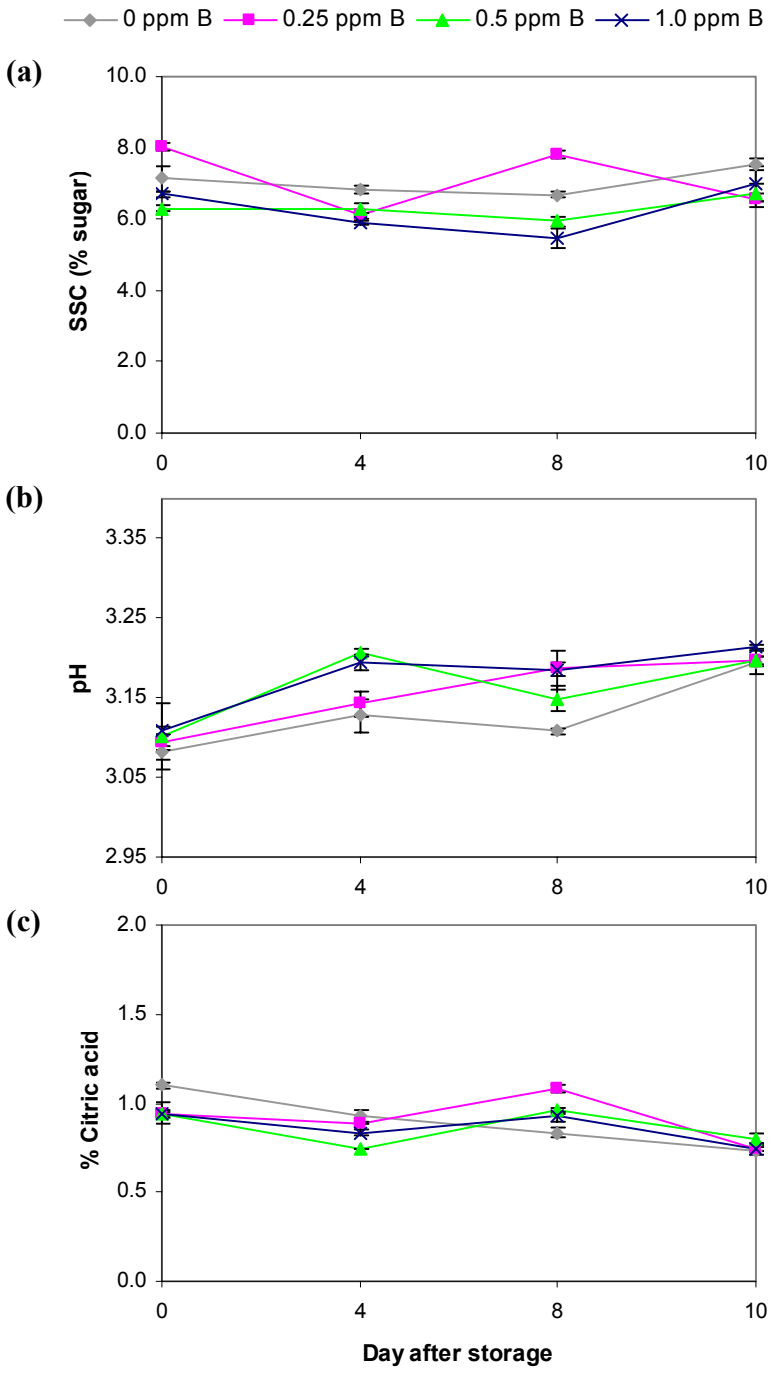


Figure 4.7 Effect of preharvest boron application on soluble solids content (a), pH (b) and titratable acidity (c) of 'Aromas' during storage at 10°C, 90±5% RH for 0 to 10 days. Data shown are means ± SE from $n = 3$.

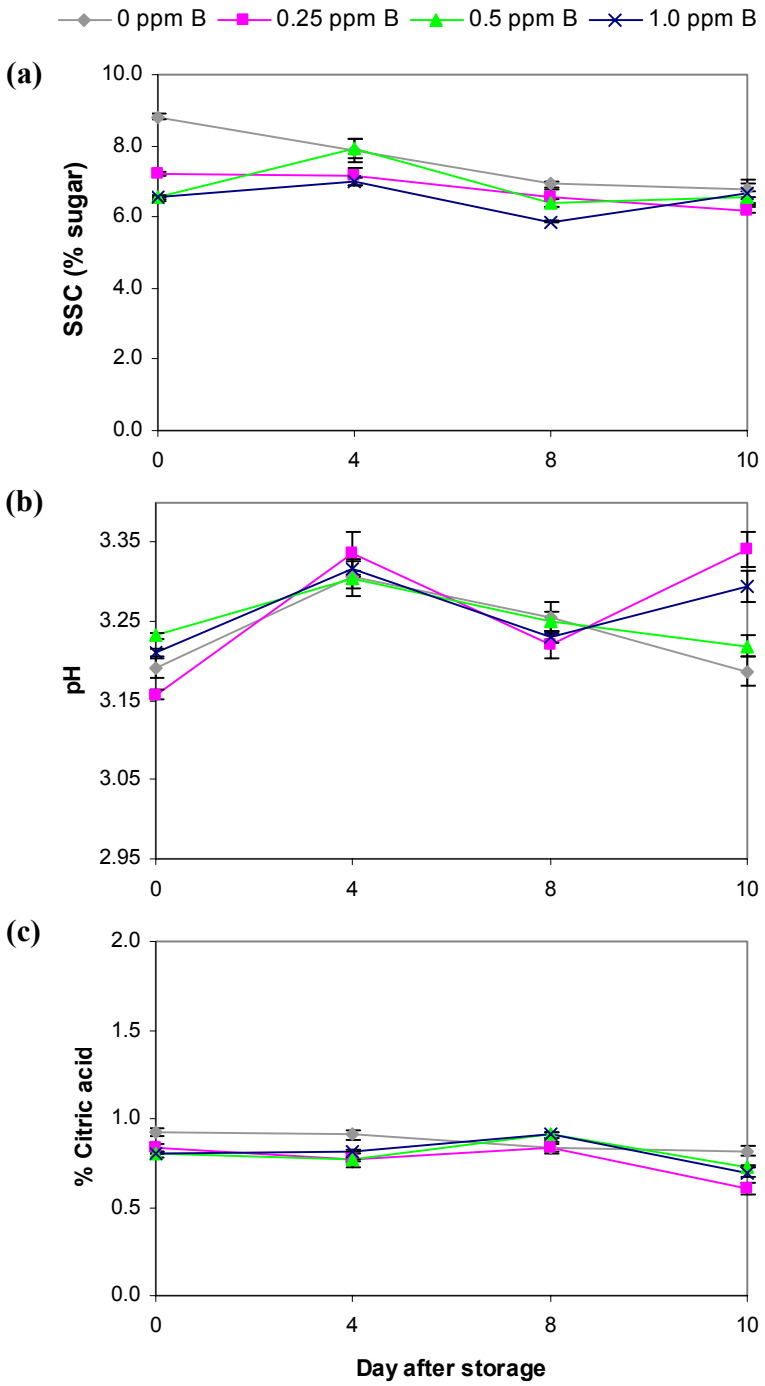


Figure 4.8 Effect of preharvest boron application on soluble solids content (a), pH (b) and titratable acidity (c) of 'Selva' during storage at 10°C, 90±5% RH for 0 to 10 days. Data shown are means ± SE from n = 3.

4.3.4 Effect on boron content in leaf tissues

Due to yield reduction during growth, there was not enough fruit to sample for nutrient analysis. However, leaf tissue analysis is the usual means for assessing plant nutrition to determine if each element is being supplied at a sufficient level. Thus the results obtained in this study were used to determine if each element was being supplied at a sufficient level and also to examine the interaction between boron and other elements.

The replicated raw data obtained from the nutrient analysis were used to calculate the mean and standard error values for 18 nutrients present in leaf tissues obtained from the cultivars 'Aromas' (Table 4.4) and 'Selva' (Table 4.5) that received four boron treatments during growth. Nutrient content determined in this study was compared with the nutrient levels for strawberry leaf tissues recommended by Reuter and Robinson (1997) (see Appendix 3).

It was determined that boron was present at sufficient levels in both 'Aromas' and 'Selva' in all four treatments, including the 0 ppm B treatment. Additionally, leaf boron content significantly increased ($P < 0.05$) with increasing boron concentration in the nutrient solutions applied into soil. With increased boron application, the concentrations of manganese, calcium and aluminium decreased in 'Aromas' leaf tissues although differences were not always significant. In particular, manganese content of 'Aromas' across the four boron treatments was less than a critical deficiency level whereas 'Selva' leaf manganese content was in the marginal range.

For both cultivars, the decreased leaf calcium concentrations with increased boron concentrations were in the marginal range. Similarly, the concentrations of potassium and phosphorus were also in the marginal range whereas the concentrations of copper, magnesium, sodium and sulphur in leaf tissues obtained from all treatments were adequate. Zinc concentration was also adequate, except for 'Selva' leaf tissues obtained from the 0 and 0.5 ppm B treatment in which leaf zinc content was slightly

lower than the deficient level. It appeared that iron concentration in leaf tissues of both ‘Aromas’ and ‘Selva’ in all treatments was deficient.

Table 4.4 Effect of preharvest boron application on foliar concentration of nutrients in 'Aromas'. The nutrient concentrations were determined using ICP-OES analysis. Leaf tissues were collected from plants that received 0, 0.25, 0.5 and 1.0 ppm B in 0.25 Hoagland's solution (400 mL per week) for 12 months. Data are presented as means ± SE from $n = 3$.

Nutrient (mg/kg)	'Aromas' leaf tissues			
	0 ppm B	0.25 ppm B	0.5 ppm B	1.0 ppm B
Iron	39.29 ± 1.92	40.26 ± 1.50	43.05 ± 1.79	41.46 ± 1.91
Manganese	25.62 ± 1.34	20.42 ± 1.29	17.18 ± 1.45	16.87 ± 2.29
Boron	28.47 ± 1.23	42.08 ± 0.78	50.95 ± 0.80	69.00 ± 2.84
Copper	9.34 ± 0.81	9.02 ± 0.30	9.77 ± 0.46	9.54 ± 0.66
Molybdenum	< 1.00	< 1.00	< 1.00	< 1.00
Cobalt	< 1.00	< 1.00	< 1.00	< 1.00
Nickel	< 1.00	< 1.00	< 1.00	< 1.00
Zinc	21.47 ± 2.12	21.17 ± 0.48	23.62 ± 0.84	22.65 ± 1.32
Calcium	10866.67 ± 417.67	10200.00 ± 550.76	8800.00 ± 550.76	9066.67 ± 536.45
Magnesium	5600.00 ± 57.74	5400.00 ± 115.47	5066.67 ± 260.34	5100.00 ± 300.00
Sodium	860.00 ± 77.67	583.33 ± 57.83	643.33 ± 141.46	453.33 ± 105.25
Potassium	17233.33 ± 768.84	19266.67 ± 433.33	18900.00 ± 251.66	18366.67 ± 491.03
Phosphorus	3333.33 ± 233.33	3433.33 ± 166.67	3866.67 ± 440.96	3633.33 ± 133.33
Sulphur	1476.67 ± 28.48	1573.33 ± 49.10	1606.67 ± 70.55	1560.00 ± 20.82
Aluminium	6.07 ± 1.20	3.76 ± 0.51	3.28 ± 0.70	2.95 ± 0.97
Cadmium	< 0.30	< 0.30	< 0.30	< 0.30
Lead	< 3.00	< 3.00	< 3.00	< 3.00
Selenium	< 10.00	< 10.00	< 10.00	< 10.00

= deficient range according to Reuter and Robinson (1997).
= marginal range according to Reuter and Robinson (1997).

Table 4.5 Effect of preharvest boron application on foliar concentration of nutrients in 'Selva'. The nutrient concentrations were determined using ICP-OES analysis. Leaf tissues were collected from plants that received 0, 0.25, 0.5 and 1.0 ppm B in 0.25 Hoagland's solution (400 mL per week) for 12 months. Data are presented as means ± SE from $n = 3$.

Nutrient (mg/kg)	'Selva' leaf tissues			
	0 ppm B	0.25 ppm B	0.5 ppm B	1.0 ppm B
Iron	37.26 ± 1.13	43.51 ± 3.32	39.07 ± 0.70	41.76 ± 0.91
Manganese	31.13 ± 7.24	32.98 ± 2.76	32.32 ± 1.46	26.09 ± 0.52
Boron	26.53 ± 0.64	54.42 ± 1.37	61.60 ± 4.05	79.04 ± 4.81
Copper	9.94 ± 0.30	9.65 ± 0.32	8.70 ± 0.85	9.58 ± 0.32
Molybdenum	< 1.00	< 1.00	< 1.00	< 1.00
Cobalt	< 1.00	< 1.00	< 1.00	< 1.00
Nickel	< 1.00	< 1.00	< 1.00	< 1.00
Zinc	19.35 ± 0.77	20.21 ± 0.79	18.11 ± 0.61	20.14 ± 0.72
Calcium	10133.33 ± 517.47	11366.67 ± 417.67	11333.33 ± 145.30	10566.67 ± 66.67
Magnesium	4566.67 ± 133.33	4900.00 ± 200.00	4800.00 ± 173.21	4500.00 ± 152.75
Sodium	406.67 ± 104.93	356.67 ± 43.72	403.33 ± 74.24	483.33 ± 258.74
Potassium	16633.33 ± 635.96	18300.00 ± 461.88	17633.33 ± 990.51	17600.00 ± 450.92
Phosphorus	3066.67 ± 120.19	3400.00 ± 115.47	2933.33 ± 202.76	2966.67 ± 88.19
Sulphur	1493.33 ± 34.80	1613.33 ± 29.63	1540.00 ± 55.68	1526.67 ± 34.80
Aluminium	3.10 ± 1.39	3.58 ± 0.43	4.24 ± 1.21	2.20 ± 0.54
Cadmium	< 0.30	< 0.30	< 0.20	< 0.30
Lead	< 3.00	< 3.00	< 3.00	< 3.00
Selenium	< 10.00	< 10.00	< 10.00	< 10.00

= deficient range according to Reuter and Robinson (1997).
= marginal range according to Reuter and Robinson (1997).

4.4 Discussion

In this present study, yield of fruit decreased over time. Initially, this was assumed to be due to the infestation by larvae of the family Pyralidae which fed on the crowns, affecting plant growth and flowering. However, yield reduction continued in the subsequent experiments, using new plants which were set up in a different glasshouse to avoid reinfestation. Relatively few flowers formed in this present study which was assumed to be the cause of yield reduction, as suggested by Neilson and Eaton (1983). Furthermore, few flowers developed into fruit and most of these died or aborted.

Death or abortion of flowers in the present study was assumed to be due to treatment with boron and inoculation with *B. cinerea*. Artificial inoculation with *B. cinerea* may cause the flowers to die if the infection is aggressive, as occurs in natural infection (Jarvis and Borecka, 1968). However, the average percentage of 'Aromas' flowers that died was higher in the inoculated plants (54.2%) than in the uninoculated control plants (38.4%) (data were pooled from all boron treatments), whereas in 'Selva', there was no difference between inoculated and uninoculated plants in terms of the average percentage of flowers that died. Boron application seemed to have no consistent effect on the number of flowers that died, as indicated by the lack of difference in the percentage of flowers that died between boron and no boron treatments, whether the flowers were inoculated with *B. cinerea* or not and for either cultivar.

It is known that boron deficiency reduces pollen germination and pollen tube growth which consequently results in yield reduction (Wójcik and Lewandowski, 2003) (see Section 1.5). In this present study, however, the concentration of boron in leaves from the 0 ppm B treatment (28.47±1.23 and 26.53±0.64 for 'Aromas' and 'Selva', respectively) was within the adequate range (25 to 50 mg per kg) according to the nutrient levels for strawberry leaf tissues recommended by Reuter and Robinson (1997). It is, therefore, likely that the lack of fruit in the 0 ppm B treatment was not

due to shortage of boron in the plants. It is possible that the amount of boron was insufficient for optimum growth of the strawberry cultivars 'Aromas' and 'Selva' and consequently affected fruit yield. In addition, the amount of this micronutrient element may have been at a deficient level but not less than a critical concentration at which the deficiency symptoms appear. Different strawberry cultivars require different amounts of boron for growth and fruit production. Wójcik and Lewandowski (2003) indicated in their review that maximum yield of 'Redcoat' strawberries corresponded with a leaf boron level of 58 to 116 mg per kg whereas for 'Midway', leaf boron content of 20 to 30 mg per kg permitted maximum fruit yield. Furthermore, boron deficiency symptoms appeared in 'Midway' strawberry when leaf boron concentration was 2 to 5 mg per kg. However, fruit yield can also be influenced by boron toxicity. Boron toxicity resulted in a dramatic reduction of yield of kiwifruit (Sotiropoulos *et al.*, 1999) and of 'Sparkle' strawberry fruit where boron toxicity symptoms on leaves were observed in the 0.5, 1.0 and 5.0 ppm B treatments (Gilbert and Robbins, 1950). In strawberry, soil boron application in some cases caused phytotoxicity because of the small difference between deficient and toxic levels of boron (Wójcik and Lewandowski, 2003). Critical levels may therefore be different for 'Aromas' and 'Selva' and need to be established in future research.

The symptom of leaf scorch (Figure 4.2a) which occurred in the boron treatments was similar to those of salt damage and boron toxicity. Although boric acid is the form of boron that plant roots absorb most efficiently (reviewed by Gupta, 1979), strawberry is sensitive to boric acid (Incitec Pivot Limited, 2003). Excessive boric acid applications to the soil can produce toxic effects in the strawberry (Latimer, 1943), causing leaf tip burn, which further depresses growth and yield (Maas, 1998). In addition, boron sometimes accumulates to toxic levels if excess is applied (Maas, 1998). In the present study, leaf boron content in all treatments where boron was added, except 'Aromas' in the 0.25 ppm B treatment, was higher than the adequate level recommended by Reuter and Robinson (1997). However, the leaf boron concentration that causes boron toxicity has not been reported for strawberry. It was

not possible in the present study to ascertain whether this symptom of phytotoxicity was due to excessive boron, salt damage, a combination of the two or some other factors.

In general, strawberry is sensitive to sodium salts, which often accumulate in the soil, resulting in salt damage (Ulrich *et al.*, 1980; Maas, 1998). Hoagland and Snyder (1933) reported that heavy irrigation applied to strawberry plants grown in beds is conducive to the accumulation of sodium salts and some cultivars are highly sensitive to even moderate concentrations of sodium. In the present study, sodium content in leaves of both 'Aromas' and 'Selva' in the boron treatments was less than the level likely to cause sodium toxicity (0.2% sodium) according to Maas (1998). In comparison with the sodium content in leaves analysed following preharvest calcium application (see Tables 3.9 and 3.11), leaf sodium content across the boron treatments in this experiment was extremely high. This indicates that sodium accumulated in this experiment and it is suggested that the two cultivars 'Aromas' and 'Selva' are relatively sensitive to salt damage, especially 'Aromas' where symptoms were more severe. However, this suggestion needs confirmation through further research.

Even though nutrient analysis suggested that 'Aromas' appeared manganese deficient and 'Selva' appeared zinc deficient, the normal deficiency symptoms were not evident. Leaf iron content was also in the deficient range even though soil analysis indicated high levels of iron in the Waikerie soil which was used in this study (see Appendix 4). Iron may not therefore be taken up by the plant or be available to the plant. Nutrient status in the plant may not always reflect the soil nutrition and, given the cultivar dependency for nutrient uptake, there is a need to further understand these interactions.

In spite of the increased boron content in leaf tissues and the variable shelf life of fruit from treated plants, postharvest quality in terms of firmness, SSC and TA of fruit of either 'Aromas' or 'Selva' was not obviously influenced by boron application.

Likewise, Wójcik and Lewandowski (2003) reported for 'Elsanta' strawberry and Singh *et al.* (2007) for 'Chandler' strawberry that boron sprays increased boron content in leaves and fruit but did not increase fruit yield or improve fruit quality. However, both of these studies revealed that application of a combination of boron and calcium increased fruit firmness, SSC and TA and decreased the incidence of grey mould. The authors proposed that boron may enhance the effectiveness of calcium as a binding agent to strengthen cell walls. As a result, fruit may be firmer and consequently less susceptible to grey mould. Dunn (2003) suggested that increasing soil boron concentrations and availability may enhance calcium uptake by strawberry plants as indicated by a significant positive correlation between calcium and boron. In the present study, however, leaf calcium content did not increase with increasing boron concentrations in the nutrient solutions applied to soil. Thus, it would be of interest to conduct experiments involving application of boron plus calcium to 'Aromas' and 'Selva'.

Due to the apparent sensitivity of strawberry to boric acid and a narrow range between deficient and toxic levels, it is important to determine the optimum concentration of boron that would enhance host resistance to grey mould and enhance fruit quality without affecting either plant development or fruit yield. In addition, further study on the application of boron by foliar sprays would also be of interest, given that Wójcik and Lewandowski (2003) reported that boron sprays appeared more successful in increasing fruit yield than application to soil, and sprays would help to avoid any accumulation of salts in the soil due to continuous application.

4.5 Conclusions

Preharvest boron application had no effect on grey mould development. For both 'Aromas' and 'Selva', grey mould did not develop on fruit, whether plants received boron or not, during storage at 10^{o}C, 90±5% RH for 7 days. Inoculation of flowers with *B. cinerea* did not result in flower death. However, fruit yields were reduced across boron treatments, including the no boron treatment. Preharvest boron

application did not influence GA, firmness, SSC or TA of fruit. However, fruit harvested from 0.25 and 1.0 ppm B treatments had a longer shelf life than fruit harvested from 0 and 0.5 ppm B treatments. Boron content in leaf tissues increased with increasing boron concentration in the nutrient solutions. Application of boron to soil may not be a suitable method of adding boron to 'Aromas' and 'Selva' plants, in that there might be an accumulation of boron and other salts in the soil which is toxic to the plants.

Chapter Five
Effect of Postharvest Calcium Treatment

5.1 Introduction

Postharvest calcium treatment is a means of applying calcium directly to a fruit and may be the best method to increase flesh calcium content (Conway *et al.*, 1994). Calcium is applied directly as a solution onto the fruit surface by dipping, pressure infiltration or vacuum infiltration. Vacuum infiltration seems to be the most effective method of increasing calcium content of fruit. However, the soft texture of strawberry prevents its use. Postharvest dipping in various calcium solutions may, therefore, be a suitable non-damaging treatment and could result in efficient calcium translocation to fruit tissues (García *et al.*, 1996).

Postharvest calcium application by dipping fruit in solutions containing calcium salts has been shown to increase fruit calcium content and to increase fruit firmness in storage. Dipping fruit in 1.0% calcium gluconate for 5 min decreased surface damage and delayed both decay due to visible fungal growth and loss of firmness in the strawberry cultivar 'Camarosa' during a storage period of 4 days at 20^{o}C (Hernández-Muñoz *et al.*, 2006). The type of fungal growth was not reported. Immersing the cultivar 'Sequoia' for 5 min in 0.5 and 1.0% calcium chloride solutions increased levels of calcium in fruit and increased postharvest storage life of fruit from 3 (control) to 21 days without any symptoms of infection or any change in external appearance (Bitencourt De Souza *et al.*, 1999). However, the pH, total soluble solids (TSS), total titratable acidity (TA), the ratio of TSS to TA, the soluble and total pectin contents and the ratio between soluble and total pectin did not significantly differ between calcium-treated and control fruit.

Dipping fruit of the cultivar 'Tudla' in 1.0% calcium chloride solution for 15 min was effective in increasing the calcium content, in controlling postharvest decay and in

increasing firmness and soluble solids content (García *et al.*, 1996). However, the use of calcium chloride may cause bitterness or changes to the flavour, which result from a residual amount of calcium chloride on the surface of the fruit (Luna-Guzmán and Barrett, 2000).

Calcium lactate represents an alternative calcium source (Luna-Guzmán and Barrett, 2000). Dipping the cultivars 'Cardinal' and 'Sunrise' in a 0.5% calcium lactate solution for 1 min improved fruit firmness and character as indicated by shear press values, reduced drained weight loss, wholeness and texture sensory ratings (Morris *et al.*, 1985).

Based on the apparent beneficial effect of postharvest calcium treatment with regards to increased shelf life and yield due to prevention of fungal decay in strawberry and the lack of research focusing on *B. cinerea* in strawberry, the research described in this chapter focused on the effect of postharvest calcium application on the development of grey mould caused by *B. cinerea* on the cultivar 'Selva'.

5.2 Materials and methods

5.2.1 Fruit materials

Fruit from the strawberry cultivar 'Selva' was harvested in the early morning from the farm (see Section 2.2). The fruit were hand-picked and harvested fully ripe. These fruit were packed in plastic containers gently and transported to the Waite Campus within 1 h after packing. After storage overnight at 4^{o}C, fruit were sorted to remove defective fruit. Individual fruit weighing 10 to 20 g were selected and randomly allocated to treatments prior to experimentation.

5.2.2 Postharvest treatments

Regardless of type of calcium solution, three concentrations (1500, 3000 and 4500 ppm Ca) were used to treat fruit. Sterile nanopure water (SNW) was used to treat the controls. All solutions contained three drops of Tween 20 (Amresco®, Ohio) per L. The general methodology for postharvest treatment with calcium solutions is summarised in Figure 5.1. Briefly, strawberry fruit were disinfested by dipping in 2% sodium hypochlorite for 1 min and allowed to dry in a laminar flow. Surface-sterilised fruit were then dipped for 5 min in either calcium solution at the concentrations mentioned before or in SNW, placed on sterile heavy-duty towel and dried in a laminar flow for 10 min prior to inoculation with *B. cinerea*.

5.2.3 Inoculation of fruit

A conidia suspension of *B. cinerea* was prepared, as previously described in Section 2.6.2, to a concentration of 10^6 conidia per mL. Fruit cheeks were wound-inoculated (approximately 0.05 mL per wound) by puncturing once to a depth of 2 mm with a 0.5-mm diameter needle previously dipped in the *B. cinerea* conidia suspension (El-Kazzaz *et al.*, 1983). Inoculated fruit were then incubated as described below.

5.2.4 Storage conditions

The treated fruit were kept in aluminium foil trays covered with plastic bags and stored at 10°C, 90±5% relative humidity (RH).

5.2.5 Effect of calcium lactate on *B. cinerea* development

To determine if calcium lactate directly affects *B. cinerea*, discs, 4.5 mm in diameter, were cut from the edge of 2-week-old V-8 agar cultures and placed on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI) supplemented with calcium lactate (BDH GPR™, England) at three concentrations: 1500, 3000 and 4500 ppm Ca. PDA without calcium lactate was used as the control. The 9-cm diameter Petri dishes were sealed with Parafilm® and incubated on a bench in the laboratory at room temperature (22 to 24°C). The colony diameter of *B. cinerea* was measured daily as

the average of the horizontal and vertical diameters. There were four replicates per treatment.

In an initial experiment, calcium lactate was prepared to the concentrations required (see Section 5.2.2). Fruit were randomly distributed into four groups of 60. Each group was assigned to one of four treatments, including the control, with three replicates, each consisting of 20 fruit. Fruit were treated with calcium solutions and were then inoculated with *B. cinerea* as described in Sections 5.2.2 and 5.2.3, respectively.

To evaluate the optimum storage duration to allow calcium to enter the fruit, fruit were treated with calcium lactate solutions as explained in Section 5.2.2 and were then stored at 10^{o}C for either 6, 12 or 24 h before inoculation according to the method described in Section 5.2.3. There were 10 single fruit per treatment. The resulting optimum storage duration (24 h) was subsequently used in a larger experiment with four replicates of 10 fruit per treatment. Calcium lactate-treated fruit were kept in the dark at 10^{o}C for 24 h prior to inoculation.

5.2.6 Effect of calcium chloride on *B. cinerea* development

The effect of calcium chloride on the growth of *B. cinerea in vitro* was determined on PDA supplemented with calcium chloride ($CaCl_2$, BDH AnalaR® Merck Pty, Ltd, Kilsyth, Victoria) at three concentrations: 1500, 3000 and 4500 ppm Ca. PDA without calcium chloride was used as the control. The methodology for incubation and measurement of fungal growth was performed as for calcium lactate (see Section 5.2.5). There were five replicates per treatment. The trial was conducted twice.

In a preliminary experiment, fruit were randomly allocated into four groups of 10 and each group was assigned to one of four treatments, including the control. Treatments were arranged in completely randomised design. Fruit were treated with calcium chloride solutions using the methods described in Section 5.2.2. After applying

calcium, treated fruit were stored at 10°C for either 10 min, 12 or 24 h before inoculation using the methods described in Section 5.2.3.

In a subsequent experiment, four replicates of 10 fruit were assigned to one of four treatments. Based on the results obtained in the preliminary experiment, calcium-treated fruit were stored at 10°C in natural light for 24 h before inoculating with *B. cinerea*.

5.2.7 Direct comparison of calcium lactate and calcium chloride in delaying *B. cinerea* development

This experiment was conducted to compare the effect on grey mould development of calcium lactate and calcium chloride concurrently. Calcium lactate and calcium chloride at three concentrations: 1500, 3000 and 4500 ppm Ca were used. After applying calcium and storage for 24 h under natural light at 10°C, calcium-treated fruit in each treatment were divided into two groups. One group was inoculated with *B. cinerea* and the other was mock-inoculated with SNW. Treatments were arragned in completely randomised design, with four replicates of 10 fruit per treatment.

5.2.8 Further evaluation of the most effective treatments from prior experiments

Based on the results from previous experiments, the two most effective treatments (calcium lactate at 3000 ppm Ca and calcium chloride at 4500 ppm Ca) were tested further. Fruit were treated with calcium solutions as described in Section 5.2.2. Fruit treated with SNW were used as the control. The methodology is summarised in Figure 5.2. Prior to inoculation with *B. cinerea*, all treated fruit were divided into two groups: one group was stored in the dark at 10°C and the other was stored in light (white fluorescence tube, 36W Daylight 9J, Crompton, Australia) at 10°C. After storage for 24 h, fruit were inoculated as previously described in Section 5.2.3 and inoculated fruit were stored at 10°C, 90±5% RH and in a cycle of 12 h light (white fluorescence tube, 36W Daylight 9J, Crompton, Australia) and 12 h dark for 7 days.

The light/dark storage periods used in this experiment were designed to imitate common storage conditions used in industry. Treatments were arranged in completely randomised design, with four replicates of 10 fruit per treatment. The trial was conducted using late-season fruit, in May 2006, as well as early-season fruit, in December 2006.

5.2.9 Botrytis fruit rot evaluation

The diameter of rot lesion was measured daily for up to 7 days after inoculation. The plastic bag was removed during measurement but the fruit still remained in the tray; fruit were examined for rot development by measuring the diameter of rot lesions in the inoculated area using calipers (digiMax, Switzerland). The rot lesion diameters were measured horizontally and vertically, and the lesion areas were calculated and recorded in mm^2.

To confirm *B. cinerea* was responsible for lesions where fruit had not been inoculated, the rot tissue was cut into segments of about 5 x 5 mm, then surface-disinfested by dipping in 2% sodium hypochlorite for 1 min and placed on PDA plates. The plates were then incubated on the laboratory bench at ambient temperature (22 to 24^{o}C) and observed daily for growth of *B. cinerea*. Where grey sporulation developed on PDA plates, a small amount of the growth was transferred to a drop of sterile water on a slide and covered with a coverslip before observing by light microscopy. *B. cinerea* was confirmed according to Maas (1998), where a conidiophore with a swollen basal cell, irregularly branched and bearing grape-like clusters of conidia was identified.

5.2.10 Statistical analysis

Data on rot lesion areas and the percentage of rotten fruit were subjected to log transformation and arcsine square root transformation, respectively, before conducting analysis of variance. Means comparisons were performed by the least significant difference (LSD) test at $P < 0.05$.

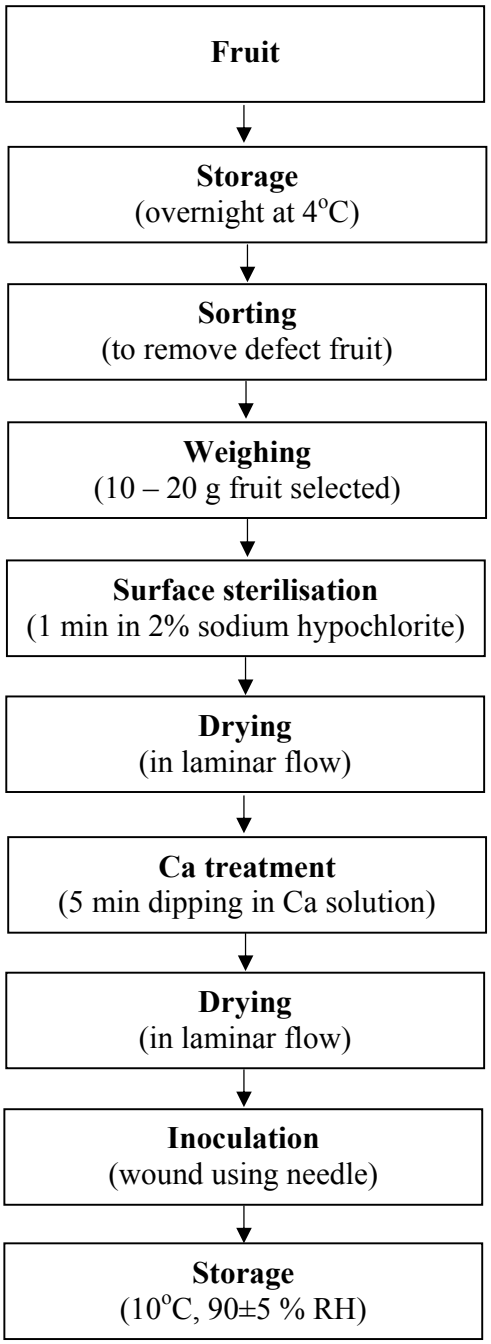


Figure 5.1 Diagram of general methodology of postharvest calcium treatment.

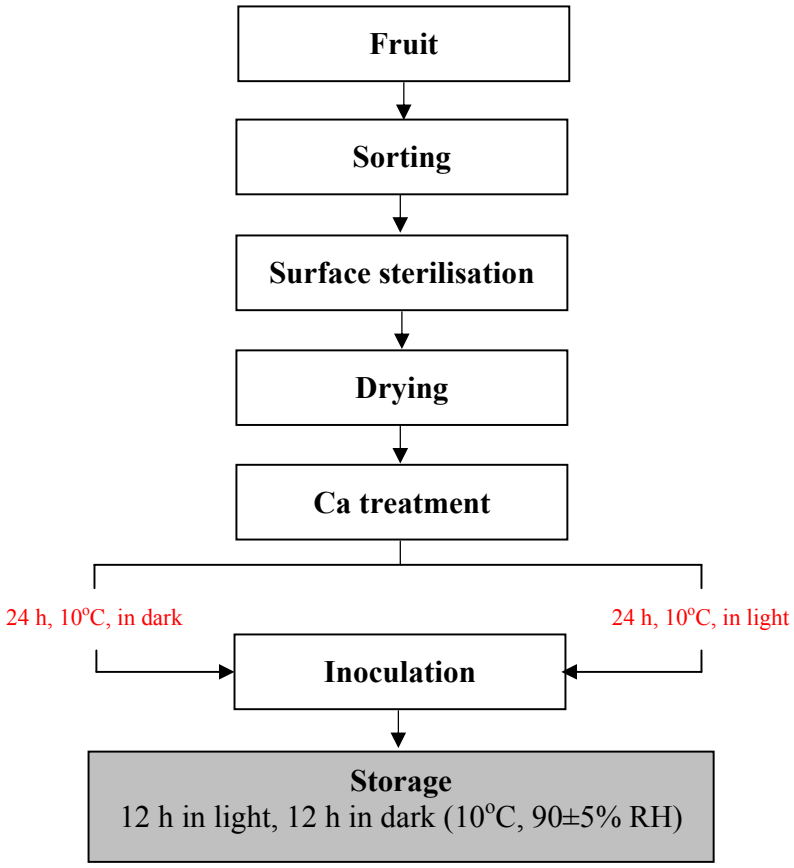


Figure 5.2 Diagram of modified methodology used to further evaluate the most effective treatments: calcium lactate at 3000 ppm Ca and calcium chloride at 4500 ppm Ca as detailed in Section 5.2.8. Prior to inoculation, calcium-treated fruit were divided into two groups: one group was stored in the dark and another was stored in light at 10°C, 90±5% RH for 24 h. After inoculation, inoculated fruit were stored at 10°C, 90±5% RH in the cycle of 12 light and 12 h dark.

5.3 Results

5.3.1 Effect of calcium lactate on *B. cinerea* development *in vitro*

Calcium lactate had no significant effect on growth of *B. cinerea in vitro*. Incorporation of calcium lactate in PDA at 1500, 3000 or 4500 ppm Ca did not significantly affect the colony diameter of *B. cinerea* on PDA (Figure 5.3).

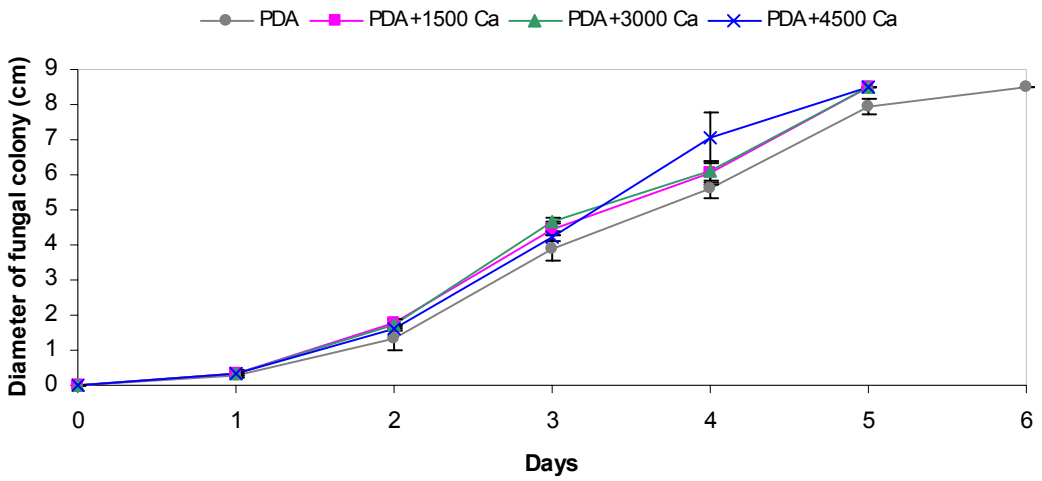


Figure 5.3 Colony diameter of *Botrytis cinerea* on potato dextrose agar amended with calcium lactate at 0, 1500, 3000 and 4500 ppm Ca. The cultures were kept in natural light at ambient temperature (22 to 24°C) and the colony diameters were measured daily for 6 days. Data shown are means ± SE from $n = 4$.

5.3.2 Effect of calcium lactate on *B. cinerea* development in fruit

In the initial trial where calcium-treated fruit were inoculated with *B. cinerea* immediately after drying in the laminar flow, calcium lactate dips did not affect the development of *B. cinerea* on strawberries in comparison with the untreated control during storage for up to 7 days at 10°C (Figure 5.4). Although, the rot lesion area in fruit in the 3000 ppm Ca treatment did not differ from the control especially between

4 and 7 days after inoculation, it was significantly greater ($P < 0.05$) for 1500 and 4500 ppm Ca treatments especially between 5 and 7 days after inoculation (Figure 5.5). However, salt accumulation was observed on the calyx of fruit treated with 4500 ppm Ca (data not shown).

Figure 5.6 shows the effect of calcium lactate treatment and different pre-inoculation storage periods on Botrytis rot lesion area. After inoculation and storage at 10^{o}C for 7 days, the area of rot lesions on the untreated control fruit did not differ among the fruit stored for 6, 12 and 24 h before inoculation. However, dipping fruit in calcium lactate solutions appeared to show some promise in delaying Botrytis rot development following the longest storage period before inoculation. Calcium-treated fruit stored for 24 h after calcium treatment and before inoculation developed slightly smaller rot lesions than those stored for 6 and 12 h. Within the storage period of 24 h between calcium treatment and inoculation, calcium-treated fruit at any concentration of calcium solutions showed smaller rot lesions than the controls, although not statistically significant.

However, in the subsequent trial, when the rot lesion development was followed for calcium-treated fruit stored in the dark for 24 h prior to inoculation with *B. cinerea*, only fruit treated with calcium lactate solution containing 1500 ppm Ca showed significantly smaller ($P < 0.05$) rot lesions than those in the control (Figure 5.7). On the other hand, the rot lesions on fruit treated with either 3000 or 4500 ppm Ca in calcium lactate solutions were significantly greater ($P < 0.05$) than those on the controls from 5 days after inoculation.

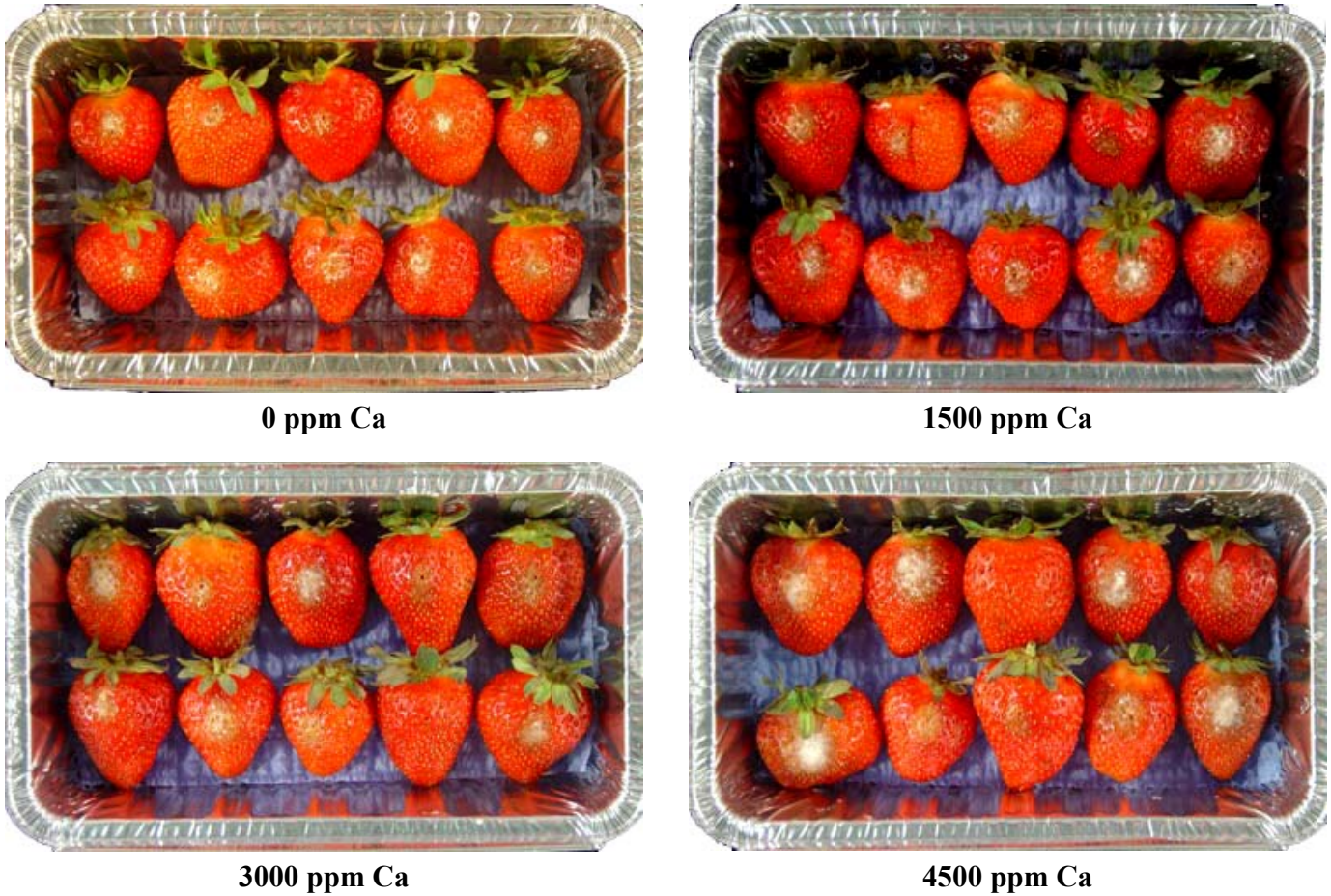


Figure 5.4 Development of rot lesion area on fruit treated with calcium lactate and storage at 10^{o}C, 90±5% RH for 7 days after inoculation with *Botrytis cinerea*.

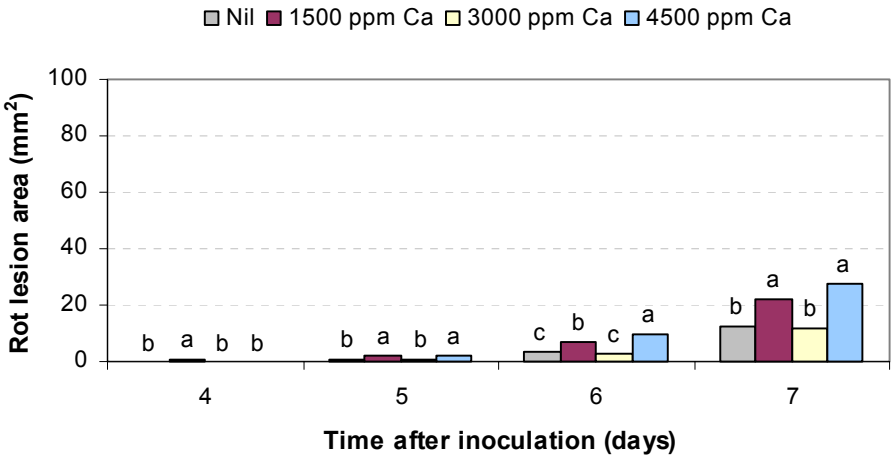


Figure 5.5 Effect of calcium lactate dips on the area of rot lesions after storage at 10°C, 90±5% RH for up to 7 days. Fruit were dipped in calcium lactate solutions at the concentration of 1500, 3000 or 4500 ppm Ca for 5 min and left to dry in a laminar flow for 10 min before inoculation with *Botrytis cinerea*. Fruit treated with sterile nanopure water were used as controls. Data shown are actual means from $n = 60$. For each time after inoculation, the different letters indicate significant differences among treatments according to LSD at $P < 0.05$. The experiment was conducted in March 2005.

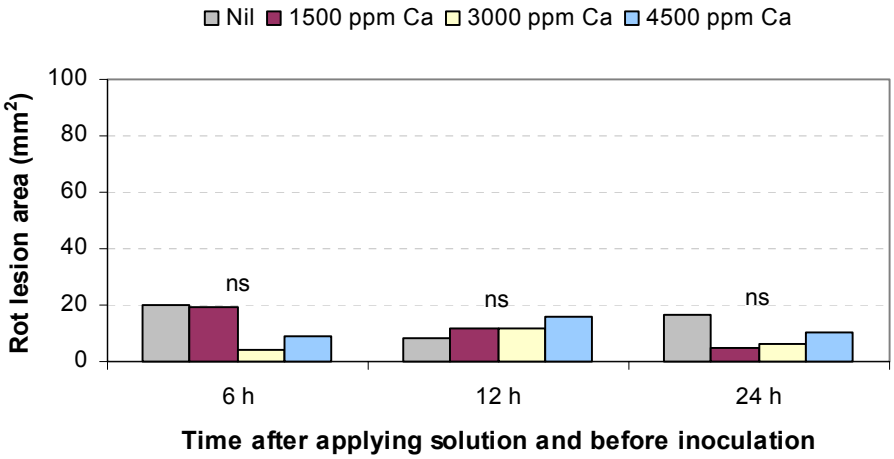


Figure 5.6 Effect of calcium lactate and storage period after calcium treatment prior to inoculation on rot lesion development, 7 days after inoculation. Fruit were dipped in calcium lactate at 1500, 3000 or 4500 ppm Ca for 5 min and stored for 6, 12 and 24 h under natural light at 10°C, 90±5% RH prior to inoculation. Fruit treated with sterile nanopure water were used as controls. Data shown are actual means from $n = 10$. For each time after applying solution and before inoculation, 'ns' indicates no significant differences among treatments according to LSD at $P < 0.05$. The experiment was conducted in November 2005.

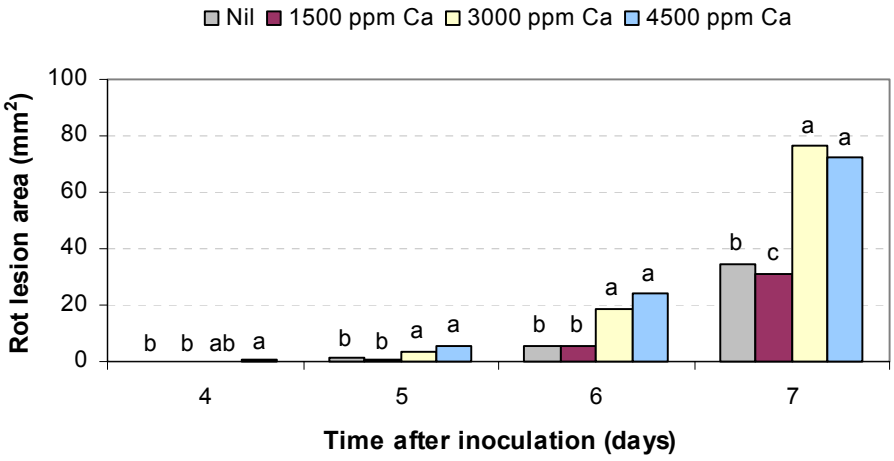


Figure 5.7 Effect of calcium lactate dips on rot lesion development after storage at 10°C, 90±5% RH for up to 7 days. Fruit were dipped in calcium lactate solutions at the concentration of 1500, 3000 or 4500 ppm Ca for 5 min and stored at 10°C in the dark for 24 h before inoculation. Fruit treated with sterile nanopure water were used as controls. Data shown are actual means from n = 30. For each time after inoculation, the different letters indicate significant differences among treatments according to LSD at $P < 0.05$. The experiment was conducted in December 2005.

5.3.3 Effect of calcium chloride on *B. cinerea* development *in vitro*

Calcium chloride had no effect on growth of *B. cinerea in vitro*. Incorporation of calcium chloride in PDA at 1500, 3000 or 4500 ppm Ca did not affect the colony diameter of *B. cinerea* on PDA (Figure 5.8).

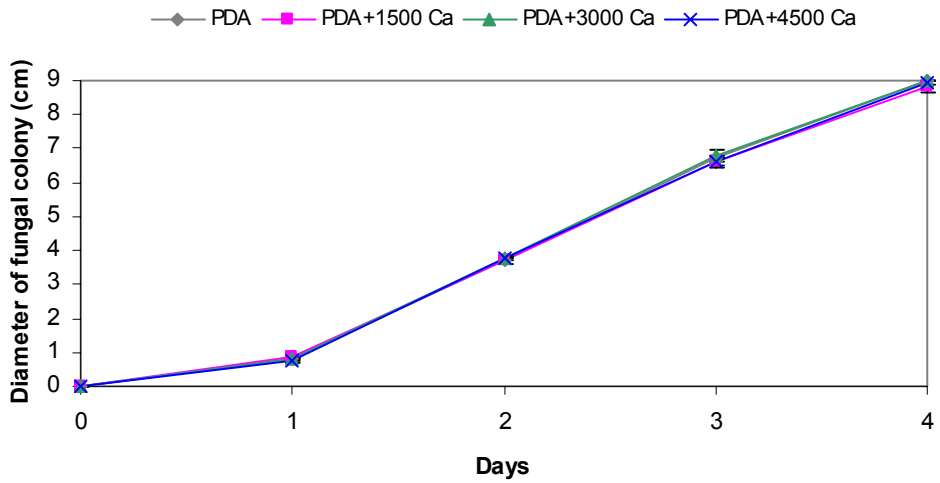


Figure 5.8 Colony diameter of *Botrytis cinerea* on potato dextrose agar amended with calcium chloride at 0, 1500, 3000 and 4500 ppm Ca. The cultures were kept in natural light at ambient temperature (22 to 24°C) and the colony diameter was measured daily for 4 days. Data shown are means ± SE from $n = 5$.

5.3.4 Effect of calcium chloride on *B. cinerea* development

In the preliminary experiment to evaluate the optimum period of pre-inoculation storage after applying calcium chloride solution, the areas of rot lesions on fruit treated with calcium chloride at 3000 and 4500 ppm Ca appeared smaller than those on the controls when the fruit were stored for 24 h after calcium treatment and before inoculation but were insignificant (Figure 5.9). In the subsequent experiment, calcium chloride treatment plus storage for 24 h prior to inoculation with *B. cinerea* had some effect in reducing rot development compared with the control (Figure 5.10). However, only rot lesion area of the 4500 ppm Ca treatment was significantly less ($P < 0.05$) than the control.

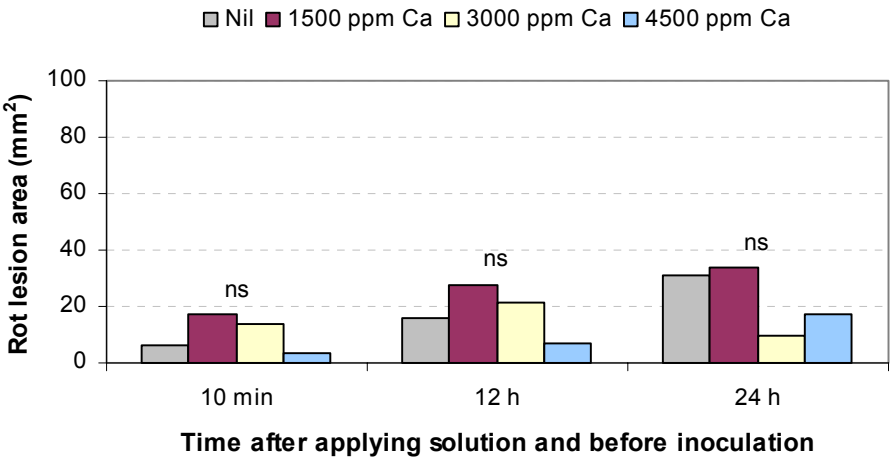


Figure 5.9 Effect of calcium chloride and storage period after calcium treatment prior to inoculation on rot lesion development, 7 days after of inoculation. Fruit were treated with calcium chloride solutions at 1500, 3000 or 4500 ppm Ca for 5 min and stored for 10 min, 12 and 24 h under natural light at 10°C, 90±5% RH prior to inoculation. Fruit treated with sterile nanopure water were used as controls. Data shown are actual means from $n = 10$. For each time after applying solution and before inoculation, 'ns' indicates no significant differences among treatments according to LSD at $P < 0.05$. The experiment was conducted in November 2005.

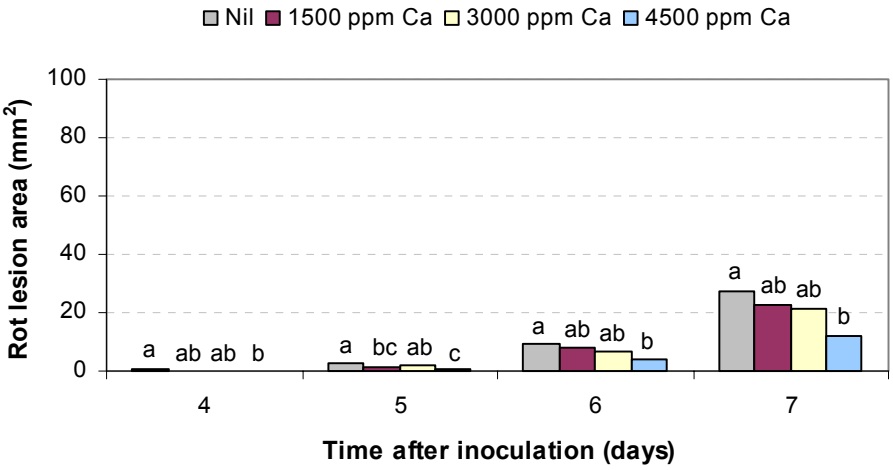


Figure 5.10 Effect of calcium chloride dips on rot lesion development after storage at 10°C for up to 7 days. Fruit were dipped in calcium chloride solutions at the concentration of 1500, 3000 or 4500 ppm Ca for 5 min and stored at 10°C, 90±5% RH under natural light for 24 h before inoculation. Fruit treated with sterile nanopure water were used as controls. Data shown are actual means from $n = 40$. For each time after inoculation, the different letters indicate significant differences among treatments according to LSD at $P < 0.05$. The experiment was conducted in March 2006.

5.3.5 Direct comparison of calcium lactate and calcium chloride in delaying *B. cinerea* development

After inoculation with *B. cinerea* and storage for 7 days at 10^{o}C under natural light, there was no significant difference between calcium treatments and the untreated control (Figure 5.11). However, there was a significant difference ($P < 0.05$) between calcium lactate and calcium chloride treatments. Rot lesions were smaller on fruit treated with 3000 ppm Ca in calcium lactate than with 3000 ppm Ca in calcium chloride.

For the mock-inoculation treatment, strawberries treated with calcium solutions or treated with SNW as a control and mock-inoculated with SNW did not show any sign of rot at the inoculated site during storage for up to 7 days at 10^{o}C under natural light. However, fruit appeared to rot near the calyx (Figure 5.12). The percentage of fruit with this rot was recorded after 7 days of storage (Figure 5.13). At day 7, the percentage of rotten fruit in 3000 ppm Ca in the calcium lactate treatment (7.5%) was significantly less ($P < 0.05$) than the control (12.5%), while all treatments of calcium chloride showed significant fewer ($P < 0.05$) fruit with rot than the control: 2.5%, 2.5% and 5.0% for 1500, 3000 and 4500 ppm Ca, respectively. The numbers of fruit that rotted in the calcium chloride treatments tended to increase with increasing concentration of calcium chloride.

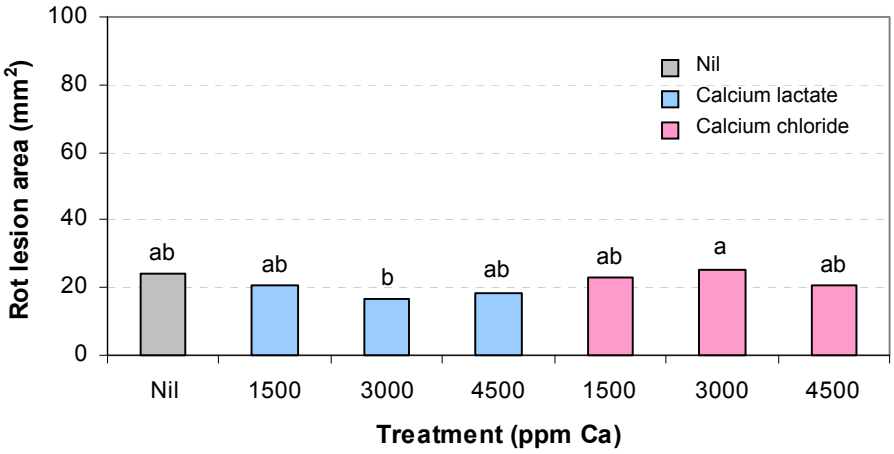
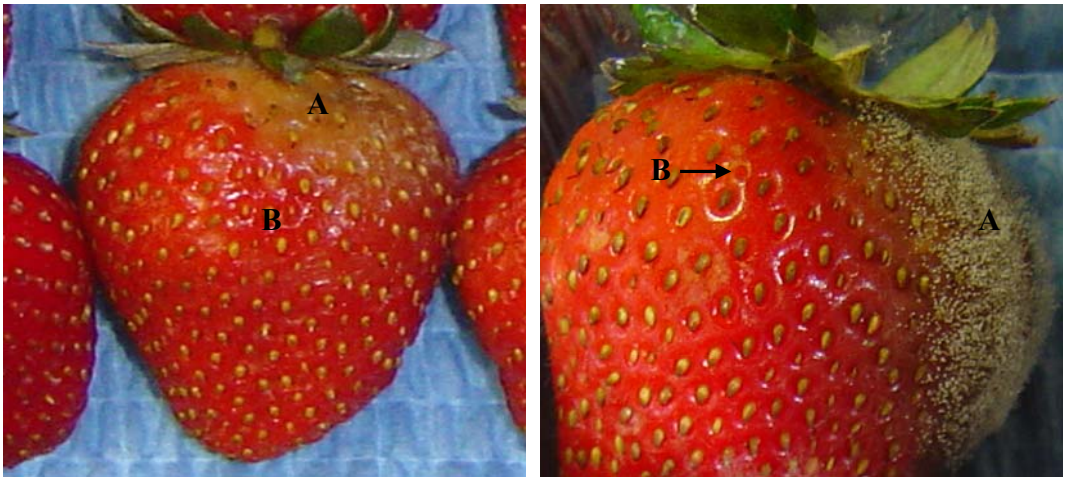


Figure 5.11 Comparison of the effect on Botrytis rot lesion area of treatment with calcium lactate (blue bars) and calcium chloride (pink bars) after 7 days of storage. Fruit were dipped for 5 min in calcium lactate solutions or calcium chloride solutions at 1500, 3000 and 4500 ppm Ca and stored at 10°C, 90±5% RH in continuous light for 24 h before inoculation with *Botrytis cinerea*. Fruit treated with sterile nanopure water were used as controls. Data shown are actual means from $n = 40$. The different letters indicate significant differences according to LSD at $P < 0.05$.



(a) **(b)**

Figure 5.12 Botrytis rot near the calyx (A) on fruit mock-inoculated with sterile nanopure water (SNW) by wounding at site B:

(a) fruit dipped in SNW (control) before mock-inoculation, after 4 days of storage at 10°C, 90±5% RH

(b) fruit dipped in calcium lactate at the concentration of 3000 ppm Ca before mock-inoculation, after 15 days of storage at 10°C, 90±5% RH.

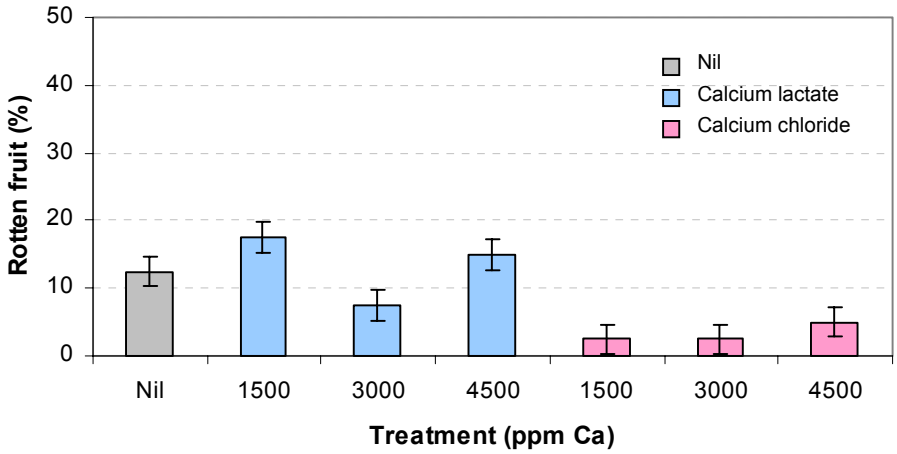


Figure 5.13 The percentage of fruit which presented visible rot near the calyx after 7 days of storage at 10°C, 90±5% RH. Fruit were dipped for 5 min in calcium lactate solutions at 1500 (CL1500Ca), 3000 (CL3000Ca), 4500 (CL4500Ca), calcium chloride solutions at 1500 (CC1500Ca), 3000 (CC3000Ca) or 4500 (CC4500Ca) ppm Ca and stored at 10°C, 90±5% RH in continuous light for 24 h before mock-inoculation with sterile nanopure water. Fruit dipped in sterile nanopure water were used as the control. Percentage data were arcsine transformed previous to the analysis of variance. Non-transfromed means are shown. Bars indicate standard error; $LSD_{0.05}$ = 2.23.

5.3.6 Further evaluation of the most effective treatments from prior experiments

The efficacy of calcium lactate at 3000 ppm Ca and calcium chloride at 4500 ppm Ca for delaying Botrytis rot development was evaluated on late-season fruit harvested in May 2006 and on early-season fruit harvested in December 2006.

On late-season fruit, calcium treatment reduced rot lesion development on calcium-treated fruit in certain conditions (Figure 5.14a). Seven days after storage at 10^{o}C, 90±5% RH in 12 h light and 12 h darkness, rot lesions on fruit treated with calcium chloride at 4500 ppm Ca were significantly smaller ($P < 0.05$) than those on the control. There was no significant difference between storage for 24 h in light and in dark prior to inoculation within treatments.

On early-season fruit, dipping fruit in 4500 ppm Ca in calcium chloride solution and keeping for 24 h in the dark prior to inoculation significantly reduced ($P < 0.05$) rot lesion areas in comparison with the control fruit stored in the dark (Figure 5.14b). However, post-calcium treatment storage for 24 h in light prior to inoculation did not affect rot lesions in any of the treatments. There was no difference between light and dark conditions during storage after calcium treatment prior to inoculation on calcium-treated fruit, whereas rot lesion area in the control fruit stored in light was significantly smaller ($P < 0.05$) than those stored in the dark for 24 h prior to inoculation.

In terms of comparison between fruit from different times of the season, the mean rot lesion area on early-season fruit appeared to be smaller than on late-season fruit, although data could not be compared statistically.

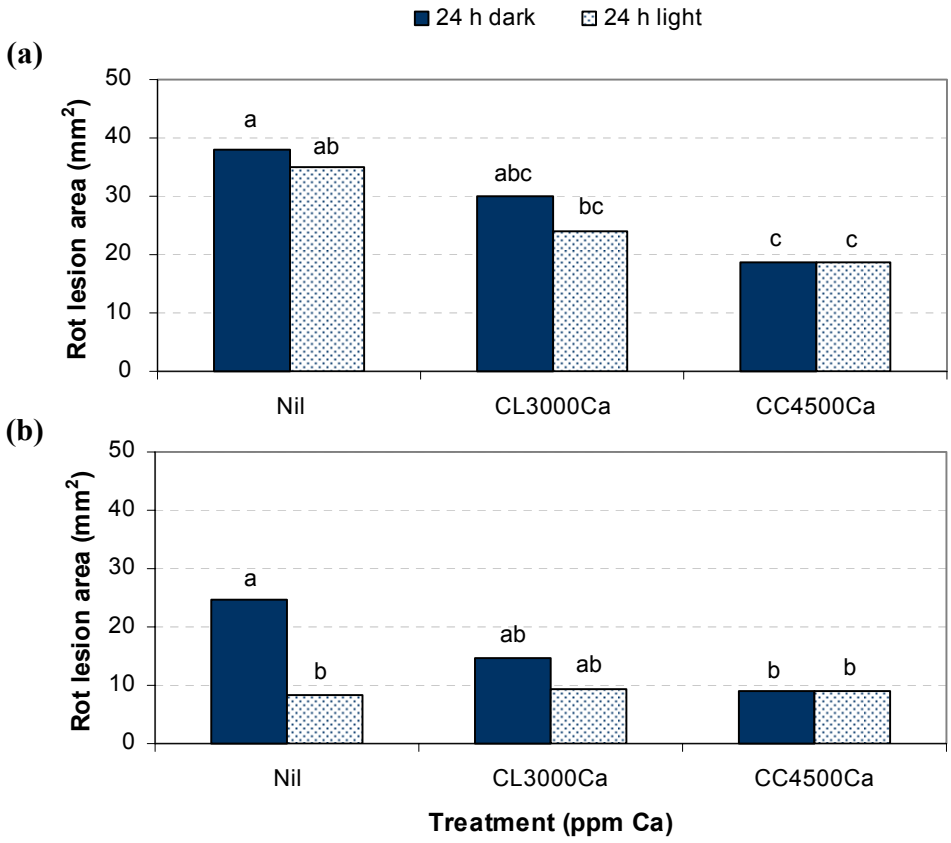


Figure 5.14 Effect of calcium lactate at 3000 ppm Ca (CL3000Ca) and calcium chloride at 4500 ppm Ca (CC4500Ca) on the development of Botrytis rot on late-season strawberries (May, 2006) (a) and on early-season strawberries (December, 2006) (b). Fruit were inoculated after dipping in calcium solutions for 5 min, then were divided into two groups; one group stored in dark and another group stored in light at 10^{o}C, 90±5% RH prior to inoculation for 24 h. Inoculated fruit were then stored under 12 h light and 12 h dark and rot lesions were measured after 7 days of storage. Fruit treated with sterile nanopure water were used as controls. Data shown are actual means from n = 40. The different letters indicate significant differences among treatments according to LSD at $P < 0.05$ in each fruit category after treatments.

5.4 Discussion

Postharvest calcium treatment had a somewhat variable effect on the development of fruit rot caused by *B. cinerea* in the strawberry cultivar 'Selva'. Calcium lactate and calcium chloride treatments sometimes provided a similar effect in delaying rot lesion expansion in comparison with the untreated control. However, calcium chloride-treated fruit generally showed slightly smaller rot lesions than those treated with calcium lactate. A similar result has been observed in fresh-cut cantaloupe, where dipping in 2.5% solutions of either calcium lactate or calcium chloride maintained firmness throughout 12 days of storage at 5^{o}C, but only calcium chloride-treated samples had a lower total plate count and yeast and mould counts than water-dipped samples (Luna-Guzmán and Barrett, 2000). The delay in decay caused by pathogens as a result of calcium treatment is thought to be due to either the role of calcium in strengthening the cell wall thus indirectly decreasing pathogen development or the calcium had a direct effect on growth of pathogens (Barkai-Golan, 2001). Germ-tube growth of *Colletotrichum gloeosporioides* and *C. acutatum*, causing apple bitter rot, was inhibited by calcium chloride and calcium propionate at 1000 ppm Ca (Biggs, 1999). The present study, however, found that calcium lactate and calcium chloride have no direct effect on colony growth of *B. cinerea in vitro*. This finding is in agreement with previous work by Chardonnet *et al.* (1997) that calcium chloride had no effect on the growth of *B. cinerea* hyphae; these authors also found that increasing concentrations of calcium chloride increased the cell wall as a proportion of the mycelia on a dry weight basis. They suggested that the calcium ion does not stimulate fungal growth, although it has a role in apical growth of hyphae.

The delay of fungal development by calcium treatment may potentially be explained by the increased calcium content in flesh leading to reinforcement of fruit tissues through maintenance of the structural integrity of the cell wall (Conway *et al.*, 1994). However, the efficiency of calcium treatment in delaying fungal growth may depend on several factors, such as calcium source, calcium concentration and the amount of calcium taken into the plant tissue. In this study, calcium solution did not infiltrate

into fruit when dipped in calcium solution amended with food dye for 5 min as indicated by the appearance of dye only on the fruit surface (data not shown). The infusion of calcium into plant tissue may be limited due to obstructions resulting from growing conditions, environmental factors (Barkai-Golan, 2001), period of time for calcium to penetrate into fruit and method of calcium application.

As described in section 5.1, active infiltration may be a better procedure than dipping to allow calcium into fruit. Vacuum or pressure infiltration procedures with calcium chloride solution were more effective in reducing decay of apple following inoculation with *Penicillium expansum* than dipping into the solution (Conway *et al.*, 1994). In a soft textured fruit like strawberry, forcing calcium into fruit by pressure might not be suitable as skin damage may occur resulting in the fruit becoming more susceptible to pathogens. Lara *et al.* (2004) found that the postharvest quality of 'Pajaro' strawberry fruit was markedly better on calcium-infiltrated fruit, whereas differences between untreated and calcium-dipped fruit were not significant. In contrast, decay caused by *B. cinerea* was less in calcium-dipped than calcium-infiltrated strawberries.

It was suggested that a storage period after dipping in calcium solution might be necessary in order to allow calcium to penetrate into fruit (Prof. Daryl Joyce, personal communication, 28 September 2005), where calcium ions bind to pectins in the middle lamella region of the cell wall and make the cell walls less accessible to fungal infection (Conway *et al.*, 1994; Moline, 1994). Subsequently, preliminary results of this study showed that the longer period of storage after calcium treatment prior to inoculation tended to reduce rot lesion area. In comparison between the storage periods of 10 min, 6 h, 12 h and 24 h, inoculation of fruit 24 h after calcium treatment delayed rot development on fruit treated with calcium lactate (Figure 5.6) and calcium chloride (Figure 5.9) in comparison with untreated fruit. However, the conditions of storage, with/without light, also affected the development of rot. Fruit treated with calcium chloride at any concentration (1500, 3000 and 4500 ppm Ca)

followed by 24 h natural light storage at 10°C prior to inoculation had smaller rot areas than those treated with SNW (Figure 5.10). In contrast, fruit treated with calcium lactate and kept in 24 h darkness at 10°C prior to inoculation did not show any efficacy of calcium treatment in delaying rot development (Figure 5.7). These results suggested that light may influence calcium absorption into fruit. In subsequent trials, however, whereas fruit treated with 3000 ppm Ca in calcium lactate and stored in 24 h light before inoculation had slightly smaller rot lesion areas than corresponding fruit stored in 24 h darkness, the untreated control also showed a similar effect (Figure 5.14). However, calcium treatment using the same conditions did not reduce the number of mock-inoculated fruit that were rotten (Figure 5.13), indicating that adding calcium into fruit did not influence latent infection by *B. cinerea* where rot lesions usually develop near the calyx (Figure 5.12).

Although the development of Botrytis rot on early-season and late-season fruit could not be compared in a single experiment due to the different growing seasons, it is suggested that rot lesion areas on early-season fruit tended to be smaller than those on late-season fruit (Figure 5.14). This may be explained by the greater fruit firmness usually observed in early-season fruit, partly due to sufficient fertiliser, thus early-season fruit may be more resistant to Botrytis infection. In addition to the softer texture increasing susceptibility to Botrytis infection in late-season fruit, as the season progresses, inoculum accumulates in the field, creating more opportunities for the fungus to infect and higher rates of quiescent infection (Wszelaki and Mitcham, 2003).

However, fruit harvested at different times of the season may respond to calcium treatment at different levels, therefore, affecting the efficiency of calcium in delaying rot development. According to the results in Figure 5.14, calcium treatment seemed to be more effective on late-season fruit than on the early-season fruit, as indicated by smaller rot lesions on calcium-treated fruit than on the corresponding untreated control. This suggests that more calcium may be taken into late-season fruit than

early-season fruit. Likewise, Conway and Sams (1985) reported that calcium absorption in 'Golden Delicious' apples picked 2 weeks before the prime harvest period and treated with calcium chloride solution was low and did not inhibit decay. In contrast, apple fruit picked 2 weeks after prime harvest time absorbed large quantities of calcium that markedly reduced decay development, but caused severe calcium injury.

The concentration of calcium solution required to decrease decay without causing surface-damage is important. Conway (1982) pointed out that the concentration of calcium in the flesh of apples dipped in calcium chloride increased as the concentration of the solution increased but not enough to reduce decay caused by *P. expansum*. However, high concentrations of calcium may cause damage to the fruit surface. Conway *et al.* (1994) suggested that the decrease in decay caused by *P. expansum* in apples was partly correlated to the increase in calcium concentration of the fruit tissue, but some superficial peel injury occurred at the higher concentrations of calcium solution. In this present study, the optimum concentration of calcium lactate and calcium chloride solutions for delaying Botrytis rot development were 3000 and 4500 ppm Ca, respectively. In addition, no visible surface damage occurred on calcium-treated fruit at any concentration of calcium solutions, but salt accumulation occurred on the calyx of fruit treated with calcium lactate at 4500 ppm Ca. Manganaris *et al.* (2007) found that calcium lactate and calcium propionate increased the calcium content of peaches considerably, but at the same time accelerated the process of flesh softening, as a result of surface-damage symptoms due to excessive salt concentration. Thus immersion of fruits in high calcium concentration sometimes may increase the risk of salt-related fruit injury due to osmotic pressure (Saftner *et al.*, 1998) and subsequently increases the risk of susceptibility to the action of fungal pathogens.

5.5 Conclusion

Postharvest treatment with calcium lactate and calcium chloride at the concentrations of 3000 and 4500 ppm Ca, respectively, were most effective in delaying Botrytis rot development on strawberry cultivar 'Selva' after 7 days of storage at 10^{o}C, 90±5% RH. Calcium lactate is a potential alternative to calcium chloride for reducing decay caused by *B. cinerea* in strawberry without providing undesirable bitterness. A storage time of at least 24 h after calcium dips prior to inoculation was required, perhaps in order to allow calcium to enter into the fruit. Fruit harvested early in the season seemed to be more resistant to *B. cinerea* than those harvested late in the season. However, calcium treatment tended to be more effective when applied to late-season fruit. The findings in this study may contribute to the development of strategies to reduce damage by *B. cinerea* in industry.

Chapter Six
Effect of Calcium and Boron on Botrytis Leaf Blight

6.1 Introduction

Infected strawberry leaf residues are a potential source of inoculum of *B. cinerea* (Maas, 1998). During the growing season, latent infection can become established in healthy leaves at any time (Sosa-Alvarez *et al.*, 1995). Once infected leaves senesce and die, the fungus becomes active, colonizes the dead tissue and eventually sporulates to produce the inoculum for subsequent infections (Braun and Sutton, 1988). Interestingly, Braun and Sutton (1987) reported that sporulation of the fungus on dead strawberry leaves is the principal source of initial inoculum in epidemics of grey mould fruit rot. Management of disease on leaves may, therefore, reduce the inoculum produced.

The manipulation of plant nutrition, such as calcium and boron, has been considered as an alternative disease control to fungicides, which can be potentially harmful to humans and the environment. Because the nutritional status of plants influences their response to pathogens, adjustment of fertilisation rates and the composition of the nutrient solution have been proposed as means for disease suppression (Yermiyahu *et al.*, 2006). High calcium rates are generally associated with reduced susceptibility to *B. cinerea* in various host plants, such as rose (Volpin and Elad, 1991), sweet basil (Yermiyahu *et al.*, 2006), bean and tomato (Elad and Volpin, 1993). However, there is little information about the effect of calcium on Botrytis leaf blight on strawberry.

Although boron has been reported to reduce diseases in several plants, such as tomato, cotton and bean (see Section 1.5), there have been no reports of its effect on Botrytis leaf blight in strawberry. Although Wójcik and Lewandowski (2003) demonstrated that sprays of boron increased boron content in leaf tissues of 'Elsanta'

strawberry, they did not investigate any subsequent effect on disease development on leaves.

The application of calcium to plants significantly increased the calcium content of leaf tissues in both 'Aromas' and 'Selva' (see Table 3.15 and 3.17). Likewise, Dunn and Able (2006) showed that the calcium content of 'Selva' leaves increased with increasing calcium concentrations applied to soil. Similarly, boron content in leaf tissues of 'Aromas' and 'Selva' increased with increasing boron concentration in fertigation nutrient solution (see Table 4.4 and 4.5). However, it is not known if the increase of calcium or boron content in leaf tissues had an effect on Botrytis blight development on strawberry leaves. Thus, the aim of the studies reported in this chapter was to determine the effect of calcium and boron on the response of the strawberry cultivars 'Aromas' and 'Selva' to *B. cinerea* and to examine the correlation between nutrient concentration in the fertigation solution, nutrient status in leaf and blight lesion size.

6.2 Materials and methods

6.2.1 Plant materials

Leaves were collected from plants of 'Aromas' and 'Selva' which had been grown in the glasshouse and used in studies on the effect of preharvest calcium application (Chapter 3) and effect of preharvest boron application (Chapter 4).

Briefly, to examine the effect of preharvest calcium nutrition, plants were grown in 15-cm diameter pots containing 2.5 kg of Golden Grove sand. A 0.25 strength Hoagland's solution was amended with calcium sulphate ($CaSO_4$, Labchem, Ajax Finechem, Seven Hills, New South Wales) at four concentrations (0, 100, 300 and 500 ppm Ca) and 100 mL of one of the solutions was applied to each plant by hand every 2 days (see Section 3.2.2.3). The experiment was conducted twice, once in June

2005 (after applying nutrient solutions for ~ 12 months) and again in September 2006 (after applying nutrient solutions for ~ 24 months).

To examine the effect of preharvest boron nutrition, methods were performed as above except that plants were grown in Waikerie sand and boric acid (H_3BO_3, BDH AnalaR®, Merck, Kilsyth, VIC) at 0, 0.25, 0.5 and 1.0 ppm B was prepared in 0.25 strength Hoagland's solution (see Section 4.2.2). The experiments were performed twice, once in December 2006 (after applying nutrient solutions for ~ 6 months) and again in July 2007 (after applying nutrient solutions for ~ 12 months).

Fully expanded, healthy leaves were harvested by detaching at the base of the petioles and transported to the laboratory just before use. Three leaflets were considered as one leaf and there were three leaves per replicate and three replicates per treatment with complete randomisation.

6.2.2 Conidia suspension and inoculation of leaf

A conidia suspension of *B. cinerea* was prepared according to the method described in Section 2.6.2 to a concentration of 10^6 conidia per mL. Detached leaves were surface sterilised by soaking in 2% sodium hypochlorite for 2 min and allowed to dry in a laminar flow before placing on individual sterile filter papers (Whatman® No.1) saturated with sterile nanopure water to maintain free moisture in 15-cm diameter Petri dishes (one leaf per dish). The leaves were wounded by puncturing with a sterile 0.5-mm diameter needle (three wounds per leaf). A disc (approximately 4.5-mm diameter X 5-mm thick) of potato dextrose agar (PDA) was then placed on each wound then 5 μL of conidia suspension (10^6 conidia per mL) was placed onto each PDA disc (Figure 6.1). Dishes were sealed with Parafilm® to maintain high relative humidity (RH). Dishes were arranged in a stack of nine for each treatment and were then incubated on a bench in the laboratory at room temperature (22 to 24^{o}C) in natural light.

6.2.3 Evaluation of Botrytis leaf blight

Inoculated leaves were observed every day for blight development for up to 7 days after inoculation. The blight lesion diameters were measured horizontally and vertically by using calipers (digiMax, Switzerland) and the mean of the two measurements was calculated and recorded in millimeters (mm).

6.2.4 Nutrient analysis

The data on calcium content and boron content in leaf tissues used in this study were obtained from the nutrient analysis reported in Chapter 3 (see Section 3.2.4) and Chapter 4 (see Section 4.2.4), respectively. The leaves subjected to nutrient analysis were collected at the same time as the second experiments on the effect of calcium and boron on Botrytis leaf blight were performed, i.e. in September 2006 and in July 2007 for calcium and boron experiments, respectively.

6.2.5 Statistical analysis

Data were statistically analysed as described in Section 2.8 and the differences between the means of the treatments determined according to the least significant difference (LSD) at $P < 0.05$. Means and standard errors were calculated using Microsoft Excel 2003. The correlation between nutrient concentration in the fertigation solution, leaf nutrient status and blight lesion size in the second experiment was determined using GenStat (6^{th} Edition, Rothamsted Experimental Station, UK).

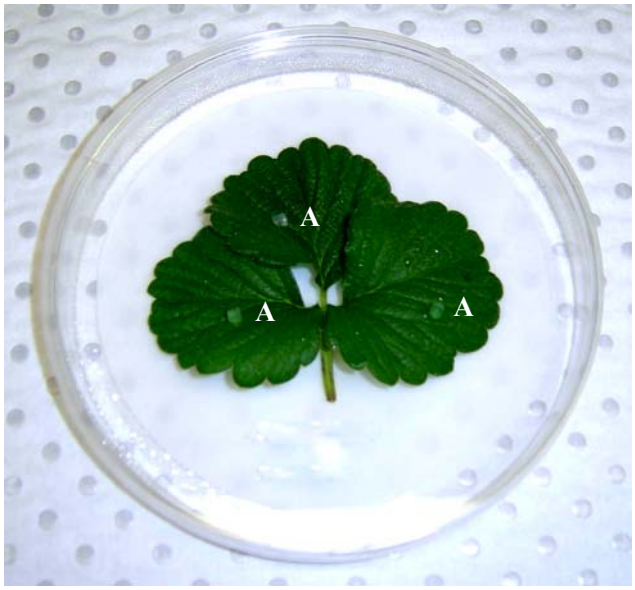


Figure 6.1 *Botrytis cinerea*-inoculated leaves in a 15-cm diameter Petri dish. A 4.5-mm diameter disc of potato dextrose agar (A) was placed on a wound on each leaflet and then a droplet of 5 µL of conidia suspension of *B. cinerea* (10^6 conidia per mL) was placed onto each disc.

6.3 Results

6.3.1 Effect of calcium on the development of Botrytis leaf blight

In general, calcium application did not influence the severity of Botrytis leaf blight in either 'Aromas' or 'Selva'. At the inoculation site, blight lesions began as small, firm, brown spots and became larger with time (Figure 6.2 and 6.3 for 'Aromas and 'Selva', respectively). However, blight lesions on 'Selva' leaves were slightly smaller than those on 'Aromas' leaves, regardless of calcium treatment, especially 7 days after inoculation, except those in the 500 ppm Ca treatment.

In 'Aromas', there was no obvious difference in blight lesion size between the two experiments, conducted in June 2005 (Figure 6.4a) and September 2006 (Figure 6.4b), and similar trends were observed in both. Generally, blight lesions appeared 3 days after inoculation in all calcium treatments and calcium did not influence blight lesion size. However, the trend suggested that by 7 days after inoculation, the lesions were smaller in the no calcium treatments than in the calcium treatments but differences were not significant.

As for 'Aromas', blight lesion diameters on 'Selva' leaves were not different between the two experiments conducted in different years (Figure 6.5). Calcium did not affect blight lesion size except in the second experiment (Figure 6.5b) where at day 7 after inoculation, the lesions on leaves from plants that received calcium were significantly smaller ($P < 0.05$) than those from plants that did not receive calcium.

6.3.2 Correlation between calcium concentration in nutrient solution, calcium content in leaf and blight lesion size

Calcium content in leaves (obtained from the nutrient analysis reported in Chapter 3) was used to determine the correlation between calcium concentration and blight lesion size (Table 6.1). The linear correlation coefficients between calcium concentration in the fertigation nutrient solution, calcium content in leaf and blight lesion size shown in Table 6.2 and 6.3 for 'Aromas' and 'Selva', respectively,

describe only the results obtained in the second experiment. For both cultivars, calcium concentration in the fertigation nutrient solution was significantly positively correlated with calcium content in the leaf ($P < 0.01$, $r = 0.744$ and $r = 0.783$ for the first and second experiments, respectively), indicating that leaf calcium status increased with increasing calcium concentration in the nutrient solution applied to plants. However, there was no correlation either between calcium concentration in the nutrient solution and blight lesion size or between calcium content in the leaf and blight lesion size.

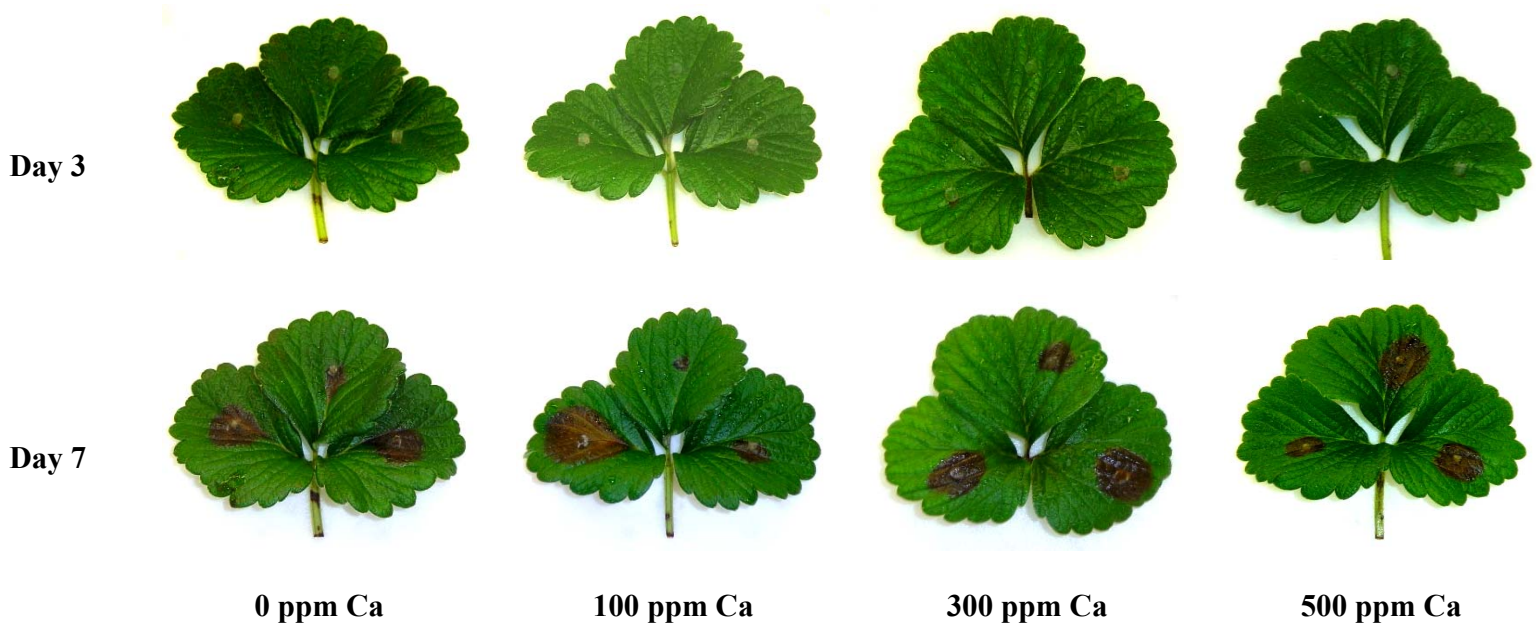


Figure 6.2 Leaves of cultivar 'Aromas' 3 and 7 days after inoculation with *Botrytis cinerea*, illustrating the effect of calcium on the severity of Botrytis leaf blight on leaves detached from plants that received 0, 100, 300 and 500 ppm Ca. The leaves were from the second experiment conducted in September 2006 and representative of both experiments.

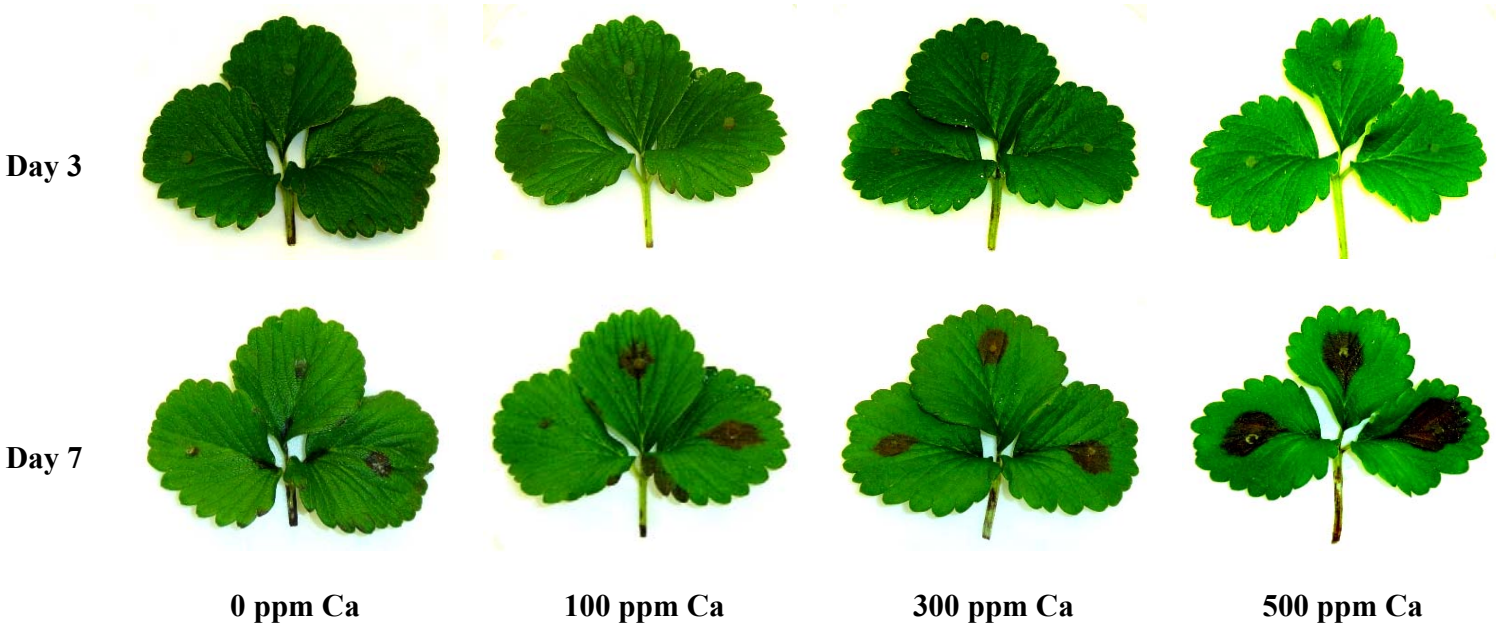


Figure 6.3 Leaves of cultivar 'Selva' 3 and 7 days after inoculation with *Botrytis cinerea*, illustrating the effect of calcium on the severity of Botrytis leaf blight on leaves detached from plants that received 0, 100, 300 and 500 ppm Ca. The leaves were from the second experiment conducted in September 2006 and representative of both experiments.

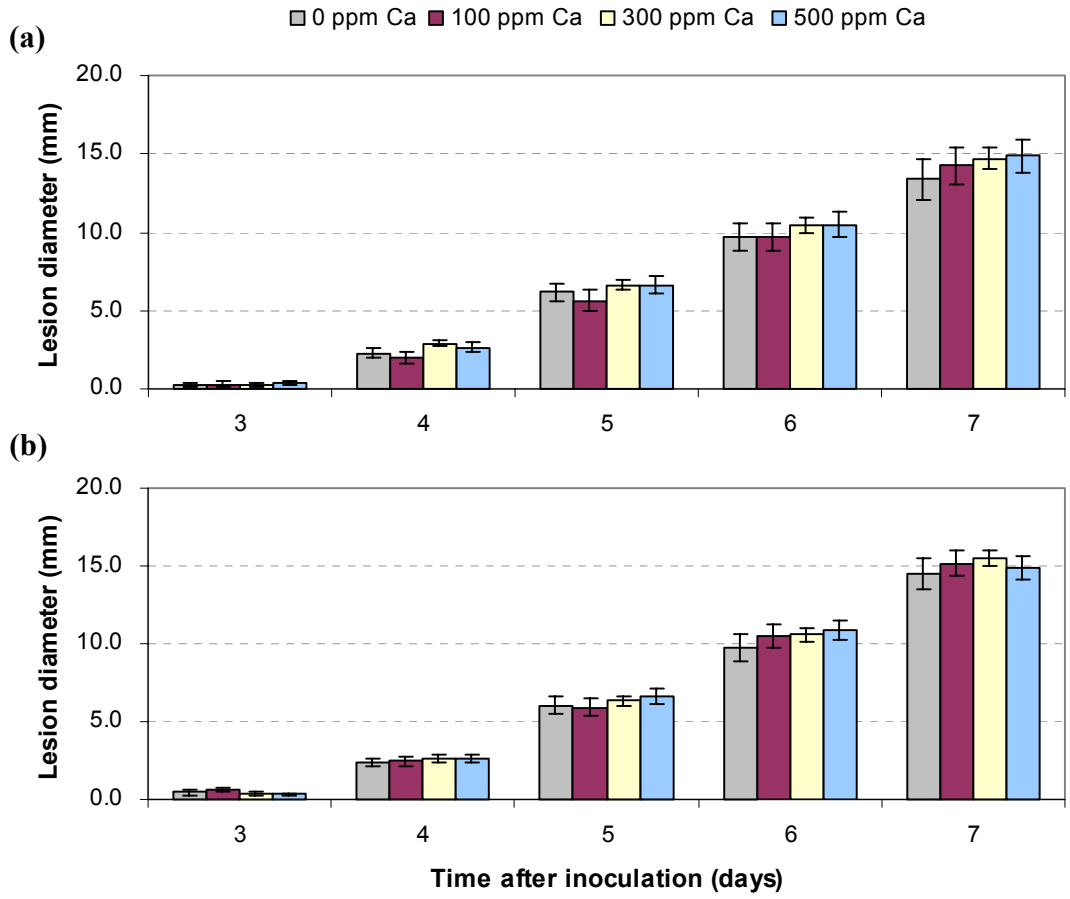


Figure 6.4 Effect of calcium, incorporated in fertigation nutrient solution, on severity of Botrytis leaf blight in 'Aromas' during 7 days after inoculation. Assessments were performed on leaves detached from the pot experiment (Section 3.2.2.3) and were conducted in June 2005 (a) and September 2006 (b). Data shown are means ± SE from $n = 9$.

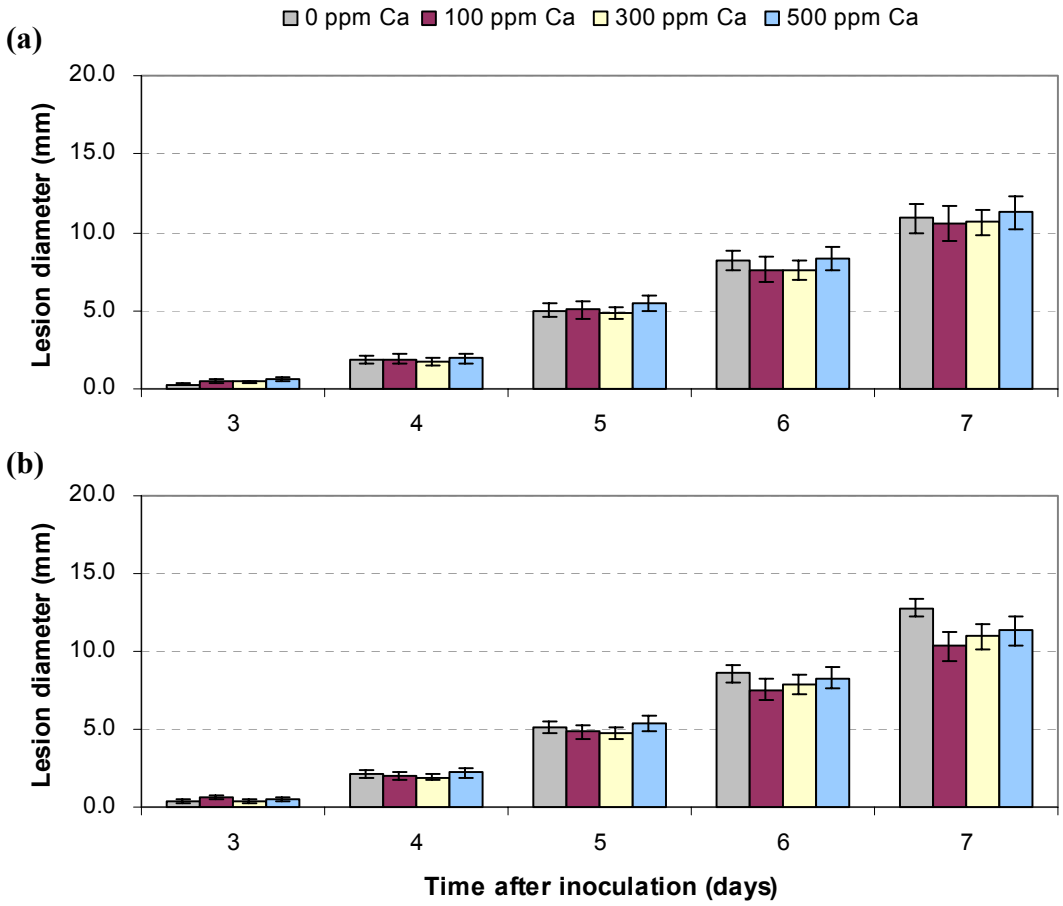


Figure 6.5 Effect of calcium, incorporated in fertigation nutrient solution, on severity of Botrytis leaf blight in 'Selva' during 7 days after inoculation. Assessments were performed on leaves detached from the pot experiment (Section 3.2.2.3) and were conducted in June 2005 (a) and September 2006 (b). Data shown are means ± SE from $n = 9$.

Table 6.1 Calcium content in 'Aromas' and 'Selva' leaves. The calcium concentration was determined using ICP-OES analysis. Data are obtained from the nutrient analysis shown in Table 3.15 and 3.17 for 'Aromas' and 'Selva', respectively, and presented as means ± SE from $n = 3$.

Calcium concentration in nutrient solution (ppm Ca)	**Calcium content in leaf tissues (mg per kg)**	
	'Aromas'	**'Selva'**
0	3933.33 ± 284.80	3700.00 ± 208.17
100	11100.00 ± 888.82	11300.00 ± 832.67
300	11266.67 ± 317.98	12833.33 ± 317.98
500	12466.67 ± 933.33	13733.33 ± 1490.34

Table 6.2 The linear correlation coefficients between calcium concentration in the fertigation nutrient solution, calcium content in leaf and blight lesion size (7 days after inoculation) in cultivar 'Aromas' in September 2006.

Correlation matrix	**Calcium concentration in nutrient solution**	**Calcium content in leaf**
Calcium content in leaf	0.744**	
Blight lesion size	0.068	0.258

** Significant at $P < 0.01$.

Table 6.3 The linear correlation coefficients between calcium concentration in the fertigation nutrient solution, calcium content in leaf and blight lesion size (7 days after inoculation) in cultivar 'Selva' in September 2006.

Correlation matrix	**Calcium concentration in nutrient solution**	**Calcium content in leaf**
Calcium content in leaf	0.783**	
Blight lesion size	-0.121	-0.311

** Significant at $P < 0.01$.

6.3.3 Effect of boron on the development of Botrytis leaf blight

In a manner similar to that previously described (Section 6.3.1), blight symptoms first appeared at the inoculation site as small, restricted brown lesions, enlarged rapidly during incubation, particularly 6 and 7 days post inoculation such that blight extended over most of the leaflet in 'Aromas' by day 7 (Figure 6.6). The blight lesions expanded slowly and did not cover the leaflet of 'Selva' 7 days after inoculation (Figure 6.7). However, blight lesions on 'Selva' leaves were slightly smaller than on 'Aromas' leaves regardless of boron treatment and the experiment.

For 'Aromas', blight lesions appeared 2 days after inoculation and significantly increased in size over time in both experiments. However, lesions tended to be larger in the first experiment (July 2007) than in the second experiment (December 2006). In the first experiment, conducted in December 2006 (Figure 6.8a), application of boron seemed to have an effect on severity of Botrytis blight in that smaller blight lesions were observed on leaves detached from plants that received additional boron during growth. In particular, leaves from plants that received 0.25 and 0.5 ppm B had significantly smaller blight lesions than those from plants that received no boron. Blight lesions in the 1.0 ppm B treatment developed more slowly than those in the 0 ppm B in the first 4 days after inoculation, but then developed quickly from day 5 such that lesions did not differ in size from those in the 0 ppm B treatment by day 7. Similarly, in the second experiment, conducted in July 2007 (Figure 6.8b), blight lesion sizes in the boron treatments were significantly smaller ($P < 0.05$) than those in the 0 ppm B treatment during the first 4 days after inoculation. However, only those in the 0.5 ppm B treatment remained significantly smaller ($P < 0.05$) than those in the 0 ppm B treatment until day 7 after inoculation.

Similar trends were observed in 'Selva' (Figure 6.9). In general, blight lesions appeared 2 days after inoculation and significantly increased ($P < 0.05$) over the incubation period. In the first experiment, conducted in December 2006, blight lesions on leaves detached from plants that received no boron significantly increased

($P < 0.05$) over the period of incubation and were significantly larger ($P < 0.05$) than those on leaves from plants that received 1.0 ppm B during the 7 days after inoculation (Figure 6.9a). At day 7, significantly smaller ($P < 0.05$) lesion sizes were observed in all boron treatments than in the 0 ppm b treatment. Similar trends were observed in the second experiment, conducted in July 2007, in that smaller blight lesions were observed with increasing boron concentration, in particular, the 1.0 ppm B treatment, in that the lesions were significantly smaller ($P < 0.05$) than in the 0 ppm B treatment from day 4 after inoculation (Figure 6.9b). From day 5, blight lesions were smaller in the boron treatments than the no boron treatment, except for the 0.25 ppm B treatment where lesions were larger.

6.3.4 Correlation between boron concentration in nutrient solution, boron content in leaf and blight lesion size

Boron content in leaves (obtained from the nutrient analysis reported in Chapter 4) was used to determine the correlation between boron concentration and blight lesion size (Table 6.4). A correlation between boron concentration and blight lesion diameter was found only in the second experiment. The linear correlation coefficients in Table 6.5 indicate that boron concentration in the fertigation nutrient solution was significantly positively correlated with boron content in leaf of 'Aromas' ($P < 0.001$, $r = 0.981$), whereas blight lesion size was not correlated with boron concentration either in the nutrient solution or in the leaf. For 'Selva', boron concentration in the fertigation nutrient solution was significantly positively correlated with boron content in leaf ($P < 0.001$, $r = 0.919$) (Table 6.6), indicating that leaf boron content strongly increased with increasing boron concentration in the fertigation nutrient solution. However, blight lesion size was significantly negatively correlated with both boron concentration in the nutrient solution and in the leaf ($P < 0.05$, $r = -0.622$ and -0.558, respectively). This indicated that blight lesion diameter on 'Selva' leaves decreased with increasing boron concentration in nutrient solution and with increasing leaf boron content.

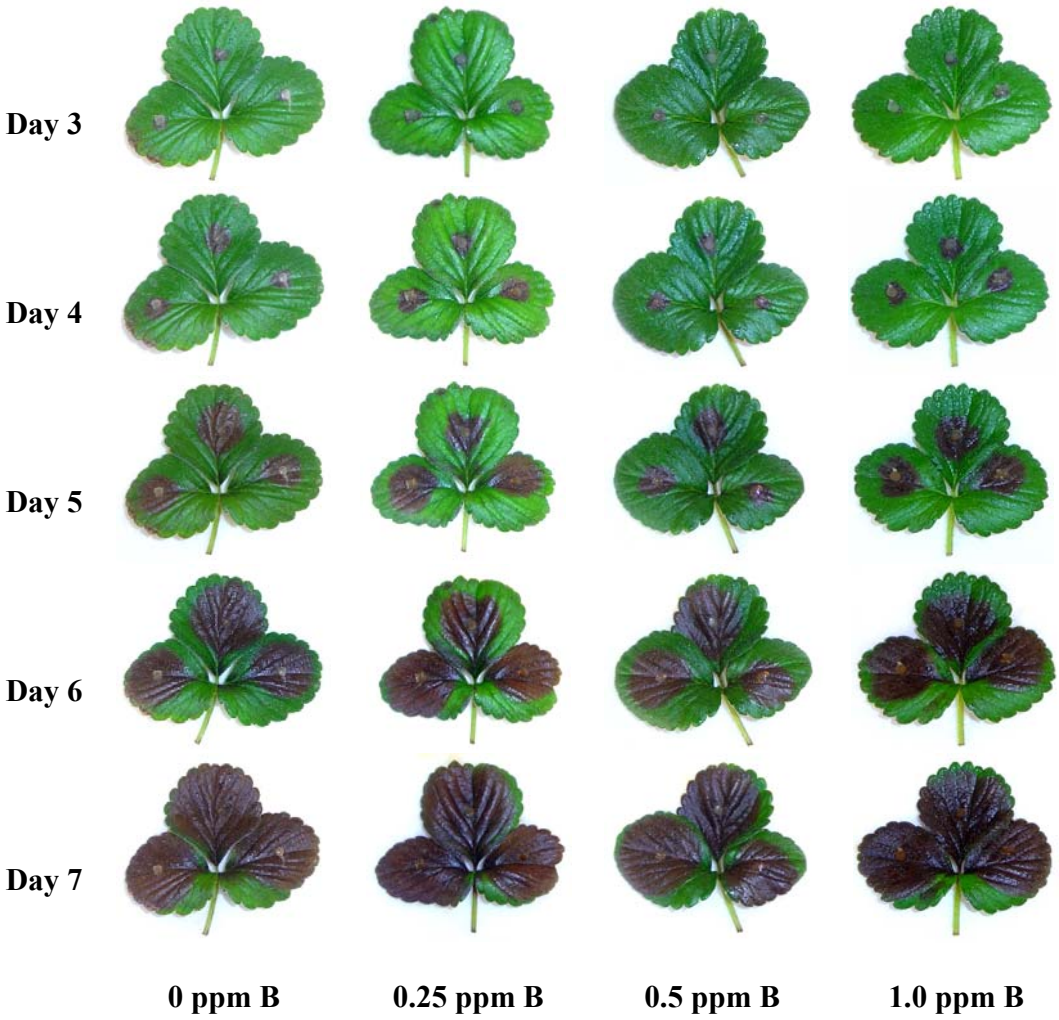


Figure 6.6 Leaves of cultivar 'Aromas' 3 to 7 days after inoculation with *Botrytis cinerea*, illustrating the effect of boron on the severity of Botrytis leaf blight on leaves detached from plants that received 0, 0.25, 0.5 and 1.0 ppm B. The leaves were from the second experiment conducted in July 2007 and representative of both experiments.

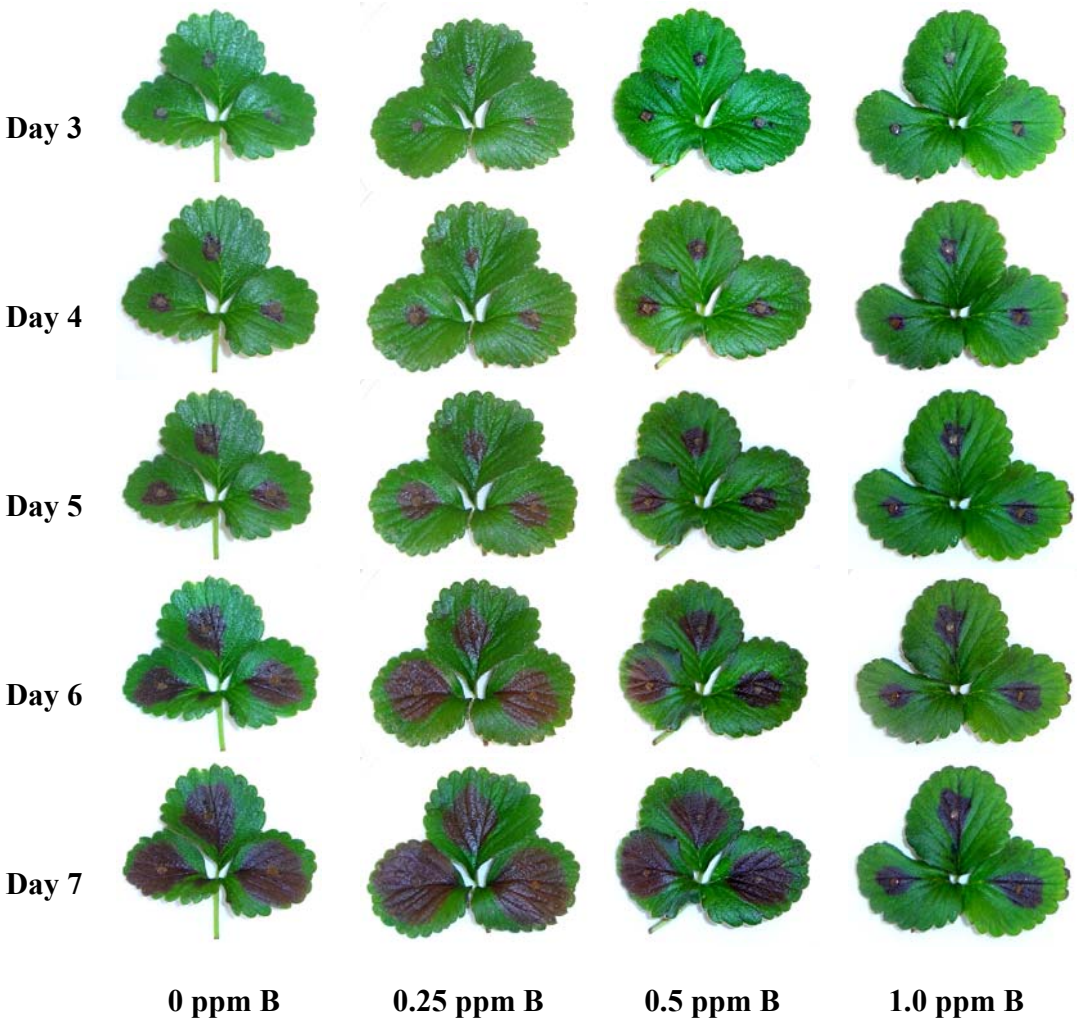


Figure 6.7 Leaves of cultivar 'Selva' 3 to 7 days after inoculation with *Botrytis cinerea*, illustrating the effect of boron on the severity of Botrytis leaf blight on leaves detached from plants that received 0, 0.25, 0.5 and 1.0 ppm B. The leaves were from the second experiment conducted in July 2007 and representative of both experiments.

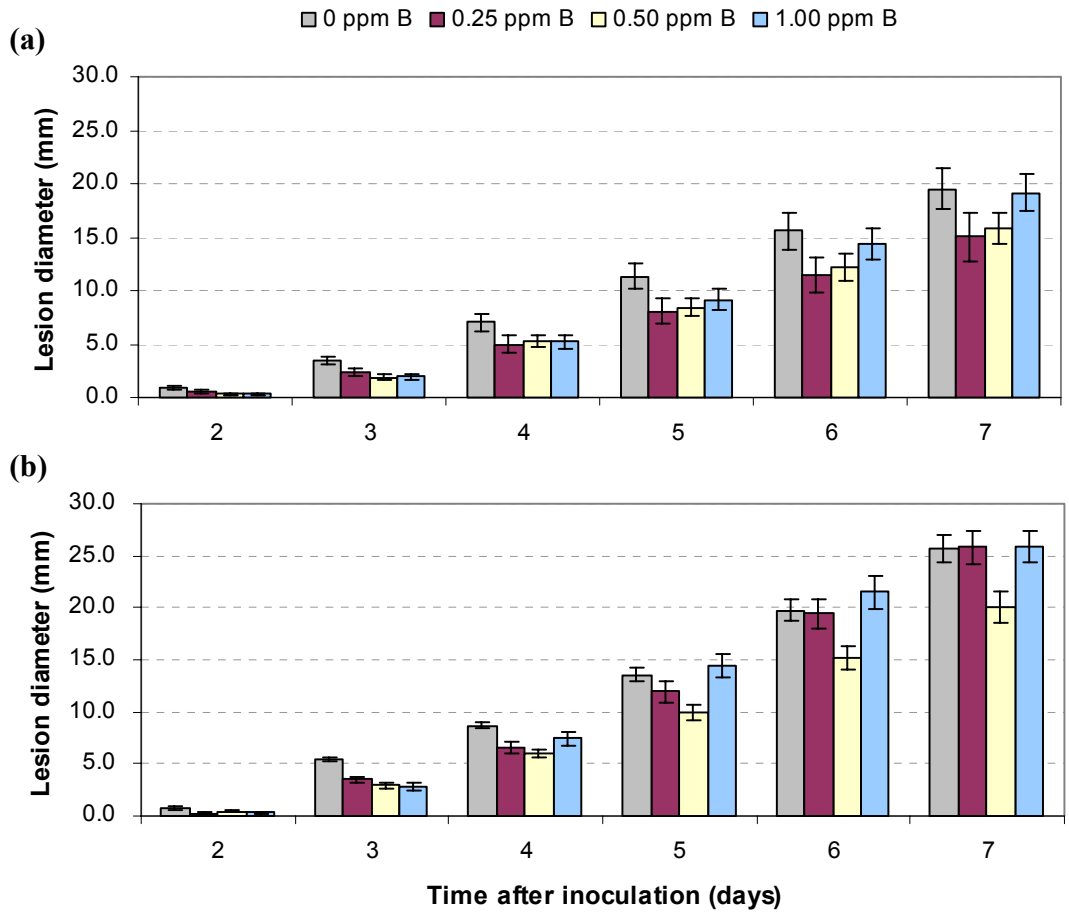


Figure 6.8 Effect of boron, incorporated in fertigation nutrient solution, on severity of Botrytis leaf blight in 'Aromas' during 7 days after inoculation. Assessments were performed on leaves detached from the pot experiment and were conducted in December 2006 (a) and July 2007 (b). Data shown are means ± SE from $n = 9$.

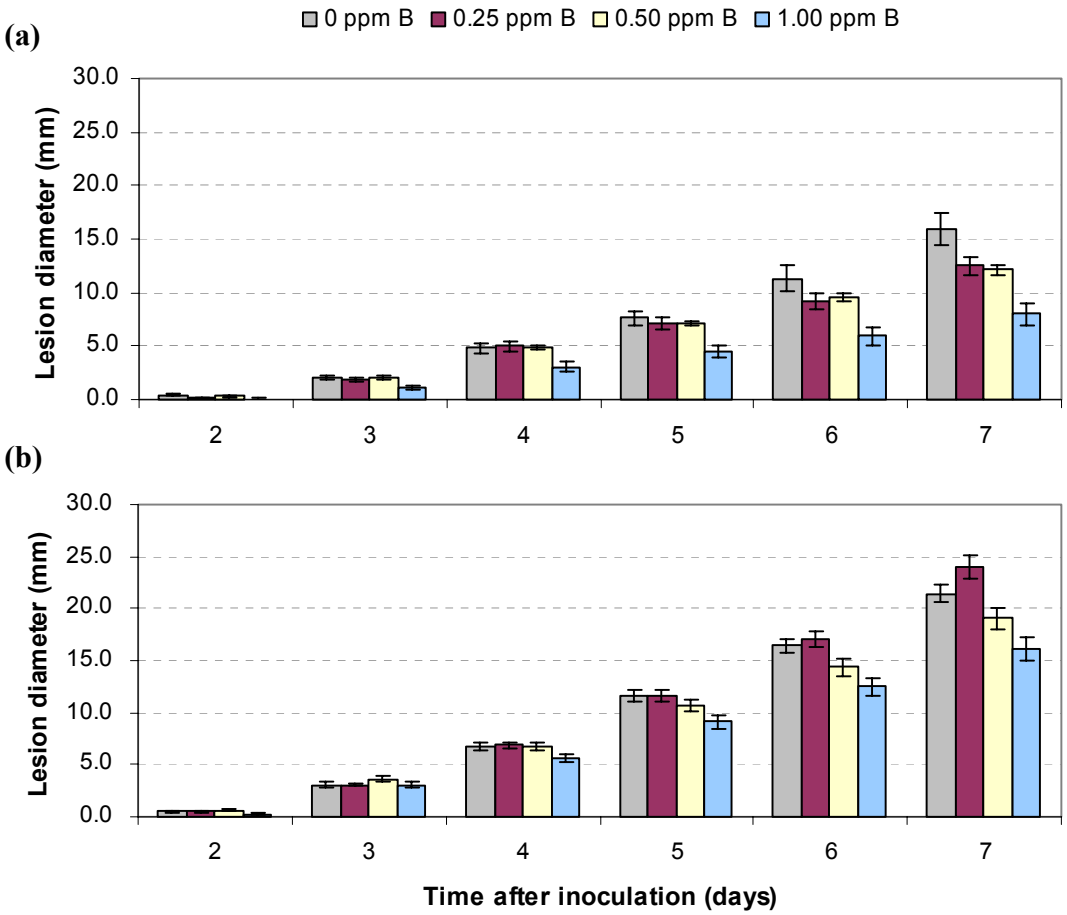


Figure 6.9 Effect of boron, incorporated in fertigation nutrient solution, on severity of Botrytis leaf blight in 'Selva' during 7 days after inoculation. Assessments were performed on leaves detached from the pot experiment and were conducted in December 2006 (a) and July 2007 (b). Data shown are means ± SE from $n = 9$.

Table 6.4 Boron content in 'Aromas' and 'Selva' leaves. The boron concentration was determined using ICP-OES analysis. Data are obtained from the nutrient analysis shown in Table 4.4 and 4.5 for 'Aromas' and 'Selva', respectively, and presented as means ± SE from $n = 3$.

Boron concentration in nutrient solution (ppm B)	**Boron content in leaf tissues (mg per kg)**	
	'Aromas'	**'Selva'**
0	28.33 ± 1.33	26.67 ± 0.67
0.25	42.00 ± 1.00	54.67 ± 1.20
0.50	51.00 ± 0.58	61.67 ± 4.26
1.00	69.00 ± 0.36	79.00 ± 4.73

Table 6.5 The linear correlation coefficients between boron concentration in the fertigation nutrient solution, boron content in leaf and blight lesion size (7 days after inoculation) in cultivar 'Aromas' in July 2007.

Correlation matrix	**Boron concentration in nutrient solution**	**Boron content in leaf**
Boron content in leaf	0.981***	
Blight lesion size	-0.046	-0.025

*** Significant at $P < 0.001$.

Table 6.6 The linear correlation coefficients between boron concentration in the fertigation nutrient solution, boron content in leaf and blight lesion size (7 days after inoculation) in cultivar 'Selva' in July 2007.

Correlation matrix	**Boron concentration in nutrient solution**	**Boron content in leaf**
Boron content in leaf	0.919***	
Blight lesion size	-0.622*	-0.558*

*, *** Significant at $P < 0.05$ and $P < 0.001$, respectively.

6.4 Discussion

Initially, several methods of leaf inoculation were trialled to induce blight symptoms. There was no blight symptom expression on inoculated leaves when only a droplet of conidia suspension was placed on a wound on the leaf (data not shown). However, inoculation of the leaf either by placing a disc of PDA containing *B. cinerea* hyphae on a wounded leaf or placing a disc of sterile PDA followed by a droplet of conidia suspension resulted in blight lesions. Thus, the latter method was performed to inoculate leaf samples in subsequent experiments. PDA may provide nutrients that assist colonisation of the wounded tissue by *B. cinerea*. The review of Botrytis infection by Holz *et al*. (2004) suggested that nutrient supplements influence the pre-penetration activities in conidia-bearing droplets on plant surfaces and also subsequent symptom development. Addition of exogenous nutrients to inoculum was required for induction of symptoms by *B. cinerea* on several plants. Strawberry leaves and cucumber cotyledons showed 100% infection when they were inoculated with conidia suspended in potato-sucrose broth whereas the conidia suspended in distilled water were unable to develop the blight lesions (Shirane and Watanabe, 1985). Similarly, addition of sugar to inoculum stimulated infection by *B. cinerea* on broad bean leaves (Harper *et al.*, 1981), plum and nectarine (Fourie and Holz, 1998).

Apart from supplementary nutrients, plant cultivar may also influence the virulence of *B. cinerea*, as indicated by lesion diameter. Although 'Aromas' is considered to be superior to 'Selva' in terms of yield, fruit quality and disease resistance (University of California, 2001a), it was observed in this present study that the 'Selva' leaf has a darker green colour and is thicker than the 'Aromas' leaf. The structure and thickness of the cuticle and the epidermal layers have been regarded as major factors of resistance against infection by *B. cinerea* in grapevine (Elmer and Michailides, 2004). This is supported by the finding in this study that leaves of 'Selva' were less susceptible to *B. cinerea* than leaves of 'Aromas' as indicated by the smaller blight lesions on 'Selva' leaves than on 'Aromas' leaves in both calcium and boron nutritional experiments.

In addition, the age of the plant may influence the response to infection by *B. cinerea*. In this study, blight lesions were larger in the second experiments in both nutrient studies. The older plants appeared to be more susceptible to infection by *B. cinerea*.

Plant response to *B. cinerea* may also be influenced by supplementation with nutrients. However, variable results with calcium have been obtained depending on plants and cultivars. In this present study, addition of calcium at the concentrations studied had no apparent benefit except a slight reduction in lesion size on 'Selva' in the second experiment (Figure 6.5b), especially at 7 days after inoculation. This finding is contradictory to previous works on rose (Volpin and Elad, 1991), sweet basil (Yermiyahu *et al.*, 2006), bean and tomato (Elad and Volpin, 1993) where application of calcium reduced susceptibility to *B. cinerea*.

Application of boron reduced blight lesion development on leaves of both 'Aromas' and 'Selva'; however, this result was consistent only in 'Selva'. Negative correlation coefficients (Table 6.5) indicated that blight lesion diameter decreased with increasing boron concentration in nutrient solution and with increasing boron content in 'Selva' leaves. These correlations, however, were examined only for the second experiment of each nutritional experiment as it coincided with the nutrient analysis as performed in Chapters 3 and 4. Thus it is suggested that the experiments should be repeated to determine if the correlations are repeatable.

Although boron appeared to reduce blight lesion expression, application of high concentrations of boron caused toxicity to the plants. In both 'Aromas' and 'Selva', marginal leaf necrosis was observed on plants that received additional boron, especially at 0.5 and 1.0 ppm. This observation is consistent with the work on 'Sparkle' strawberry by Gilbert and Robbins (1950) in that marginal necrosis of the leaves occurred at 0.5 and 1.0 ppm B. Similar results were obtained for 'Crusader' strawberry by Mason and Guttridge (1974) that boron toxicity occurred on plants fed with nutrient solutions containing 0.5 ppm B and above. This suggests that it is

important to investigate the optimum boron concentration applied to plants that can enhance host resistance to *B. cinerea* without causing toxicity.

However, it has been reported that boron toxicity is significantly influenced by calcium concentration in the soil or nutrient solutions and Ca/B ratio in plant tissues in which high calcium concentrations improve tolerance of plants to boron toxicity (Sotiropoulos *et al.*, 1999). Additionally, a combination of calcium and boron application may contribute to the effectiveness of calcium in strengthening the cell walls. Sprays of calcium plus boron significantly increased levels of calcium and boron in leaves and fruit, increased fruit firmness and decreased the incidence of grey mould in 'Elsanta' strawberry (Wójcik and Lewandowski, 2003) and 'Chandler' strawberry (Singh *et al.*, 2007). Thus, further study on the application of calcium plus boron is recommended as the combination influence on *B. cinerea* development on leaves in 'Aromas' and 'Selva'.

It is noted here that only one isolate of *B. cinerea* was used in this present study. *B. cinerea* is a very variable fungus. Chardonnet *et al.* (2000) reported that the inhibitory activity of calcium on *B. cinerea* also depended on isolate. So it would be wise to test other isolates in future studies. Furthermore, investigation of sporulation of the fungus on dead leaves from nutrition experiments would be worth pursuing as conidia produced on leaf residues are a primary source of inoculum in the field. In addition, further investigation should be conducted on leaves at different ages, at different times of year and from plants growing in the field.

6.5 Conclusion

Supplementary nutrients were required for artificial inoculation of leaves with *B. cinerea*. Increasing calcium concentration in the fertigation nutrient solution increased calcium content in leaves but did not influence blight development in either cultivar. However, leaves from 'Selva' plants that received calcium had somewhat smaller blight lesions at day 7 after inoculation than those from plants that received no calcium. Application of boron reduced blight lesion development on leaves of 'Selva', and increasing boron concentration in the fertigation nutrient solution was correlated with increased boron content in leaves and decreased blight lesion size at 7 days after inoculation.

Chapter Seven
General Discussion

The improvement of fruit quality and reduction of disease development through the manipulation of plant nutrients such as calcium and boron has been reported for several plants. In strawberry, calcium application has been reported to maintain postharvest quality and reduce disease development in some cultivars whereas boron application was not successful. However, research focused on the effect of these two elements on grey mould development in strawberry is limited. This study was designed to provide knowledge of how calcium and boron might contribute to the improvement of fruit quality and the reduction of susceptibility to grey mould, particularly for the South Australian-grown cultivars 'Aromas' and 'Selva'.

Preharvest application of calcium and boron to soil through fertigation did not improve postharvest quality of either 'Aromas' or 'Selva' fruit. However, the incidence of grey mould development on fruit was reduced following the preharvest calcium application. Postharvest calcium application delayed development of Botrytis fruit rot somewhat and calcium lactate was a potential alternative to calcium chloride for reducing the decay caused by *B. cinerea*. Calcium was shown to be immobile within the plants in that the amount of calcium in leaf tissues was always significantly higher than in fruit tissues regardless of the amount of calcium applied. However, the increased calcium in leaves did not influence the development of Botrytis leaf blight. Even though preharvest application of boron had no effect on Botrytis fruit rot, it appeared to reduce the size of lesions of Botrytis leaf blight on 'Selva'. This might be expected, given that the leaf boron content increased in response to preharvest boron application. The cultivar dependency for this response may be linked to other resistance mechanisms, given that boron did appear to reduce the lesions early (up to 4 days) after inoculation in 'Aromas'. However, the application of boron must be tightly controlled because strawberries do appear to be boron-sensitive and this also is

cultivar dependent. The knowledge obtained in this study suggests that the improvement of fruit quality and disease management may be determined, in part, by the amount of calcium and boron that can be successfully taken into the fruit.

7.1 Calcium and boron mobility may affect postharvest quality

External appearance, firmness, soluble solid contents (SSC), pH and titratable acidity (TA) are parameters used to determine quality of fruit. Although shrivel, loss of shine and calyx browning were slightly reduced by calcium application, there was no impact on other parameters. Boron application had no significant effect on any quality parameters. This indicates that preharvest application of calcium sulphate and boric acid did not improve fruit quality to a great extent nor slow fruit ripening and senescence processes in either 'Aromas' or 'Selva'. However, this may have been due to a lack of mobilisation of these two elements into the fruit.

Following calcium application, the amount of calcium increased in leaf tissues proportionally to that applied (r = 0.744 and 0.783 for 'Aromas' and 'Selva', respectively) but did not increase in fruit tissues, indicating that there is an uneven distribution of calcium in plants. This result has implications for calcium application in the strawberry industry, suggesting that combined with low mobility of calcium into the fruit and poor solubility of calcium sulphate in water, application of calcium sulphate to soil through fertigation is not likely to be successful in improving fruit quality.

In South Australia, strawberry growers have encountered calcium deficiency although additional calcium has been applied. Applying supplementary calcium is unlikely to be of benefit if calcium uptake by plants and/or calcium distribution in plants is limited. In the same plant, the calcium content in leaves is often higher than that in fruit, suggesting it may be due to poor calcium distribution rather than low calcium uptake (Conway *et al.*, 1994). Thus, the efficiency of calcium in improving fruit

quality and disease management through spray application of calcium directly to flowers and developing fruit merits further investigation.

As for calcium, the amount of boron in leaves increased with increasing boron concentration applied to the plants (r = 0.981 and 0.919 for 'Aromas' and 'Selva', respectively). Due to the lack of data on boron content in fruit, no conclusions could be made concerning boron mobility in plant tissues for 'Aromas' and 'Selva'. However, boron was relatively immobile within 'Elsanta' (Wójcik and Lewandowski, 2003) and 'Chandler' strawberry plants (Singh *et al.*, 2007) as indicated by higher boron content in leaves than in fruit. Furthermore, Gupta (1979) suggested that boron is not readily translocated from older to younger plant tissue. Thus, further research needs to be conducted to determine if application of boric acid through fertigation increases fruit boron content in 'Aromas' and 'Selva'.

However, there appears to be a narrow range between boron deficiency and toxicity, with evidence of damage via both in this study. Damage included leaf burning as well as flower abortion and death which consequently affected plant growth and yield. Given that strawberry is sensitive to boron and different strawberry cultivars require different amounts of boron for growth and fruit production, further research should be conducted to identify the most suitable range of boron to apply to 'Aromas' and 'Selva' strawberry plants to enhance plant growth and improve postharvest quality of fruit.

7.2 Impact of calcium and boron on grey mould

The development of Botrytis fruit rot is a major limitation to the storage life of strawberries and the preharvest application of calcium and boron provided mixed results. No definite conclusions could be made concerning the influence of boron application on grey mould development on fruit as, even though preharvest calcium sulphate application did not influence postharvest quality greatly, it prolonged shelf life of fruit of both 'Aromas' and 'Selva' by reducing the incidence of Botrytis fruit

rot. Conway *et al.* (1994) and Moline (1994) suggested that calcium taken into the fruit tissues is probably incorporated into the cell walls of fruit, conferring reduced susceptibility to fungal infection. This role, however, can be realised only if sufficient amounts of calcium can be translocated to the fruit.

In this study, given that calcium was not mobilised into fruit, the decrease of Botrytis fruit rot could be due to a reaction of calcium in combination with other nutrient elements such as boron, giving strengthened cell walls or tolerance. The success of application of calcium plus boron in increasing calcium and boron content in leaves and fruit, increasing fruit firmness and decreasing the incidence of grey mould has been reported for 'Elsanta' strawberry (Wójcik and Lewandowski, 2003) and 'Chandler' strawberry (Singh *et al.*, 2007). With this knowledge, further research is recommended to determine the effect of calcium plus boron in maintaining fruit firmness and delaying decay caused by grey mould in the cultivars 'Aromas' and 'Selva'. If it is found to be beneficial, such a strategy would assist strawberry growers to reduce losses due to Botrytis fruit rot.

The reduction in Botrytis fruit rot due to increased calcium in the leaf may also be due to a number of other reasons. Preharvest calcium application may have contributed to preventing infection in other plant parts such as flowers and leaves. Indeed, calcium application slowed the development of rot in uninoculated fruit and fruit developed from inoculated flowers (Chapter 3) suggesting that it may prevent or reduce latent infection of flowers. However, calcium did not decrease Botrytis leaf blight (Chapter 6) and so it seems unlikely that preharvest calcium has its effect through the reduction of leaf inoculum. The increase of calcium may have also prevented other nutrient element deficiencies, suggesting a role in ensuring the nutrient status of the plant is maintained and its ability to tolerate pathogens enhanced.

Although boron did not affect postharvest development of Botrytis fruit rot, the increased leaf boron content following boron application delayed the development of Botrytis leaf blight on 'Selva' leaves, suggesting a potential influence of boron in reducing the production of primary inoculum of *B. cinerea*. As infected strawberry leaf residues are a primary source of inoculum of *B. cinerea* (Maas, 1998), management of disease on leaves may reduce the inoculum produced and consequently, fruit rot in the field.

Because there appears to be a correlation between nutrient mobility and levels in leaves and fruit for calcium and boron, consideration of postharvest application is warranted.

7.3 Factors influence the amount of calcium taken into fruit during postharvest application

Due to the low mobility of calcium within the plant, postharvest calcium treatment may be the best method of increasing fruit calcium content (Conway *et al.*, 1994). However, the efficiency of postharvest calcium treatment in delaying fungal growth is likely to depend on the amount of calcium taken into the fruit during treatment. In this present study, factors such as calcium concentration, storage conditions (light and dark), period of time for calcium to penetrate into fruit and time of harvest influenced the development of Botrytis fruit rot, suggesting that these factors may influence the amount of calcium taken into fruit. To test this hypothesis, further research should be conducted to measure calcium in fruit after calcium treatment and after storage to determine if calcium really does change with storage time, with storage in light and dark, and with time of harvest.

Given the results showing smaller rot lesions on calcium-treated fruit than the corresponding untreated control, postharvest calcium application to late-season fruit is recommended to industry. However, the effectiveness of postharvest calcium application on Botrytis rot on fruit harvested at different times of the season needs to

be confirmed using fruit harvested at regular intervals throughout a single season and repeated over several years. If postharvest calcium application is found to be beneficial at certain times of the year, such knowledge would provide a strategy for industry to manage grey mould in fruit effectively, efficiently and with minimal chemical use.

7.4 Cultivar comparison

'Selva' is widely grown and produced in South Australia whereas 'Aromas' is a new cultivar, considered to be superior to 'Selva' for productivity and fruit quality (Shasta Nursery, 2006). In addition, 'Aromas' is relatively resistant to a number of diseases whereas 'Selva' is susceptible to disease and to infestation by two-spotted mite and red spider mite (Shasta Nursery, 2006). In this study, however, there were few differences between the two cultivars, with only fruit firmness and SSC differing.

Preharvest calcium application resulted in SSC of 'Selva' (6 – 7%) slightly higher than that of 'Aromas' (5 – 6%), indicating 'Selva' to be sweeter than 'Aromas' (Chapter 3). However, SSC of both cultivars were lower than the recommended acceptable level for strawberry which is 7% (Mitcham *et al.*, 2007). In the experiment on preharvest boron application, there was no difference in SSC between the two cultivars (Chapter 4), and only the SSC of fruit of both cultivars in the 0 ppm B treatment were above the acceptable level of 7% (according to Mitcham *et al.*, 2007). Fruit firmness of 'Aromas' was slightly greater than that of 'Selva' in both preharvest calcium and boron applications. Given its lower SSC and greater firmness, 'Aromas' may be more resistant to infection and disease development but have a less desirable flavour than 'Selva'.

However, slightly higher leaf boron content in 'Selva' than in 'Aromas' and the success in decreasing Botrytis leaf blight only in 'Selva' after boron application indicates a cultivar-related difference in boron response. This suggests that nutrition as a means of a disease control may be cultivar dependent.

7.5 Further research

Given the relative immobility of calcium in plants and the sensitivity of strawberry to boron toxicity, a number of further research possibilities exist for studying the effect of calcium and boron on strawberry fruit quality and disease development.

First, further research should be conducted to determine the influence of factors such as environment, plant age and soil on the uptake of calcium and boron to strawberry plants. Soil factors such as soil type, texture and pH are perhaps the primary factors determining plant nutrient uptake (Gupta, 1993). The changes in calcium and boron content with leaf age indicate that stage of plant growth may also influence calcium and boron uptake. With age, calcium content in strawberry leaves (Chiu and Bould, 1976) and boron content in corn leaves and tassels increased (Clark, 1975), but boron concentration decreased in broccoli, brussels sprouts and cauliflower (Gupta and Cutcliffe, 1973). In addition, plant calcium and boron uptake may also be affected by environmental factors such as temperature and soil moisture. At higher temperature and moist soil conditions, more calcium and boron are taken up by the plants than at lower temperatures and in dry soil (Klein and Ferguson, 1987; Gupta, 1993; Maas, 1998). Thus, the effect of these factors on calcium and boron uptake represents a significant area for future research.

To follow calcium and boron uptake and distribution in the plants, another potential area for further research would be analysis of uptake and distribution of calcium and boron in the plant using new technologies in imaging, such as labelling with radioactive isotopes of calcium and boron, and binding to fluorescent dyes followed by expose to fluorescent light (Till Photonics, 2007). An image is thus created which can be analysed according to intensity, ultimately reflecting the nutrient status.

However, a breeding program to produce new cultivars which are more efficient in calcium uptake and distribution and also to develop cultivars with boron efficiency

and tolerant of boron toxicity is perhaps a long-term solution for strawberry production in terms of improving fruit quality and disease resistance.

7.6 Conclusions

Calcium and boron showed a potential in delaying grey mould development in fruit and leaves, respectively, and may have the potential to improve postharvest quality if calcium and boron are taken into the fruit. Postharvest calcium treatment tended to be more effective in delaying development of Botrytis fruit rot when applied to late-season fruit. This finding may provide a basis for application in industry to reduce losses due to Botrytis fruit rot. However, it is likely that calcium and boron are relatively immobile within the plants. A breeding program to develop calcium efficient, boron efficient and boron toxicity tolerant cultivars may provide a long-term strategy for management of Botrytis grey mould and leaf blight of strawberry.

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Appendix 1

Papers published

Naradisorn, M., Klieber, A., Sedgley, M., Scott, E. and Able, A. J. 2006. Effect of preharvest calcium application on grey mould development and postharvest quality in strawberries. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 147-150.

Poster presented

Naradisorn, M., Sedgley, M., Scott, E. and Able A. J. Effect of calcium lactate on grey mould development in strawberry. *Australasian Postharvest Horticulture Conferences 2005*, 27-30 September 2005, Rotorua, New Zealand. Page 50.

Appendix 2

V-8 juice agar

V-8 juice	900 mL
Dextrose	15 g
Agar*	20 g

Fill up with distilled water to 1000 mL.

Adjust pH to 6.5 using 1M NaOH.

Autoclave for 20 min at 121^{o}C.

Pour into sterile Petri dishes once cool.

* Recommend to add agar after adjusting pH.

Appendix 3

Recommended nutrient levels for strawberry leaf tissues

From Reuter and Robinson (1997)

NOTE: This table is included on page 184 of the print copy of the thesis held in the University of Adelaide Library.

Appendix 4

Analyses of sand used in experiments

Nutrient	Concentration (mg/kg)		
	Mount Compass*	Golden Grove	Waikerie
Iron	480	0.06	570
Manganese	n/a	0.09	0.2
Boron	n/a	< 0.05	< 0.08
Copper	n/a	< 0.02	< 0.1
Molybdenum	n/a	< 0.02	< 0.2
Cobalt	n/a	< 0.02	< 0.1
Nickel	n/a	< 0.03	< 0.3
Zinc	n/a	0.12	< 0.06
Calcium	n/a	11.5	8.0
Magnesium	n/a	4.0	14.0
Sodium	n/a	3.2	9.8
Potassium	17.0	85.5	< 2.0
Phosphorus	3.0	< 0.1	1.9
Sulphur	2.8	6.0	1.7
Aluminium	2.0	0.18	123
Cadmium	n/a	0.009	< 0.06
Lead	n/a	n/a	< 0.7
Selenium	n/a	n/a	< 2.0

n/a = data not available.

* Data obtained from Yusuf Genc.