Effect of Calcium and Boron Nutrition on Grey Mould of Capsicum (*Capsicum annuum* L.) and Fruit Quality

By

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Table of Contents

Abstract

Capsicum (*Capsicum annuum* L.) is mostly cultivated in humid and warm conditions, which increases disease development, particularly grey mould caused by *Botrytis cinerea*. Infection of capsicum fruit by *B. cinerea* often occurs preharvest but symptoms of grey mould are not usually visible until after harvest making the pathogen difficult to control. Appropriate fertilisation that ensures calcium (Ca) and boron (B) is sufficient in plant tissues, especially in fruit, has been suggested as an alternative to fungicides for disease management. This research studied the infection pathway of *B. cinerea* and the effect of Ca and B on grey mould development and quality of fruit in two capsicum cultivars (cv. Aries and cv. Papri Queen).

Botrytis cinerea infected capsicum preharvest and flowers often died when inoculated at anthesis. The number of dead flowers increased when inoculum concentration increased. The extent of grey mould development on fruit inoculated preharvest was not affected by timing of inoculation [at anthesis, 3 days after anthesis (DAA) or 6 DAA], but was dependent on inoculum concentration and cultivar. When capsicum fruit were inoculated after harvest, grey mould developed most rapidly in red (R) fruit from cv. Aries and breaker red (BR) fruit from cv. Papri Queen. An inoculation of 10^6 conidia mL^{-1} caused more disease on fruit than 10^4 or 10^5 conidia mL^{-1}. Cv. Aries was more susceptible to *B. cinerea* than cv. Papri Queen regardless of whether inoculation occurred before or after harvest.

The effect of both soil and foliar application of boron (B), at different concentrations, on grey mould development and fruit quality of capsicum was examined. Preharvest B application, from transplanting to harvest when fruit were mature and red, using 0.05 or 0.1 mM H_3BO_3 via soil amendment or 2.0 or 7.0 mM H_3BO_3 as a foliar spray increased B concentration in leaves and fruit of both cultivars. However, soil application was more effective than foliar application in increasing B concentration in plant tissues. Foliar application of B at low concentrations (0.025 or 0.075 mM H_3BO_3) did not increase B concentration in plant tissue. Increasing B concentration in leaf and fruit tissue reduced grey mould

development on fruit inoculated with *B. cinerea* preharvest compared to the control, but did not affect grey mould development on red fruit inoculated with *B. cinerea* postharvest. Preharvest soil application of B increased shelf life of fruit, but did not affect quality of fruit including water content, firmness, total soluble solid content (TSSC) and titratable acidity (TA) at harvest or during storage. Symptoms of B toxicity were observed on leaves from plants that received high B concentration (0.1 mM H_3BO_3) in the soil, but no effect was observed on fruit.

Preharvest application of calcium (Ca) via soil amendment [1.5, 4.0 or 8.0 mM $Ca(NO_3)_2$] or as a foliar spray [0.5 or 1.0 % w/v mM $Ca(NO_3)_2$] increased Ca concentration in leaves, but did not increase Ca concentration in fruit, regardless of cultivar. Soil Ca application appeared to increase Ca concentration in leaf tissue more effectively than the Ca foliar spray. Ca concentration in leaf tissue from cv. Aries was significantly higher than in leaf tissue from cv. Papri Queen when plants received the same amount of Ca, regardless of application method. Ca treatment did not affect quality of fruit at harvest or during storage. Preharvest application of Ca reduced grey mould development on fruit that had been inoculated with *B. cinerea* preharvest, but did not reduce grey mould in fruit inoculated postharvest. Symptoms of Ca deficiency were observed on plants that received no Ca or low Ca concentration [1.5 mM $Ca(NO_3)_2$] from transplant to fruiting.

Dipping and vacuum infiltration with calcium chloride ($CaCl_2.2H_2O$) did not increase Ca concentration in flesh after treatment, but vacuum infiltration did increase Ca concentration in flesh after 10 days of cool storage (10°C). Ca treatment after harvest did reduce grey mould development on fruit, but did not affect the quality of fruit during storage. A directly inhibitory effect of Ca on fungal growth was responsible for reducing grey mould development on fruit.

In conclusion, capsicum was most sensitive to infection by *B. cinerea* at anthesis and high inoculum concentrations caused a greater disease incidence in capsicum fruit, regardless of whether inoculation occurred preharvest or after harvest. Reducing inoculum concentration, especially during flowering, is therefore recommended to reduce losses in capsicum. Preharvest application of Ca or B

may be used as an alternative method to reduce grey mould on capsicum fruit, but they had no effect on fruit quality. Postharvest application of Ca could also be recommended for cv. Aries fruit before or during storage for controlling grey mould on fruit. Findings in this research may therefore provide basic knowledge for management of *B. cinerea* in the capsicum industry.

Declaration

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Abbreviation

Abbreviation	Full term
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Communities
ASTA	American Spice Trade Association
B	boron
BR	breaker red
°Bx	°Brix
°C	Degrees Celsius
Ca	calcium
$CaCl_2$	calcium chloride
$Ca(NO_3)_2$	calcium nitrate
$CuSO_4$	copper sulphate
cv.	cultivar
DAA	days after anthesis
DAH	days after harvest
DPI	days post-inoculation
DG	deep green
DW	dry weight
EDTA	ethylenediaminetetraacetic acid
et al.	and others
e.g.	for example
FW	fresh weight
FAO	Food and Agricultural Organisation
Fe^{3+}-EDTA	Ethylenediaminetetraacetic acid iron (III)
Fig	Figure
g	gram
h	hour
H_3BO_3	boric acid
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometer
kg	kilogram

kgf	kilogram force
kGy	kiloGray
KCl	potassium chloride
KNO_3	potassium nitrate
KH_2PO_4	potassium dihydrogen orthophosphate
KOH	potassium hydroxide
K_2SO_4	potassium sulphate
L	litre
LSD	Least Significant Difference
$MgSO_4$	magnesium sulphate
$MnSO_4$	manganese sulphate
mg	milligram
min	minute
mL	millilitre
mm	millimetre
mm^2	square millimetre
mt	million tones
N	Newton
NaH_2PO_4	sodium phosphate dibasic
NH_4NO_3	ammonium nitrate
$(NH_4)_6Mo_7O_{24}$	ammonium molybdate tetrahydrate
PDA	Potato dextrose agar
PE	pectinesterase
PG	polygalacturonase
pH	power of hydrogen (negative log of H^+ concentration)
ppm	parts per million
RH	relative humidity
RO	reserve osmosis
SE	standard error
sec	seconds
TSSC	total soluble solid content
TA	titratable acidity

UC	University of California
UV	ultraviolet
$ZnSO_4$	zinc sulphate
w/v	weight by volume
µM	micromoles per litre
µL	microlitre
%	percentage

CHAPTER ONE

Introduction

Capsicum (*Capsicum annuum* L.), also called pepper, is a warm-season crop that is a member of the *Solanaceae* family. Capsicum fruit is the major source of red food colourant and pungency for spice production and can also have medicinal uses [Food and Agricultural Organisation (FAO), 2013]. For the period from 2001 to 2011 capsicum production increased by 17% such that by 2011 there was over 29.61 million tones (Mt) and 1.84 million hectares of cultivation (FAO 2013). However, capsicum fruit are susceptible to fruit rots, especially grey mould caused by *Botrytis cinerea* Pers. [teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel], which causes high postharvest losses (20 - 25%) (Fallik et al. 1996). *B. cinerea* is therefore a significant concern for growers and marketing operations in many countries.

Botrytis cinerea also causes grey mould disease in a wide range of economically important plants, and it has traditionally been considered as a non-specialised necrotrophic fungus that multiplies on debris of a broad range of plant species. Following infection during flowering and/or the early stages of fruit development, *B. cinerea* may remain latent until environmental conditions are suitable and ripening causes physiochemical and biochemical changes in the fruit, leading to the development of grey mould (Droby and Lichter 2004), making fruit unmarketable (Utkhede and Mathur 2003). Fungicide application is the most commonly-used method to control fungal pathogens preharvest and postharvest. However, fungicides may be toxic to human health and damage the environment. Alternative means of control, such as nutrient application to plants, could minimise the use of fungicides. The use of nutrient application might be a potential way to control diseases as well as improving plant growth and fruit quality (Fallahi et al. 1997; Hansch and Mendel 2009).

Calcium (Ca) and Boron (B) are essential nutrients for plant growth. Adequate Ca in plants has been demonstrated to increase cell wall strength and thickness. Calcium may also increase plant defence responses to infection by fungi (Benhamou 1996). The role of B in maintaining the structural integrity of plant

membranes is well known (Sams 1999; Hewett 2006). There are some previous studies describing the positive effects of these two nutrients on disease development in plants and in fruits after harvest, such as bean and tomato (Elad and Volpin 1993), table grape (Amiri et al. 2009) and strawberry (Wójcik and Lewandowski 2003; Singh et al. 2007). However, there is a lack of understanding about the effect of Ca and B on grey mould of capsicum and quality of capsicum fruit. Therefore, this research aimed to investigate the effect of Ca and B nutrition on grey mould of capsicum and fruit quality.

CHAPTER TWO

General literature review

2.1 Capsicum

Capsicums (*Capsicum annuum* L.) originated in South and Central America (Burt 2005). Capsicums are primarily classified as one of three cultivar types: capsicum, paprika and chilli. Capsicum is a sweet non-pungent fruit often called bell pepper and used for fresh consumption. Paprika is non-pungent and used to produce spice, while chillies are smaller and hotter than non-pungent capsicum (Bosland et al. 1996 in Klieber 2000) and are used in both spice and sauces (Rajput and Paruleke 1998).

Capsicums are planted world-wide and the production has increased steadily with international trade being valued at approximately US$3.1 billion (FAO 2013). Capsicum normally grow in warm conditions with 16°C to 21°C on average being best for fruit setting, high yields and good quality fruit (Burt 2005). Asia produced two-thirds of world's capsicum in 2011 (20.51 Mt) (FAO 2013). Capsicum are grown throughout Australia in tropical and subtropical areas (primarily in Queensland, South Australia and Victoria) with the total capsicum production in 2012 of 36,600 tonnes (Australian Bureau of Statistic, 2013). Capsicums can be grown on many different kinds of soil with a wide range of pH (5 - 9) but its optimum is well-drained soil at a pH of 5.5 to 6.5 (Burt 2005).

Capsicum is self-pollinating and fruit are normally mature (fully grown and light green in colour) approximately 30 to 35 days after anthesis, with fruit taking a further 20 to 25 days to turn red depending on season (Burt 2005; Rajput, 1998). Harvesting at the light green stage affects fruit quality because fruit colour does not change properly to red, while fruit harvested at the breaker stage (40% to 50% red surface colouration) will normally ripen and develop colour sufficiently (Pham 2007). Postharvest changes in fruit will be discussed in the next section.

2.2 Postharvest changes in capsicum

Based on their postharvest physiology and biochemical changes, fruit have been divided into two groups: climacteric and non-climacteric fruit (Biale 1981). In

climacteric fruit, respiration peaks before ripening and this is associated with significant changes in sugar content, colour and texture. Ethylene production also increases sharply to reach a peak and remains at a relatively high rate throughout the ripening period (Rhodes 1980). The autocatalytic activity of ethylene plays an important role in the coordination and completion of the ripening process of climacteric fruit (Giovannoni 2001). In contrast, there is no peak in respiration or ethylene production in non-climacteric fruit and ripening processes are not sensitive to ethylene. Capsicum is difficult to classify as either climacteric or non-climacteric due to variation amongst cultivars and species. Saltveit (1997) did not consider capsicums to be climacteric because there was a lack of the typical increase in respiration and ethylene production during ripening. Limited ethylene production has been reported in the paprika-type cultivar, cv. Papri Queen, and the bell pepper, cv. Aries (Pham 2007), while fruit from the chilli cv. Changjiao did not respond to ethylene or have increased respiration during ripening (Lu et al. 1990). Even though bell pepper from cv. Maor at the green and red stages had a significant increase in ethylene, there was very low respiration during storage (Lurie et al. 1986). Together, these results suggest that most capsicum is probably non-climacteric.

Changes in colour, firmness and sugar content during ripening of capsicum fruit on the plant have also been widely investigated. Chlorophyll commonly decreases and is absent from tissues when the fruit turns fully red (Mendez and Mosquera 2002). Firmness has also been shown to decline during ripening due to the breakdown of the fruit cell walls (Brady 1987). These changes are caused by the increasing activity of hydrolase enzymes, including polygalacturonase (PG) and pectinesterase (PE) (Sethu et al. 1996). The hydrolase enzymes, especially PG, are reported to be absent or inactive when fruit are not ripe, but to have high activities when fruit are ripe (Harpster et al. 1997). During ripening, the neutral sugars decreased dramatically, from 25% initially (1st day of harvest) to 15% (21st day of harvest) (Sethu et al. 1996). The change in total soluble solid content (TSSC) in capsicum fruit also depends on ripening stage (Pham 2007). The TSSC of fruits which ripened on the plant increased greatly and reached a peak when fruit turned

full red and was higher than for fruits harvested at the deep and light green stages in Papri Queen, Aries and Caysan cultivars.

Postharvest loss in capsicum is related to shriveling (weight loss), nutrient reduction and rot caused by diseases. Although weight loss occurs, the extent of this is limited because peppers have a thick waxy layer on their epidermis (Kissinger et al. 2005). Disease is therefore the main factor limiting the shelf life of capsicum (Sharma et al. 2009). Most postharvest diseases are caused by fungi, including white mould caused by *Fusarium solani*, black mould caused by *Alternaria alternata* and grey mould caused by *Botrytis cinerea* (Barkai-Golan 2001). Several species of bacteria, such as *Bacillus polymyxa* and *Erwinia carotovora* ssp. *carotovora*, also cause rotting, which may occur in fruit from any region growing crops (Snowdon 1991). Under suitable conditions, fungi germinate on the surface of the host to penetrate into the host tissues and to develop there (Barkai-Golan 2001). Seedlings and ripening fruit were reported to be susceptible to infection by *Colletotrichum truncatum* and infected leaves served as a potential primary inoculum source for fruit infection (Ranathunge et al. 2012). Seed, placenta and pericarp can also be infected by fungi causing a latent infection where symptoms are not observed until postharvest (Elad and Shtienberg 1995). *B. cinerea* is considered to be the most important disease in many countries and efficacy of fungicides is being lost because of development of resistance. Development of alternative means to control fungi may therefore be essential to contribute to sustainable production.

2.3 Grey mould

Grey mould (also called Botrytis fruit rot or ash mould) caused by *Botrytis cinerea* Pers. [teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel], causes many different symptoms. *B. cinerea* causes soft rot associated with destruction and water-leaking of parenchyma tissues and then a grey mass of conidiophores and conidia appears rapidly. These are the most common symptoms on leaves and fruits with soft tissues (Williamson et al. 2007). Grey mould also might occur anywhere on the fruit, including the stem-end and blossom-end (Snowdon 1991) (Fig 2.1).

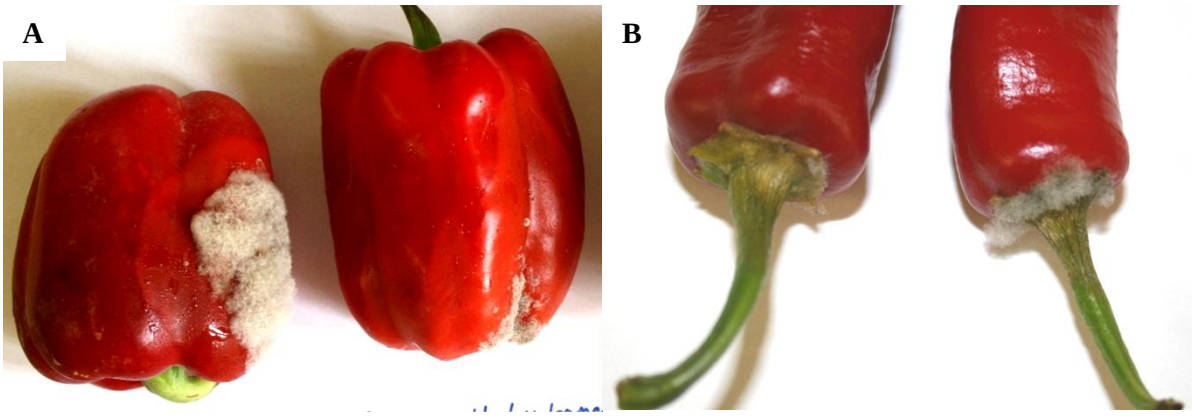

Fig. 2.1 Grey mould on capsicum fruit from cv. Aries (**A**) and cv. Papri Queen (**B**)

In capsicum fruit, infected tissue is water-soaked and brown-grey, and grey-brown conidia can develop accompanied by small black sclerotia (Snowdon 1991). *Botrytis cinerea* infects tissues at many developmental stages, even seedlings, and remains latent for long periods before causing rot quickly when environmental conditions are optimal and the host physiology changes (Droby and Lichter 2004). The life cycle for *B. cinerea* can be divided into two main modes depending on the weather and time of year (Fig 2.2). In the winter, the fungus can persist in dead organs or the soil as resting bodies (sclerotia and mycelia) or in infected plant debris (Snowdon 1991). In early spring when the weather is warmer, the mycelia and sclerotia become active and germinate to produce conidiophores on the surfaces of the infected plant debris. The conidia are disseminated from conidiophores by wind, rain-splash, irrigation (Maas 1998) and by human hands to fruit (Plakidas 1964; Barkai-Golan 2001). In the summer, the fungus does not enter a resting period, but produces conidiophores directly on infected tissue. In the presence of moisture, conidia on infected organs produce fresh mycelia which quickly spread in the tissue, causing cells to collapse and the tissue to be destroyed. Conidia commonly infect susceptible tissue first by germination and form appressoria which allow penetration via wounds. Mycelia then spread to other tissues when resistance of the fruit to the pathogen decreases (discussed in more detail in Section 2.3.2).

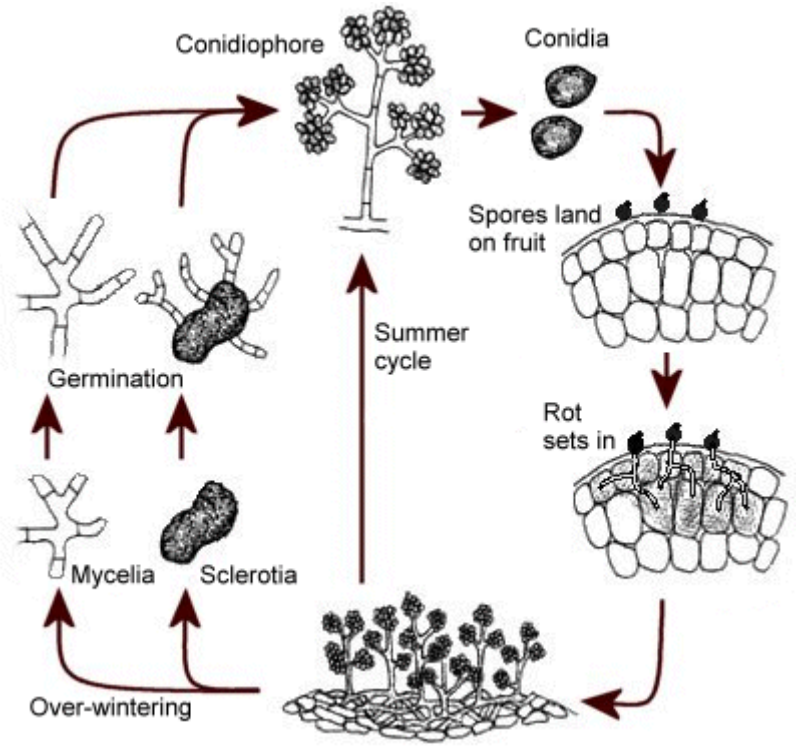

Fig. 2.2 Lifecycle of *Botrytis cinerea* [modified from (Winedoctor 2010)]

B. cinerea re-enters the reproductive stage by generating conidiophores again and releases fresh conidia. This process is constantly repeated during appropriate conditions in the summer and the fungus only enters the over-wintering cycle when cooler conditions occur (Williamson et al. 2007).

2.3.1 Development of grey mould disease

Conidia of *B. cinerea* are released from conidiophores by air currents in humid weather and usually develop on dead plant organs and spread quickly into susceptible plant parts, especially wounded tissues. In presence of nutrients and moisture on wound sites, conidia germinate and hyphae colonise plant tissue causing cell death. Moderate temperature (15 to 25°C) with high humidity conditions or a wet surface are optimal for grey mould to develop (Maas 1998). However, a number of conditions have been shown to slow disease development, including cold temperature or hot and dry weather (Snowdon 1991; Williamson et al. 2007). Light also has effects on *B. cinerea* at different wavelengths. Elad and Shtienberg (1995) indicated that sporulation in *B. cinerea* was reduced by increasing the ratio of blue to ultraviolet (UV) light which passed through

polyethylene film. In addition, fungal development decreases at low oxygen and high carbon dioxide concentration. Mycelial growth of *B. cinerea* and *Fusarium roseum* was inhibited by over 50% when the oxygen level was 4%, and germination of *B. cinerea* was inhibited by more than 90% in an atmosphere of 16% carbon dioxide compared to ambient conditions (Wells and Uota 1970). Because the carbon dioxide level is high inside capsicum fruit (~ 30 mg kg^{-1} h^{-1}) (Villavicencio et al. 1999), *B. cinerea* may favour development in the outer tissues of capsicum fruit.

2.3.2 Infection pathways

Experiments in previous research have confirmed that flowering is the most important time for *B. cinerea* to infect plants and cause subsequent infection of fruit such as strawberry (Jarvis and Borecka 1968) and grapes (Keller et al. 2003). Conidia can germinate in drops of free water on the petal or any part of the flower, and then penetrate through the senescing parts, into the edge of the receptacle where they form mycelia that then become dormant as a latent infection (Barkai-Golan 2001). During fruit ripening the cell wall becomes softer due to activity of the enzymes, PG and PE (Brady 1987). Mycelium of *B. cinerea* then invades and develops in the fruit, causing rot after harvest (Williamson et al. 2007). When tomato fruit were infected by *B. cinerea* during flowering, grey mould only developed at the stem-end when fruit were stored (Lavy-Meir et al. 1989). *B. cinerea* also can infect fruit directly in the field via airborne conidia (Barkai-Golan 2001), especially where fruit have been damaged during harvest, handling and transport causing wounds and bruises. However, the infection pathway of *B. cinerea* in capsicum is not well understood and it is not known if the flower stage is the most susceptible to *B. cinerea*. As such, the activity of *B. cinerea* in capsicum was investigated in this study.

2.4 Disease control methods

Because capsicum is mostly grown in glasshouses or warmer regions in the field, both of which favour development of *B. cinerea*, grey mould needs to be controlled. Which methods are used depends on cultural practice and the desired quality of products. However, three major methods of disease control commonly

exist; physical or cultural, chemical and biological methods (Elad and Shtienberg 1995).

2.4.1 Physical or cultural methods

As noted above, grey mould is stimulated by moderate temperature and high humidity. Thus in crop management, an open canopy is necessary to provide good air movement and high light interception so that free water dries as soon as possible. Avoiding rainfall during the blossom period by covering the crop with plastic can reduce disease in strawberry by 90% compared with open field plants (Williamson et al. 2007). In addition, removal of plant debris after each growing season can reduce the amount of inoculum (Elad and Shtienberg 1995). Hence, removal of inoculum and making conditions unsuitable for survival of the pathogen are effective ways to minimise infection by *B. cinerea*.

Physical methods after harvest, such as heat treatment, ionising radiation and UV illumination, are used to kill and inhibit fungi and bacteria on fruit. Heated fruit were often harder than non-heated fruits during storage (Klein and Lurie 1991), helping fruit to resist pathogen infection. In a study by Fallik et al. (1996), the authors treated red sweet pepper by dipping these fruit in hot water at 50°C for 3 minutes. Decay development caused by *B. cinerea* was completely inhibited in both naturally-infected fruit and artificially-inoculated fruit. However, heat damage was shown in fruit when dipping at a temperature hotter than 50°C for more than 3 min.

Ionising radiation may be harmful for living cells of pathogens and it may also prevent decay by delaying ripening and senescence (Barkai-Golan 2001). Barkai-Golan et al. (1971) found that radiation of 2 kGy from CO^{60} (cobalt) extended the shelf-life of strawberry naturally infected by *B. cinerea* from 3 to 10 days at 15°C. UV at low doses has a germicidal activity that can reduce diseases in a wide range of fruits and vegetables (Wilson et al. 1994). Additionally, UV treatment has been found to delay the ripening process in some commodities, such as tomato and peach (Liu et al. 1993), leading to an indirect reduction in their susceptibility to infection. Inoculated berries treated with UV-C doses of 0.125-0.5 kJ m^{-2} had a

significantly lower incidence of infection and a reduction of *B. cinerea* compared with control berries (Nigro et al. 1998).

2.4.2 Chemical methods

Although chemical methods of disease control include fungicides (compounds lethal to fungi) and fungistatins (compounds that inhibit fungal growth), fungicide application is more common on seed or plant surfaces either to kill fungal spores or prevent their germination (Persley 1993). Pre-harvest fungicide application is an effective way to reduce infections initiated in the field (Barkai-Golan 2001) and to prevent the formation of latent infections in the young fruit (Sommer et al. 1973). However, preharvest fungicides do not protect fruit if wounded during postharvest handling, so fungicide application is also needed in the postharvest period. Postharvest fungicide application should be done as soon as possible after harvest to prevent mycelial growth in the host tissue (Barkai-Golan 2001).

However, there are rising concerns about increased chemical use in agricultural systems because of the development of strains of fungi resistant to chemicals (Sharma et al. 2009). Using chemicals might be potentially harmful for human health and damage the environment. Therefore, alternative and more environmentally friendly methods such as biological control are now necessary to reduce fungicide use in agricultural systems.

2.4.3 Biological methods

Biological control (biocontrol) has become a focus for research to reduce the presence of fungicide residues in fresh food crops and to slow the development of pathogens resistant to major fungicides. Biological control has been used to antagonise preharvest and postharvest pathogens (Barkai-Golan 2001) and occurs via four main modes of action: the secretion of an antibiotic compound by the antagonist; competition with pathogens for nutrients at wound sites on the plant tissue; secretion of enzymes to injure the pathogen; and induction of host defence mechanisms (Droby et al. 1992; Wilson et al. 1994). The effectiveness of a biological agent depends on factors such as time of application, presence of moisture in the wound, and the number of pathogen conidia or antagonist concentration. Smilanick (1994) stated that biocontrol should be applied to the

wound site prior to the arrival of the pathogen. The antagonist yeast *Candida oleophila* controlled *B. cinerea* more effectively when applied to a fresh wound than a dry wound (Mercier and Wilson 1995), and the greatest antagonist activity of *Trichoderma* was observed at the highest concentration of the antagonist and the lowest inoculum levels of the pathogens (Mortuza and Ilag 1999). Similarly, an increase in the concentration of yeast *Saccharomyces cerevisiae* from 10^5 to 10^7 cells mL^{-1} significantly enhanced their antagonistic activity to *B. cinerea* in table grapes (Nally et al. 2012). Schena (1999) indicated that application of the yeast-like fungus, *Aureobasidium pullulans,* at 10^8 and 10^7 cells mL^{-1} controlled *B. cinerea, Rhizopus stolonifer* and *Aspergillus niger* on table grape, and *B. cinerea* and *R. stolonifer* on cherry and tomato. When wound sites in apple fruit were inoculated with *Candida oleophila* prior to inoculation with *B. cinerea,* the percentage of fruit with grey mould after 14 days storage was significantly reduced compared with the control (Mercier and Wilson 1995). The application of the yeasts *Rhodotorula rubra* and *Candida pelliculosa* to wounded tomato before inoculation with *B. cinerea* was reported to reduce the diameter of lesions by more than 60% compared to the control after 1 week (Dal Bello et al. 2008).

In addition, host protection plays a significant role in reducing the incidence of disease. Plant have the ability to detect invading pathogens and respond by producing toxic chemicals or defence-related proteins against the pathogen (Ferreira et al. 2007). Capsidiol, a phytoalexin which is a natural defence mechanism to protect against disease infection (Egea et al., 1996). However, the ability of the host to protect against infection differs between crops and cultivars. All capsicum cultivars grown in Australia are susceptible to *B. cinerea*. Although some level of control is available by keeping the humidity low in the glasshouse (Conrad, L., Seminis Sales, Monsanto, Personal Communication, March 2010), with no true resistance, other alternative methods must be sought.

An adequate supply of nutrients, especially micronutrients, is important for plant growth, fruit quality and disease resistance. The application of nutrients to plants to improve their resistance is therefore considered to be a biological method to reduce pathogen diseases. Calcium induces the signaling pathways that lead to plant defence responses such as the production of lignin-like polymers that cannot

be easily degraded therefore preventing the pathogen from spreading (Benhamou 1996). Calcium (Ca) and boron (B) have been demonstrated to improve fruit quality and reduce disease incidence in crops, such as bean and tomato (Elad and Volpin 1993), table grape (Amiri et al. 2009), and strawberry (Wójcik and Lewandowski 2003; Singh et al. 2007). However, quality of sweet pepper cv. Orlando at harvest, including firmness, TSSC or acidity was not affected by Ca or potassium nutrient treatments (Rubio et al. 2010). There has been limited research on the effect of Ca and B on grey mould development and fruit quality in capsicum. The role of these elements will be discussed below.

2.5 Calcium and boron application to control disease

Both Ca and B are essential nutrients for plant growth, but they also have a role in protecting plants from disease. Calcium is an essential component of the plant cell wall and confers structural rigidity and firmness (Maas 1998; Easterwood 2002). Moreover, adequate Ca may increase the resistance of the plant to diseases because the Ca^{2+} forms cross-bridges between adjacent pectic acids or between pectic acids and other polysaccharides which makes the cell wall more resistant to the action of the pathogen's pectolytic enzymes (Preston 1979; Conway et al. 1994a). Calcium is thought to have a key role in the induction of plant defence responses (Benhamou 1996). The role of B is linked to cell wall synthesis by cross-linking of cell wall polysaccharides and the structural integrity of biomembranes as well (Marschner 1995; Hansch and Mendel 2009). Therefore, fruit becomes less susceptible to attack by the pathogen. Furthermore, application of Ca and/or B significantly deceased the activity of the fruit softening enzymes PG and PC (Dong et al. 2009). As a result, the fruit may display better resistance to grey mould development.

Calcium and B concentration in plant tissue may be less than optimal because of some major factors. Firstly, both Ca and B are immobile in plants and poorly translocated to actively growing tissues and fruits. Therefore, Ca and B content was often higher in older tissues than growing tissues and higher in the leaves than in fruit (Gupta 1979; Reuter and Robinson 1997). Moreover, Ca is taken up less effectively than some other nutrients, such as potassium (K), sodium (Na) and

ammonium (NH_4) (Kirby and Pilbeam 1984). Boron uptake was less by roots of aged plants than young plants (Marschner 1995). These factors cause blossom-end rot and blackheart in celery, tomato and pepper (Geraldson 1957). In addition, environmental factors also affect the uptake of Ca and B. Increasing soil pH and wet winters both cause B deficiency (Mengel and Kirkby 1987). Salinity and high temperature are also reported to reduce Ca and B content in plant tissues (Mengel and Kirkby 1987; Taylor and Locascio 2004).

The interaction of Ca and B may affect plant growth and disease resistance. A high Ca concentration in soil caused a B deficiency in tomato (Geraldson 1957; Dong et al. 2005), while B sprays increased the mobility of Ca and the Ca concentration of apple fruit as well (Shear and Faust 1971). Boron sprays during blossoming effectively reduced bitter pit in apple, which is caused by Ca deficiency (Dunlap and Thompson, 1959 in Mengel and Kirkby, 1987). In addition, B has a role in keeping Ca within the wall which is important for maintenance of cell wall integrity. The host would therefore have reduced susceptibility to the pathogen (Stangoulis and Graham 2007).

Botrytis cinerea often infects fruit in the preharvest period as a latent pathogen (see section 2.3.2) and grey mould symptoms are evident only in the postharvest period. In order to minimise pathogen development, it is necessary to ensure Ca and B are present at a high enough level in both the preharvest and postharvest period.

2.5.1 Preharvest application

Calcium and B deficiencies cause serious problems in the plant. Calcium deficiency was related to poor germination of seed, reduction in tissue growth and absence of fruit in tomato and paper (Taylor and Locascio 2004). Calcium deficiency also led to reduced firmness of tissue due to dissolution of the cell wall (Mengel and Kirkby 1987), increased blackheart in celery and blossom-end rot in watermelon, pepper, eggplant and tomato (Taylor and Locascio 2004). Boron deficient plants have abnormal development of fruit and restricted flower and fruit development (Jones 1998). In some plants, inadequate B causes fruit to be very small and of poor quality (Mengel and Kirkby 1987).

The literature suggests a wide range of optimal concentrations for Ca in plants due to variation in requirement among plant species. Generally, Ca concentrations in plants and in fruit were from 0.1 to 5% and 0.2 to 0.3% of the dry weight of the tissue, respectively (Taylor and Locascio 2004). In bell pepper, the concentrations of Ca in the youngest mature leaf for deficient, adequate and high range of Ca content are 1.00 - 1.29%, 1.30 - 2.80% and >2.80%, respectively (Reuter and Robinson 1997). In contrast, B has a very narrow range between deficiency (23 – 24 mg kg^{-1}), adequately (25 – 75 mg kg^{-1}) and toxicity (>75 mg kg^{-1}) (Reuter and Robinson 1997).

Preharvest Ca and B applications have been demonstrated to reduce grey mould development and/or improve fruit quality in some crops (Table 2.1). Soil application of Ca has occurred at concentrations ranging from 1.0 to 8.0 mM and for B was from 0.01 to 0.2 mM. However, B has been reported to be toxic in capsicum at concentrations higher than 1 mg kg^{-1} (Nabi et al. 2006). Some salts containing Ca or B were used in preharvest applications. Calcium nitrate [$Ca(NO_3)_2$] is often used in industry or in research as a source of Ca because it is more soluble than calcium sulphate ($CaSO_4$) or calcium chloride ($CaCl_2$), and boric acid (H_3BO_3) is most commonly used as a boron-containing compound for soil application.

Foliar Ca and B applications have been used widely as an effective method to increase Ca and B concentration in fruit due to immobility of these elements when applied to soil. Calcium chloride or $Ca(NO_3)_2$ has most commonly been applied as a Ca-containing compound and Ca concentrations of foliar spraying solutions have a range from 0.8 to 2 % $CaCl_2$ or 1 - 2% $Ca(NO_3)_2$ (Table 2.1). The B-containing compound most commonly used in foliar sprays is H_3BO_3 at concentrations ranging from 0.025 to 0.1%. The first time of spraying is often when the petals drop, with later applications at 7-day intervals in strawberry and 14-day intervals in pepper (El-Tohamy et al. 2006; Singh et al. 2007).

Preharvest Ca and B applications have been reported to increase marketable yield of fruit, improve quality, appearance and reduce blossom-end rot in capsicum

Table 2.1 The effect of preharvest Ca and B application in some fruit crops

Application method	Crop	Treatment	Effect	References
Soil amendment	Bean and tomato	1-3 mM $CaCl_2$ or $Ca(NO_3)_2$	Decreased severity of grey mould Reduced severity of fruit ghost spots	(Elad and Volpin 1993)
	Apple	27 mg B kg^{-1} soil (H_3BO_3)	Increased fruit yield and higher fruit soluble solid content	(Wojcik et al. 2008)
	Sweet pepper (four cultivars)	$Ca(NO_3)_2$ at low, medium and high level (1.5, 4 and 8 mM)	Increased marketable yield and improved fruit appearance at 4 and 8 mM	(Rubio et al. 2010)
		B at rate of 0, 1, 2, 4, and 8 mg B kg^{-1} soil (H_3BO_3)	Maximum crop biomass at ~1 mg B kg^{-1} Toxic for plants at higher than 1 ppm	(Nabi et al. 2006)
Foliar spray	'Asgari' grape	$CaCl_2$ at 0, 0.8, 1.2, 1.6 and 2% w/v	Significantly improved berry firmness, colour and appearance associated with increase of Ca concentration Reduced *Botrytis* infection and berry drops Leaf injury (lesions) was sometime observed at high Ca level (2% w/v) but not observed on cluster or fruit	(Amiri et al. 2009)
	Anna apple	0.0, 0.025, 0.05, and 0.1 % H_3BO_3	Increased in fruit yield and improved fruit physical properties and decreased in severity of blossom-end rot	(Khalifa et al. 2009)
	Strawberry	– $CaCl_2$ at rate of 2 kg Ca $ha^{-1}spray^{-1}$ – 150 g B (H_3BO_3) $ha^{-1}spray^{-1}$ – Ca + B in combination at same levels	Significantly reduced albinism and grey mould for Ca and Ca + B treatments Significantly decreased malformation in B-treated fruits Increased fruit firmness and reduction in disorder incidence compared to B alone and control	(Singh et al. 2007)
	Pepper	$CaCl_2$ at 1 and 2 % w/v	Improved yield	(El-Tohamy et al. 2006)
		$Ca(NO_3)_2$ 1 - 2% + 0.5% Tween 20	Significantly increased Ca concentration in leaf, fruit and reduced blossom-end rot	(Schon 1993)

(Schon 1993; Keinan et al. 2000; Rubio et al. 2010). However, the effect of Ca fertilisation on grey mould of capsicum is not well understood. Boron fertilization at 1 mg kg^{-1} H_3BO_3 was the critical level for growth of capsicum (Nabi et al. 2006), but the effect of B on grey mould of capsicum and fruit quality remains unclear. Foliar application of Ca at 1.0 or 2.0% $CaCl_2$ improved growth parameters of capsicum plants (plant height, number of branches and fresh weight of plants) and yield significantly compared to the control (El-Tohamy et al. 2006). However, the effect of foliar application of Ca on quality of capsicum fruit has not been reported. Therefore, this study focused on the effect of preharvest Ca and B application on grey mould development and fruit quality in capsicum.

2.5.2 Postharvest application

Both Ca and B have limited mobility in the plant phloem and the lowest concentration is found in the fruit (Mengel and Kirkby 1987). Postharvest application has been an effective way to increase concentration of nutrients in fruit. Postharvest Ca application is a way of applying Ca directly to harvested fruit via the standard practice of dipping fruit in solution at ambient pressure (normal dipping) or active infiltration (pressure infiltration or vacuum infiltration) (Conway et al. 1994a). Calcium chloride has been commonly used as a dipping solution to increase firmness for whole fruit and fresh-cut commodities (Martín-Diana et al. 2007). However, B is not commonly applied postharvest due to toxicity of inorganic boron-containing compounds to human health if adults consume more than 13 mg B per day (Nielsen 1997).

Normal dipping of fruit in a $CaCl_2$ solution increased Ca concentration of the skin and outer layers of the fruit, but an active infiltration procedure was more effective in increasing Ca concentration in flesh of apples (Conway et al. 1994a). Increasing Ca concentration in apples by vacuum infiltration reduced incidence of bitter pit and the quality of fruit during storage was better than fruit that were dipped (Scott and Wills 1977). Conway (1982) examined the effect of postharvest Ca application on decay of ‘Delicious’ apples. Fruit were treated with a range of $CaCl_2$ solutions (0, 2, 4, 6 and 8%) by dipping, vacuum infiltration or pressure infiltration for 2 min. Pressure infiltration of 8% $CaCl_2$ resulted in the least decay

and the highest concentration of Ca. Vacuum infiltration also increased Ca concentration in flesh, but did not reduce decay. However, tissue damage prevents the use of active infiltration of Ca in some soft fruit, such as capsicum, so dips are the preferred method to improve postharvest quality of soft fruit and control decay. For example, dipping in 1% $CaCl_2$ for 1 min recommended for diced Roma tomato fruit to increase Ca concentration in fruit, enhance firmness and decrease soluble pectin (Magee et al. 2003).

Calcium treatment delayed decay caused by pathogens due to either Ca increasing cell wall strength or directly inhibiting the pathogen growth itself (Barkai-Golan 2001). Grey mould development in the strawberry cultivar 'Selva' was reduced significantly by normal dipping in calcium lactate solution (1500 mg kg^{-1} Ca) or $CaCl_2$ solution (4500 mg kg^{-1} Ca) (Naradisorn 2008). Although the chloride anion from calcium chloride could have a potential effect on fungal growth, the inhibitory effect of Ca cation on conidial germination and germ-tube elongation of *B. cinerea* has been confirmed *in vitro* (Wisniewski et al. 1995).

Postharvest Ca treatment has potential benefits in reducing grey mould development and extending fruit shelf life in some fruits, as discussed above. However, the effect of this element on the storage life of capsicum fruit and grey mould development is not well understood. Thus, its effect on postharvest quality and grey mould development in capsicum fruit was examined in this study.

2.6 Literature summary and aims of study

Capsicum plants are susceptible to *B. cinerea* which causes grey mould. Grey mould is responsible for the main losses during the preharvest and postharvest periods. The flowers and wound sites on fruit have been demonstrated to be the most susceptible to infection by *B. cinerea* in crops, such as grape, strawberry and tomato. However, how *B. cinerea* infects the capsicum plant is unclear. Therefore, aim of this study was to conduct a comprehensive study of how *B. cinerea* infects capsicum fruit in both preharvest and postharvest periods (Chapter 3, Le at all. 2013).

Fungicide application has been used to control pathogens and diseases in both the preharvest and postharvest periods. Using fungicide may cause a risk to human health and the environment. The method of Ca and B application has been shown a potential to improve fruit quality and decrease grey mould development in a number of fruit crops. However, an understanding of the effect of Ca and B on grey mould of capsicum and fruit quality remains unclear. Therefore, this study aimed to examine the effect of preharvest and postharvest application of Ca (Chapter 5 and 6) and preharvest application of B (Chapter 4) on postharvest quality and grey mould development on capsicum fruit.

CHAPTER THREE

Infection pathway of *Botrytis cinerea* in capsicum fruit

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CHAPTER FOUR

Effect of preharvest boron applications

4.1 Introduction

Capsicum is mostly cultivated in the glasshouse or in the field in warmer regions, both of which favour development of disease on fruit, particularly grey mould which is caused by *Botrytis cinerea*. *Botrytis cinerea* infects flowers and young fruit but then remains latent until ripening, such that grey mould symptoms are usually not visible until after harvest (Chapter 3), making the pathogen difficult to control. In addition, because the cultivars of bell pepper most commonly grown in Australia are susceptible to *B. cinerea*, control options may be necessary. Fungicide application has been the most commonly used method to control this pathogen both preharvest and postharvest. However, using fungicides raises a risk to human health and may damage the environment or allow development of strains of fungi resistant to chemicals (Elad and Shtienberg 1995; Sharma et al. 2009). Therefore, alternative means to control *B. cinerea* are necessary to reduce the use of chemicals in agricultural practice. Nutrient deficiency, especially micronutrients have been demonstrated to reduce resistance of plants or elevate pathogen development (Marschner 1995). Ensuring adequate nutrition may therefore be an important way to support resistance of plants to *B. cinerea*. Boron (B) is a key element that contributes to resistance of plants (Conway et al. 1994a; Marschner 1995).

An adequate B level in the plant has been demonstrated to reduce disease incidence in fruit, due to the involvement of B in the structural integrity of biomembranes and in cell wall synthesis by cross-linking of cell wall polysaccharides (Marschner 1995; Hansch and Mendel 2009). When B deficiency occurs in plants, cell wall and membrane structure may be compromised and the cell wall becomes more susceptible to attack by the pathogen (Cakmak and Römheld 1997). Although B accumulates in growing tissues of plants, B concentration is often higher in the leaves than in fruit (Gupta 1979; Reuter and Robinson 1997) because B is phloem immobile (Blevins and Lukaszewski 1998). Furthermore, B has a very narrow range between deficiency and toxicity. Mengel

and Kirkby (1987) indicated that soils with less than 1 ppm water soluble B may not supply enough B for plant growth, while values above 5 ppm may be toxic. Therefore, providing B at an optimal level is important. Postharvest B application is not commonly used due to toxicity of inorganic boron-containing compounds to human health if adults consume more than 13 mg B per day (Nielsen 1997). Preharvest B application to the soil or as a foliar spray has been commonly used to achieve optimal yield, greater postharvest quality of fruit and less disease severity in a number of crops including apples (Peryea and Drake 1991; Omaima and Karima 2007; Wojcik et al. 2008; Khalifa et al. 2009), peaches (Sotiropoulos et al. 2010; Thomidis and Exadaktylou 2010; Kavvadias et al. 2012), strawberries (Naradisorn et al. 2006; Singh et al. 2007) and tomatoes (Huang and Snapp 2004). Boron fertilisation by the addition of 1 ppm boric acid to soil was critical for capsicum to grow healthy (Nabi et al. 2006), but the role of this element in grey mould development and fruit quality remains unclear. The effect of preharvest application method by soil fertigation or foliar spray on postharvest quality of fruit and disease in capsicum fruit is also unknown. Boron concentration in 'Conference' pear fruit was significantly increased by foliar B spray compared to that using a soil B application (Wojcik and Wojcik 2003). Foliar B spray may also be a more effective method to increase B concentration in capsicum fruit tissue, particularly in cases when soil application causes phytotoxicity. Therefore, the aim of this research was to examine the effect of preharvest soil or foliar B application on grey mould development and postharvest quality of capsicum fruit.

4.2 Materials and methods

4.2.1 Plants and growth conditions

Capsicum cv. Aries and cv. Papri Queen were used in this study as per Chapter 3. Seeds supplied by Monsanto® and Fairbanks Seeds (Australia), respectively were sown in 6-pot trays (12 cm length x 8 cm width) in UC potting mix (University of California at Davis) (Baker 1957) to germinate before transplanting to the larger individual pots (15 cm diameter) containing Mount Compass sand. The sand was passed through a 2-mm stainless steel sieve to remove organic matter and other debris and washed with reverse osmosis (RO) water and then air-dried in the glasshouse. Sieved Mount Compass sand (2.5 kg) was weighed into each

individual pot, into which young seedlings at the four leaf stage (6 to 7 weeks) were planted. Soil B applications were initiated immediately after transplanting seedlings. Foliar B application was initiated when plants had started flowering.

Capsicum plants were grown in a greenhouse as per Chapter 3. Pest and insects control were implemented when necessary (usually at ~ 2 months before flower buds and during fruit development). Spider mites mostly appeared on capsicum plants of the experiments and two insecticides were used for control them including Omite® at 2 g L^{-1} - group 12C insecticide, Chemtura Australia Pty Ltd or Secure® at 0.3 ml L^{-1} - group 13A insecticide, Crop Care Australasia Pty Ltd.

4.2.2 Nutrient solutions for B application

4.2.2.1 Soil application

The basal nutrient solution for all treatments was modified from Hoagland's solution (Hoagland and Arnon 1938) as described by Rubio et al. (2010) in the following form: 6.0 mM KNO_3, 4.0 mM $Ca(NO_3)_2$, 1 mM $MgSO_4.7H_2O$, 1 mM KH_2PO_4 and 0.05 mM KCl. The micronutrients without B were applied similarly in all the treatments, in the following form: 0.002 mM $MnSO_4.H_2O$, 0.002 mM $ZnSO_4.7H_2O$, 0.0005 mM $CuSO_4.5H_2O$, 0.0005 mM $(NH_4)_6$ $Mo_7O_{24}.4H_2O$ and 0.02 mM Fe^{3+}-EDTA. Boric acid (H_3BO_3) was used as the source of B to obtain three B concentrations of 0.00, 0.05 and 0.1 mM H_3BO_3 in the Hoagland's solution. The pH was kept within the range of 5.5 to 6.0 by adding 1 µM or 0.5 µM KOH. The Hoagland's solution at three B concentrations with micronutrients (200 mL) was applied manually to each plant by pouring into the soil every 2 days after transplanting, while reverse osmosis (RO) water was used to maintain soil moisture, particularly on hot days during summer. RO water was used as a control (0.0 mM H_3BO_3). Nutrient analysis showed that B concentration in control plants was at adequate level (Table 4.1), which suggested contamination of the treatment with B. The RO water was tested and found to have a B concentration of 0.08 ppm, but this was only known after finishing the experiments.

Twenty plants for each cultivar per treatment were divided into five replicate groups, with four plants per treatment in each group arranged randomly in the

greenhouse. The experiment was conducted twice using the same plants: plants were kept after the first experiment for a repeat experiment.

4.2.2.2 Foliar B application

The Hoagland's solution plus the micronutrient without B, as described in Section 4.2.2.1, was applied to all treatments after transplanting. Plants treated with 0.1 mM H_3BO_3 in the soil showed symptoms of toxicity on their leaves (Fig 4.1), therefore lower B concentrations were chosen for the first foliar application trial to avoid toxicity. Three B concentrations; 0, 0.025 and 0.075 mM H_3BO_3, were initially trialled. Capsicum plants were sprayed at weekly intervals by using a hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L per ha). Treatment with RO water was the control (0 mM H_3BO_3). Higher B concentrations were selected for the second foliar application trial after analysing the nutrient status of plant tissue at the end of the first trial. The contamination of the RO water with B resulted in high B concentration in control plants and B concentration was not significantly different among the three B treatments. Therefore, plants were maintained after harvest for a second trial. In the second trial, 0.0, 2.0 or 7.0 mM H_3BO_3 was sprayed on to plants as described earlier. Twenty plants for each cultivar per treatment were divided into three treatment groups for three B concentration applications. Each group including 60 plants was arranged randomly in the greenhouse.

4.2.3 Nutrient analysis of leaves and fruit

Nutrient concentration of leaves and fruit were measured using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) at Waite Analytical Services (WAS), Waite Campus, University of Adelaide (Wheal et al. 2011). Youngest mature leaves (~ 20 g) and three replicate fruit at the red stage were randomly collected at harvest from a total of 20 replicated plants per treatment for each cultivar for nutrient analysis. Samples were placed in a paper bag and dried at 80°C ± 3°C in an oven until the moisture of samples was constant after weighing twice. The dry samples were ground into fine grade powder by an electric mill (Kika-werke GMBH, Germany) before providing them to WAS.

Each sample was analysed in triplicate and nutrient concentrations in plant tissues were expressed relative to dry weight (DW).

4.2.4 Inoculation of fruit preharvest or postharvest

4.2.4.1 Isolation, maintenance and culture of *B. cinerea*

Isolation, maintenance and culture of *B. cinerea* were carried out as described in Chapter 3.

4.2.4.2 Pre-harvest inoculation

Previous research showed that flowers of capsicum died at a greater rate when they were inoculated with 10^6 conidia mL^{-1}, particularly in cv. Aries, which appeared more susceptible than cv. Papri Queen (Table 1, Chapter 3). Moreover, there was no significant difference in the development of grey mould on fruit derived from flowers inoculated at 0, 3 or 6 days after anthesis (DAA) (Fig 1 and 2, Chapter 3). Therefore, in order to minimise the death of flowers following inoculation, this research used flowers at 3 DAA.

Fruit on multiple flowering nodes on each plant were randomly selected and tagged before direct application of 100 µL of conidial suspension onto the stem-end of small fruit by using a pipette. Development of grey mould on fruit cv. Papri Queen was much less than on fruit from cv. Aries (Fig 1 and 2, Chapter 3). In order to ensure grey mould development on fruit from cv. Papri Queen after harvest, a suspension of 10^6 conidia mL^{-1} was used for inoculation of fruit from cv. Papri Queen, while a suspension of 10^4 conidia mL^{-1} was used for cv. Aries. Conidial suspension was prepared as per Chapter 3. There were 15 or more replicate young fruit on 15 or more plants per treatment to ensure that at least 10 red fruit were obtained for each treatment in each experiment to assess grey mould development on fruit after harvest.

4.2.4.3 Postharvest inoculation of fruit

Symptomless, uniform red fruit (100% red with low intensity) were harvested from treated plants for postharvest inoculation. Postharvest inoculation was conducted as described by Fallik et al. (1996) and as per Section 'Inoculation by *B. cinerea*' in Chapter 3. Briefly, fruit were surface-sterilised by dipping in 2% sodium hypochlorite for 1 min and then air-dried and rinsed briefly with sterile water. The surface-sterilised fruit were wounded on opposite sides to a depth of 1.5 mm by puncturing them with the point of a sterile 1.5 mm diameter nail. Each wound site was inoculated with 40 µL of suspension containing 10^5 conidia mL^{-1}. Ten fruit per treatment in each experiment were used in this study.

4.2.5 Assessment of grey mould development and postharvest quality in fruit

4.2.5.1 Grey mould development on fruit

Symptomless red fruit derived from young fruit inoculated preharvest were harvested to assess development of grey mould during storage using the protocols described in Section 'Assessment of grey mould development' in Chapter 3. The fruit were monitored daily and the number of fruit exhibiting rot was recorded. Rot development was quantified by measuring lesion area on individual fruit by measuring the length and width of decayed areas using digital callipers (digiMax, Switzerland) and multiplying them.

The correlation between lesion area on fruit and B concentration in leaves and fruit of capsicum cv. Aries and cv. Papri Queen was determined based on 12 individual measurements of grey mould development on fruit and B concentration in leaves and fruit from experiments involving soil or foliar B application. Lesion area on fruit derived from young fruit inoculated preharvest was measured at 28 days after harvest (DAH), while lesion area on fruit inoculated postharvest was measured at 10 DAH. Boron concentration in leaves and fruit was analysed at harvest as described as in Section 4.2.2.3.

4.2.5.2 Postharvest quality of fruit

Healthy fruit were harvested at full red (100% red with low intensity) from 20 treated plants for assessment of postharvest quality during storage at 10°C at five time points: 0, 5, 10, 15 and 23 DAH. Firstly, 10 fruit per treatment were randomly collected to measure length and width by using a digital calliper and then weighed (using a balance; Adventurer™, OHAUS, USA). Three fruit for each time-point per treatment were then selected randomly and washed three times in RO water to remove soil, dust particles and pesticide residues prior to placing in clear plastic containers (30 cm length x 20 cm width x 10 cm height) and stored at 10°C.

Shelf life was assessed by using a 9-point general appearance (GA) scale (9 = the best condition and 1 = the worst condition) as described by Able et al. (2002). When fruit reached a GA of 5.5, they were considered to have reached the end of their storage life. General Linear Regressions were fitted to data using GenStat (14th Edition, VSN International Limited, UK) and shelf life was predicted as the time to reach a GA score of 5.5 using a third degree polynomial.

Fruit firmness was determined by measuring penetration force in kilogram-force (kgf) by a Fruit Pressure Tester (FT 110, Italy) equipped with a 4.5-mm diameter plunger tip. A puncture test was performed on four sides of each fruit for cv. Aries and two sides of each fruit for cv. Papri Queen by holding the 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 line inscribed on the tip. Firmness (N cm^{-2}) was calculated by using following formula:

$$\text{Firmness (N cm}^{-2}) = \frac{\text{kgf x 9.807}}{\pi \text{ x r}^2}$$

Where: 1 kgf cm^{-2} = 9.807 N cm^{-2}

r^2 is radius of plunger tip

After measuring the firmness, the stem-end and seeds were removed from individual fruit and the remains of whole fruit was cut into small pieces (2 cm length x 5 mm width) for assessment of total soluble solids content (TSSC),

titratable acidity (TA) and extractable colour. Small pieces (20 g) were randomly collected for TSSC and TA and 10 g for extractable colour.

TSSC was determined by using a hand-held refractometer (Stanley Limited, Switzerland). Small pieces of fruit were ground using a porcelain mortar and pestle prior to wrapping with cheesecloth and squeezing by hand. Juice samples were then filtered through mira-cloth to make sure a clear juice was available for measurement. A drop of juice sample was placed on the glass surface of the refractometer using a micropipette. Two measurements per fruit were taken and the TSSC was read and presented as ºBrix.

TA was determined by taking 6 g of the juice sample, prepared as described above, diluting to 50 mL with nanopure water and using 0.1 N NaOH to titrate to pH 8.1. Each fruit was titrated in triplicate. Results were expressed as percentage of citric acid in the juice which is the predominant organic acid in capsicum (Rubio et al. 2010) using the formula:

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

Where acid milliequivalent factor for citric acid = 0.064 (Garner et al. 2005)

Extractable colour was measured based on the standard method of the American Spice Trade Association (ASTA) and presented as ASTA units [Association of Official Analytical Communities (AOAC 1997)] and American Spice Trade Association (ASTA 1985)]. Ten g of tissue pieces from each fruit, prepared as for TSSC measurement, were placed in a paper bag and dried at 40°C in an oven until the moisture of samples was constant after weighing twice. The dry weight of samples was used to calculate water content in fruit (for only the experiment of foliar application with high B concentration) as per the following formula:

$$\mathrm{Water\ content\ (\%)} = \frac{\mathrm{Fresh\ weight\ (g) - dry\ weight\ (g)}}{\mathrm{Total\ fresh\ weight\ (g)}} \times 100\%$$

The dry samples were then ground into fine grade powder as described above. The ground powder was kept in sealed plastic bags and stored in the dark at 4°C using a water absorbent (silica gel) for no longer than 2 weeks.

Seventy mg of the ground sample was placed in a 100 mL volumetric flask with acetone. The flask was tightly stoppered, shaken and placed in the dark at room temperature (22°C) for 16 h. The supernatant (containing the colour extract) was transferred to 1 mL cuvettes and the absorbance at 460 nm (A_{460}) measured using a spectrophotometer (SP8001, Metertech). The final ASTA value for each measurement was calculated using the following formula:

$$\text{ASTA} = \frac{A_{460} \text{ x } 16.4}{\text{Sample dry weight (g)}}$$

4.2.6 Statistical analysis and photography

Experiments were conducted twice and designed with no blocking using the factors of cultivar and B concentration. Data from repeated experiments when B was applied to the soil were statistically similar and therefore combined for analysis. Data were subjected to repeated measurements analysis of variance (ANOVA) using GenStat (14^{th} Edition, VSN International Limited, UK) and means of the treatments compared using the Least Significant Difference (LSD) at a significance level of $P \leq 0.05$. Means and standard errors were determined using Microsoft Excel. Photography was by a digital camera (Canon D500, Japan).

4.3 Results

4.3.1 Effect of B applied to soil on nutrient status of plant tissues, postharvest quality of fruit and grey mould development on capsicum

Toxicity symptoms were first observed on the leaves of plants from cv. Aries, which had received a soil application of 0.1 mM H_3BO_3 in the first experiment when plants had flower buds (Fig 4.1A). Symptoms (where older leaf margins turned to a faded brown) on leaves of the same plants became greater in the repeat experiment (Fig 4.1B). Toxicity symptoms did not show on leaves of plants treated with 0.05 mM H_3BO_3 in either experiment. Toxicity symptoms were not observed on leaves from cv. Papri Queen in any experiments. There were also no toxicity symptoms on flowers or fruit of any plants, regardless of whether B was applied to the soil or via foliar application.

4.3.1.1 Boron concentration in leaf and fruit tissues

Boron concentration in leaf and fruit tissues significantly increased with increasing B concentration in the nutrient solution applied into the soil (Table 4.1). B concentration in leaves and fruit from plants treated with 0.1 mM H_3BO_3 was significantly higher ($P<0.001$) than B concentration in leaves and fruit from plants treated with 0 or 0.05 mM H_3BO_3 for both cultivars. The B concentration in leaves and fruit from plants treated with 0.05 mM H_3BO_3 was significantly more than that in control plants. B concentration in leaf tissues of control plants (0 mM H_3BO_3) unexpectedly had B present at levels considered to be adequate for growth (23 - 24 mg kg^{-1}) (Reuter and Robinson 1997). Boron concentration in leaf tissue from both cultivars treated with 0.05 or 0.1 mM H_3BO_3 was above those usually regarded as toxic for capsicum (40 - 100 mg kg^{-1}) (Reuter and Robinson 1997). There were no significant differences in other nutrients, regardless of cultivar or B concentration (Appendix A.2). However, copper concentration in leaves from any of the three concentrations, regardless of cultivar, appeared deficient compared to that considered to be adequate (6 - 25 mg kg^{-1}) (Reuter and Robinson 1997).

4.3.1.2 Postharvest quality of fruit

Length, width and weight were not significantly different among fruit from plants treated with the three B concentrations, regardless of cultivar (Fig 4.2A, B).

The shelf life of fruit from cv. Aries plants treated with 0.1 mM H_3BO_3 in the soil was significantly longer ($P = 0.024$) than that from plants treated with less boron (Fig 4.3A), while shelf life of fruit grown in soil treated with 0.0 or 0.05 mM H_3BO_3 was not significantly different from each other. For cv. Papri Queen, there was no significant difference in the shelf life of fruit from plants treated with three B concentrations (Fig 4.3B). The shelf life of fruit from cv. Papri Queen was significantly shorter than for fruit from cv. Aries.

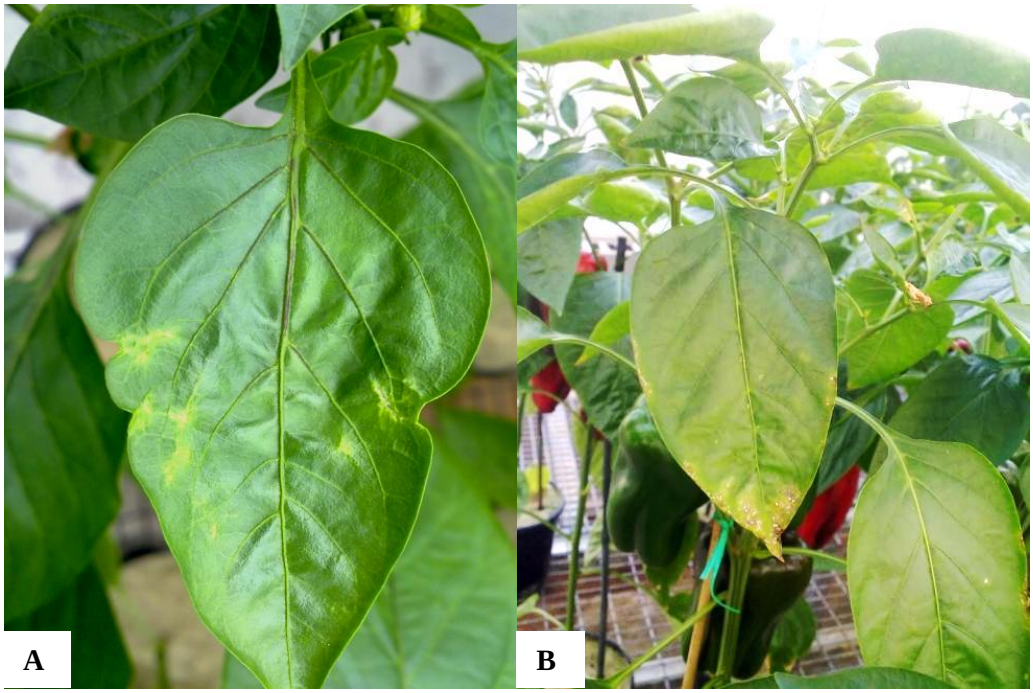


Fig. 4.1 Toxicity symptoms on the leaves of plants of cv. Aries growing in soil to which 0.1 mM H_3BO_3 had been applied. (**A**) Plants at 60 days after transplanting at flower bud stage in the first experiment; (**B**) Plants at 180 days after transplanting at the harvest stage in the repeat experiment

Table 4.1 Effect of soil boron application on B concentrations in leaf and fruit tissues of cv. Aries and cv. Papri Queen. Other nutrients are in a table in Appendix A.2. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that received 0, 0.05 or 0.1 mM H_3BO_3 in Hoagland's solution (200 mL per plant every 2 days). Data are presented as means ± SE from n = 6 from two experiments. For each cultivar, means with the same letters in each column were not significantly different as determined using the LSD (P<0.05)

Cultivar	**B treatment (mM H_3BO_3)**	**B concentration (mg kg^{-1} DW)**	
		Leaf	**Fruit**
Aries	Control	55.73 ± 8.50^{a}	10.84 ± 0.21^{a}
	0.05	169.12 ± 21.65^{b}	14.28 ± 0.27^{b}
	0.1	287.50 ± 18.87^{c}	17.85 ± 0.37^{c}
	LSD (P value)	*55.32 (<0.001)*	*2.16 (<0.001)*
Papri Queen	Control	50.59 ± 4.66^{a}	11.74 ± 0.16^{a}
	0.05	193.19 ± 32.91^{b}	14.28 ± 0.22^{b}
	0.1	305 ± 40.31^{c}	18.35 ± 0.43^{c}
	LSD (P value)	*96.50 (<0.001)*	*2.16 (<0.001)*

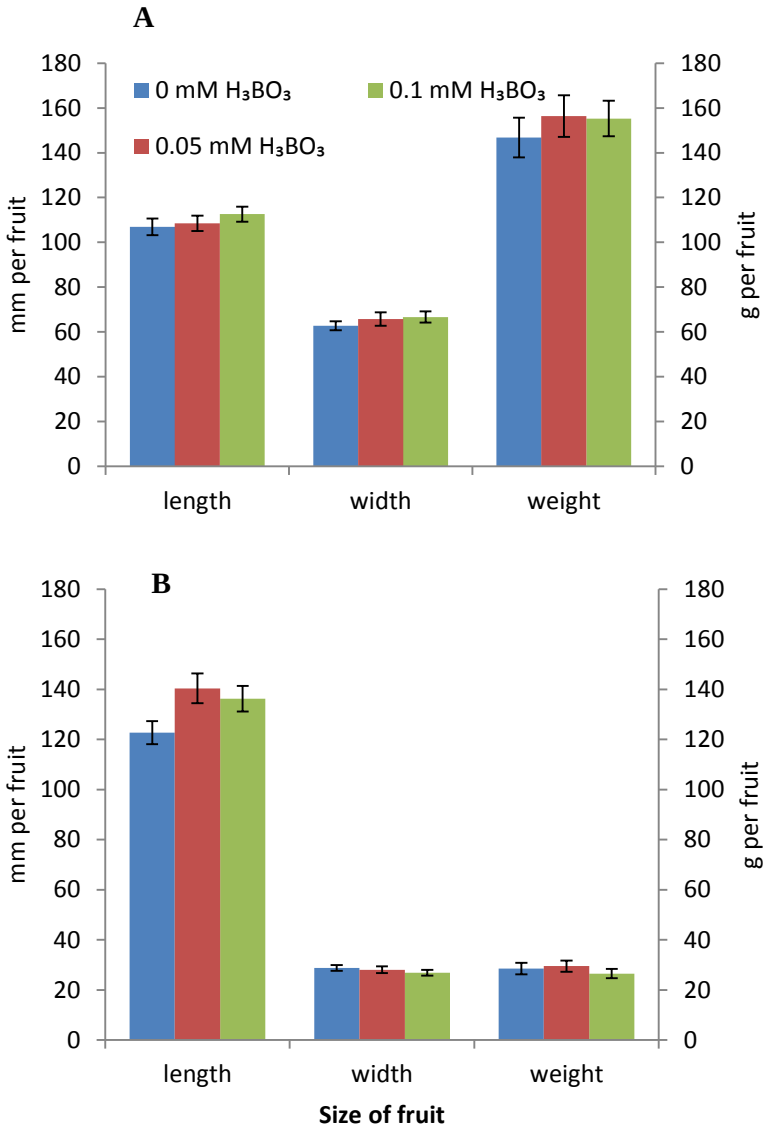


Fig. 4.2 Length, width and weight of fruit from cv. Aries (**A**) and cv. Papri Queen (**B**) when plants received a soil application of 0, 0.05 or 0.1 mM H_3BO_3 in Hoagland's solution (200 mL per plant every 2 days) from transplant to harvest of red fruit. Data are means ± SE from n = 20 across two experiments

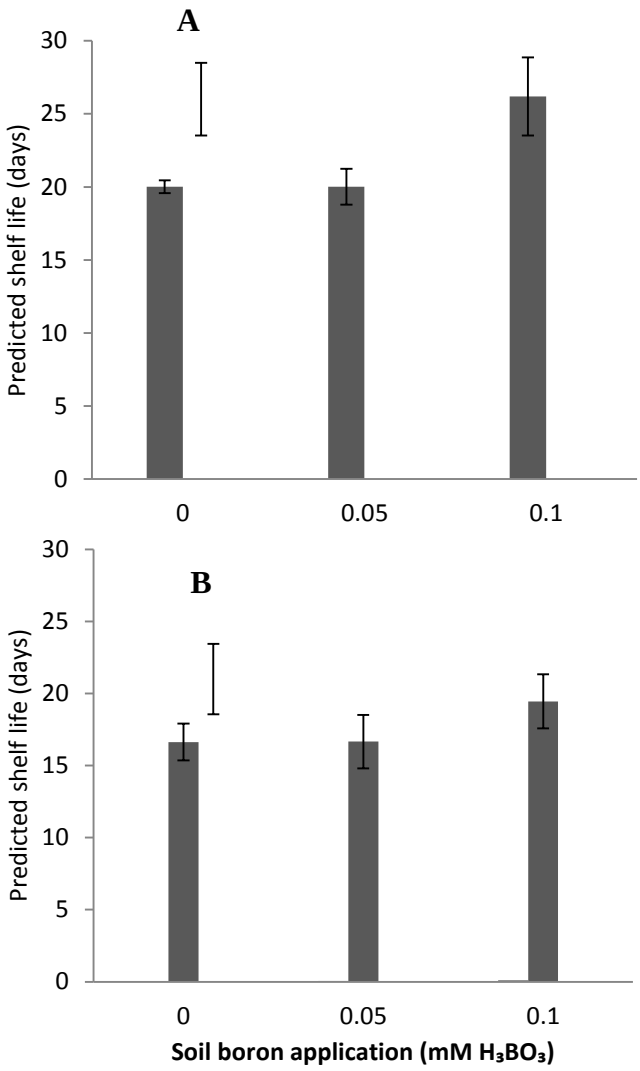


Fig. 4.3 Effect of soil B application on shelf life of fruit from cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90%. Fruit were harvested from plants that received a soil application of 0, 0.05 or 0.1 mM H_3BO_3 in Hoagland's solution (200 mL per plant every two days) from transplant to harvest of red fruit. Shelf life of fruit was predicted at a general appearance (GA) of 5.5 by using a third degree polynomial in GenStat. Data are means ± SE from n = 6 across two experiments. LSDs ($P \leq 0.05$) for among individual boron concentrations are shown

Extractable colour of fruit from both cultivars increased significantly during storage, regardless of soil treatment. Extractable colour of fruit was not significantly different among the three B concentration treatments (Fig 4.4A, B). Fruit from cv. Papri Queen had a significantly greater extractable colour than fruit from cv. Aries.

Firmness of fruit from cv. Aries significantly decreased ($P<0.001$) from 10 DAH to 23 DAH during storage compared with that of fruit at harvest in all three B treatments (Fig 4.4C), while firmness of fruit from cv. Papri Queen decreased significantly ($P = 0.002$) at 5 DAH but then significantly increased from 10 DAH to 23 DAH (Fig 4.4D).

TSSC in fruit from cv. Aries increased significantly ($P<0.001$) at 10 DAH compared to that at harvest. Fruit from the control treatment showed a further significant increase in fruit at 23 DAH, while there was no futher change in TSSC in fruit from plants treated with 0.05 or 0.1 mM H_3BO_3 (Fig 4.4E). For cv. Papri Queen, initial TSSC in fruit at harvest was not significantly different among B treatments, but significantly different at 10 DAH when TSSC in fruit from control plants was significantly lower ($P = 0.009$) than that from plants treated with two B concentrations (Fig 4.4F). However, at 23 DAH TSSC in fruit from control plants was significantly higher than that from plants treated with 0.1 mM H_3BO_3, but the same with that from plants treated with 0.05 mM H_3BO_3. TSSC in fruit from cv. Papri Queen was significantly higher than in fruit from cv. Aries.

TA in fruit during storage was not significantly different among B concentration treatments for both cultivars (Fig 4.4G, H). For cv. Aries, TA in fruit from plants treated with 0 or 0.05 mM mM H_3BO_3 increased significantly ($P<0.001$) from 5 DAH to 23 DAH, while TA in fruit from plants treated with 0.1 mM H_3BO_3 increased significantly from 5 DAH to 15 DAH but then there was no change at 23 DAH. TA in fruit from cv. Papri Queen decreased significantly ($P<0.001$) at 5 DAH but then significantly increased from 10 DAH to 23 DAH.

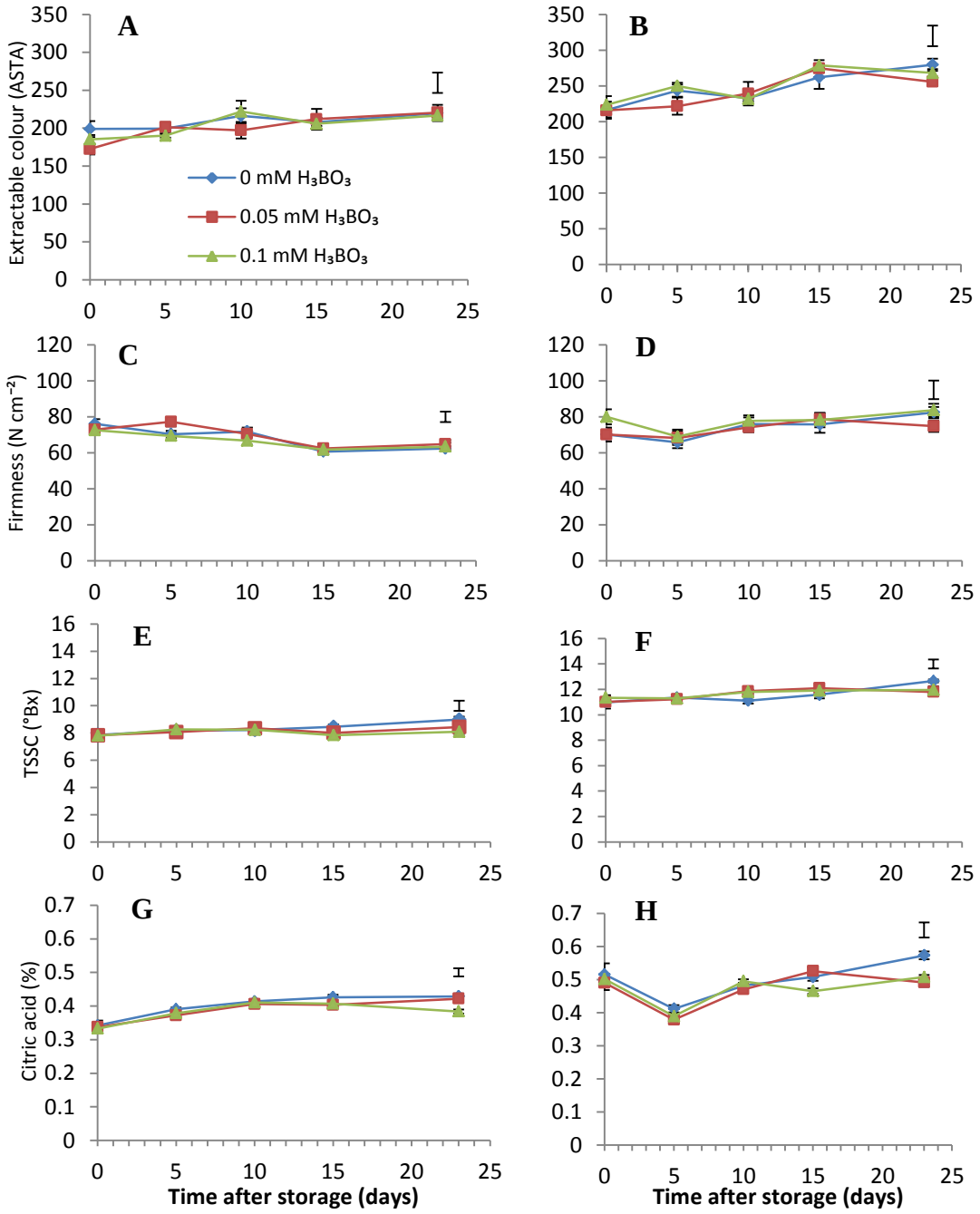


Fig. 4.4 Effect of soil B application on postharvest quality of fruit during storage at 10°C and relative humidity of >90%: extractable colour (**A** and **B**); firmness (**C** and **D**); TSS (**E** and **F**) and TA (**G** and **H**) for cv. Aries and cv. Papri Queen, respectively. Fruit were harvested from plants that received a soil application of 0, 0.05 or 0.1 mM H_3BO_3 in Hoagland's solution (200 mL per plant every 2 days) from transplanting to harvest of red fruit. Data are means ± SE from n = 6 across two experiments. LSDs ($P \leq 0.05$) are shown for among individual B concentration for each time-point during storage

4.3.1.3 Grey mould development on fruit derived from inoculated young fruit

Grey mould was not observed on fruit at harvest in either cv. Aries or cv. Papri Queen. Grey mould development on fruit was mostly observed 14 days after harvest (DAH) and then increased significantly ($P<0.001$) during storage, regardless of cultivar or B treatment (Fig 4.5).

For cv. Aries, lesion area on fruit from plants treated with 0.1 mM H_3BO_3 was significantly smaller ($P = 0.009$) than that from plants treated with 0.0 mM H_3BO_3 from 18 DAH to 28 DAH, whereas lesion area on fruit from plants treated with 0.05 mM H_3BO_3 was similar to that from plants treated with 0.1 mM H_3BO_3, but was significantly smaller than that from control plants at 18 DAH (Fig 4.5A). The number of fruit exhibiting rot was not significantly different among B treatments (Fig 4.5B). For cv. Papri Queen, lesion area on fruit from plants treated with 0.1 mM H_3BO_3 was significantly smaller ($P = 0.039$) than that from plants treated with 0.0 mM H_3BO_3 at 28 DAH, but was the same with that from plants treated with 0.05 mM H_3BO_3 (Fig 4.5C). The percentage of fruit exhibiting rot from plants that did not receive B was significantly larger ($P = 0.039$) than those that received B (Fig 4.5D). Lesion area on fruit from cv. Papri Queen, regardless of B treatment, was significantly smaller ($P<0.001$) than that from cv. Aries.

Time to reach 50% of maximum lesion area on fruit from plants treated with 0.1 mM H_3BO_3 was significantly longer ($P = 0.001$ for cv. Aries and $P = 0.006$ for cv. Papri Queen) than that from control plants for both cultivars (Fig 4.6A and 4.6B). Time to reach 50% maximum lesion area on fruit from plants treated with 0.05 mM H_3BO_3 was not significantly different from that from control plants or plants treated with 0.1 mM H_3BO_3 for cv. Aries, but was significantly longer than that from control plants for cv. Papri Queen.

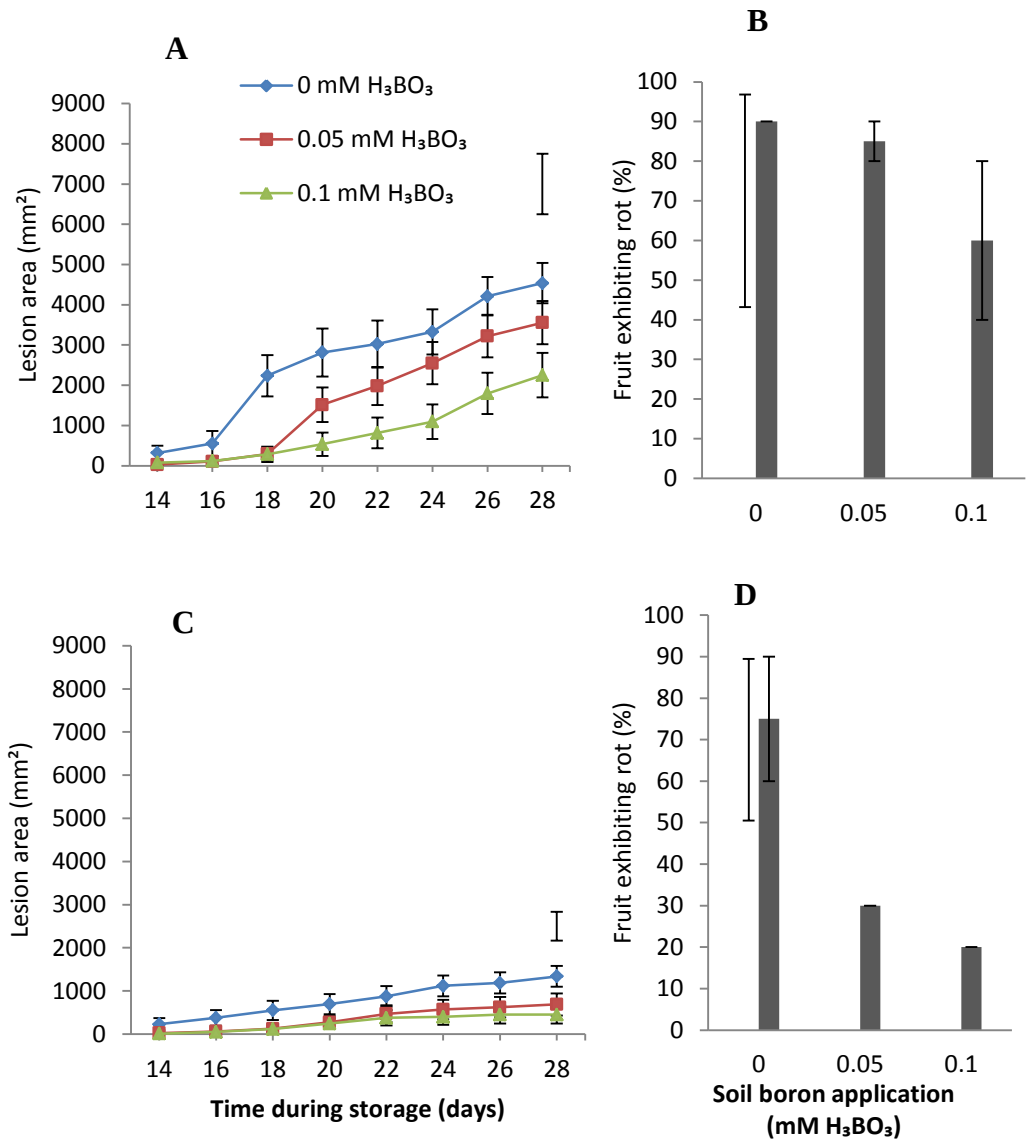


Fig. 4.5 Effect of soil B application on grey mould development and the number of fruit of capsicum cv. Aries (**A** and **B**) and cv. Papri Queen (**C** and **D**) exhibiting rot during storage at 10°C and relative humidity of >90%. Fruit were derived from young fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA when plants received a soil application of 0, 0.05 or 0.1 mM H_3BO_3 in Hoagland's solution (200 mL per plant every 2 days) from transplant to harvest red fruit. Data are means ± SE from n = 20 across two experiments. LSDs ($P \leq 0.05$) are shown for among individual boron concentrations for each time-point during storage

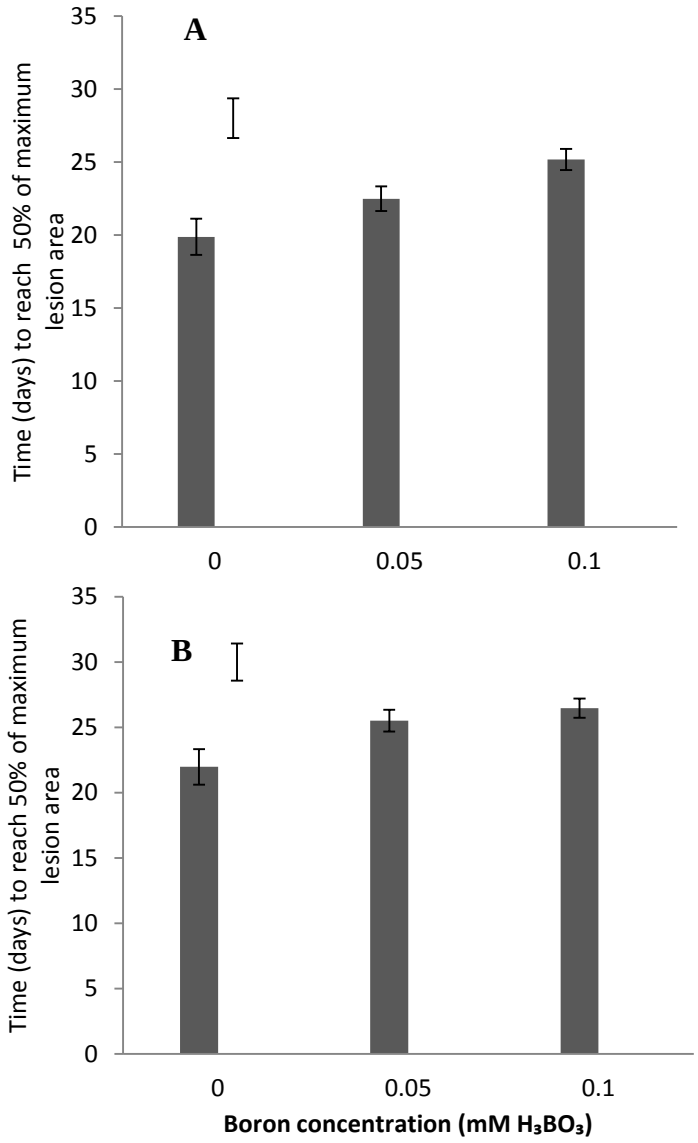


Fig. 4.6 Time after harvest for lesions to reach 50% of maximum size on fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% for 28 days when plants received a soil application of 0, 0.05 or 0.1 mM H_3BO_3 in Hoagland's solution (200 mL per plant every 2 days) from transplant to harvest red fruit. Fruit were derived from young fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA. Data are means ± SE from n = 20 across two experiments. LSDs ($P \leq 0.05$) for among individual B concentrations at 28 days after harvest are shown

4.3.1.4 Grey mould development on fruit inoculated postharvest

Symptoms of grey mould on fruit which were inoculated postharvest were observed 4 days post-inoculation (DPI) for both cultivars, with 100% of fruit exhibiting rot after 10 DPI. Lesion area on fruit was significantly different among B treatments for both cultivars (Fig 4.7A and 4.7B). Lesion area on fruit from plants which were treated with either B concentration was significantly smaller than that from control plants from 8 to 10 DPI for cv. Aries ($P = 0.013$) and at 10 DPI for cv. Papri Queen ($P<0.001$). However, lesion area was similar on fruit from plants of cv. Aries which were treated with either B concentration. For cv. Papri Queen, lesion area on fruit from plants treated with 0.1 mM H_3BO_3 was significantly smaller than that from plants treated with 0.05 mM H_3BO_3.

4.3.2 Effect of B as a foliar application on nutrient status of plant tissues, postharvest quality of fruit and grey mould development

4.3.2.1 Foliar application at lower B concentrations

Symptoms of B toxicity or deficiency were not observed on leaves or fruit from plants treated with the three B concentrations of 0, 0.025 or 0.075 mM H_3BO_3 as a foliar spray from flowering to harvest of red fruit.

B concentration was not significantly different in leaves or fruit from the control or plants sprayed with B and all values in the leaves were above the critical level for deficiency (25 -75 mg kg^{-1}) (Reuter and Robinson 1997) (Table 4.2). There were no significant differences in other nutrients in leaf and fruit tissues, regardless of cultivar or B concentration (Appendix A.3).

Length, width and weight were not significantly different among fruit from plants treated with the three B concentrations, regardless of cultivar (Fig 4.8A, B).

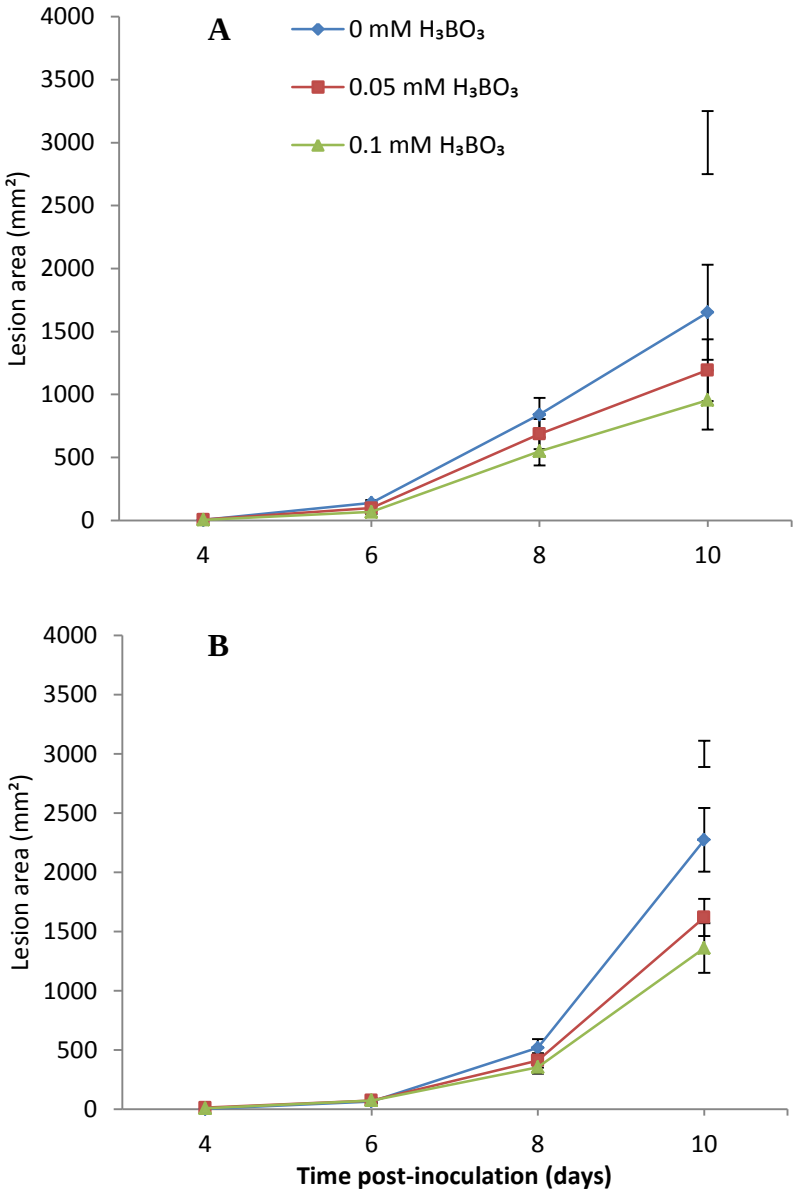


Fig. 4.7 Effect of soil B application on grey mould development on postharvest-inoculated fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% when plants received a soil application of 0, 0.05 or 0.1 mM H_3BO_3 in Hoagland's solution (200 mL per plant every 2 days) from transplant to harvest red fruit. Fruit were wounded both sides and inoculated postharvest with 40 µL suspension of 10^5 conidia mL^{-1}. Data are means ± SE from n = 20 across two experiments. LSDs ($P \leq 0.05$) are shown for among individual B concentrations for each time-point post-inoculation

Table 4.2 Effect of foliar B application on nutrient status in capsicum plant tissues in the first trial (low B concentrations). Other nutrients are in a table in Appendix A.3. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that received a foliar application: 0.0, 0.025 or 0.075 mM H_3BO_3 from flowering to harvest of red fruit. Plants were watered with Hoagland's solution without B (200 mL per plant for every 2 days). Data are presented as means ± SE from n = 3. There were no significant difference between B treatments within tissue types (P values are shown)

Cultivar	**B treatment (mM H_3BO_3)**	**B concentration (mg kg^{-1} DW)**	
		Leaf	**Fruit**
Aries	Control	87.54 ± 0.60	12.67 ± 0.99
	0.025	72.34 ± 0.98	10.77 ± 0.4
	0.075	76.23 ± 1.4	10.16 ± 0.32
	P value	*0.06*	*0.074*
Papri Queen	Control	66.79 ± 0.60	11.40 ± 0.47
	0.025	67.92 ± 1.41	11.57 ± 0.36
	0.075	77.53 ± 0.49	11.51 ± 0.33
	P value	*0.07*	*0.95*

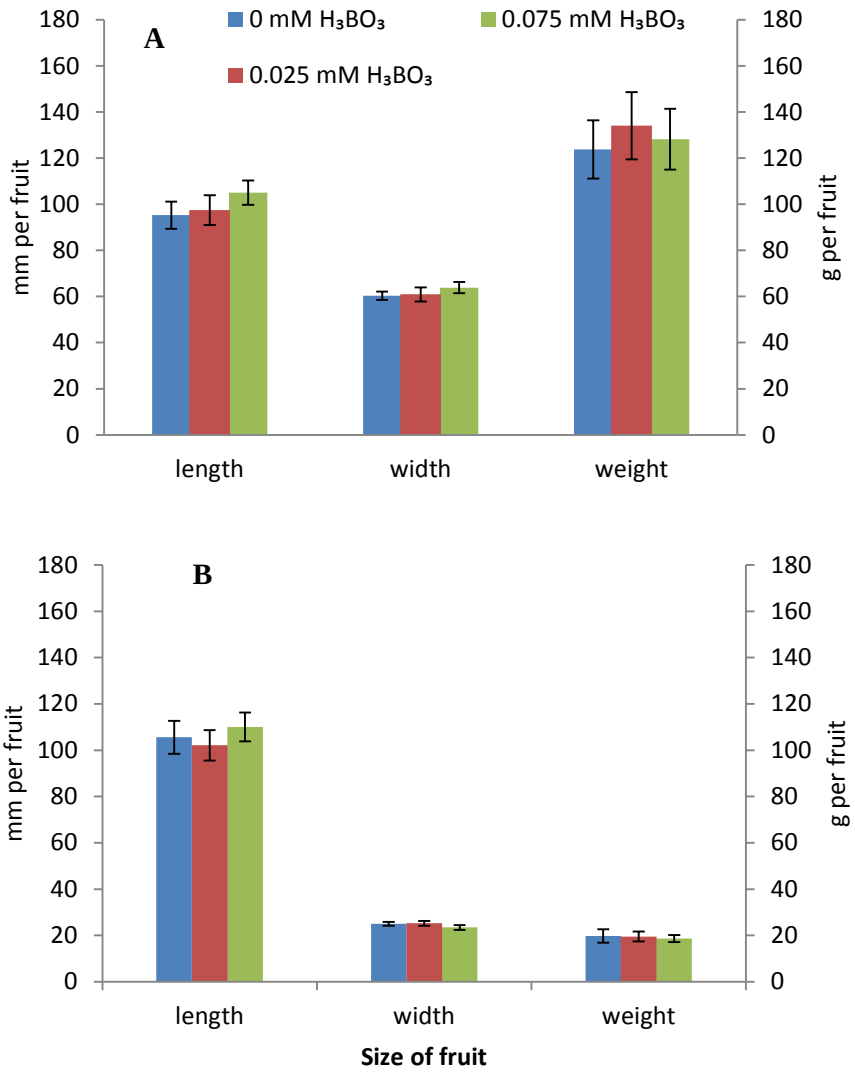


Fig. 4.8 Length, width and weight of fruit from cv. Aries (**A**) and cv. Papri Queen (**B**) when plants received a foliar application of 0, 0.025 or 0.075 mM H_3BO_3 in the first trial (lower B concentrations). Plants were sprayed from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Data are means ± SE from n = 10

The shelf life of fruit from plants that had B applied to them as a foliar spray appeared to be longer than those from control plants, but the difference was not significant, regardless of cultivar (Fig 4.9A, B). The shelf life was similar for fruit of cv. Aries and fruit of cv. Papri Queen, regardless of B concentration.

Application of B as a foliar spray did not affect extractable colour of fruit during storage regardless of cultivar (Fig 4.10A, B). Extractable colour increased steadily during storage and was significantly higher at 10 DAH than at harvest, regardless of B treatment or cultivar.

Firmness of fruit during storage was not significantly different among three B treatments, regardless of cultivar (Fig 4.10C, D). Firmness of fruit from cv. Aries decreased significantly ($P<0.001$) from 10 DAH to 23 DAH, while firmness of fruit from cv. Papri Queen increased significantly ($P<0.001$) from 10 DAH to 23 DAH.

TSSC in fruit of both cultivars generally increased steadily from 10 DAH to 23 DAH (Fig 4.10E, F). For cv. Aries at 5 DAH, TSSC in fruit from plants treated with 0.075 mM H_3BO_3 as a foliar spray was significantly lower ($P = 0.038$) than that for fruit from plants treated with 0 mM or 0.025 mM H_3BO_3 (Fig 4.10E). At 15 DAH TSSC in fruit from plants treated with 0.025 mM H_3BO_3 was significantly higher ($P = 0.038$) than that of fruit from control plants, but the same as that for fruit from plants treated with 0.075 mM H_3BO_3. For cv. Papri Queen, foliar B application had no effect on TSSC in fruit during storage (Fig 4.10F).

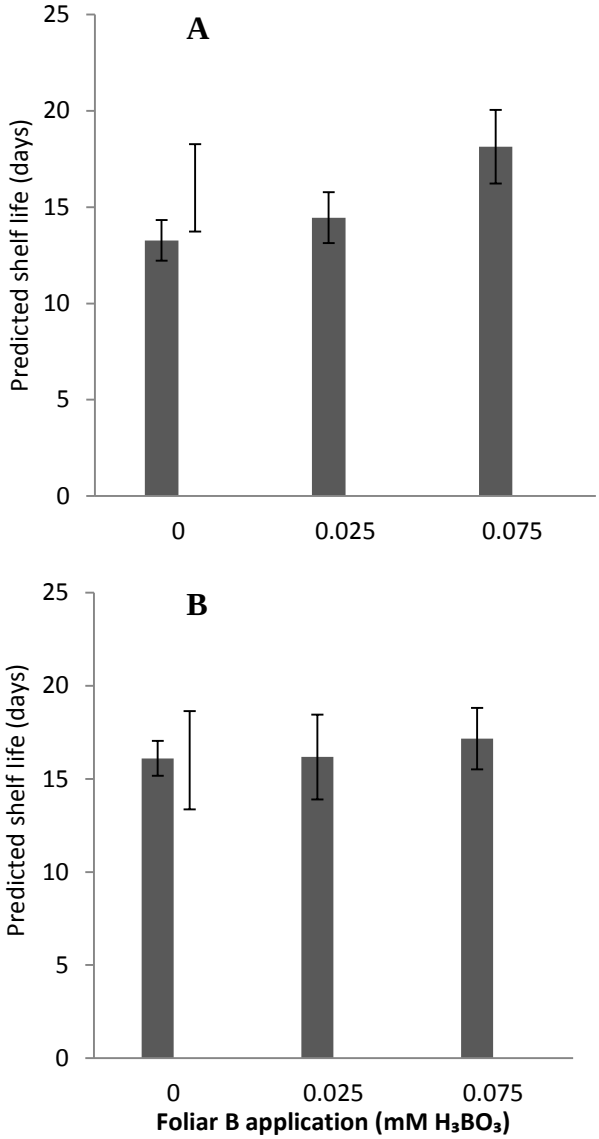


Fig. 4.9 Effect of foliar B application on shelf life of fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% in the first trial (lower B concentrations). Fruit were harvested from plants that received a foliar application of 0, 0.025 or 0.075 mM H_3BO_3. Plants were sprayed from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Shelf life of fruit was predicted at a general appearance (GA) of 5.5 by using a third degree polynomial in GenStat. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) for among individual boron concentrations are shown

TA in fruit during storage was not significantly different among B concentration treatments, regardless of cultivar (Fig 4.10G, H). For cv. Aries, TA in fruit increased significantly ($P<0.001$) from 5 DAH to 10 DAH but then did not change from 15 DAH to 23 DAH, while TA in fruit from cv. Papri Queen increased significantly ($P<0.001$) from 10 DAH to 23 DAH.

Lesion area on fruit derived from young fruit that had been inoculated preharvest was not significantly different for cv. Aries, regardless of B concentration used (Fig 4.11A). However, for cv. Papri Queen, lesion area on fruit from plants sprayed with either 0.075 or 0.025 mM H_3BO_3 was significantly smaller ($P<0.001$) from 20 DAH to 28 DAH compared to those from control plants (Fig 4.11C). The percentage of fruit exhibiting rot from plants that were sprayed with 0.075 mM H_3BO_3 appeared to be lower than those that were not sprayed or sprayed with lower B concentration, regardless of cultivar (Fig 4.11B, D).

Time to reach 50% of maximum lesion area on fruit derived from young fruit that had been inoculated preharvest was not significantly different among three B treatments for cv. Aries (Fig 4.12A). In contrast, for cv. Papri Queen time to reach 50% of maximum lesion area on fruit from control plants was significantly shorter ($P<0.001$) than that on fruit from plants sprayed with 0.025 or 0.075 mM H_3BO_3 (Fig 4.12B). However, time to reach 50% of maximum lesion area was similar on fruit between plants sprayed with either B concentration.

Lesion area on fruit inoculated postharvest was not significantly different, regardless of B concentration or cultivar (Fig 4.13A, B).

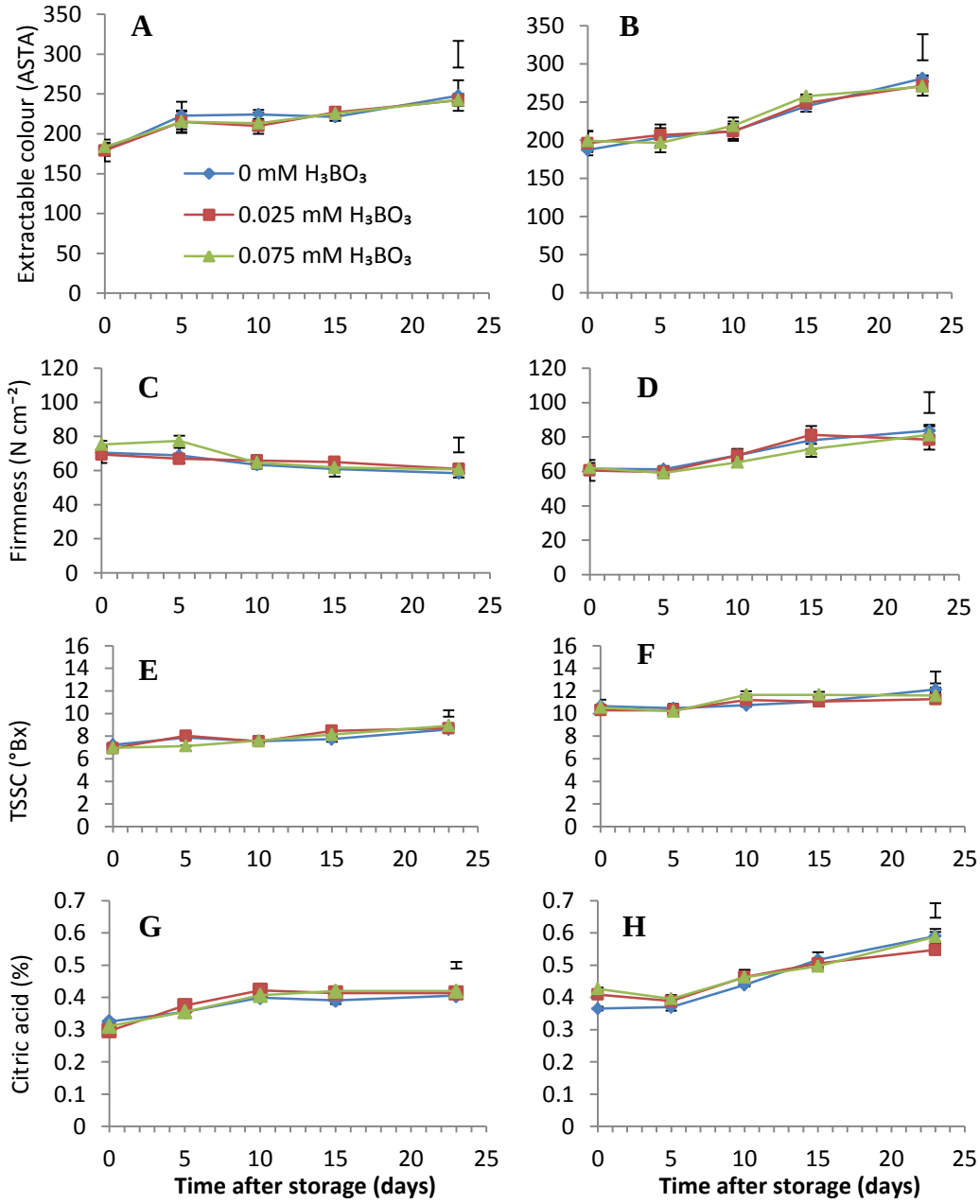


Fig. 4.10 Effect of foliar B application on postharvest quality of fruit during storage at 10°C and relative humidity of >90%: extractable colour (**A** and **B**); firmness (**C** and **D**); TSS (**E** and **F**) and TA (**G** and **H**) for cv. Aries and cv. Papri Queen, respectively in the first trial (lower B concentrations). Fruit were harvested from plants that received a foliar application of 0, 0.025 or 0.075 mM H_3BO_3 from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Data are means ± SE from n = 3. LSDs ($P \leq 0.05$) are shown for among individual B concentration for each time-point during storage

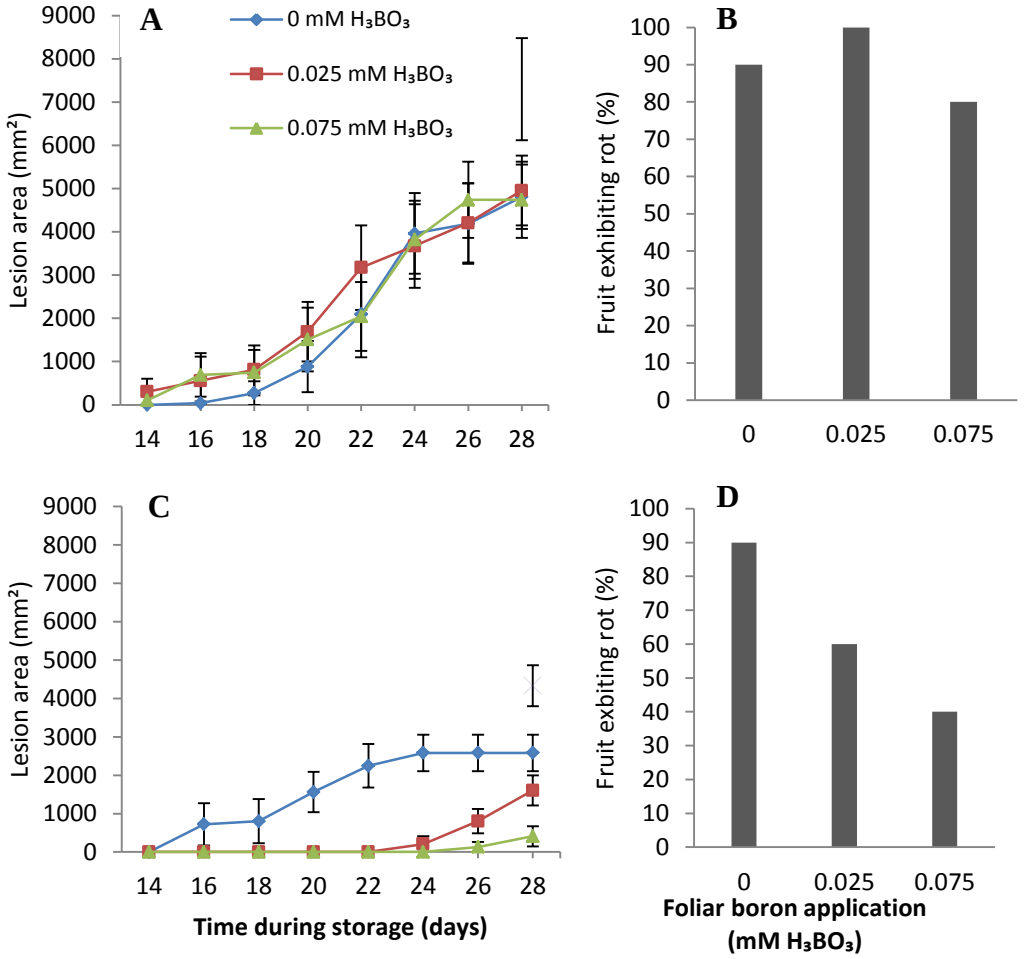


Fig. 4.11 Effect of foliar B application on grey mould development and percentage of fruit exhibiting rot of capsicum cv. Aries (**A** and **B**), cv. Papri Queen (**C** and **D**) during storage at 10°C and relative humidity of >90% in the first trial (lower B concentrations). Fruit were derived from young fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA when plants received a foliar application of 0, 0.025 or 0.075 mM H_3BO_3. Plants were sprayed with foliar B from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual boron concentration for each time-point during storage

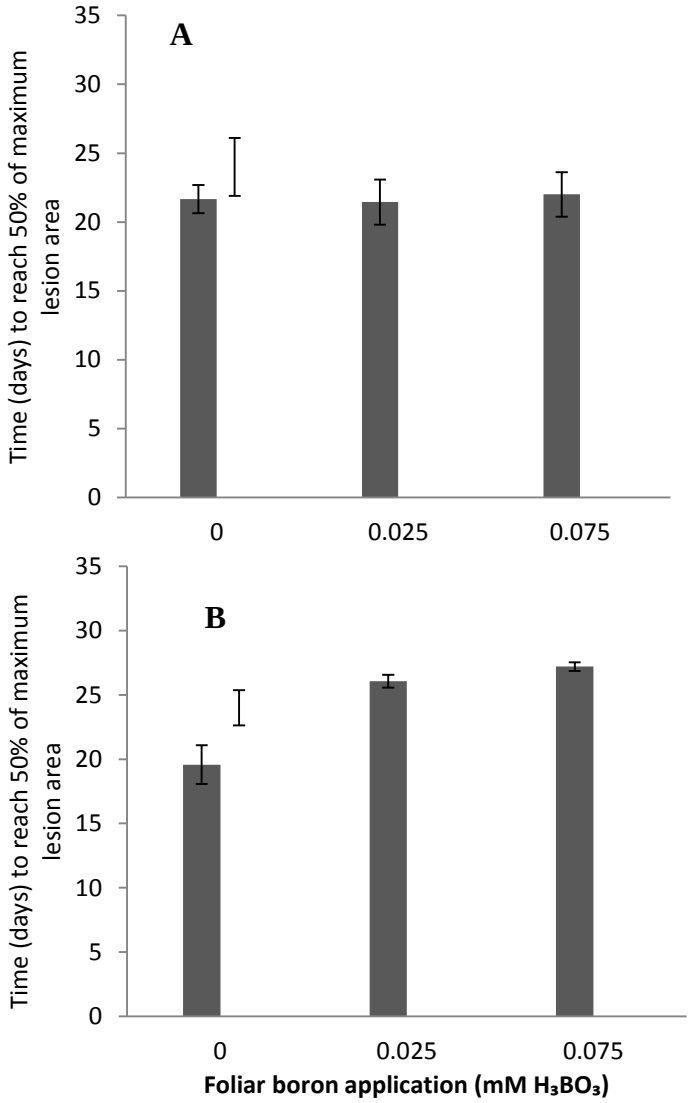


Fig. 4.12 Time after harvest for lesions to reach 50% of maximum size on fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% for 28 days in the first trial (lower B concentration) when plants received a foliar application of 0, 0.025 or 0.075 mM H_3BO_3. Plants were sprayed with foliar B from flowering to harvest at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Fruit were derived from young fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) for among individual B concentrations at 28 days after harvest are shown

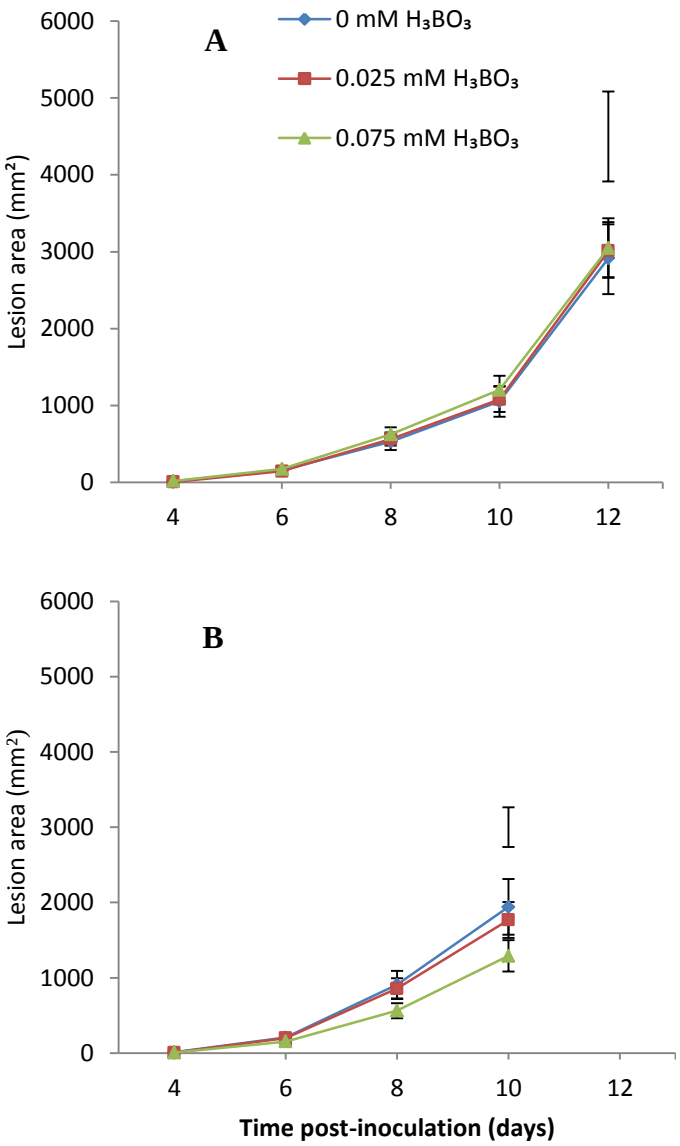


Fig. 4.13 Effect of foliar B application on grey mould development on postharvest-inoculated fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% in the first trial (lower B concentrations) when plants received a foliar application of 0, 0.025 or 0.075 mM H_3BO_3. Plants were sprayed with foliar B from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Fruit were wounded both sides and inoculated postharvest with 40 µL suspension of 10^5 conidia mL^{-1}. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual B concentrations for each time-point post-inoculation

4.3.2.2 Foliar application at higher B concentrations

Symptoms of B toxicity or deficiency were not observed on leaves and fruit from plants treated with three higher B concentrations of 0, 2.0 or 7.0 mM H_3BO_3 from flowering to harvest of red fruit.

B concentration in leaf tissues from control plants indicated the plants were deficient and spraying with 2.0 or 7.0 mM H_3BO_3 significantly increased the boron concentration in leaves of both cultivars. Boron concentration in leaves from plants sprayed with 7.0 mM H_3BO_3 was significantly greater ($P<0.001$) than that of leaves from plants sprayed with 2.0 mM H_3BO_3 (Table 4.3). For cv. Aries, B concentration in fruit from plants treated with 7.0 mM H_3BO_3 was significantly higher ($P = 0.048$) than that from control plants, but was not significantly different between plants sprayed with 2.0 mM H_3BO_3 and control plants. For cv. Papri Queen, spraying plants with 2.0 or 7.0 mM H_3BO_3 did not affect B concentration in the fruit. There were no significant differences in other nutrients in the leaves and the fruit, regardless of cultivar or B concentration (Appendix A.4).

Length, width and weight were not significantly different among fruit from plants treated with the three B concentrations, regardless of cultivar (Fig 4.14A, B).

The shelf life of fruit was not significantly different among the three B applications, regardless of cultivar (Fig 4.15A, B).

Extractable colour in fruit increased steadily during storage for all three B applications, regardless of cultivar. Extractable colour in fruit from cv. Papri Queen was significantly greater than that for fruit from cv. Aries from 15 to 23 DAH. Application at 2.0 or 7.0 mM H_3BO_3 as a foliar spray had no effect on extractable colour of fruit during storage, regardless of cultivar (Fig 4.16A, B).

Table 4.3 Effect of foliar B application on nutrient status in capsicum plant tissues in the second trial (higher B concentrations). Other nutrients are in a table in Appendix A.4. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that received a foliar application: 0.0, 2.0 or 7.0 mM H_3BO_3 from flowering to harvest of red fruit. Plants were watered with Hoagland's solution without B (200 mL per plant for every 2 days). Data are presented as means ± SE from n = 3. For each cultivar, means with the same letters in each column were not significantly different as determined using the LSD (P<0.05)

Cultivar	**B treatment (mM H_3BO_3)**	**B concentration (mg kg^{-1} DW)**	
		Leaf	**Fruit**
Aries	Control	*14.98 ± 0.01[a]	4.99 ± 0.54[a]
	2.0	32.86 ± 0.41[b]	5.61 ± 0.24[ab]
	7.0	54.98 ± 0.43[c]	6.77 ± 0.34[b]
	LSD (P value)	*1.55 (<0.001)*	*1.37 (0.048)*
Papri Queen	Control	*18.38 ± 0.28[a]	4.05 ± 0.3[a]
	2.0	25.50 ± 0.02[b]	4.35 ±0.27[a]
	7.0	32.83 ± 0.19[c]	4.33 ± 0.26[a]
	LSD (P value)	*0.88 (<0.001)*	*0.95 (0.70)*

**deficient as stated by Reuter and Robinson (1997)*

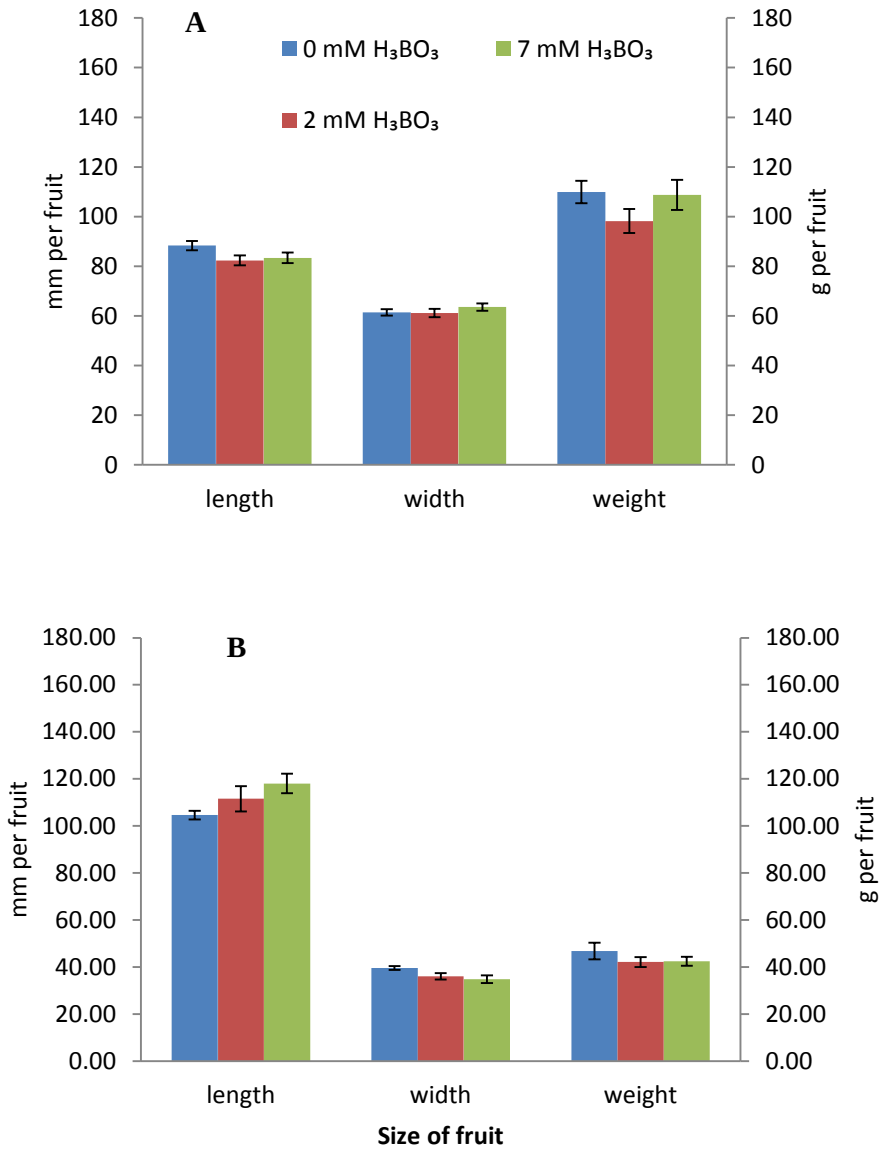


Fig. 4.14 Length, width and weight of fruit from cv. Aries (**A**) and cv. Papri Queen (**B**) when plants received a foliar application of 0, 2 or 7 mM H_3BO_3 in the second trial (higher B concentrations). Plants were sprayed from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Data are means ± SE from n = 10

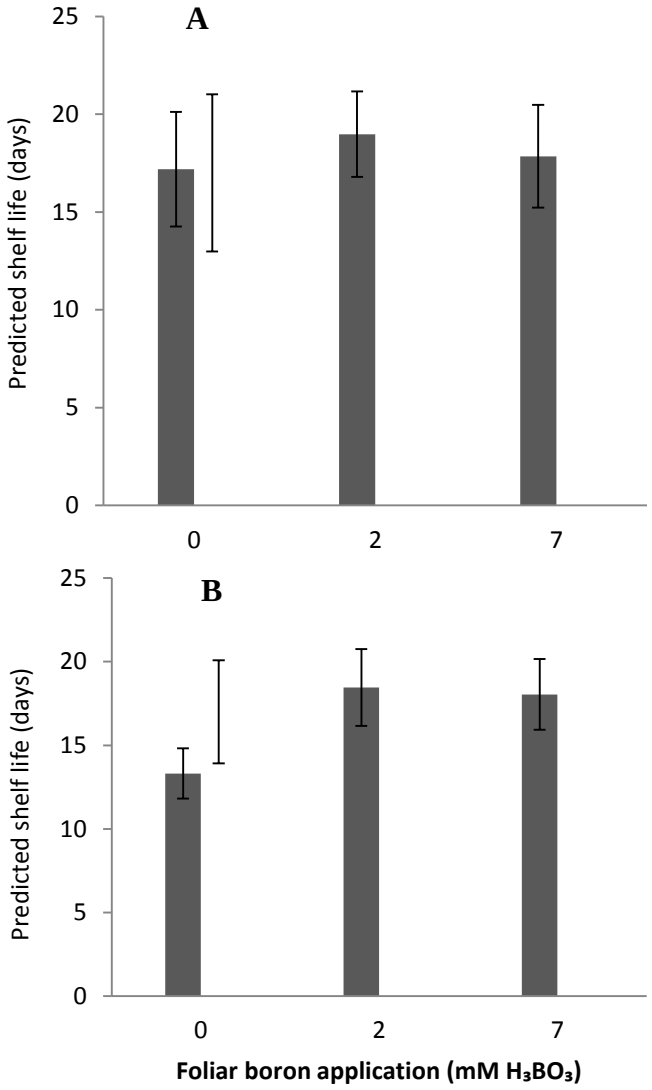


Fig. 4.15 Effect of foliar B application on shelf life of fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% in the second trial (higher B concentrations). Fruit were harvested from plants that received a foliar application of 0, 2 or 7 mM H_3BO_3. Plants were sprayed from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Shelf life of fruit was predicted at a general appearance (GA) of 5.5 by using a third degree polynomial in GenStat. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) for among individual boron concentrations are shown

Firmness of fruit during storage was not affected by application at 2.0 or 7.0 mM H_3BO_3 as a foliar spray, regardless of cultivars (Fig 4.16C, D). Firmness of fruit from cv. Aries decreased significantly (P =0.001) at 10 DAH but then did not change at 15 DAH and significantly increased at 23 DAH. In contrast, firmness of fruit from cv. Papri Queen increased significantly (P<0.001) from 10 DAH to 15 DAH but then remained constant to 23 DAH.

Water content in fruit at 23 DAH was significantly lower (P<0.001) than that in fruit at harvest from 10 DAH to 23 DAH, regardless of cultivar or B treatment (Fig4.16E, F). Water content in fruit (53.89%) from cv. Aries was significantly greater (P = 0.047) than that in fruit (47.24%) from cv. Papri Queen, regardless of B concentration (Fig 4.16E, F). Foliar B application did not affect water content in fruit from both cultivars.

For fruit from cv. Aries at 0 DAH, TSSC in fruit from control plants was significantly greater (P = 0.006) than that for fruit from plants sprayed with 2.0 mM H_3BO_3, but the same with that for fruit from plants sprayed with 7.0 mM H_3BO_3 (Fig 4.16G). At 23 DAH, TSSC in fruit from control plants was significantly (P = 0.006) lower than that from plants sprayed with either of the two B concentrations. For fruit from cv. Papri Queen, TSSC in fruit was not affected by application 2.0 or 7.0 mM H_3BO_3 as a foliar spray (Fig 4.16H).

Titratable acidity (TA) in fruit during storage, regardless of cultivar, was not significantly different among B concentrations (Fig 4.16K, I). For cv. Aries, TA in fruit decreased significantly (P<0.001) at 5 DAH then increased at 10 DAH, followed by a steady increase from 15 DAH to 23 DAH. For cv. Papri Queen, TA in fruit increased significantly (P<0.001) from 10 DAH to 23 DAH. Amount of acid in fruit from cv. Papri Queen was generally higher than in fruit from cv. Aries.

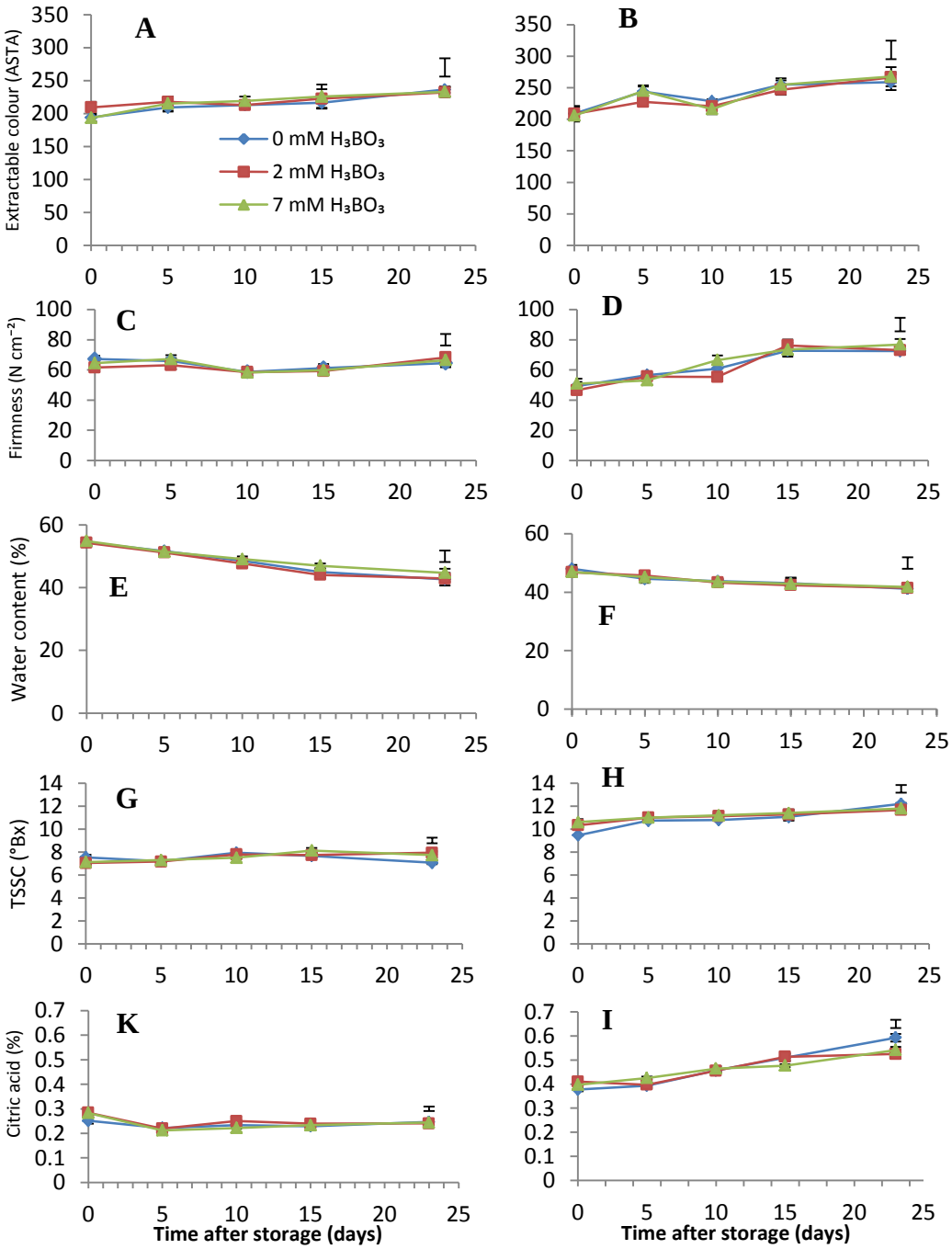


Fig. 4.16 Effect of foliar B application on postharvest quality of fruit during storage at 10°C and relative humidity of >90%: extractable colour (**A** and **B**); firmness (**C** and **D**); water content (**E** and **F**); TSSC (**G** and **H**) and TA (**K** and **I**) for cv. Aries and cv. Papri Queen, respectively in the second trial (higher B concentrations). Fruit were harvested from plants that received a foliar application of 0, 2 or 7 mM H_3BO_3 from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Data are means ± SE from n = 3. LSDs ($P \leq 0.05$) are shown for among individual B concentration for each time-point during storage

When considering grey mould development on fruit derived from young fruit that had been inoculated preharvest, lesion area on fruit from plants sprayed with 7.0 mM H_3BO_3 was significantly smaller (P = 0.037) from 18 DAH to 22 DAH than that from plants sprayed with 2.0 mM H_3BO_3 for cv. Aries (Fig 4.17A). For cv. Papri Queen, there was no significant difference for lesion area on fruit, regardless of the B concentration used on plants (Fig 4.17C). The percentage of fruit exhibiting rot from plants that were sprayed with 7.0 mM H_3BO_3 appeared to be lower than those that were not sprayed or sprayed with lower B concentration, regardless of cultivar (Fig 4.17B, D).

Time to reach 50% of maximum lesion area on fruit derived from young fruit that had been inoculated preharvest was not significantly different for cv. Aries, regardless of B concentration used on plants (Fig 4.18A). In contrast, for cv. Papri Queen, the time to reach 50% of maximum lesion area on fruit from control plants was significantly shorter (P = 0.013) than that for fruit from plants sprayed with 2.0 or 7.0 mM H_3BO_3 (Fig 4.18B). However, time to reach 50% of maximum lesion area was similar on fruit of plants sprayed with either B concentration.

The lesion area on fruit inoculated postharvest was not significantly different, regardless of B application on plants or cultivar (Fig 4.19A, B).

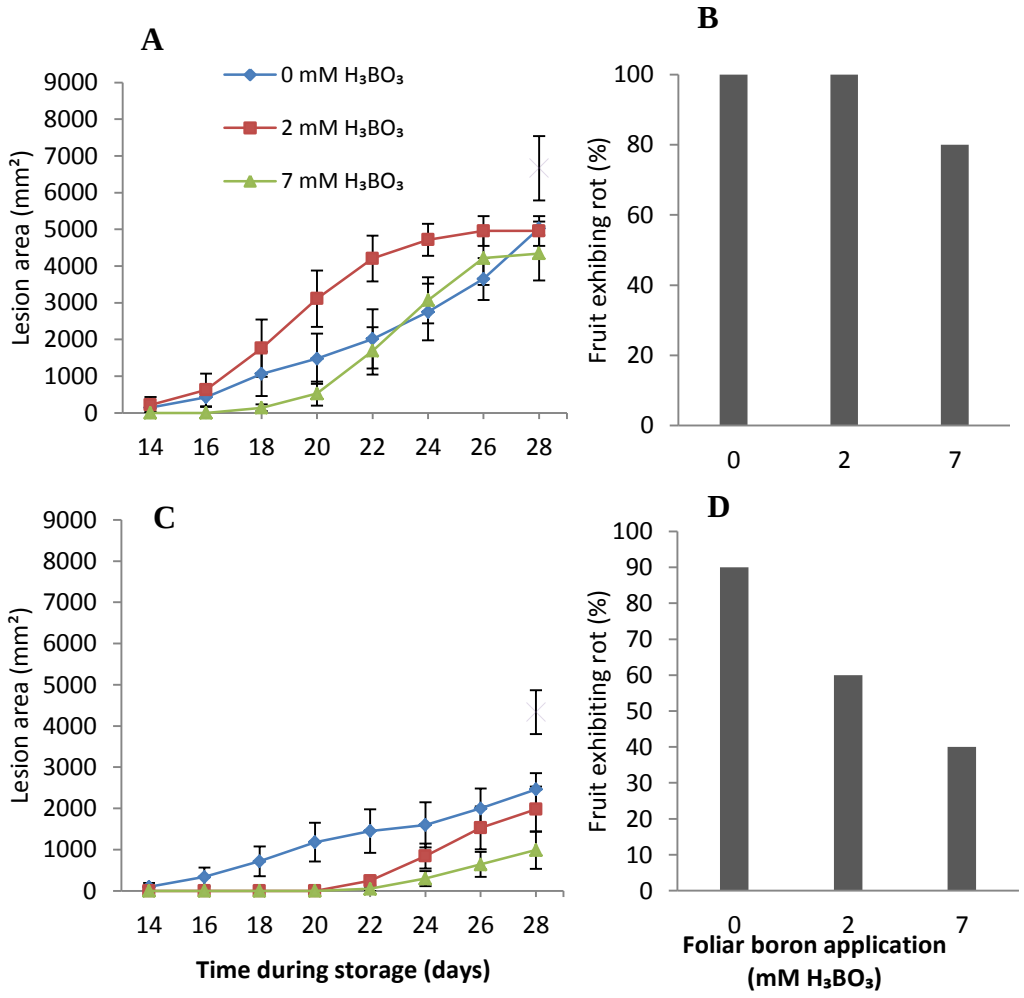


Fig. 4.17 Effect of foliar B application on grey mould development and percentage of fruit exhibiting rot of capsicum cv. Aries (**A** and **B**), cv. Papri Queen (**C** and **D**) during storage at 10°C and relative humidity of >90% in the second trial (higher B concentrations). Fruit were derived from young fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA when plants received a foliar application 0, 2 or 7 mM H_3BO_3. Plants were sprayed foliar boron from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual boron concentration for each time-point during storage

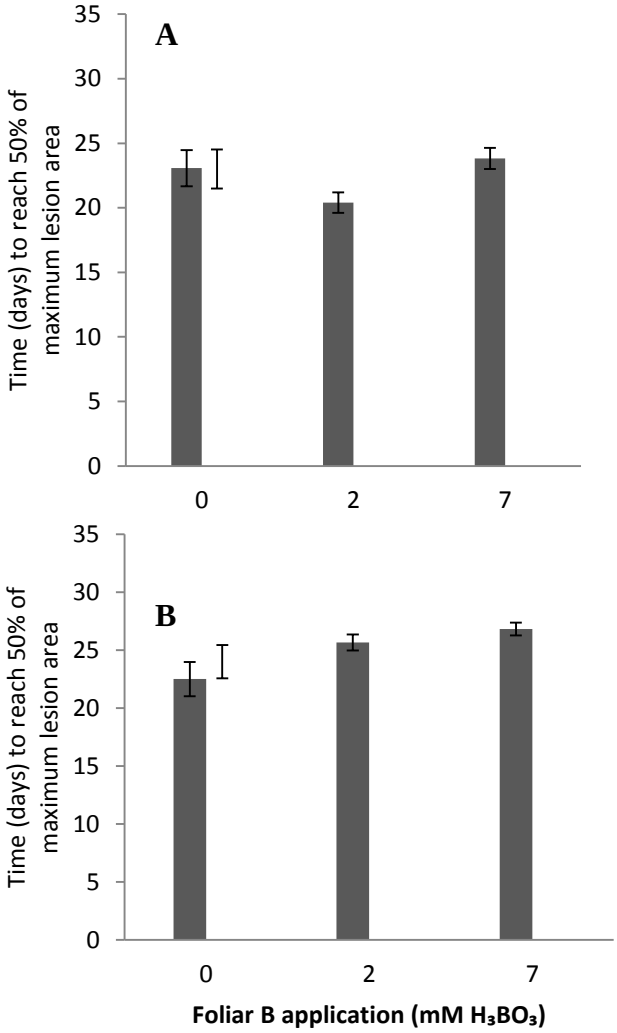


Fig. 4.18 Time after harvest for lesions to reach 50% of maximum size on fruit of capsicum cv. Aries (**A** and **B**) and cv. Papri Queen (**C** and **D**) during storage at 10°C and relative humidity of >90% for 28 days in the second trial (higher B concentrations) when plants received a foliar application of 0, 2 or 7 mM H_3BO_3. Plants were sprayed foliar boron from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Fruit were derived from young fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) for among individual boron concentrations at 28 DAH are shown

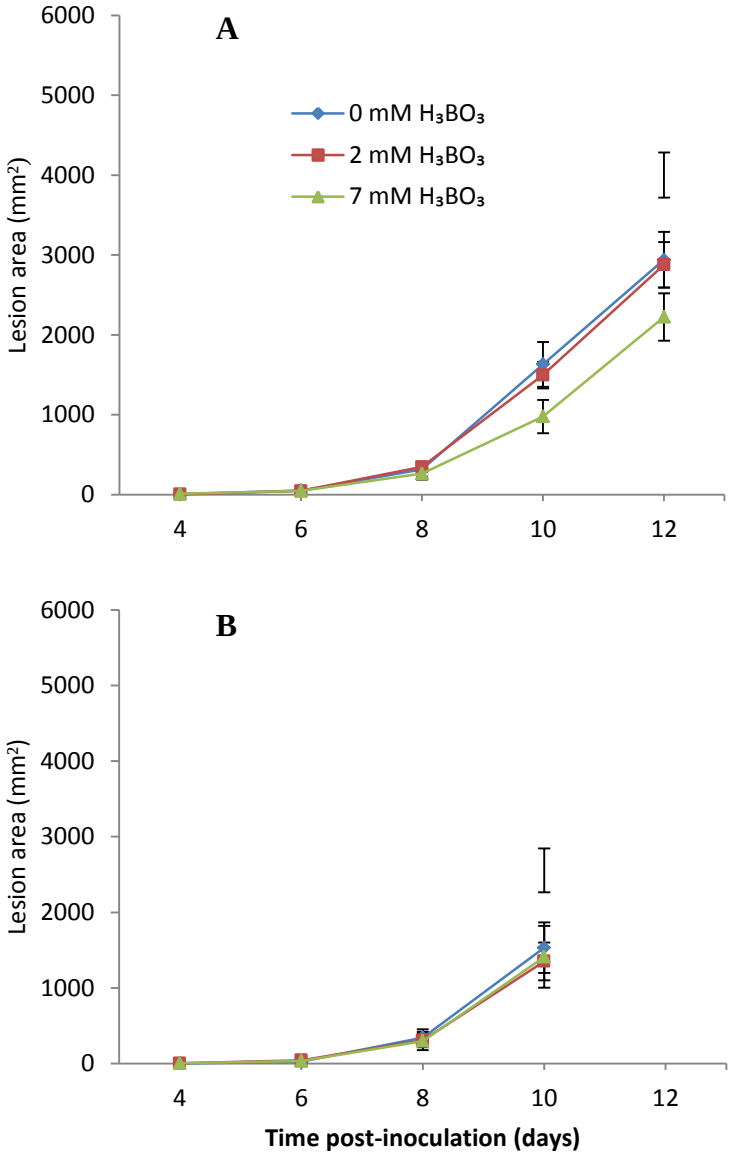


Fig. 4.19 Effect of foliar B application on grey mould development on postharvest-inoculated fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% in the second trial (higher B concentrations) when plants received a foliar application of 0, 2 or 7 mM H_3BO_3. Plants were sprayed foliar boron from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Fruit were wounded both sides and inoculated postharvest with 40 µL suspension of 10^5 conidia mL^{-1}. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual boron concentrations for each time-point post-inoculation

4.3.3 Negative correlation between lesion area on fruit and boron concentration on leaf and fruit tissues

The correlation between grey mould development on fruit derived from young fruit that had been inoculated preharvest and B concentration in leaves or fruit of capsicum cv. Aries and cv. Papri Queen was determined from 12 individual measurements of lesion area on fruit at 28 DAH and B concentration in leaves (Fig 4.20A) or in fruit (Fig 4.20B). Data from soil and foliar B application were used to build the trendline. Higher concentrations of B in the leaves and the fruit were associated with less severe disease, regardless of cultivar. However, there was only a significant negative correlation between B concentration in the leaves (R^2 =0.95) or in the fruit (R^2 =0.68) from cv. Aries and grey mould development on fruit.

When grey mould development was assessed on fruit inoculated postharvest, there was also a negative relationship between grey mould development on fruit and B concentration in leaf or fruit tissue (Fig 4.21A, B). However, there was only a significant correlation between B concentration in the fruit (R^2 =0.85) from cv. Papri Queen and grey mould development on fruit.

4.4 Discussion

In the present research, shelf life of fruit from B-treated plants from cv. Aries was longer than that from control plants, but preharvest B application did not affect yield or postharvest quality of fruit. Boron concentration in leaves and fruit from plants that received a soil B application was significantly higher than that from plants that received a foliar B application. Increasing B concentration in plant tissues resulted in restricting grey mould development on fruit, regardless of whether inoculation occurred before or after harvest.

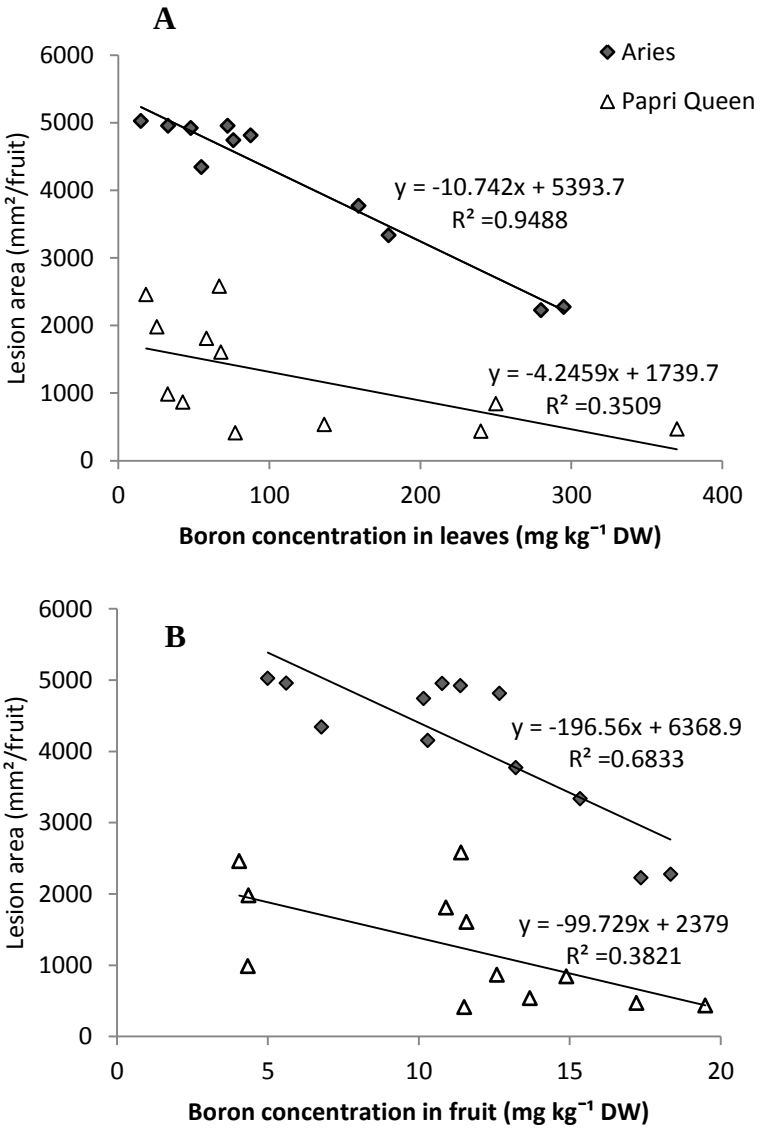


Fig. 4.20 The negative correlation between grey mould development on fruit from latent infection and B concentration in leaves (**A**) and fruit (**B**) of capsicum cv. Aries and cv. Papri Queen. Lesion area at 28 DAH on fruit (at 10°C and relative humidity of >90%) derived from young fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA. Young mature leaves and red fruit for nutrient analysis were collected at harvest. Data was collected from twelve individual measurements of grey mould development on fruit and B concentration in leaves or fruit in the experiments involving soil and foliar B application

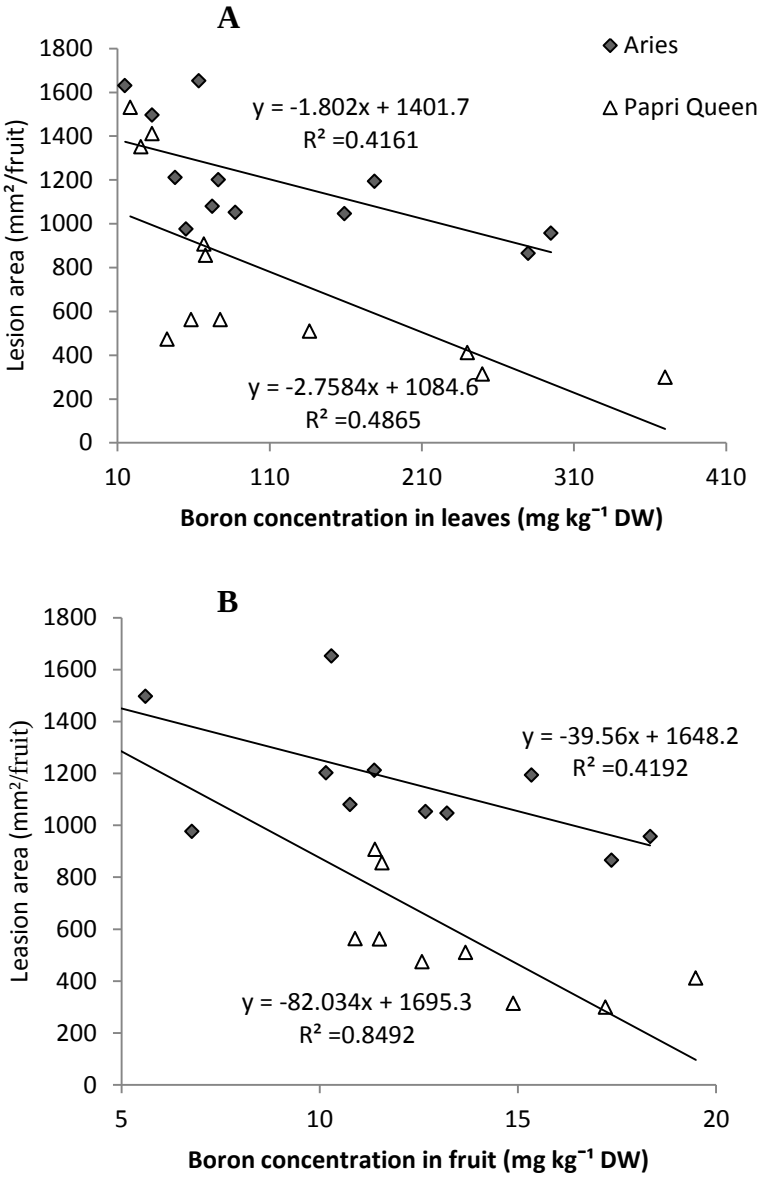


Fig. 4.21 The negative correlation between grey mould development on postharvest-inoculated fruit and B concentration in leaves (**A**) or fruit (**B**) of capsicum cv. Aries and cv. Papri Queen. Lesion area at day 10 on fruit (at 10°C and relative humidity of >90%) that were inoculated postharvest. Fruit were wounded on opposite sides and inoculated with 40 µL suspension of 10^5 conidia mL^{-1}. Young mature leaves and red fruit for nutrient analysis were collected at harvest. Data was collected from 12 individual measurements of grey mould development on fruit and B concentration in leaves or fruit in the experiments involving soil and foliar B application

The shelf life of fruit was related to water loss during storage which depends on surface area, epicuticular wax content and initial water content of fruit (Lownds et al. 1993; Kissinger et al. 2005). Water loss of pepper fruit has been shown to be positively correlated with initial water content and surface area, but negatively correlated with the amount and type of epicuticular wax (Lownds et al. 1993). In the present research, small fruit and low initial water content in fruit from cv. Papri Queen prevented water loss during storage. As a result, shelf life of fruit from cv. Papri Queen was not significantly different among B treatments. In contrast, fruit from cv. Aries of plants treated with boron generally had a longer shelf life than those from control plants, particularly when B was applied to the soil during growth of plants. Fruit from B-treated plants were observed to see less shrivelled during storage than those from control plants. However, water loss of fruit from B-treated plants (18.48%) was not significantly different compared with those from control plants (21.65%) after 23 days of storage. Foliar application of B with the highest rate of B (300 mg L^{-1}) was reported to lower the rate of defect formation in tomato fruit compared to a control (Huang and Snapp 2004). Boron may possibly decrease the cuticular micro-cracking on the fruit surface because B is believed to affect physiology of fruit when B binds apoplastic proteins to the cis-hydroxyl groups of membranes and cell walls (Dugger 1973) or when B interferes with manganese-dependent enzymatic reactions (Blevins and Lukaszewski 1998). In the present research, B-treated fruit may have less cracks and smaller cracks than control fruit. After harvest, softening in fruit that occurs due to a change of cell-wall carbohydrate metabolism and TSSC and TA of capsicum fruit increased during storage resulting from increasing activity of the degrading enzyme PG (Sethu et al. 1996). In this research, TSSC and TA from plants of the cv. Aries treated with B were generally lower than those from control plants after storage which means that postharvest quality of fruit from B-treated plants was better than those from control plants. Boron sprays before full bloom lowered TSSC in pear fruit cv. Conference than control plants due to reducing membrane permeability significantly after ripening (Wojcik and Wojcik 2003). Moreover, pear fruit cv. Conference from B-treated plants was also reported to have a lower respiration rate at harvest and during storage than those from control plants (Xuan et al. 2002). Ethylene is known as a ripening

hormone that can affect cell wall structure. Increasing ethylene production in fruit was associated with higher membrane permeability (Brady 1987). Lower ethylene production may have contributed to reducing membrane permeability in fruit from B-treated plants although ethylene production by capsicum fruit is low during storage due to its non-climacteric nature (Pham 2007). Therefore, more research is necessary to examine the effect of preharvest B application on membrane permeability of capsicum fruit.

In the present research, preharvest application of B, regardless of whether B was applied to the soil or as a foliar spray, did not affect extractable colour or firmness of fruit at harvest or during storage. The results were in agreement with previous research that showed quality parameters of fruit from control strawberry plants (Singh et al. 2007) or control pear plants did not differ with fruit from B-treated plants (Wojcik and Wojcik 2003).

Boron concentration in the leaf, regardless of whether B was applied to the soil or foliage, was significantly higher than B concentration in fruit. Boron uptake by roots of plants in the form of boric acid is believed to be a passive process (Brown et al. 2002). After uptake by roots, B is loaded in to the xylem for transporting to the growing point of stems and leaves, with this process being driven by the transpiration stream (Brown and Shelp 1997). Thus, nutrients in the xylem are mainly accumulated in the sites which have the highest transpiration rate (such as large leaves). In addition, B has been considered to be immobile in the phloem, with phloem transport being independent of transpiration. Nutrient movement in the phloem mainly supplies requirements of fruit and seeds, which do not have water loss (Brown and Shelp 1997). The results from the present research about nutrient status of capsicum plants confirmed the lack of mobility of B in phloem. In the present study, preharvest B application as a soil fertiliser was more effective than foliar B application for increasing B status in leaves and fruit. Similarly, spraying B before full bloom did not increase leaf and fruit B concentration of ‘Conference’ pear (Wojcik and Wojcik 2003). Boron concentration in leaves and in fruit from the first trial was significantly higher than that from the second trial. Leaf B

concentration from control plants was deficient in the second trial, but was adequate in the first trial, even though B concentration in the nutrient solution for control plants was the same in both trials. In addition, foliar spraying with 2.0 and 7.0 mM H_3BO_3 in the second trial increased leaf B concentration significantly but it was still significantly lower than leaf B concentration in the first trial, regardless of cultivar. Many species of plants maintain B concentration in the old leaves, while young leaves do not receive sufficient B for growth (Tanaka and Fujiwara 2008). In addition, B leaf concentration is not only related to transpiration rate but also to uptake rate by roots (Wojcik et al. 2008). The observation that B concentration in leaves from control plants was adequate in the first trial, but was deficient in the second trial probably reflects lower B uptake by roots as plants aged.

Boron is an important micronutrient for normal growth, yield and fruit quality. However, there is a narrow range between B deficiency and toxicity. In this study, capsicum plants that received a soil application at 0.1 mM H_3BO_3 showed symptoms of B toxicity in leaves, but this did not affect yield or quality of fruit as evidenced by the non-significant effect of length, width and weight of fruit as well as the number of fruit. This result was in agreement with research conducted by Eraslan et al. (2007) that examined the effect of a range of B concentrations regarded as toxic in capsicum plants. The fresh weight and dry weight were only significantly reduced at B concentration of 2483 mg kg^{-1}. In the present research, the highest B concentration (287.50 mg kg^{-1} for cv. Aries and 305 mg kg^{-1} for cv. Papri Queen) was much less than 2483 mg kg^{-1}. In addition, leaf B concentration from control plants in the second trial of B foliar application (14.98 mg kg^{-1} for cv. Aries and 18.38 mg kg^{-1} for Papri Queen) was deficient, based on the critical value of 20 mg kg^{-1} suggested by Reuter and Robinson (1997), but no obvious effect of deficiency on yield or quality of fruit was observed.

Preharvest application of B significantly decreased grey mould development following inoculation with *B. cinerea* preharvest and less fruit from B-treated plants exhibiting rot harvested than those harvested from control plants. The results of the present research showed a negative correlation between lesion area on fruit and B

concentration in leaves and fruit. Boron treatment was reported to also reduce infection of peaches by *Monilinia laxa*, which causes brown rot (Thomidis and Exadaktylou 2010) and to decrease the rate of defects on tomato fruit (Huang and Snapp 2004). Preharvest foliar application of B reduced blossom end rot, caused by Ca deficiency, on "Anna" apple fruit significantly (Khalifa et al. 2009). The higher concentration of B in fruit might maintain cell wall structure and reduce membrane permeability (Wojcik and Wojcik 2003). Given boron's role in reducing cuticle degradation (Blevins and Lukaszewski 1998), cell wall synthesis and maintaining cell wall structure by cross-linking of cell wall poly-saccharides (Hansch and Mendel 2009), infection by fungi may therefore be minimised by having adequate levels of B in the tissues, even though B concentration in the fruit was lower than in the leaves.

4.5 Conclusion

Preharvest soil application of B could be recommended for capsicum plants as an environmentally friendly method to reduce grey mould growth on fruit during storage as well as to improve the shelf life of fruit. Applying B to the soil was more effective than foliar sprays in terms of increasing B concentration in the leaves and the fruit at the early stages of plant development. However, foliar application at appropriate concentrations may be necessary when plants have a larger shoot mass to ensure B remains in the plant tissues at adequate levels.

CHAPTER FIVE

Effect of preharvest calcium applications

5.1 Introduction

Calcium (Ca) is an essential component of the plant cell wall that produces structural rigidity by forming cross-bridges between adjacent pectic acids or between pectic acids and other polysaccharides (Maas 1998; Easterwood 2002). However, Ca concentrations in fruit are often low because of the immobility of Ca in the phloem sap (Marschner 1995; White 2001). Calcium deficiency may cause physiological disorders of fruit including blossom-end rot in tomato and pepper (Geraldson 1957), splitting and spoilage in cherry (Meheriuk et al. 1991) or increased susceptibility to *Botrytis cinerea* (Schwab et al. 1993).

Botrytis cinerea, which causes serious loss in more than 200 crops worldwide (Williamson et al. 2007), may infect flowers or young developing fruit during preharvest and remain latent until environmental conditions are suitable for resumption of growth and ripening causes physiochemical and biochemical changes in the fruit, especially after harvest, leading to the development of grey mould (Droby and Lichter 2004). Ensuring that Ca is present at a high enough level in plant tissue, especially fruit, may therefore be necessary to reduce pathogen infection. Calcium ions appear to play a key role in the induction of the plant defence responses (Benhamou 1996). For example, phytoalexins thought to be antifungal, are produced by cultured soybean cells when treated with Ca^{2+} *in vitro* (Stäb and Ebel 1987) while intracellular Ca^{2+} appears necessary for β-1,3-glucan synthase to form callose, which re-enforces plant cell walls (Kauss et al. 1989). Moreover, increased Ca concentration in fruit may enhance cell wall resistance to the action of the pathogen's pectolytic enzymes including polygalacturonase (PG) and pectinesterase (PE) (Preston 1979; Conway et al. 1994a). Therefore, many studies have been focussed on increasing Ca concentration in the skin and flesh of fruit, including strawberry (Chéour et al. 1990; Naradisorn et al. 2006), peach (Manganaris et al. 2005; Elmer et al. 2007), sweet cherry (Ippolito et al. 2005) and table grape (Amiri et al. 2009), to improve postharvest quality of fruit and reduce fungal infection.

Fungicides are the most common way of reducing grey mould but alternative methods are necessary to minimise chemical use in fresh food crops (Sharma et al. 2009). Preharvest Ca application, by soil amendment or foliar spray, is one potential strategy to improve the quality of fruit and reduce disease incidence in fruit. Calcium chloride ($CaCl_2$) is not commonly used in the fruit industry because high chloride ion concentrations in plant tissues can be toxic to the plant (Xu et al. 1999). Calcium nitrate [$Ca(NO_3)_2$] is therefore preferred for use in research as a source of Ca because it is more soluble than other Ca-containing salts and not toxic at high concentrations. Calcium applied to soil at concentrations ranging from 1.0 to 8.0 mM $Ca(NO_3)_2$ was an effective method to increase Ca in fruit such as tomato (Elad and Volpin 1993), strawberry (Nam et al. 2006) and sweet pepper (Rubio et al. 2010). However, high temperature and relative humidity may affect Ca uptake by plants (Tadesse et al. 2002) and immobility of Ca within the plant may cause inefficient distribution of Ca in fruit (Conway et al. 1994a). Thus, soil fertilisation with Ca may not result in a sufficient increase of Ca in fruit. Application of Ca as a foliar spray may be an alternative way to increase Ca concentration in fruit. Spraying Ca during fruit development increased Ca in some fresh fruit, such as peach (Manganaris et al. 2005), strawberry (Singh et al. 2007), table grape (Amiri et al. 2009) and apple (Khalifa et al. 2009). However, the process of Ca penetration in fruit is not well understood (Serrano et al. 2004). Lenticels, cracks, stomata or surface discontinuities could allow Ca penetration (Glenn et al. 1985; Christensen 1995).

In previous studies, capsicum plants have been shown to respond positively to increased Ca concentration, regardless of whether Ca was applied to the soil or as a foliar spray. Increasing Ca in the root medium from 1.5 to 4 mM increased the marketable yield of sweet pepper (cv. Orlando) from 1.67 to 2.38 kg $plant^{-1}$ (Rubio et al. 2010). Weekly foliar sprays with 1% w/v $Ca(NO_3)_2$ for sweet peppers (cv. Hungarian Wax) increased Ca concentration in leaves as well as fruit, and were the most effective method in reducing blossom-end rot (Schon 1993), a physiological disorder commonly associated with Ca deficiency (Marcelis and Ho 1999). Increasing Ca in nutrient solution was effective against *B. cinerea* of sweet paper plants (Yoon et al. 2010). However, the role of Ca in postharvest quality of capsicum

fruit from other cultivars and grey mould development in capsicum has not been reported. Therefore, the research reported in this chapter aimed to examine the effect of preharvest soil or foliar Ca application on postharvest quality of fruit and grey mould development in capsicum fruit from cv. Aries and cv. Papri Queen.

5.2 Methods and materials

5.2.1 Plants and growth conditions

Capsicum plants of cv. Aries and cv. Papri Queen were used in these experiments. All plants were grown in a greenhouse at the Waite Campus of the University of Adelaide as per Section 4.2.1.

5.2.2 Nutrient solution for Ca application

5.2.2.1 Soil application

Initially, the basal nutrient solution was modified from Hoagland's solution (Hoagland and Arnon 1938) as described by Rubio *et al.* (2010) to obtain four Ca treatments: 0.0, 1.5, 4.0 or 8.0 mM Ca (Table 5.1). Calcium nitrate [$Ca(NO_3)_2$] was used as the source of Ca. Micronutrient solutions were also prepared and applied to plants in all the treatments as described in Section 4.2.2.1. Hoagland's solution (200 mL) in the form of four Ca concentrations, plus micronutrients, was applied manually to each plant by pouring into the soil every 2 days, while reverse osmosis (RO) water was used to maintain soil moisture, particularly for hot days during summer.

A pilot experiment showed that application of nutrient solution containing 0 or 1.5 mM Ca from when seedlings had been transplanted to individual pots was not appropriate for growth of either cultivar, due to Ca deficiency (Fig 5.1). Consequently, there were not enough red fruit for subsequent experiments. Therefore, in the first trial, plants received 4.0 mM Ca in Hoagland's solution until the plants had flowers, after which one of the three concentrations (1.5, 4.0 or 8.0 mM Ca) was applied until fruit were harvested at the red stage (100% with low intensity) (60 - 65 days after anthesis). However, nutrient analysis from the first trial

showed that the Ca concentration in leaf and fruit tissues was not significantly different among the three Ca concentration treatments and all were in the adequate range (Reuter and Robinson 1997) (Table 5.2). Therefore, a new set of plants was not used, but based on continued growth of the plants used in the first experiment Plants received 4.0 mM Ca in Hoagland's solution until the plants had flowers and then they were treated with one of the three Ca concentrations: 0.0, 1.5 or 4.0 mM Ca until fruit were harvested at the red stage.

For each cultivar, there were twenty plants per treatment divided into five replicate groups. For each group, there were four plants per treatment arranged randomly in the greenhouse.

5.2.2.2 Foliar application

Plants that were treated with a foliar Ca application continued to be watered with basal nutrient solution containing no Ca and in which 4.0 $Ca(NO_3)_2$ was replaced by 6.0 mM NH_4NO_3 (Section 5.2.2.1). Capsicum plants were sprayed from flowering to harvest of red fruit (five - seven sprays) as described in Section 4.2.2.2 but with $Ca(NO_3)_2$. Three Ca concentrations: 0.5, 0.75 or 1.0% w/v $Ca(NO_3)_2$ were initially trialled. Results showed that Ca concentration in leaf or fruit tissue was not significantly different among the three Ca treatments and Ca concentration in leaves was within the critical range (Table 5.4). Calcium concentrations: control, 0.5 or 1.5% $Ca(NO_3)_2$ were therefore selected for the second foliar application trial (Table 5.5). However, spraying plants with 1.5% w/v $Ca(NO_3)_2$ caused leaf necrosis, so Ca concentration treatment at 1.5% w/v $Ca(NO_3)_2$ was adjusted to 1.0% w/v $Ca(NO_3)_2$ after the first spray. For each cultivar, there were twenty plants per treatment divided into three treatment groups which were arranged randomly in the greenhouse.

Table 5.1 Concentration of nutrients (mM) in Hoagland's solution to form four Ca treatments which were applied to the soil preharvest

Nutrient source	0.0 mM Ca	1.5 mM Ca	4.0 mM Ca	8.0 mM Ca
KNO_3	6	5	6	0
$Ca(NO_3)_2$	0	1.5	4	7
NH_4NO_3	6	6	0	0
K_2SO_4	0	0	0	3
$MgSO_4.7H_2O$	1	1	1	1
$NaH_2PO_4.2H_2O$	0	0	0	1
KH_2PO_4	1	1	1	0
KCl	0.05	0.05	0.05	0.05
$CaCl_2.6H_2O$	0	0	0	1

5.2.3 Nutrient analysis

Youngest mature leaves (~ 20 g) were randomly collected at harvest from a total of 20 treated plants and three replicate red fruit per treatment for each cultivar were randomly collected from a total of 20 treated plants for nutrient analysis. Nutrient analysis was conducted as described in Section 4.2.3.

5.2.4 Inoculation of fruit preharvest or postharvest

5.2.4.1 Isolation, maintenance and culture of *B. cinerea*

The isolation, maintenance and culture of *B. cinerea* were as described in Chapter 3. Production of conidia for use as inoculum (at 10^4,10^5, 10^6 conidia mL^{-1}) was as per Chapter 3.

5.2.4.2 Preharvest inoculation of young developing fruit

Fifteen young developing fruit [3 days after anthesis (DAA)] on multiple flowering nodes on each plant were randomly selected, tagged and inoculated as described as in Section 4.2.4.2.

5.2.4.3 Postharvest inoculation of fruit

Ten symptomless, uniform red fruit (100% red with low intensity) were harvested per treatment for each cultivar from a total of 20 treated plants for postharvest inoculation as described in Section 4.2.4.3.

5.2.5 Assessment of grey mould development and postharvest quality in fruit

5.2.5.1 Grey mould development on fruit

Assessment of development of grey mould during storage used the protocols described in Section ‘Assessment of grey mould development’ in Chapter 3. The fruit were monitored daily and the number of fruit exhibiting rot was recorded. Rot development was quantified as lesion area on individual fruit by measuring the

length and width of decayed areas using digital callipers (digiMax, Switzerland), and multiplying them.

The correlation between lesion area on fruit and Ca concentration in leaves and fruit of capsicum cv. Aries and cv. Papri Queen was determined based on 12 individual measurements of grey mould development on fruit and Ca concentration in leaves and fruit from experiments involving soil or foliar Ca application. Lesion area on fruit derived from young developing fruit inoculated preharvest was measured at 28 days after harvest (DAH), while lesion area on fruit inoculated postharvest were measured at 10 days post-inoculation (DPI).

5.2.5.2 Postharvest quality of fruit

To assess postharvest quality of fruit, fruit were assessed at harvest and then at 5, 10, 15 and 23 days after harvest. Three fruit at each time-point per treatment (a total of 15 fruit) were used to assess shelf life, water content, extractable colour, firmness, total soluble solid content (TSSC) and titratable acidity (TA) as per Section 4.2.5.1. Soil application of Ca in both trials and foliar application of Ca in the first trial showed that extractable colour and TA in fruit were not affected by Ca treatment. Therefore, extractable colour and TA were not measured in the second trial of foliar application.

5.2.6 Statistical analysis and photography

Experiments were designed randomly using the factors of cultivar and Ca concentration. Data were subjected to repeated measurements analysis of variance (ANOVA) using GenStat and means of the treatments compared using the LSD at a significance level of ≤ 0.05. Means and standard errors were determined using Microsoft Excel. Photography was by a digital camera (Canon D500, Japan).

5.3 Results

5.3.1 Symptoms of Ca deficiency in plants treated with low Ca concentration

Symptoms of Ca deficiency such as yellow leaves and dwarfing were observed for plants that received a soil application of 0.0 or 1.5 mM Ca, regardless of cultivar (Fig 5.1A, B). No mature fruit survived on plants treated with 0.0 mM Ca from transplant to fruiting. Fruit died at an early stage from 7 to 10 DAA. Most fruit (80 - 85%) also died and very few fruit survived until red stage when plants were treated with 1.5 mM Ca. Symptoms of Ca deficiency on dying fruit from both cultivars were observed as likely blossom-end rot (Fig 5.1C, D).

5.3.2 Effect of Ca applied to soil on nutrient status of plant tissues, postharvest quality and grey mould development on capsicum fruit

5.3.2.1 Soil application of Ca in the first trial (1.5, 4.0 or 8.0 mM Ca)

Regardless of cultivar, Ca concentration in leaf tissues increased with increasing Ca concentrations applied to the soil (Table 5.2). When plants were treated with 4.0 or 8.0 mM Ca, Ca concentration in the leaves from cv. Aries was greater than that in leaves from cv. Papri Queen. For cv. Aries, Ca concentration in leaves from plants treated with 8.0 mM Ca was significantly higher ($P<0.001$) than that in leaves from plants treated with the two lower Ca concentrations and Ca concentration in leaves from plants treated with 4.0 mM Ca was significantly greater than that in leaves from plants treated with 1.5 mM Ca. For cv. Papri Queen, Ca concentration in leaves from plants treated with 1.5 mM Ca was significantly lower ($P<0.001$) than that in leaves from plants treated with 4.0 or 8.0 mM Ca, which were similar to each other. However, Ca concentration in fruit, regardless of cultivar, was not significantly different among plants treated with any of the three Ca concentrations (Table 5.2). There were no significant differences in other nutrients in leaf or fruit tissues, regardless of cultivar or Ca concentration (Appendix A.5). However, manganese concentration in leaves from both cultivars appeared deficient compared to that considered to be adequate (26 - 100 mg kg^{-1}), regardless of cultivar or Ca treatment (Reuter and Robinson 1997).

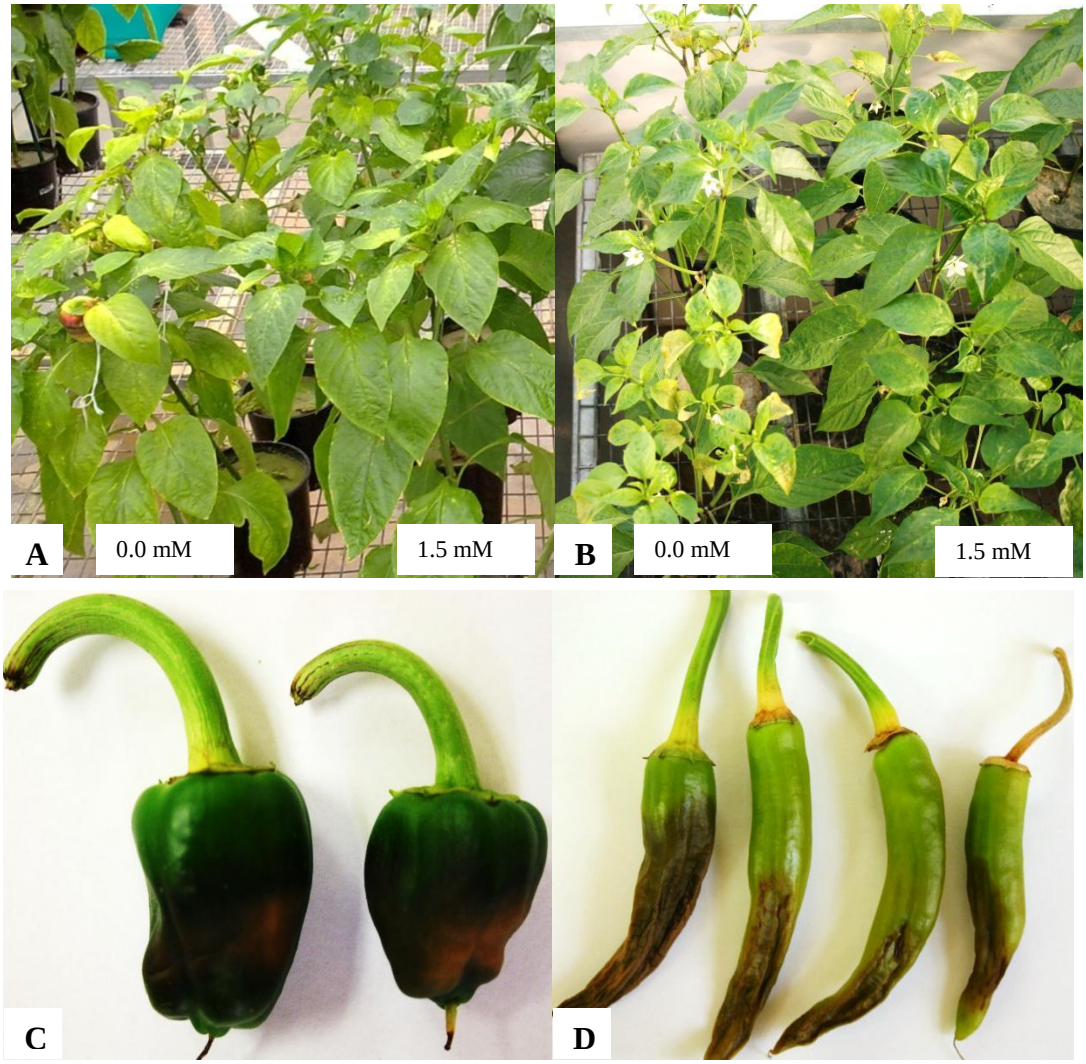


Fig. 5.1 Symptoms of Ca deficiency in capsicum plants and fruit from cv. Aries (**A**, **C**) and cv. Papri Queen (**B**, **D**) in the pilot experiment. Plants received a soil application of 0.0 or 1.5 mM Ca from transplant to fruiting (A, B). Fruit from cv. Aries (C) and cv. Papri Queen (D) when capsicum plants received 1.5 mM Ca from transplant to fruiting. Representative images are shown

Table 5.2 Effect of soil Ca application on nutrient status in capsicum plant tissues in the first trial. Other nutrients are detailed in table in Appendix A.5. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that received a soil application of 1.5, 4.0 or 8.0 mM Ca in Hoagland's solution (200 mL per plant every 2 days) from flowering to harvest of red fruit. Data are presented as means ± SE from n = 3. For each cultivar, means with the same letters in each column were not significantly different as determined using the LSD (P<0.05)

Cultivar	**Ca treatment [mM $Ca(NO_3)_2$]**	**Ca concentration (mg kg^{-1} DW)**	
		Leaf	**Fruit**
Aries	1.5	10250.00 ± 50.00[a]	993.33 ± 150.26[a]
	4.0	25000.00 ± 0.00[b]	1120.67 ± 153.67[a]
	8.0	34000.00 ± 1000.00[c]	1096.67 ± 8.82[a]
	LSD (P value)	*2601.70 (<0.001)*	*(0.75)*
Papri Queen	1.5	7850.00 ± 550.00[a]	836.67 ± 92.80[a]
	4.0	20500.00 ± 500.00[b]	1053.33 ± 48.07[a]
	8.0	21000.00 ± 0.00[b]	1226.67 ± 221.69[a]
	LSD (P value)	*1931.40 (<0.001)*	*(0.23)*

Calcium treatment did not significantly affect shelf life of fruit, regardless of cultivar (Fig 5.2A, B). The shelf life of fruit cv. Aries was similar to those from cv. Papri Queen, regardless of Ca treatment

Extractable colour in fruit generally increased during storage and was significantly higher at the end of storage than that in fruit at harvest (Fig 3A, B). For cv. Aries, application of Ca to the soil did not significantly affect extractable colour of fruit during storage (Fig 5.3A). In contrast, extractable colour of fruit from cv. Papri Queen plants treated with 8.0 mM Ca was significantly higher ($P = 0.004$) at 5 DAH, but significantly lower at 23 DAH than that in fruit from plants treated with lower Ca concentrations (Fig 5.3B). At the end of storage, extractable colour in fruit from cv. Papri Queen was significantly greater ($P = 0.01$) than that in fruit from cv. Aries.

Firmness of fruit was not significantly affected by soil application of Ca, regardless of cultivar (Fig 5.3C, D). Firmness of fruit from cv. Aries significantly decreased ($P<0.001$) at 5 DAH but significantly increased at 10 DAH and then no change was observed at 23 DAH. Firmness of fruit from cv. Papri Queen increased significantly ($P<0.001$) from 5 DAH to 10 DAH but then remained constant from 15 to 23 DAH.

TSSC in fruit from cv. Aries increased during storage and was significantly higher ($P<0.001$) at 23 DAH than that in fruit at harvest (Fig 5.3E). Soil application of Ca did not affect the TSSC in fruit from cv. Aries at harvest or during storage. In contrast, soil application of Ca significantly affected TSSC in fruit from cv. Papri Queen (Fig 5.3F). TSSC in fruit from plants treated with 4.0 or 8.0 mM Ca increased steadily during storage and was significantly higher ($P<0.001$) at 23 DAH than that at harvest. TSSC in fruit from plants treated with 4.0 mM Ca was significantly higher than that in fruit from plants treated with 8.0 mM Ca at harvest, but was the same during storage. TSSC in fruit from plants treated with 1.5 mM Ca fluctuated and was significantly lower ($P = 0.011$) than that in fruit from plants treated with higher Ca concentrations at 0 DAH, 10 DAH and 23 DAH. TSSC in fruit from cv. Papri Queen was significantly greater ($P = 0.001$) than that in fruit from cv. Aries, regardless of Ca treatment.

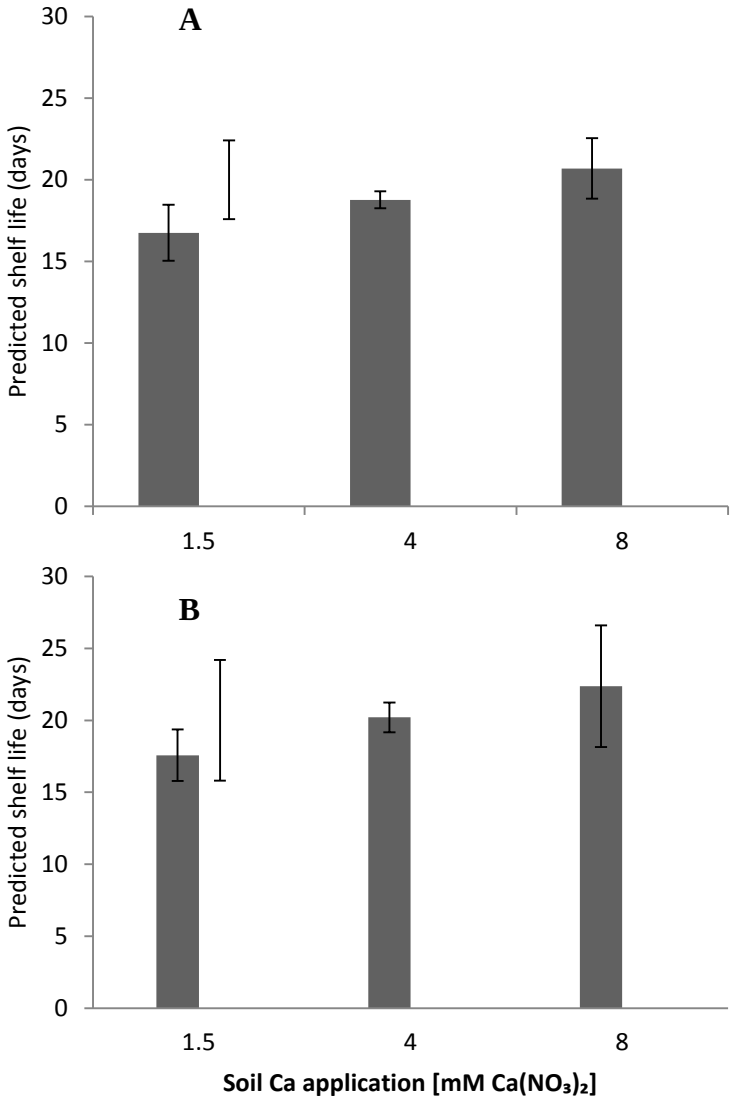


Fig. 5.2 Effect of soil Ca application on shelf life of fruit from cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% in the first trial. Fruit were harvested from plants treated by watering with 1.5, 4 or 8 mM $Ca(NO_3)_2$ in Hoagland's solution at a rate of 200 mL per plant every 2 days from flowering to harvest of red fruit. Shelf life of fruit was predicted at a general appearance (GA) of 5.5 by using a third degree polynomial in GenStat (Section 4.2.4.2). Data are means ± SE from n = 3. LSDs ($P \leq 0.05$) for among individual Ca concentrations are shown

TA in fruit from cv. Aries fluctuated during storage, but was significantly higher ($P<0.001$) at the end of storage than that in fruit at harvest, regardless of Ca concentration treatment (Fig 5.3G). For cv. Aries, TA in fruit was not significantly different at harvest or at the end of storage among Ca concentration treatments. However, TA in fruit from plants treated with 8.0 mM Ca was significantly higher ($P<0.001$) at 10 DAH, but significantly lower at 15 DAH than that in fruit from plants treated with lower Ca concentrations. At 15 DAH, TA in fruit from plants treated with 1.5 mM Ca was significantly higher than that in fruit from plants treated with higher Ca concentrations. For cv. Papri Queen, TA in fruit from plants treated with 1.5 mM Ca was significantly ($P<0.001$) lower than that in fruit from plants treated with higher Ca concentrations from 0 DAH to 10 DAH and 23 DAH (Fig 5.3H). TA in fruit from cv. Papri Queen was significantly greater ($P<0.001$) than that in fruit from cv. Aries, regardless of Ca treatment.

When considering grey mould development on fruit derived from young developing fruit that had been inoculated preharvest, lesion area on fruit from plants treated with 4.0 mM Ca was significantly smaller ($P = 0.021$) by the end of the experiment than on fruit from plants treated with 1.5 mM or 8.0 mM Ca for cv. Aries (Fig 5.4A). The percentage of fruit exhibiting rot from plants that were treated with 4.0 mM Ca appeared to be lower than those from plants that were treated with 1.5 mM or 8.0 mM Ca (Fig 5.4B). For Papri Queen, lesion area on fruit was not significantly different among fruit from plants treated with different Ca concentrations (Fig 5.4C). The percentage of fruit exhibiting rot from plants that were treated with 8.0 mM Ca appeared to be lower than those from plants that were treated with 1.5 mM or 4.0 mM Ca (Fig 5.4D). Lesions on fruit from cv. Aries were significantly larger than those on fruit from cv. Papri Queen, regardless of Ca treatment.

Although time to reach 50% of maximum lesion area on fruit appeared to be one day different, soil application did not delay the time for grey mould to reach 50% of maximum lesion, regardless of cultivar (Fig 5.5A, B).

The lesion area on fruit inoculated postharvest was not significantly different, regardless of Ca application to plants or cultivar (Fig 5.6A, B).

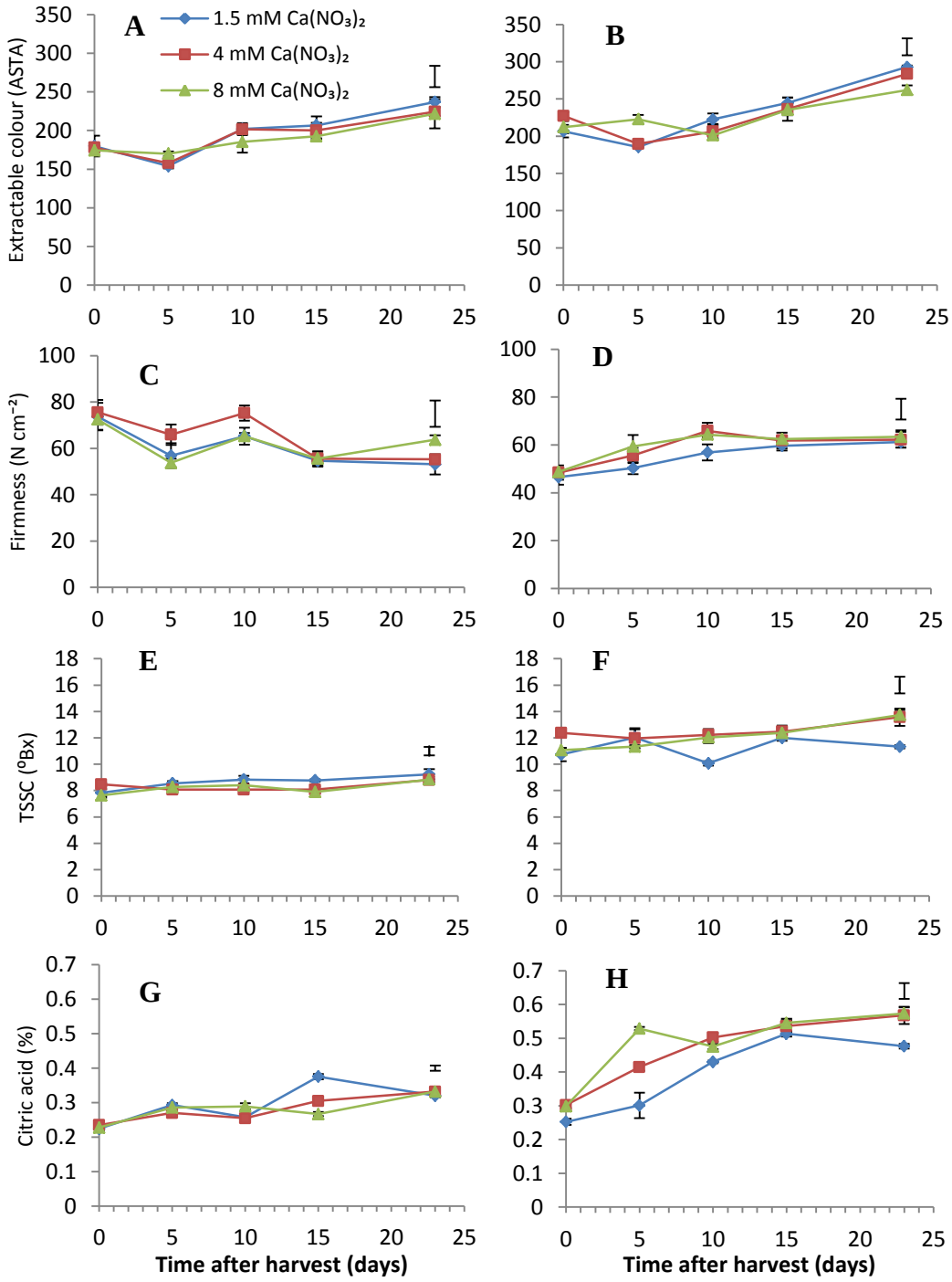


Fig. 5.3 Effect of soil Ca application on postharvest quality of fruit during storage at 10°C and relative humidity of >90%: extractable colour (**A** and **B**); firmness (**C** and **D**); TSS (**E** and **F**) and acidity (**G** and **H**) for cv. Aries and cv. Papri Queen, respectively in the first trial. Fruit were harvested from plants treated by watering with 1.5, 4 or 8 mM $Ca(NO_3)_2$ in Hoagland's solution at a rate of 200 mL per plant every 2 days from flowering to harvest of red fruit. Data are means ± SE from n = 3. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point during storage

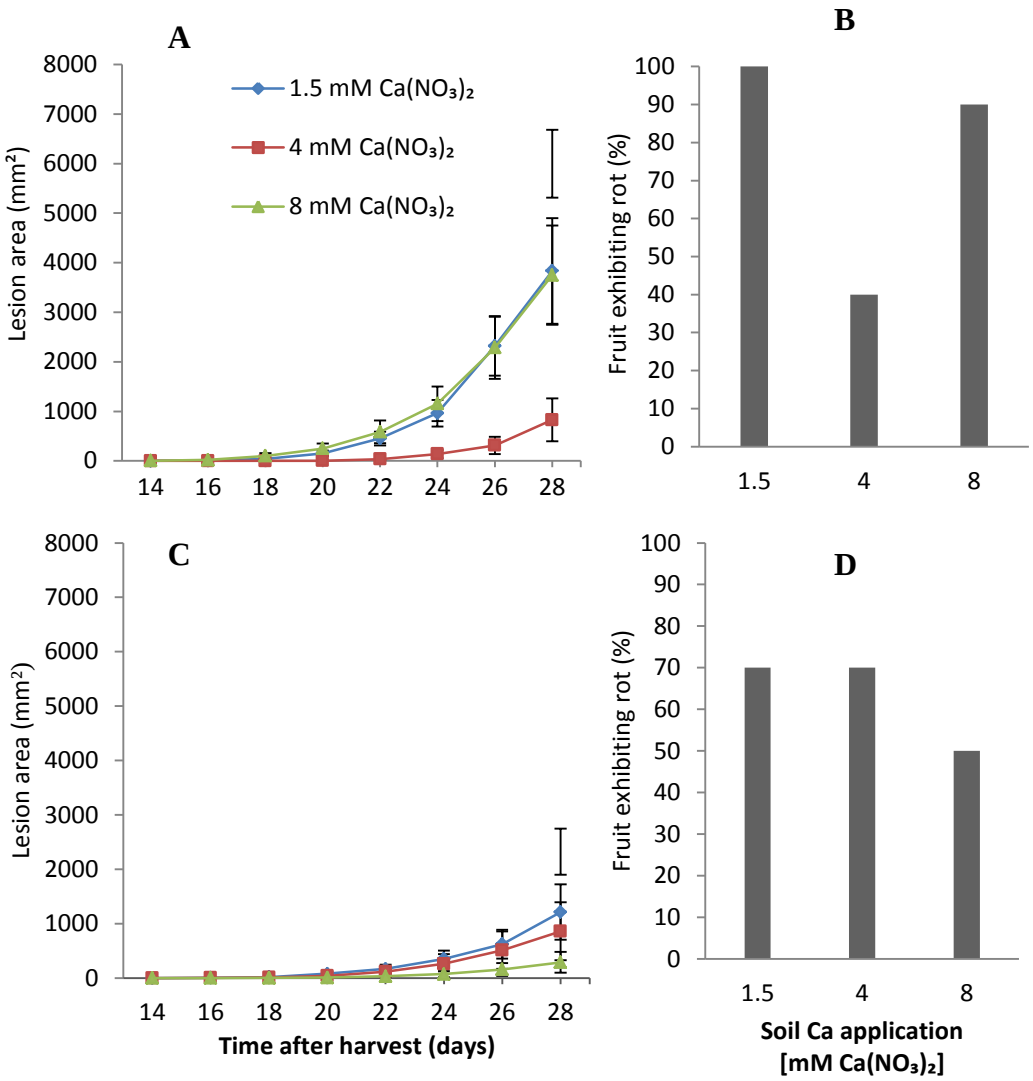


Fig. 5.4 Effect of soil Ca application on grey mould development and the number of fruit of capsicum cv. Aries (**A** and **B**) and cv. Papri Queen (**C** and **D**) exhibiting rot during storage at 10°C and relative humidity of >90% in the first trial. Fruit were derived from young developing fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA when plants received a soil application of 1.5, 4 or 8 mM $Ca(NO_3)_2$ in Hoagland's solution at a rate of 200 mL per plant every 2 days from flowering to harvest red fruit. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point during storage

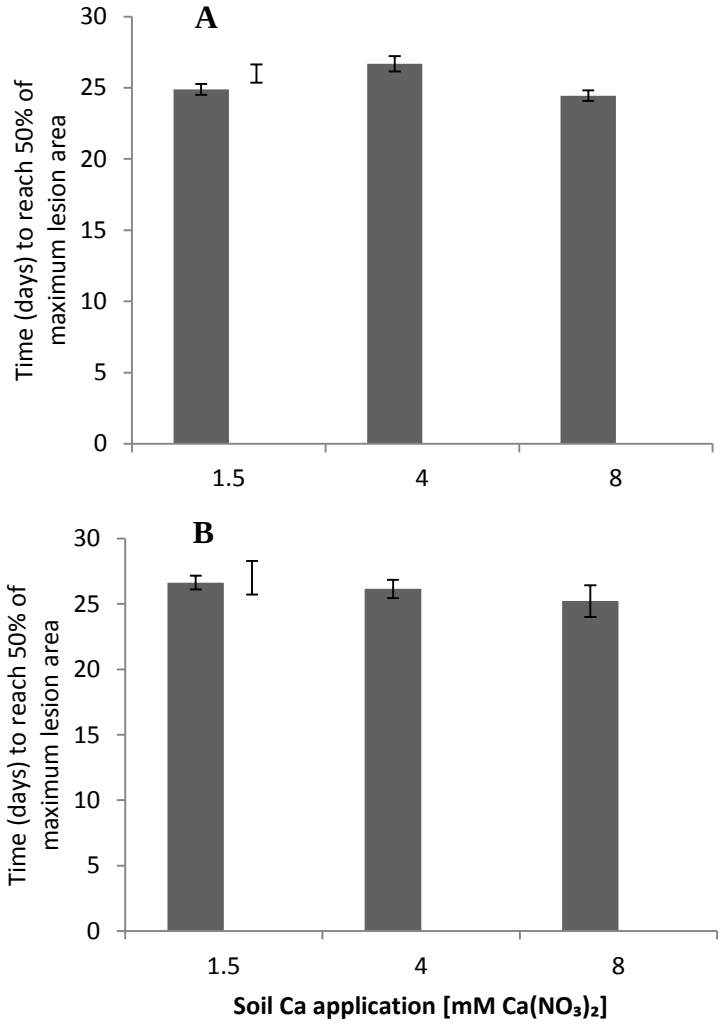


Fig. 5.5 Time after harvest for grey mould lesions to reach 50% of maximum size on fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% for 28 days in the first trial. Fruit were derived from young developing fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA when plants received a soil application of 1.5, 4 or 8 mM $Ca(NO_3)_2$ in Hoagland's solution at a rate of 200 mL per plant every 2 days from flowering to harvest of red fruit. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) for among individual Ca concentrations at 28 days after harvest are shown

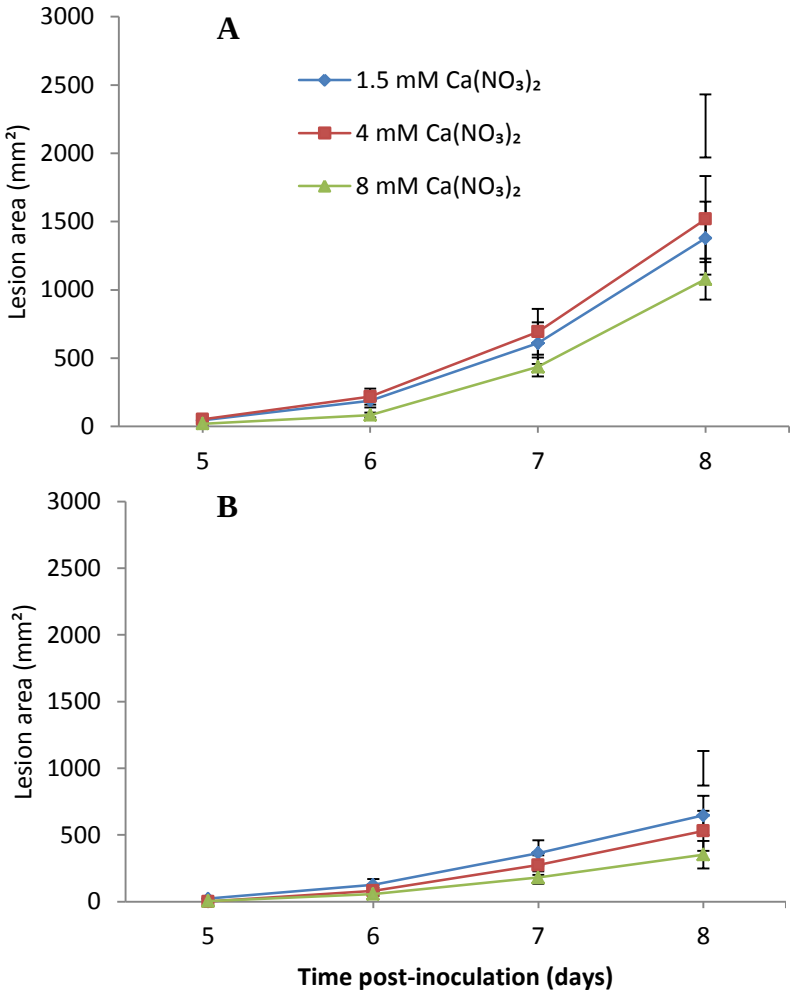


Fig. 5.6 Effect of soil Ca application on grey mould development on postharvest-inoculated fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% in the first trial when plants received a soil application of 1.5, 4 or 8 mM $Ca(NO_3)_2$ in Hoagland's solution at a rate of 200 mL per plant every 2 days from flowering to harvest red fruit. Fruit were wounded on opposite sides and inoculated postharvest with 40 µL suspension of 10^5 conidia mL^{-1}. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point post-inoculation

5.3.2.2 Soil application of Ca in the second trial (0.0, 1.5 or 4.0 mM Ca)

Ca concentration in leaf tissues was significantly different among plants treated with the three Ca concentrations: 0.0, 1.5 or 4.0 mM Ca, from flowering to harvest of red fruit (Table 5.3). The Ca concentration in leaf tissues from plants treated with 4.0 mM Ca was significantly greater (P = 0.001) than that from plants treated with 1.5 mM Ca or control plants and Ca concentration in leaf tissues from control plants was significantly lower than that from plants treated with 1.5 mM Ca, regardless of cultivar. Calcium concentration in leaf tissue from control plants, regardless of cultivar, appeared deficient compared to levels considered to be adequate (100000 - 37000 mg kg^{-1} for cv. Aries and 4000 - 10000 mg kg^{-1} for cv. Papri Queen) (Reuter and Robinson 1997). However, symptoms of Ca deficiency were not observed. Calcium concentration in fruit, regardless of cultivar, was not significantly different among plants treated with any of the three Ca concentrations. There were no significant differences in other nutrients in leaf or fruit tissues, regardless of Ca concentration (Appendix A.6). However, concentration of copper in leaf tissues from both cultivars appeared deficient compared to that considered to be adequate (6 - 25 mg kg^{-1}) (Reuter and Robinson 1997).

For cv. Aries, length and width of fruit were not significantly different among fruit from plants treated with the three Ca concentrations (Fig 5.7A). Weight of fruit from plants treated with Ca was similar but was significantly heavier (P = 0.02) than those from control plants. For cv. Papri Queen, length, width and weight were not significantly different among fruit from plants treated with the three Ca concentrations (Fig 5.7A, B). The number of fruit was similar on plants treated with different Ca concentrations, regardless of cultivar (data not shown).

The shelf life of fruit was not significantly different, regardless of cultivar (Fig 5.8A, B). The shelf life of fruit from cv. Aries was similar to that for fruit from cv. Papri Queen.

Table 5.3 Effect of soil Ca application on nutrient status in capsicum plant tissues in the second trial. Other nutrients are detailed in Appendix A.6. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that received a soil application: 0.0, 1.5 or 4.0 mM Ca in Hoagland's solution (200 mL per plant every 2 days) from flowering to harvest of red fruit. Data are presented as means ± SE from n = 3. For each cultivar, means with the same letters in each column were not significantly different as determined using the LSD (P<0.05)

Cultivar	**Ca treatment [mM $Ca(NO_3)_2$]**	**Ca concentration (mg kg^{-1} DW)**	
		Leaf	**Fruit**
Aries	Control	*9450.00 ± 450.00[a]	726.67 ± 43.72[a]
	1.5	24500.00 ± 500.00[b]	903.33 ± 103.33[a]
	4.0	48000.00 ± 2000.00[c]	983.33 ± 48.42[a]
	LSD (P value)	*5483.00 (<0.001)*	*(0.1)*
Papri Queen	Control	*3600.00 ± 1000.00[a]	966.67 ± 179.01[a]
	1.5	18350.00 ± 950.00[b]	943.33 ± 117.24[a]
	4.0	28500.00 ± 1500.00[c]	1456.67 ± 79.65[a]
	LSD (P value)	*5295.10 (0.001)*	*(0.056)*

**deficient as stated by Reuter and Robinson (1997)*

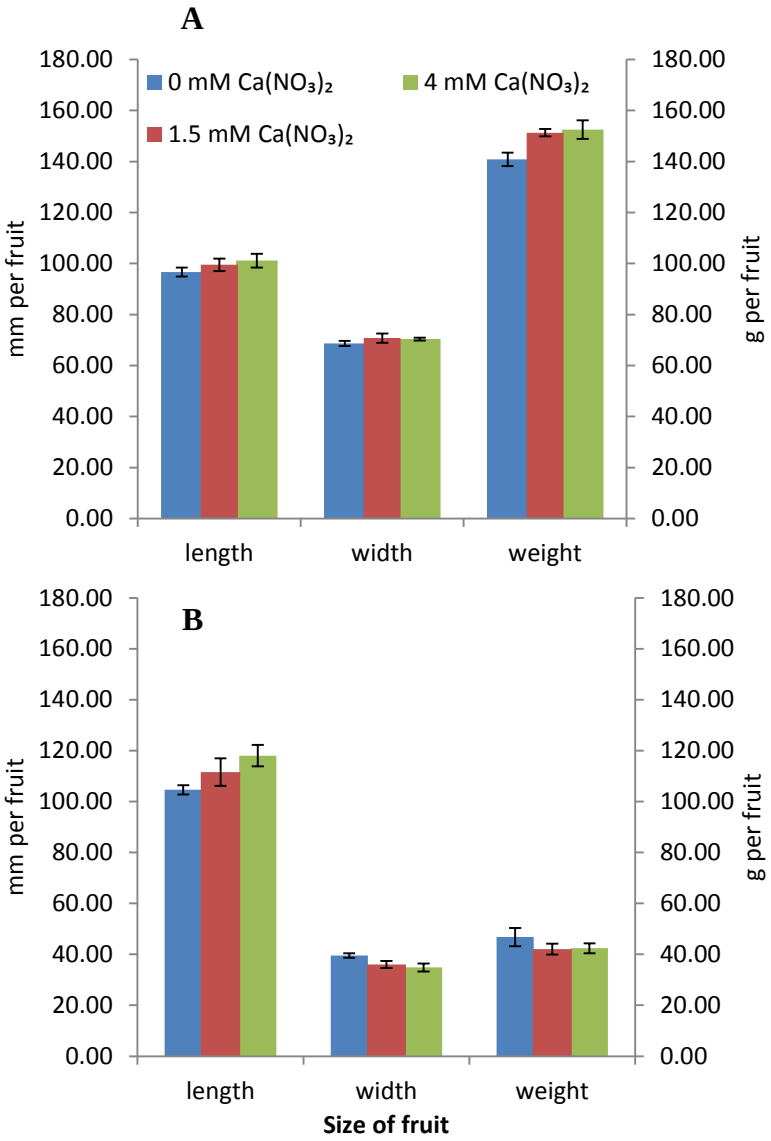


Fig. 5.7 Length, width and weight of fruit from cv. Aries (**A**) and cv. Papri Queen (**B**) when plants received a soil application of 0, 1.5 or 4 mM $Ca(NO_3)_2$ in the Hoagland's solution at a rate of 200 mL per plant every 2 days from transplant to harvest of red fruit. Data are means ± SE from n = 10

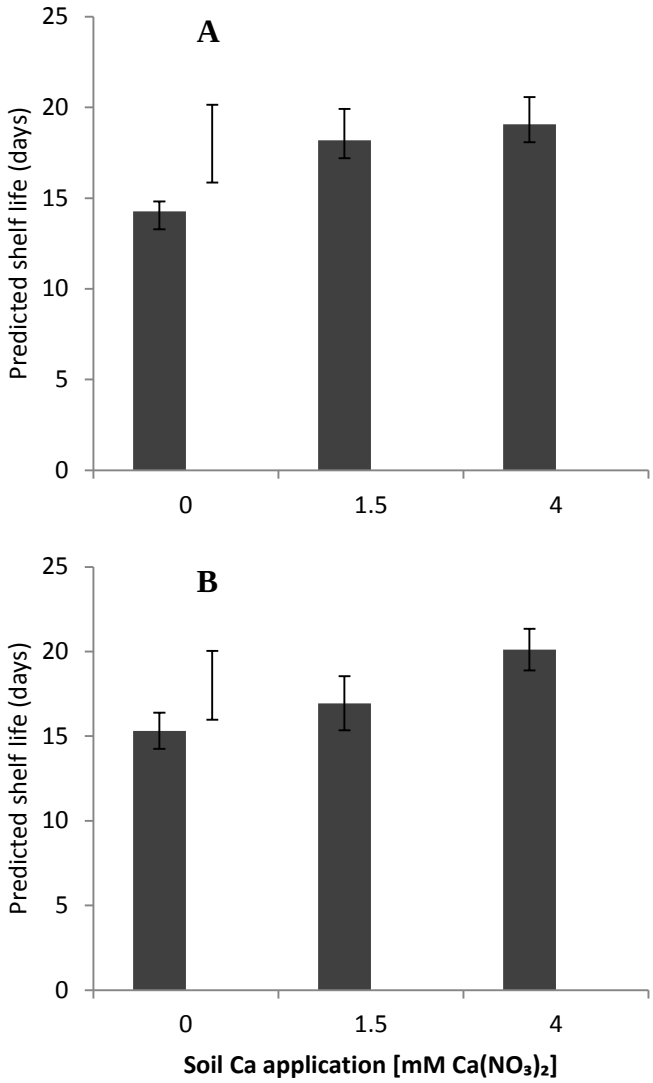


Fig. 5.8 Effect of soil Ca application on shelf life of fruit from cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% in the second trial. Fruit were harvested from plants that received a soil application of 0, 1.5 or 4 mM $Ca(NO_3)_2$ in Hoagland's solution at a rate of 200 mL per plant every 2 days from flowering to harvest of red fruit. Shelf life of fruit was predicted at a general appearance (GA) of 5.5 by using a third degree polynomial in GenStat. Data are means ± SE from n = 3. LSDs ($P \leq 0.05$) for among individual Ca concentrations are shown

Extractable colour in fruit increased steadily during storage and was significantly higher at the end of storage than that in fruit at harvest, regardless of Ca concentration treatment or cultivar (Fig 5.9A, B). Soil application of Ca did not affect extractable colour in fruit at harvest or during storage, regardless of cultivar. At the end of storage, extractable colour in fruit from cv. Papri Queen was significantly greater ($P = 0.01$) than that in fruit from cv. Aries, regardless of Ca treatment.

Firmness of fruit at harvest or during storage was not affected by soil application of Ca, regardless of cultivar (Fig 5.9C, D). Firmness of fruit from cv. Aries did not significantly change during storage, regardless of the Ca concentration applied to soil. In contrast, firmness of fruit from cv. Papri Queen increased steadily during storage and was significantly higher at 23 DAH than at harvest.

Water content in fruit decreased steadily during storage and was significantly lower ($P = 0.02$) at 23 DAH than that in fruit at harvest, regardless of cultivar or Ca treatment (Fig 5.9E, F). Soil application of Ca did not affect water content during storage in both cultivars.

TSSC increased steadily during storage and was significantly higher at 23 DAH than at harvest, regardless of Ca concentration treatment or cultivar (Fig 5.9G, H). Soil application of Ca did not affect TSSC in fruit at harvest or during storage, regardless of cultivar. TSSC in fruit from cv. Papri Queen was significantly greater ($P = 0.01$) than that in fruit from cv. Aries, regardless of Ca treatment.

For cv. Aries, TA in fruit from plants treated with 4.0 mM Ca was significantly lower ($P = 0.007$) at 10 DAH than that in fruit from plants treated with lower Ca concentrations (Fig 5.9I). For cv. Papri Queen, TA in fruit increased significantly ($P<0.001$) from 5 DAH to 15 DAH but remained constant from 15 to 23 DAH, regardless of which Ca concentration was applied to soil (Fig 5.9K). TA in fruit from cv. Papri Queen was significantly greater ($P<0.001$) than that in fruit from cv. Aries, regardless of Ca treatment.

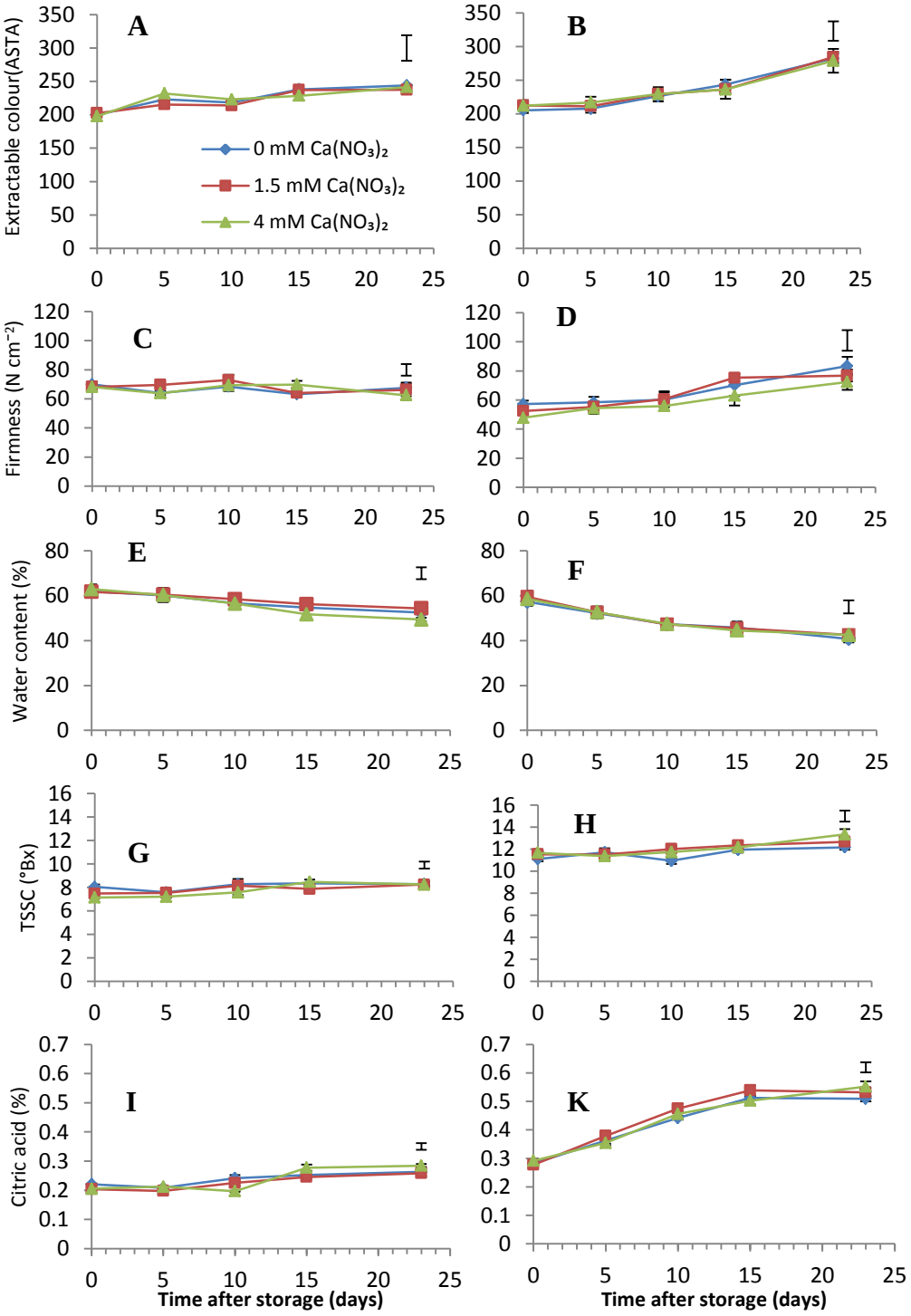


Fig. 5.9 Effect of preharvest soil Ca application on postharvest quality of fruit during storage at 10°C and relative humidity of >90%: extractable colour (**A** and **B**); firmness (**C** and **D**); water content (**E** and **F**); TSSC (**G** and **H**) and TA (**I** and **K**) for cv. Aries and cv. Papri Queen, respectively in the second trial. Fruit were harvested from plants that received a soil application of 0, 1.5 or 4 mM $Ca(NO_3)_2$ in Hoagland's solution at a rate of 200 mL per plant every 2 days from flowering to harvest of red fruit. Data are means ± SE from n = 3. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point during storage

When considering grey mould development on fruit derived from young developing fruit that had been inoculated preharvest, for cv. Aries, lesion area on fruit from plants treated with 4.0 mM Ca via soil application was significantly smaller ($P = 0.02$) than lesion area on fruit from plants treated with 1.5 mM Ca at 18 DAH and lesion area on fruit from control plants at 20 DAH (Fig 5.10A). Lesion area on fruit from plants treated with 1.5 mM Ca application to the soil was similar to lesion area on fruit from control plants. For cv. Papri Queen, lesion area on fruit from plants treated with the 4.0 mM Ca soil application was significantly smaller ($P = 0.01$) than those on fruit from control plants at 22 DAH and from 20 DAH to 24 DAH for plants treated with 1.5 mM Ca (Fig 5.10C). The percentage of fruit exhibiting rot from plants that were treated with 4.0 mM Ca appeared to be lower than control plants or those that were treated with the lower Ca concentration, regardless of cultivar (Fig 5.10B, D).

Time to reach 50% of maximum lesion area on fruit derived from young fruit that had been inoculated preharvest was not significantly different among the three Ca treatments, regardless of cultivar (Fig 5.11).

The lesion area on fruit inoculated postharvest was not significantly different for cv. Aries, regardless of Ca application to plants (Fig 5.12A). For cv. Papri Queen, lesion area on fruit from control plants was significantly larger ($P<0.001$) than that on fruit from plants treated with Ca from 8 DPI to 10 DPI (Fig 5.12B). Lesion area on fruit from plants treated with 1.5 mM Ca was also significantly larger ($P<0.001$) than that on fruit from plants treated with 4.0 mM Ca at 10 DPI.

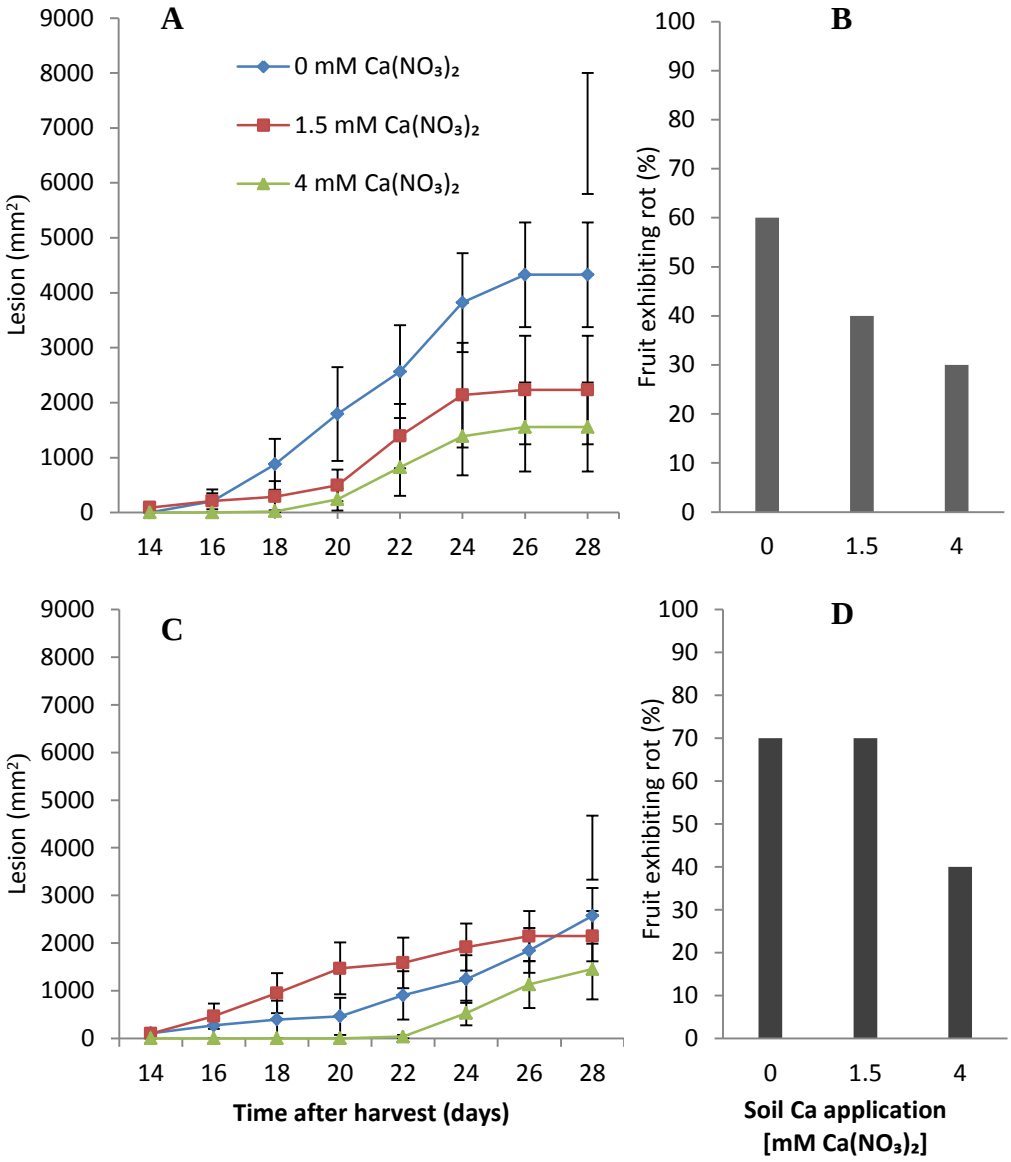


Fig. 5.10 Effect of soil Ca application on grey mould development and the number of fruit of capsicum cv. Aries (**A** and **B**) and cv. Papri Queen (**C** and **D**) exhibiting rot during storage at 10°C and relative humidity of >90% in the second trial. Fruit were derived from young developing fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA when plants received a soil application of 0, 1.5 or 4 mM $Ca(NO_3)_2$ in Hoagland's solution at a rate of 200 mL per plant every 2 days from flowering to harvest red fruit. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point during storage

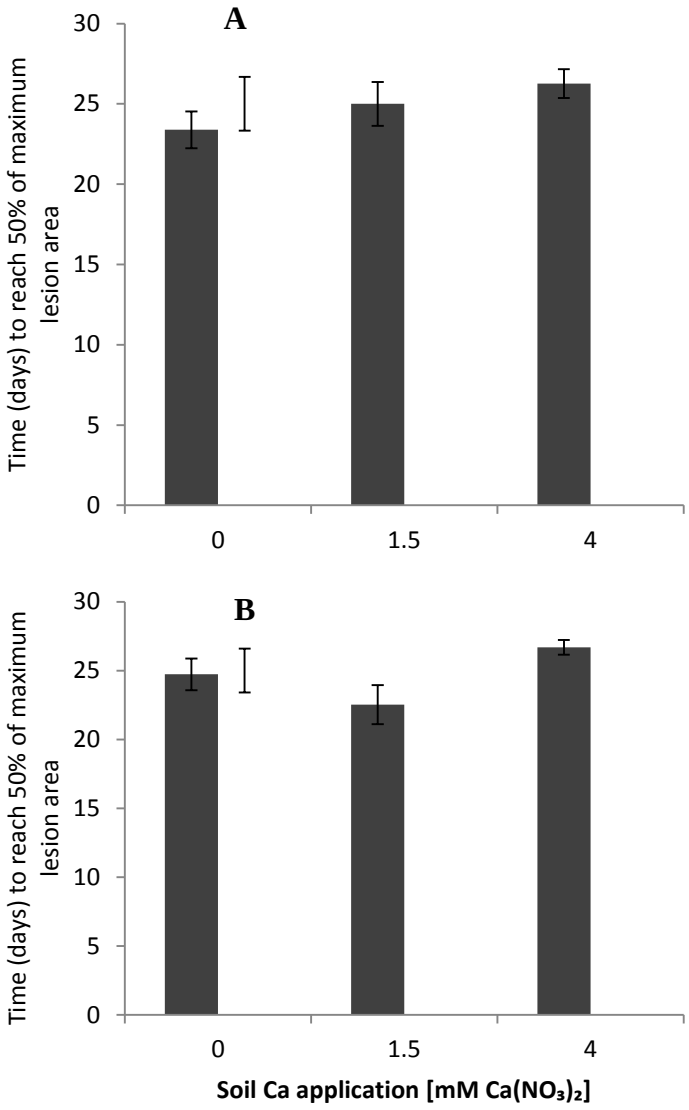


Fig. 5.11 Time after harvest for grey mould lesions to reach 50% of maximum size on fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% for 28 days in the second trial. Fruit were derived from young fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA when plants received a soil application of 0, 1.5 or 4 mM $Ca(NO_3)_2$ in Hoagland's solution at a rate of 200 mL per plant every 2 days from flowering to harvest red fruit. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) for among individual Ca concentrations at 28 days after harvest are shown

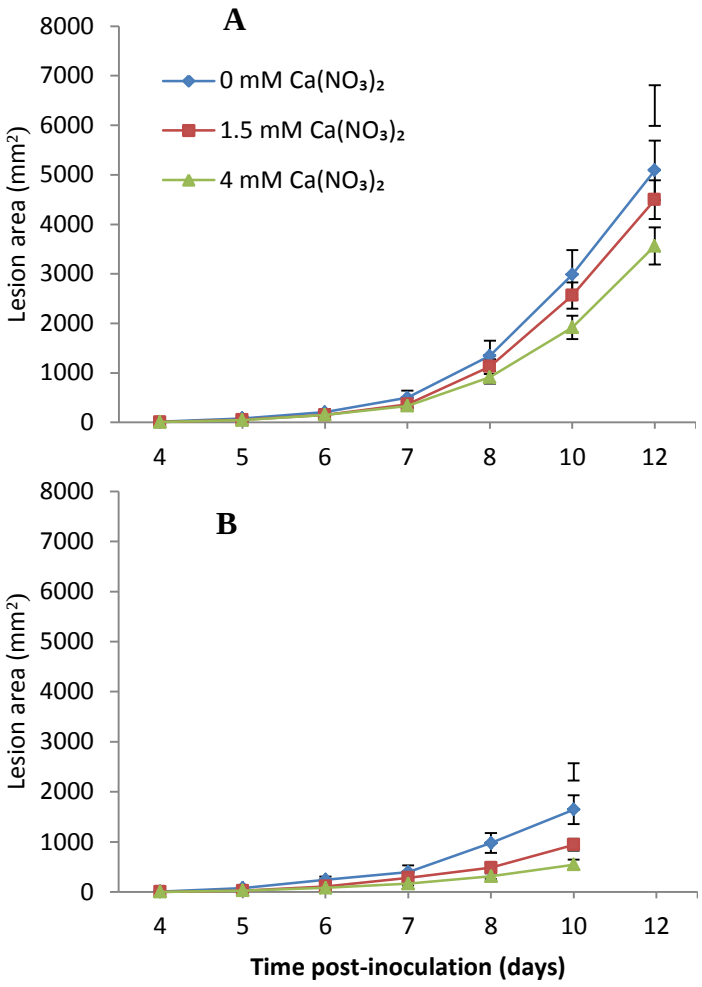


Fig. 5.12 Effect of soil Ca application on grey mould development on postharvest-inoculated fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% in the second trial when plants received a soil application of 0, 1.5 or 4 mM $Ca(NO_3)_2$ in Hoagland's solution at a rate of 200 mL per plant every 2 days from flowering to harvest red fruit. Fruit were wounded on opposite sides and inoculated with 40 µL suspension of 10^5 conidia mL^{-1}. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point post-inoculation

5.3.3 Effect of Ca as a foliar application on nutrient status of plant tissues, postharvest quality and grey mould development on capsicum fruit

5.3.3.1 Foliar application of Ca in the first trial [0.5, 0.75 or 1% $Ca(NO_3)_2$]

Ca concentration in leaf and fruit tissues was statistically similar among plants sprayed with the three Ca concentrations: 0.5, 0.75 or 1.0% w/v $Ca(NO_3)_2$, regardless of cultivar (Table 5.4). There were no significant differences in other nutrients in leaf or fruit tissue, regardless of cultivar or Ca concentration (Appendix A.7). No symptoms of Ca deficiency were observed and they appeared all the same (data not shown). However, leaf concentration of copper (6.15 - 8.50 mg kg^{-1}) and zinc (14.23 - 20.00) from cv. Papri Queen, regardless of Ca concentration applied, appeared to be lower than the range considered adequate (8 - 15 mg kg^{-1} for copper and 20 - 60 mg kg^{-1} for zinc, respectively) (Reuter and Robinson 1997).

The shelf life of fruit was not significantly different among fruit from plants sprayed with the three Ca concentrations, regardless of cultivar (Fig 5.13A, B).

Extractable colour in fruit from cv. Aries was not significantly different among the three Ca applications at harvest or during storage. Extractable colour significantly increased ($P<0.001$) at 10 DAH then remained constant to the end of storage, regardless of Ca application (Fig 5.14A, B). For cv. Papri Queen, extractable colour in fruit from plants sprayed with 1.0 % w/v $Ca(NO_3)_2$ was significantly lower ($P = 0.032$) than that from plants sprayed with 0.5% w/v $Ca(NO_3)_2$ at 23 DAH. At the end of storage, extractable colour in fruit from cv. Papri Queen was significantly greater ($P = 0.01$) than that in fruit from cv. Aries, regardless of Ca treatment.

Table 5.4 Effect of foliar Ca application on nutrient status in capsicum plant tissues in the first trial. Other nutrients are detailed in Appendix A.7. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that received a foliar application: 0.5, 0.75 or 1.0% w/v $Ca(NO_3)_2$ from flowering to harvest of red fruit. Plants were watered with Hoagland's solution without Ca (200 mL per plant for every 2 days). Data are presented as means ± SE from n = 3. There were no significant difference between Ca treatments within tissue types (P values are shown)

Cultivar	**Ca treatment [% w/v $Ca(NO_3)_2$]**	**Ca concentration (mg kg^{-1} DW)**	
		Leaf	**Fruit**
Aries	0.5	16050.00 ± 1150.00	700.00 ± 90.74
	0.75	17750.00 ± 2250.00	753.33 ± 89.69
	1.0	22500.00 ± 1500.00	613.33 ± 44.10
	P value	*0.15*	*0.48*
Papri Queen	0.5	12450.00 ± 1550.00	586.67 ± 33.33
	0.75	16450.00 ± 1650.00	446.67 ± 38.44
	1.0	17100.00 ± 1100.00	593.33 ± 35.28
	P value	*0.19*	*0.16*

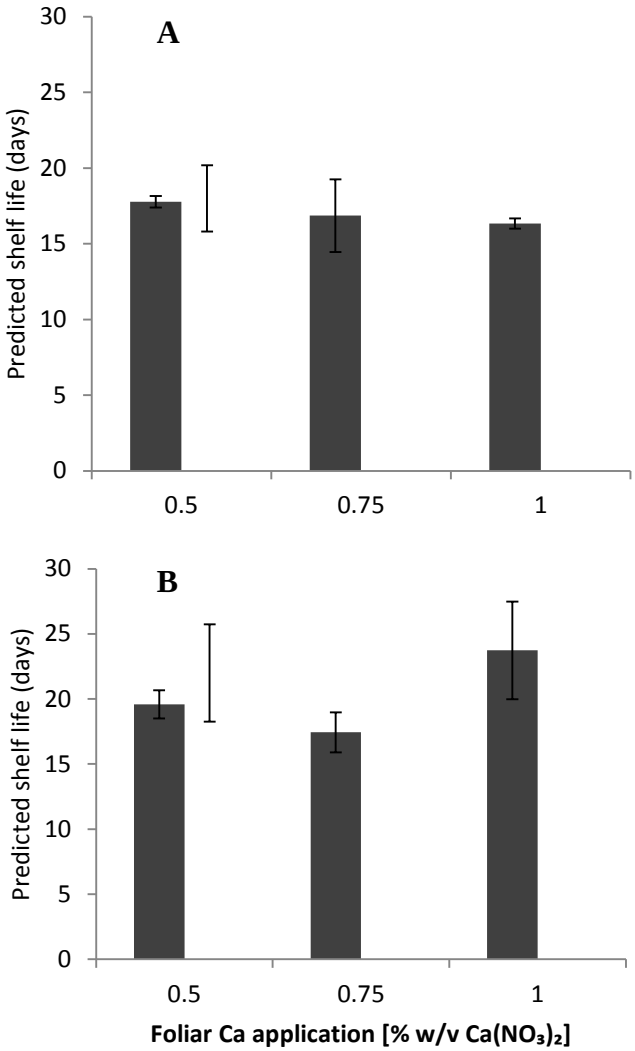


Fig. 5.13 Effect of foliar Ca application on shelf life of fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% in the first trial. Fruit were harvested from plants that received a foliar application of 0.5, 0.75 or 1% w/v $Ca(NO_3)_2$. Plants were sprayed from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Shelf life of fruit was predicted at a general appearance (GA) of 5.5 by using a third degree polynomial in GenStat. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) for among individual Ca concentrations are shown

Firmness of fruit during storage was not affected by foliar application of Ca, regardless of cultivar (Fig 5.14C, D). For cv. Papri Queen, firmness of fruit increased during storage and was significantly higher ($P<0.001$) at 23 DAH than that at harvest.

TSSC in fruit was not significantly different among the three Ca concentrations applied as a foliar spray, regardless of cultivar (Fig 5.14E, F). For cv. Aries, TSSC in fruit significantly ($P = 0.002$) decreased at 23 DAH compared to harvest, while TSSC in fruit from cv. Papri Queen increased steadily during storage and was significantly higher ($P<0.001$) at 23 DAH than at harvest, regardless of Ca concentration. TSSC in fruit from cv. Papri Queen was significantly greater ($P = 0.001$) than that in fruit from cv. Aries, regardless of Ca treatment.

For cv. Aries, TA in fruit was also not significantly different among the three Ca concentrations applied as a foliar spray (Fig 5.14G). TA in fruit significantly increased ($P<0.001$) at 5 DAH then decreased steadily to 23 DAH. For cv. Papri Queen, TA in fruit from plants sprayed with 0.5% w/v $Ca(NO_3)_2$ was significantly higher ($P<0.001$) than that in fruit from plants sprayed with higher Ca concentrations at 5 DAH (Fig 5.14H). At 15 DAH, TA in fruit from plants sprayed with 1.0% w/v $Ca(NO_3)_2$ was significantly lower than that in fruit from plants sprayed with lower Ca concentrations. At 23 DAH, TA in fruit from plants sprayed with 0.75% was significantly lower than that from plants sprayed with 0.5% or 1.0% w/v $Ca(NO_3)_2$. TA in fruit from cv. Papri Queen was significantly greater ($P<0.001$) than that in fruit from cv. Aries, regardless of Ca treatment.

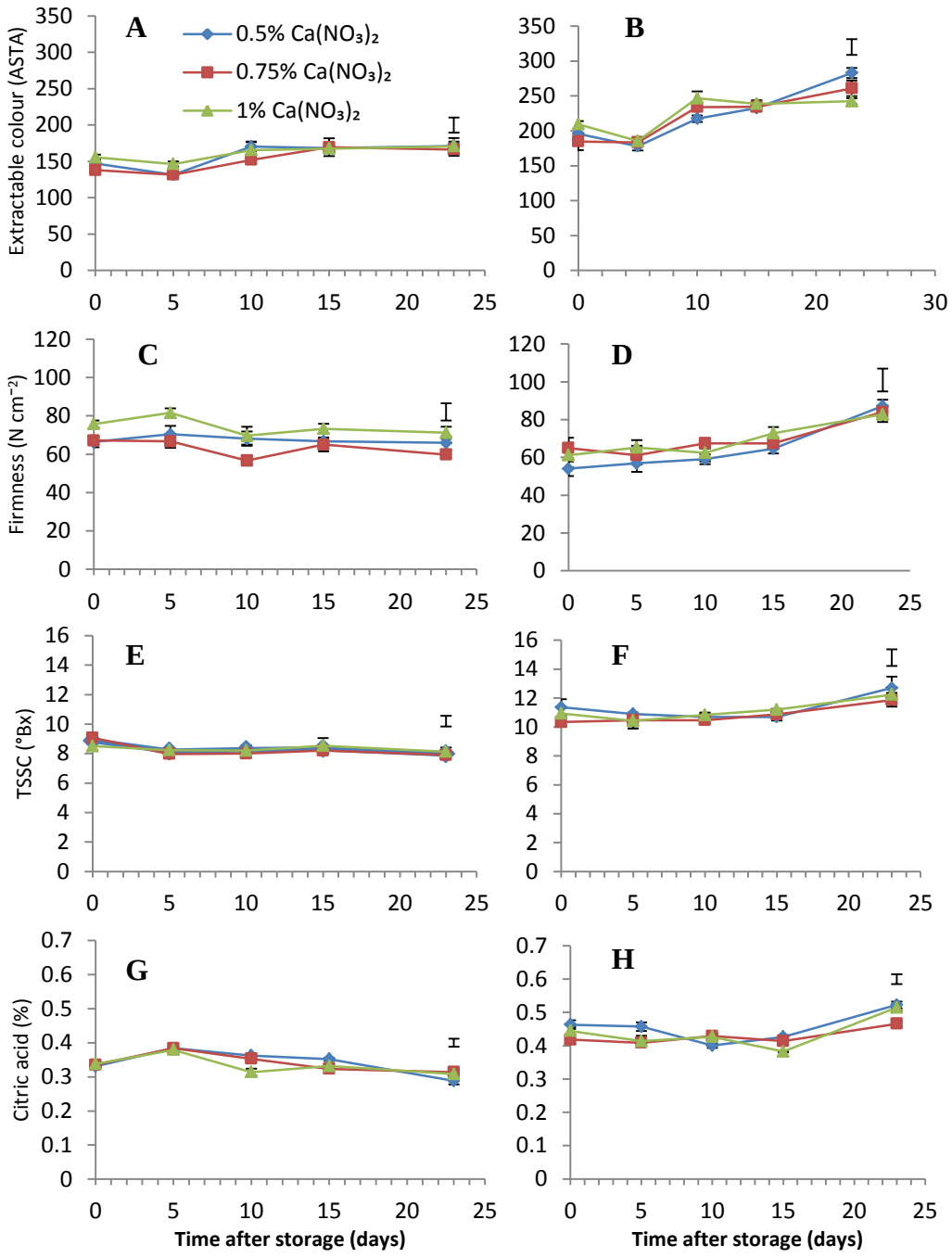


Fig. 5.14 Effect of foliar Ca application on postharvest quality of fruit during storage at 10°C and relative humidity of >90%: extractable colour (**A** and **B**); firmness (**C** and **D**); TSS (**E** and **F**) and acidity (**G** and **H**) for cv. Aries and cv. Papri Queen, respectively in the first trial. Fruit were harvested from plants that received a foliar application of 0.5, 0.75 or 1% w/v $Ca(NO_3)_2$ from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Data are means ± SE from n = 3. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point during storage

When considering grey mould development on fruit derived from young fruit that had been inoculated preharvest, lesion area on fruit from plants sprayed with 0.75% w/v $Ca(NO_3)_2$ was significantly smaller (P = 0.001) from 24 DAH to 28 DAH than that on fruit from plants sprayed with 0.5 or 1.0% w/v $Ca(NO_3)_2$ for cv. Aries (Fig 5.15A). Lesion area was similar on fruit from plants sprayed with 0.5 and 1.0% w/v $Ca(NO_3)_2$. The percentage of fruit exhibiting rot from plants sprayed with 0.75% w/v $Ca(NO_3)_2$ appeared to be lower than those from plants sprayed with 0.5 or 1.0% w/v $Ca(NO_3)_2$ (Fig 5.15B). Foliar application of Ca did not significantly affect lesion area on fruit from cv. Papri Queen (Fig 5.15C). The percentage of fruit exhibiting rot appeared to be similar for plants sprayed with any of the three Ca concentrations (Fig 5.15D). Lesion area on fruit from cv. Aries was significantly larger (P = 0.001) than lesion area on fruit from cv. Papri Queen, regardless of Ca concentration applied.

Time to reach 50% of maximum lesion area was not significantly different for fruit from plants sprayed with any of the three Ca concentrations, regardless of cultivar (Fig 5.16A, B).

The lesion area on fruit inoculated postharvest was not significantly different during storage, regardless of Ca concentration and whether applied as a foliar spray or cultivar (Fig 5.17A, B).

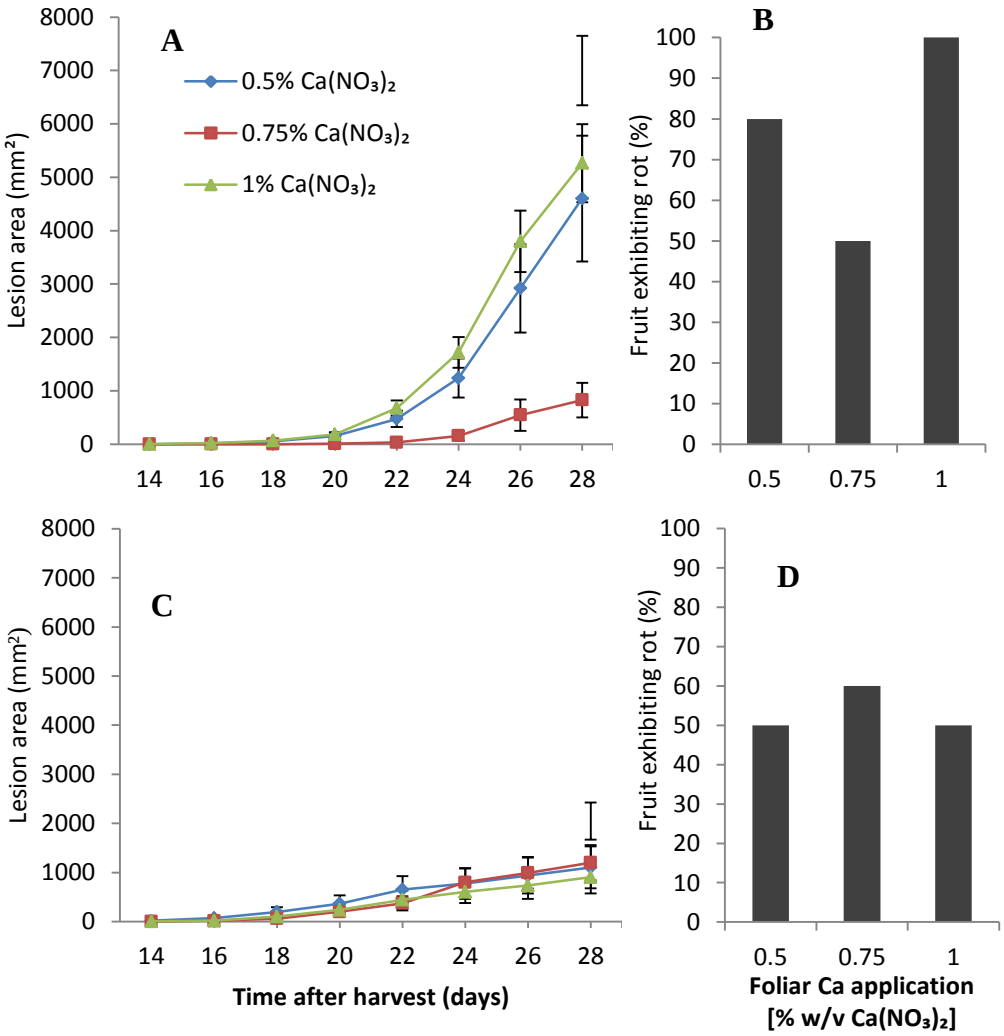


Fig. 5.15 Effect of foliar Ca application on grey mould development and percentage of fruit exhibiting rot of capsicum cv. Aries (**A** and **B**), cv. Papri Queen (**C** and **D**) during storage at 10°C and relative humidity of >90% in the first trial. Fruit were derived from young developing fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA when plants received a foliar application of 0.5, 0.75 or 1% w/v $Ca(NO_3)_2$. Plants were sprayed with foliar Ca from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point during storage

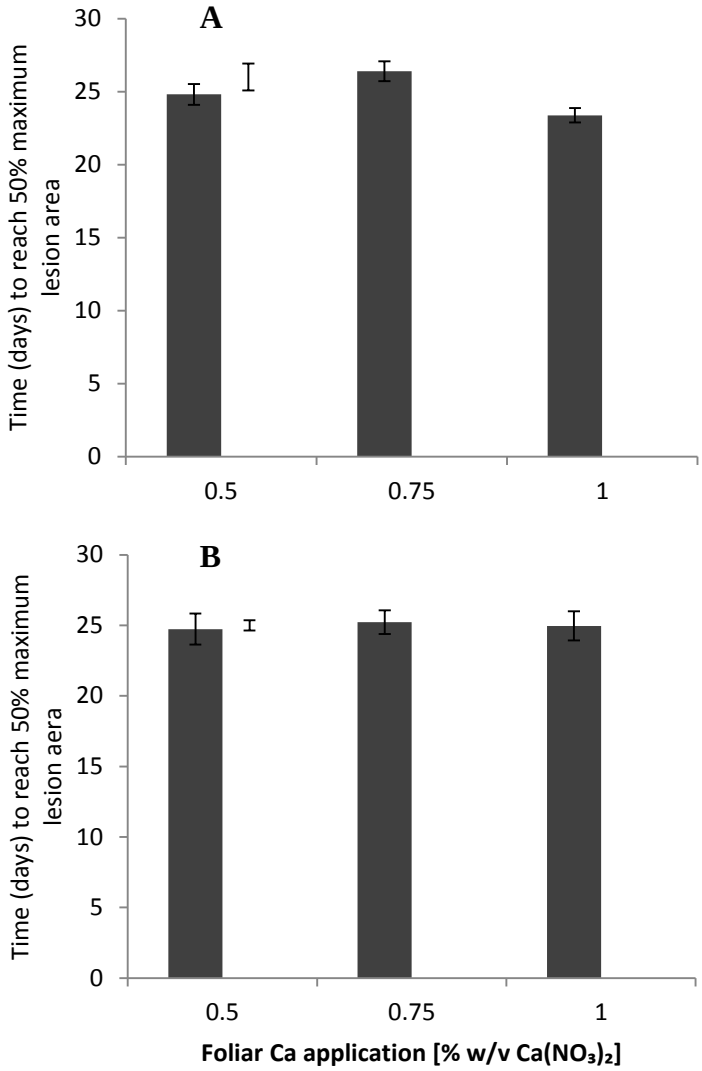


Fig. 5.16 Time after harvest for grey mould lesions to reach 50% of maximum size on fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% for 28 days in the first trial. Fruit were derived from young fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA when plants received a foliar application of 0.5, 0.75 or 1% w/v $Ca(NO_3)_2$. Plants were sprayed with foliar Ca from flowering to harvest at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) for among individual Ca concentrations at 28 days after harvest are shown

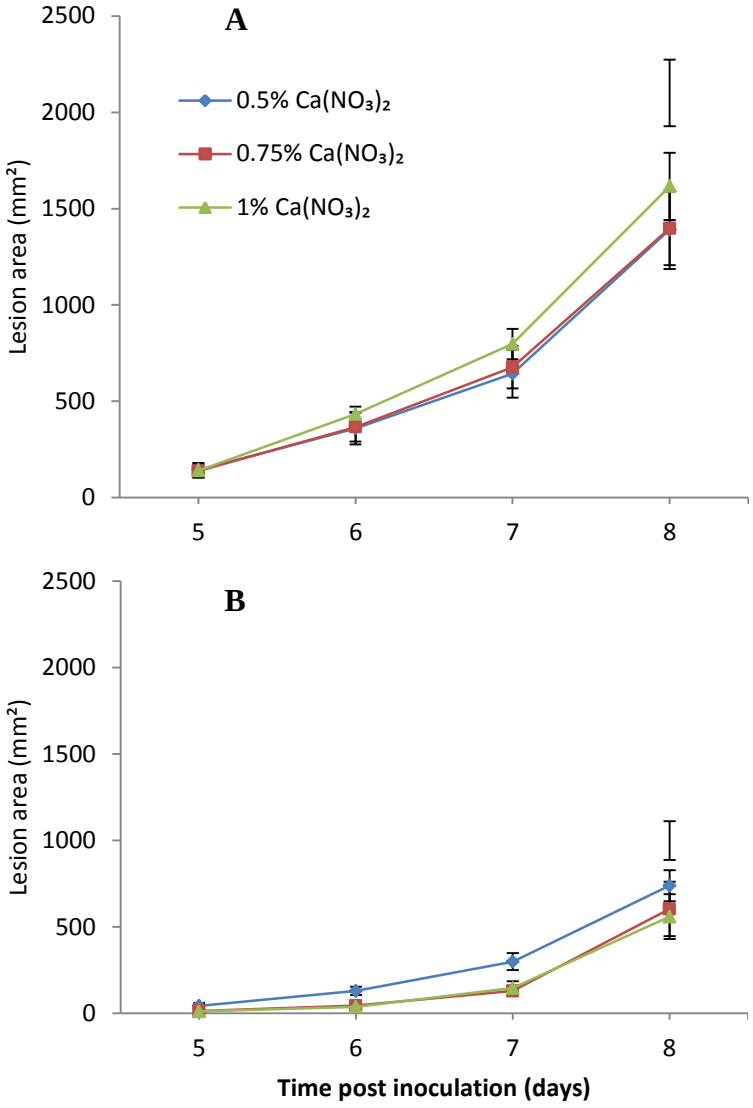


Fig. 5.17 Effect of foliar Ca application on grey mould development on postharvest-inoculated fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% in the first trial when plants received a foliar application of 0.5, 0.75 or 1% w/v $Ca(NO_3)_2$. Plants were sprayed with foliar Ca from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Fruit were wounded on opposite sides and inoculated postharvest with 40 µL suspension of 10^5 conidia mL^{-1}. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point post-inoculation

5.3.3.2 Foliar application of calcium in the second trial [0.0, 0.5 or 1% w/v $Ca(NO_3)_2$]

Ca concentration in leaf tissues was significantly different among plants sprayed with three Ca concentrations: 0.0, 0.5 or 1.0% w/v $Ca(NO_3)_2$ from flowering to harvest of red fruit (Table 5.5). Calcium concentration in fruit, regardless of cultivar, was not significantly different among plants sprayed with any of the three Ca concentrations.

Regardless of cultivar, Ca concentration in leaf tissues from plants sprayed with 1.0% w/v $Ca(NO_3)_2$ was significantly greater ($P<0.001$) than that in leaf tissues from plants sprayed with 0.5% w/v $Ca(NO_3)_2$ or control plants. Calcium concentration in leaf tissues from control plants was significantly lower than that in leaf tissues from plants sprayed with 0.5% w/v $Ca(NO_3)_2$. Calcium concentration in leaf tissue from control plants, regardless of cultivar, appeared deficient compared to that considered to be adequate (100000 - 37000 mg kg^{-1} for cv. Aries and 4000 – 10000 mg kg^{-1} for cv. Papri Queen) (Reuter and Robinson 1997). However, symptoms of Ca deficiency on leaves and fruit were not observed. There were no significant differences in other nutrients in leaf or fruit tissues, regardless of cultivar or Ca concentration (Appendix 5.5). However, concentration of copper in leaf tissues from both cultivars appeared deficient compared to that considered to be adequate (6 - 25 mg kg^{-1}) (Reuter and Robinson 1997).

The shelf life of fruit was not significantly different among the three Ca applications, regardless of cultivar (Fig 5.18A, B).

Table 5.5 Effect of foliar Ca application on nutrient status in capsicum plant tissues in the second trial. Other nutrients are detailed in Appendix A.8. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that received a foliar application: 0.0, 0.5 or 1.0% w/v $Ca(NO_3)_2$ from flowering to harvest of red fruit. Plants were watered with Hoagland's solution without Ca (200 mL per plant for every 2 days). Data are presented as means ± SE from n = 3. For each cultivar, means with the same letters in each column were not significantly different as determined using the LSD (P<0.05)

Cultivar	**Ca treatment [% w/v $Ca(NO_3)_2$]**	**Ca concentration (mg kg^{-1} DW)**	
		Leaf	**Fruit**
Aries	Control	*5150.00 ± 50.00[a]	485.00 ± 25.00[a]
	0.5	12600.00 ± 200.00[b]	470.00 ± 70.00[a]
	1.0	19250.00 ± 50.00[c]	640.00 ± 80.00[a]
	LSD (P value)	*551.20 (<0.001)*	*(0.26)*
Papri Queen	Control	*3550.00 ± 50.00[a]	485 ± 45.00[a]
	0.5	8350.00 ± 50.00[b]	675.00 ± 35.00[a]
	1.0	13250.00 ± 250.00[c]	785.00 ± 2.5.00[a]
	LSD (P value)	*373.20 (<0.001)*	*(0.35)*

**deficient as stated by Reuter and Robinson (1997)*

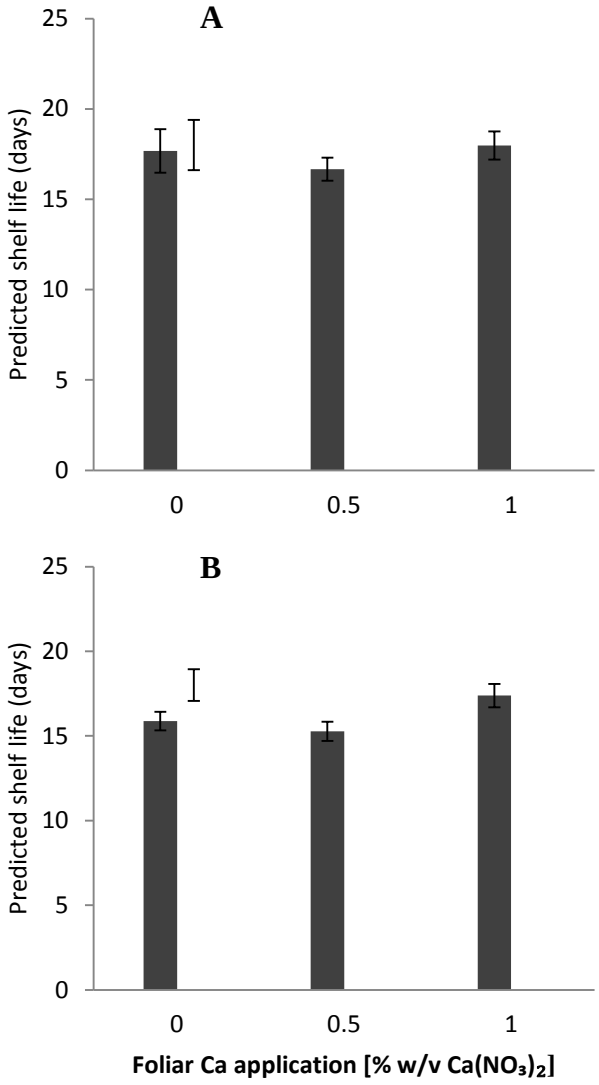


Fig. 5.18 Effect of foliar Ca application on shelf life of fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% in the second trial. Fruit were harvested from plants that received a foliar application of 0, 0.5 or 1% w/v $Ca(NO_3)_2$ in the second trial. Plants were sprayed from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Shelf life of fruit was predicted at a general appearance (GA) of 5.5 by using a third degree polynomial in GenStat. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) for among individual Ca concentrations are shown

Firmness of fruit during storage was not affected by Ca application as a foliar spray, regardless of cultivar (Fig 5.19A, B). For cv. Aries, firmness of fruit from plants sprayed with 1.0% w/v $Ca(NO_3)_2$ remained constant from 0 DAH to 10 DAH, while firmness of fruit from control plants or plants sprayed with 0.5% w/v $Ca(NO_3)_2$ significantly decreased ($P<0.001$) at 5 DAH to 10 DAH compared to that at harvest. Firmness of fruit then significantly increased at 15 DAH then decreased at 23 DAH, regardless of Ca concentration applied to plants. For cv. Papri Queen, firmness of fruit remained constant from 0 DAH to 15 DAH but then significantly increased at 23 DAH, regardless of Ca concentration.

TSSC in fruit was not significantly different among the three Ca concentrations applied as a foliar spray, regardless of cultivar (Fig 5.19C, D). For cv. Aries, TSSC in fruit significantly ($P<0.001$) decreased by 10 DAH compared to at harvest but significantly increased at 15 DAH and remained constant at 23 DAH, regardless of Ca concentration. For cv. Papri Queen, TSSC in fruit remained constant during storage, regardless of Ca concentration. TSSC in fruit from cv. Papri Queen was significantly greater ($P<0.001$) than that in fruit from cv. Aries, regardless of Ca treatment.

When considering grey mould development on fruit derived from young developing fruit that had been inoculated preharvest, for cv. Aries, lesion area on fruit from plants sprayed with 0.5 or 1.0% w/v $Ca(NO_3)_2$ was significantly smaller ($P<0.001$) than that on fruit from control plants from 22 DAH to 28 DAH (Fig 5.20A). Lesion area on fruit from plants sprayed with 0.5% w/v $Ca(NO_3)_2$ was significantly larger than that on fruit from plants sprayed with 1.0% w/v $Ca(NO_3)_2$ from 26 DAH to 23 DAH. However, foliar application of Ca did not significantly affect lesion area on fruit from cv. Papri Queen (Fig 5.20C). The percentage of fruit exhibiting rot from plants that were sprayed with 1.0% w/v $Ca(NO_3)_2$ appeared to be lower than those that were not sprayed or sprayed with lower Ca concentration, regardless of cultivar (Fig 5.20B, D).

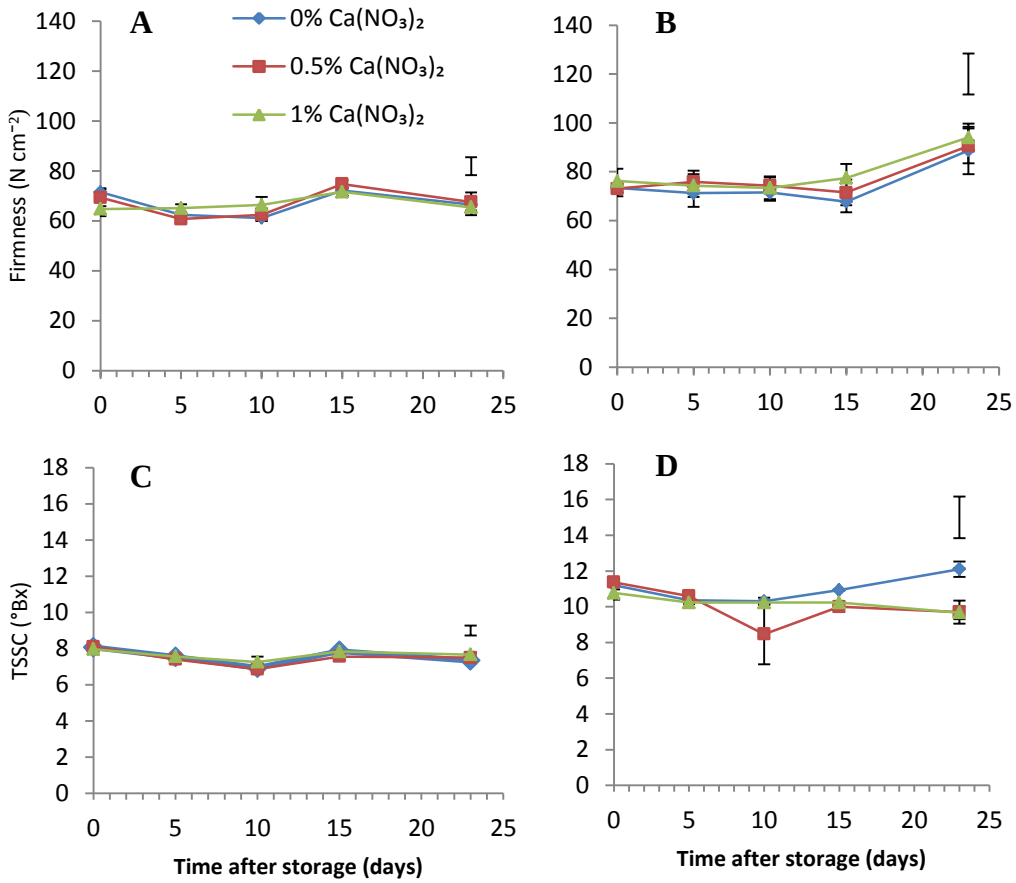


Fig. 5.19 Effect of foliar Ca application on postharvest quality of fruit during storage at 10°C and relative humidity of >90%: firmness (**A** and **B**); TSSC (**C** and **D**) for cv. Aries and cv. Papri Queen, respectively in the second trial. Fruit were harvested from plants that received a foliar application of 0, 0.5 or 1% w/v $Ca(NO_3)_2$ from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Data are means ± SE from n = 3. LSDs ($P \leq 0.05$) are shown for among individual Ca concentration for each time-point during storage

Foliar sprays of Ca delayed the time for grey mould to reach 50% of maximum lesion area on fruit compared to control for cv. Aries (Fig 5.21A), but did not affect time to reach 50% of maximum lesion area on fruit from cv. Papri Queen (Fig 5.21B).

The lesion area on fruit inoculated postharvest was not statistically significant during storage, regardless of cultivar or Ca concentration applied as a spray (Fig 5.22A, B).

5.3.4 Correlation between lesion area on fruit and calcium concentration on leaf and fruit tissues

The correlation between grey mould development on fruit inoculated preharvest and Ca concentration in leaves or fruit from cv. Aries and cv. Papri Queen was determined from 12 individual measurements of lesion area on fruit at 28 DAH and Ca concentration in leaves (Fig 5.23A) or in fruit (Fig 5.23B). Data from soil and foliar application of Ca were used to build the trend line. The correlation was not significant between Ca concentration in the leaves or in the fruit and lesion area on fruit, regardless of cultivar.

When grey mould development was assessed at 10 DPI on fruit inoculated postharvest, there was no significant correlation between Ca concentration in leaves and lesion area on fruit from cv. Aries or from cv. Papri Queen (Fig 24A). Similarly, higher Ca concentration in fruit was not significantly correlated with smaller lesion area on fruit from cv. Aries or from cv. Papri Queen (Fig 24B).

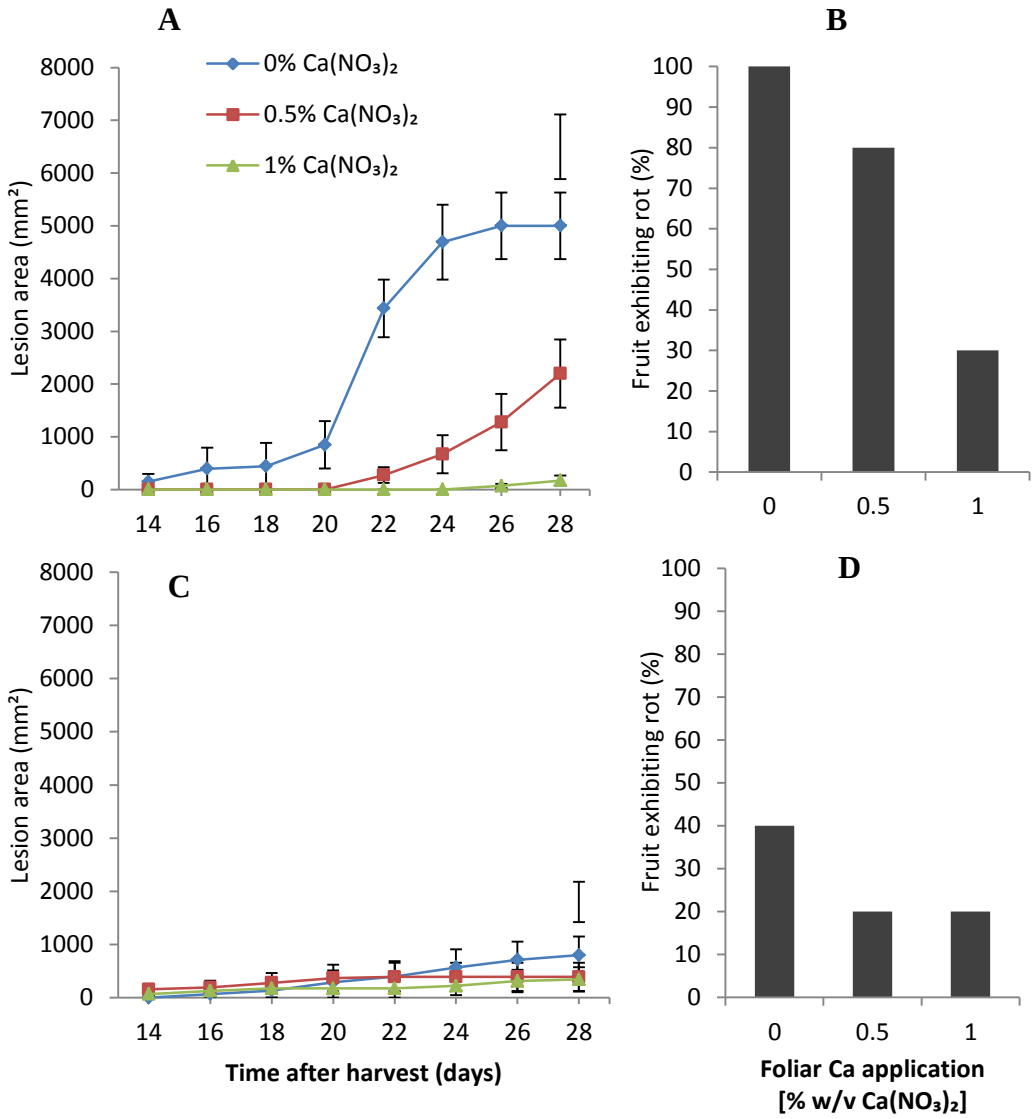


Fig. 5.20 Effect of foliar Ca application on grey mould development and percentage of fruit exhibiting rot of capsicum cv. Aries (**A** and **B**), cv. Papri Queen (**C** and **D**) during storage at 10°C and relative humidity of >90% in the second trial. Fruit were derived from young developing fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA when plants received a foliar application of 0, 0.5 or 1% w/v $Ca(NO_3)_2$. Plants were sprayed with foliar Ca from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual Ca concentration for each time-point during storage

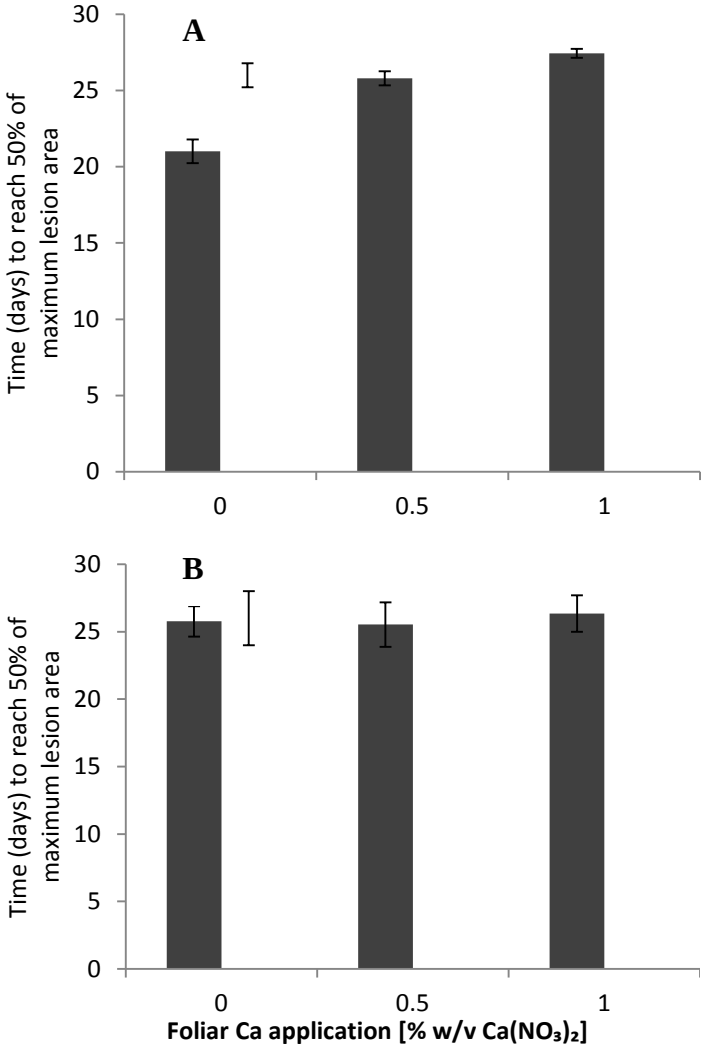


Fig. 5.21 Time after harvest for grey mould lesions to reach 50% of maximum size on fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% for 28 days in the second trial when plants received a foliar application of 0, 0.5 or 1% w/v $Ca(NO_3)_2$. Plants were sprayed with foliar Ca from flowering to harvest at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Fruit were derived from young fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) for among individual Ca concentrations at 28 days after harvest are shown

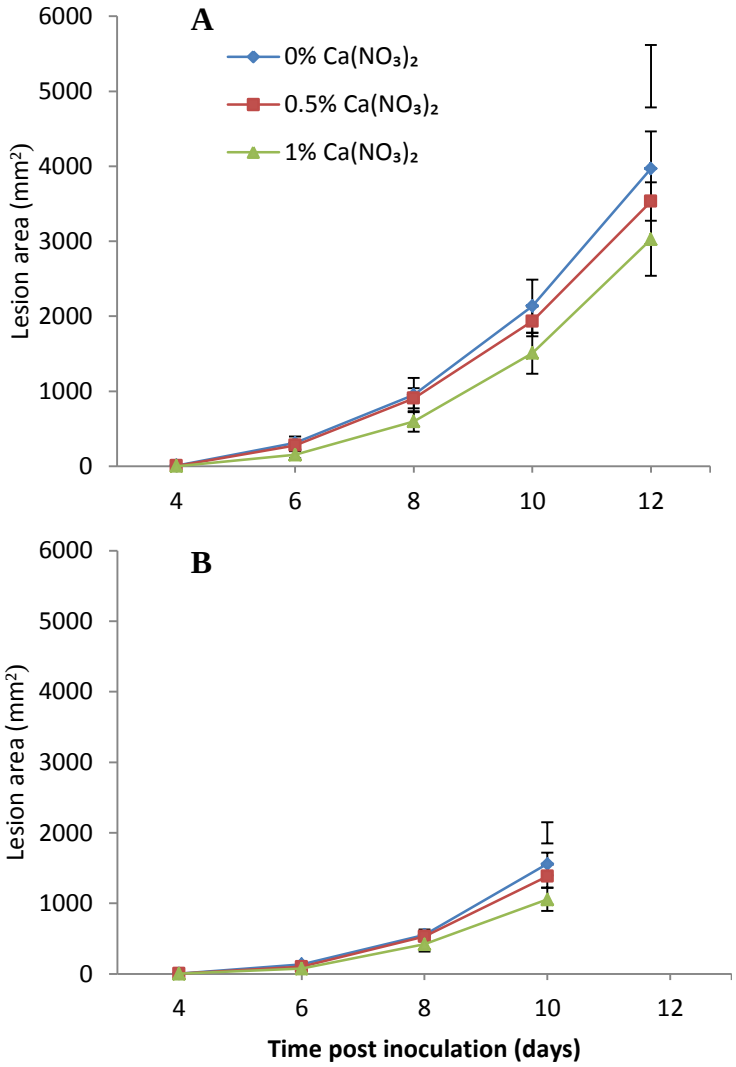


Fig. 5.22 Effect of foliar Ca application on grey mould development on postharvest-inoculated fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90% in the second trial when plants received a foliar application of 0, 0.5 or 1% w/v $Ca(NO_3)_2$. Plants were sprayed foliar Ca from flowering to harvest of red fruit at weekly intervals by hand-held sprayer pump at an average rate of 200 mL m^{-2} (equivalent to 2000 L ha^{-1}). Fruit were wounded on opposite sides and inoculated postharvest with 40 µL suspension of 10^5 conidia mL^{-1}. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point post-inoculation

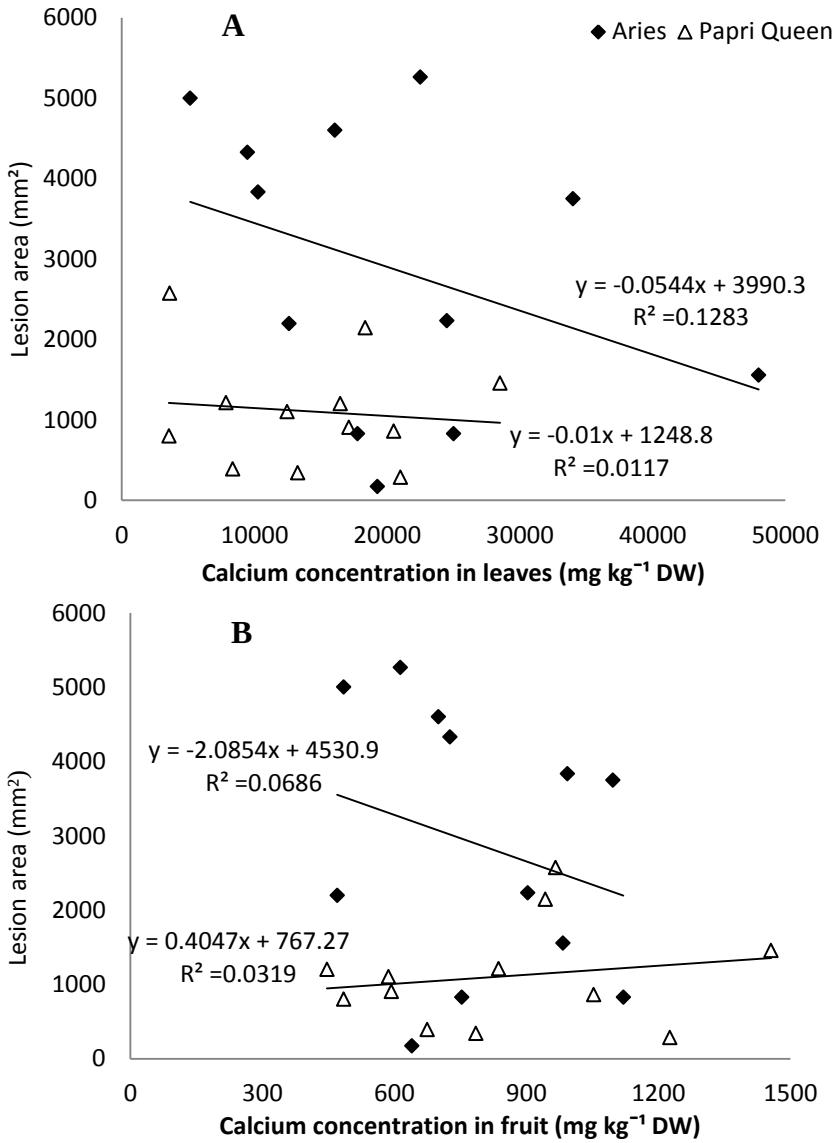


Fig. 5.23 The correlation between grey mould development on fruit inoculated preharvest and Ca concentration in leaves (**A**) and in fruit (**B**) of capsicum cv. Aries and cv. Papri Queen. Lesion area was determined at 28 DAH on fruit (at 10°C and relative humidity of >90%) derived from young developing fruit inoculated with 100 µL suspension of 10^4 conidia mL^{-1} for cv. Aries and 10^6 conidia mL^{-1} for cv. Papri Queen at 3 DAA. Data was collected from twelve individual measurements of grey mould development on fruit and Ca concentration in leaves or fruit in the experiments involving soil and foliar application of Ca

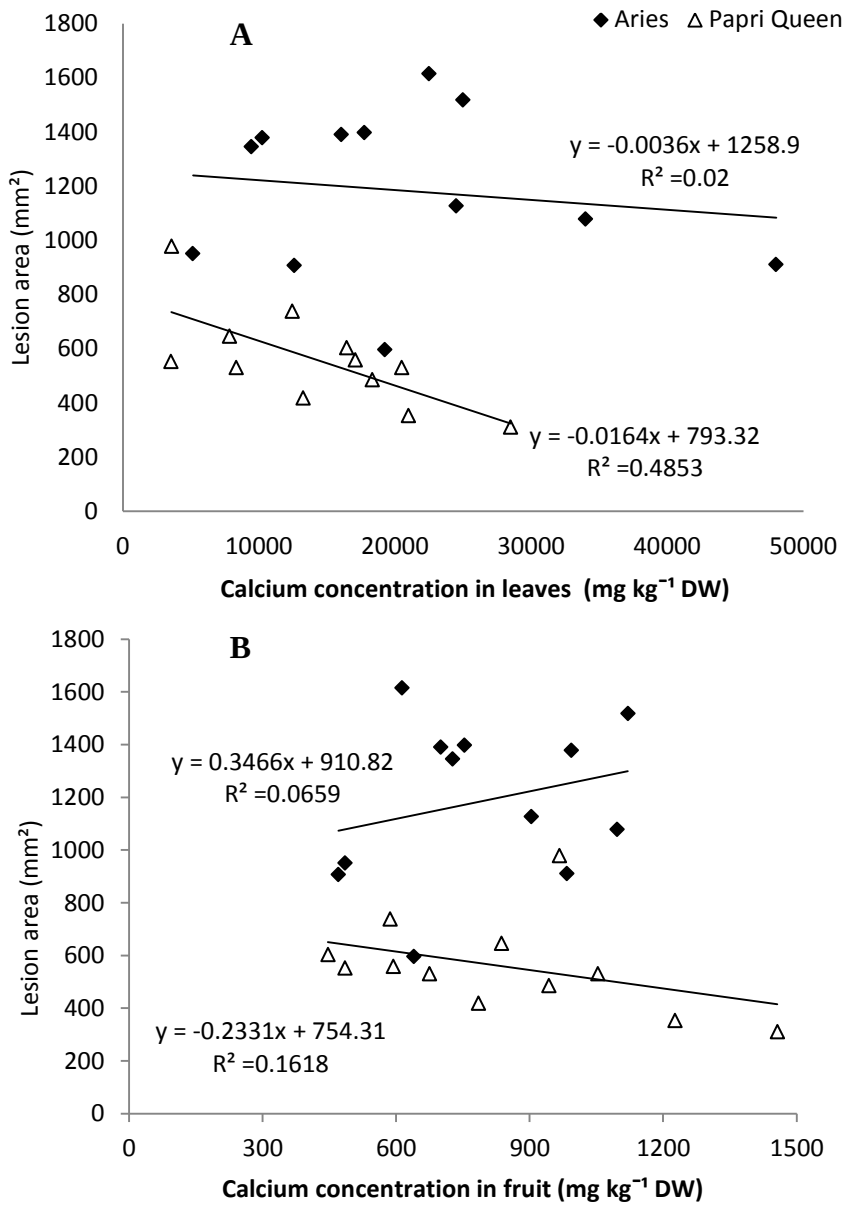


Fig. 5.24 The correlation between grey mould development on fruit inoculated postharvest and Ca concentration in leaves (**A**) or fruit (**B**) of capsicum cv. Aries and cv. Papri Queen. Lesion area was determined at 10 days post incoulation on fruit (at 10°C and relative humidity of >90%) that were inoculated postharvest. Fruit were wounded on opposite sides and then inoculated with 40 µL suspension of 10^5 conidia mL^{-1}. Data was collected from 12 individual measurements of grey mould development on fruit and Ca concentration in leaves or fruit in the experiment involving soil and foliar application of Ca

5.4 Discussion

Preharvest application of Ca did increase Ca concentration in leaf tissues significantly, but did not increase Ca concentration in fruit tissues, regardless of cultivar or application method. For both cultivars, preharvest application of Ca did not affect quality parameters of fruit at harvest or during storage. Lesion area on fruit from Ca-treated plants was significantly smaller than that on fruit from control plants when fruit were derived from young developing fruit inoculated with *B. cinerea* preharvest. However, when fruit were wounded and inoculated with *B. cinerea* postharvest, lesion area on fruit from Ca-treated plants was similar to that on fruit from the control plants.

Ca concentration did not change in fruit tissue, regardless of application method or Ca concentration treatments. Calcium has been demonstrated to be xylem mobile, but phloem immobile (Mengel and Kirkby 1987; White and Broadley 2003). Therefore, Ca uptake into the plant from the soil is reliant on transpiration rate but, because fruit have a very low transpiration rate, very little Ca translocates to fruit (White and Broadley 2003). The finding in this research confirms phloem immobility of Ca in capsicum plants and/or the low level of transpiration by fruit that may not have many stomata or if they do, stomata are unlikely to be open (Peschel et al. 2003). Given the inability to increase fruit Ca via soil fertilisation as observed in this study and by others (Rubio et al. 2010), foliar application is thought to have potential because Ca can penetrate into fruit due to natural openings including stomata, lenticels and cracks (Harker and Ferguson 1988). Spraying peach with 0.12% w/v Ca in the form of Ca-EDTA at weekly intervals from 6 or 12 weeks before harvest until commercial ripening increased Ca concentration in peach fruit significantly (Manganaris et al. 2005). Calcium concentration in tomato fruit increased significantly when plants were sprayed with 0.5 $CaCl_2$ + 20 mg L^{-1} NAA at 4 days-intervals from 18 days after transplanting to harvest (Dong et al. 2005). When surfactants such as Tween 20 are added to solutions used to spray plants, Ca uptake into fruit is increased significantly due to altered permeability of the cuticles (Harker and Ferguson 1991). Calcium concentration in capsicum fruit did not increase significantly after foliar application in this study perhaps because the natural waxy structure of the

skin (Govindarajan and Salzer 1985) did not allow Ca penetration. Adding surfactants to the solution before spraying onto capsicum plants may therefore increase Ca concentration in to the fruit and should be examined in further research.

Ca concentration in leaf tissues from capsicum cv. Aries was significantly greater than that from capsicum cv. Papri Queen, while Ca concentration in fruit appeared to be similar. Calcium uptake is dependent on transpiration rate as noted earlier and this is related to the total of leaf area per plant. Capsicum cv. Aries was observed to have larger leaves than those from cv. Papri Queen, while leaves of capsicum cv. Papri Queen were observed to be waxier than those from cv. Aries. These reasons may result in better Ca uptake by cv. Aries than cv. Papri Queen. In addition, Ca uptake ability of each cultivar could rely on the number and type of Ca transporter in the root (Demarty et al. 1984). Calcium transporter in the root of different cultivars of capsicum has not been reported and needs further research.

Length and width of fruit from both cultivars as well as weight of fruit from cv. Papri Queen were not affected by Ca treatments via soil. Therefore, the size of fruit could not be used to predict Ca deficiency in capsicum plants, but poor development of flowers and fruit was caused by Ca deficiency as observed in this research. For cv. Aries, weight of fruit from Ca-treated plants was significantly greater than that from the control. Higher Ca concentration in the root medium could have caused greater vegetative growth which affected the fruit weight (Rubio et al. 2010). Calcium may be linked to an increase of leaf photosynthetic efficiency which possibly increased number and weight of fruit per plant in tomato (Lopez and Satti 1996).

Preharvest application of Ca did not affect quality parameters of fruit including shelf life, extractable colour, firmness and TSSC at harvest or during storage, regardless of cultivar. The results in this research were in agreement with a previous report that quality of sweet pepper fruit (cv. Orlando) at harvest was not affected when plants were fertilised with different Ca concentrations (Rubio et al. 2010). Preharvest Ca spray did not affect TSSC and TA in ‘Asgari’ table grapes (Amiri et al. 2009). Quality of fruit is lost during storage due to cell-wall

breakdown and increasing membrane permeability (Brady 1987). Preharvest application of Ca was expected to maintain postharvest quality of capsicum fruit by increasing Ca concentration in the flesh and therefore stabilising the cell-wall membrane and reducing tissue break down by the linkage of the pectic substances of the cell wall (Demarty et al. 1984). Indeed, preharvest application of Ca has been shown to increase Ca concentration in fruit leading to increased firmness and shelf life of kiwifruit (Gerasopoulos et al. 1996) and strawberry fruit (Singh et al. 2007). In the present research, when plants received enough Ca to grow and produce red fruit, postharvest quality of fruit was similar because Ca concentration in fruit was not significantly different among the different Ca concentration treatments preharvest, regardless of cultivar.

Most of the observations in this research showed that when applying higher concentration of Ca to capsicum plants appeared to reduce grey mould development on fruit, regardless of cultivar. Preharvest Ca treatment was also reported to reduce grey mould in crops such as tomato (Elad and Volpin 1993) and strawberry (Chéour et al. 1990; Naradisorn et al. 2006). Firstly, Ca is known to be essential for the induction of plant defence (Benhamou 1996). Antifungal substances including phytoalexins and phenolics have been found in cell walls when Ca was applied to cultured soybean cells (Stäb and Ebel 1987). Increased Ca^{2+} intracellular concentration is closely linked to the establishment of lignin-like compounds at sites of potential fungal penetration to provide a further barrier preventing pathogen spread and enzyme-catalysed degradation (Benhamou 1996). In the present research, lower disease incidence on fruit from Ca-treated plants may be due to the role of Ca in plant defence responses to reduce infection of capsicum fruit by *B. cinerea* preharvest. However, there was no significant relationship between the highest Ca concentration in plant tissues and the lowest amount of disease. In the present research, $Ca(NO_3)_2$ was used as source of Ca, increased nitrate concentration in nutrient solution may effect on disease incidence on fruit. The high nitrate concentration in nutrient solution may increase susceptibility of plant to disease (Snoeijers et al. 2000). This may be linked to defence-related compounds in plants treated with different nitrate concentrations. Soluble phenols, rutin and α-tomatine in tomato have been shown to be lower at

higher N availability (Hoffland et al. 1999). For cv. Aries in the first trial of soil Ca application, fruit from plants treated with the highest $Ca(NO_3)_2$ showed greater grey mould incidence than fruit from plants treated with moderate $Ca(NO_3)_2$, but grey mould development was similar to that on fruit from plants treated with low $Ca(NO_3)_2$. This could be due to the negative effect of nitrate overlapping the positive effect of Ca on plant defence responses leading to greater grey mould on fruit. This could be due to the negative effect of nitrate overlapping the positive effect of Ca on plant defence responses leading to greater grey mould on fruit (Snoeijers et al., 2000).

For Papri Queen, increasing Ca concentration in the leaf tissues appeared to not affect grey mould development on fruit. Also lesion area on fruit from cv. Papri Queen was significantly smaller than that on fruit from cv. Aries, regardless of Ca concentration in leaf or fruit tissues. Although Ca deficiency developed in cv. Papri Queen, grey mould lesions on fruit were still smaller than those on fruit from cv. Aries at adequate Ca levels which reflects that Ca is not the only factor contributing to plant defence response to pathogen infection. Others factors including pathogenesis-related proteins in different cultivars of capsicum, as noted in Chapter 3, may play a role in restricting fungal growth.

Lesion area on fruit inoculated postharvest was not significantly different among Ca concentration treatments, regardless of cultivar or application method. Wounding fruit probably caused cell death and failure of natural defence by the fruit allowing grey mould to develop rapidly. Antifungal substances may be present in fruit from Ca-treated plants, but rapid growth of *B. cinerea* on wounded fruit may have overcome any inhibitory effect.

5.5 Conclusion

An increase of Ca concentration applied to plants via soil amendment or foliar spray resulted in reducing grey mould development on fruit derived from young developing fruit inoculated with *B. cinerea* preharvest. Therefore, preharvest application of Ca might be recommended for capsicum industry to reduce grey mould caused by *B. cinerea* postharvest and not affect the quality of fruit.

However, preharvest application of Ca could not protect fruit from growth of *B. cinerea* when fruit were wounded and inoculated postharvest.

CHAPTER SIX

Effect of postharvest calcium dips or vacuum infiltration

6.1 Introduction

Postharvest losses of capsicum fruit are mainly due to shrivelling and infection by *Botrytis cinerea*. Even though fruit may be infected by *B. cinerea* during development, grey mould does not develop until after harvest, when storage conditions and the changes due to ripening are suitable for its growth (Droby and Lichter 2004). Fungicide application is the most commonly-used method to control fungal pathogens preharvest and postharvest. However, fungicides may be toxic for human health and damage the environment. Alternative means of disease control, such as nutrient application, may be a safer solution that can also improve fruit quality (Fallahi et al. 1997; Hansch and Mendel 2009).

Calcium (Ca) is known to have an important role in reducing softening and fungal growth in fruit because Ca strengthens cell walls by cross-linking the carboxyl groups of polyuronide chains in the pectins found in the middle lamella (Sams 1999). Calcium also stabilises cell membranes and increases cell turgor pressure (Mignani et al. 1995; Picchioni et al. 1995). Therefore, increasing Ca content in fruit, via preharvest application of Ca, may be an effective way to extend the postharvest shelf life in a wide range of soft fruit and vegetables, such as capsicum. However, Ca is immobile in plant tissues and only small amounts are translocated from leaves to fruit, so that Ca content in fruit is often low (Chamel 1989) (Chapter 5). Alternative methods are therefore needed to increase Ca content and these may include the postharvest application of Ca-containing salts, such as calcium chloride ($CaCl_2$), using dips and vacuum or pressure infiltration. Calcium content increased 270% in peel and 74% in flesh when peach fruit were dipped in 62.5 mM $CaCl_2$ (Manganaris et al. 2007). Dipping strawberry fruit in 1% calcium chloride solution increased Ca content of fruit, maintaining firmness and controlling postharvest decay caused by *B. cinerea* (García et al. 1996; Hernandez-Munoz et al. 2008). Even though the chloride anion from $CaCl_2$ could potentially affect fungal growth, the Ca cation has been shown to be responsible for the inhibition of growth of *B. cinerea* and *Penicillium expansum in vitro*

(Wisniewski et al. 1995). $CaCl_2$ has been mostly used as a dipping solution to increase firmness and as a preservative agent for whole fruit and fresh-cut commodities (Martín-Diana et al. 2007).

Although the Ca concentration of the skin and outer layers of the fruit is increased with dipping, active infiltration appears to be a more effective way to ensure Ca concentration increases in flesh (Conway et al. 1994a; Klein et al. 1997). However, tissue damage prevents the use of active infiltration of Ca in some soft fruit and so dips are the preferred method to improve postharvest quality of soft fruit and control decay. Dipping whole kiwifruit with 1, 2 or 3% w/v $CaCl_2$ solution for 25 min maintained firmness of fruit slices compared to the control (Beirão-da-Costa et al. 2008). In addition, when diced Roma tomato fruit were dipped in 1% $CaCl_2$ for 1 min, the Ca concentration in fruit increased more than 300%, while firmness was enhanced and soluble pectin was decreased (Magee et al. 2003). Dipping fruit in 1% $CaCl_2$ solution for 2 min reduced postharvest rots of sweet cherries significantly compared to the control (Ippolito et al. 2005).

However, the effect of postharvest treatment with Ca on grey mould development and the storage life of capsicum has not been reported. Thus, this research examined the effect of postharvest Ca application on quality and grey mould development in capsicum fruit. Both dips and vacuum infiltration were investigated.

6.2 Materials and methods

6.2.1 Plant material and growth conditions

Capsicum plants of cv. Aries and cv. Papri Queen were used in this research and all plants were grown in UC potting mix at the Waite Campus of the University of Adelaide as previously described in Chapter 3. Pests were controlled as per Section 4.2.1.

6.2.2 Isolation, maintenance and culture of *B. cinerea*

The isolate of *B. cinerea* described in Chapter 3 was used to produce conidia for use as inoculum (at 10^5 conidia mL^{-1}) as per Chapter 3.

6.2.3 Effect of calcium chloride on growth of *B. cinerea in vitro*

To examine the effect of $CaCl_2$ on fungal growth *in vitro*, *B. cinerea* was cultured on potato dextrose agar (PDA, Difco, USA) amended with 100 mg L^{-1} streptomycin (Sigma, St Louis, MO) and various concentrations of calcium chloride ($CaCl_2.2H_2O$): 0, 2, 4, 6 or 8% w/v. Briefly, a disc (1 cm diameter) from the edge of a 2-week old culture of *B. cinerea* was placed in the centre of a 8.5-cm Petri dish containing the amended PDA before incubation at 22 - 24°C under a 12 h light/12 h dark cycle. Colonies in PDA were monitored daily and diameter was measured by using a digital calliper (digiMax, Switzerland). Five replicate plates were prepared for each concentration of $CaCl_2$ and the experiment was conducted twice. The daily rate of hyphal growth was also calculated.

6.2.4 Postharvest calcium dips

To study the effect of postharvest Ca dips on grey mould development and postharvest quality of fruit, $CaCl_2.2H_2O$ was used as the Ca-containing compound because it has been used most often by other authors (see Section 6.1). Symptomless, uniform-sized red fruit were harvested from plants of capsicum cv. Aries and cv. Papri Queen. Before treatment, fruit were surface-sterilised by dipping in 2% sodium hypochlorite for 1 min, rinsing with reverse osmosis (RO) water and air-drying at room temperature in a laminar flow. Fruit were then treated with four concentrations: 0, 2, 5 or 8% w/v $CaCl_2.2H_2O$. Dipping was conducted by placing fruit in the respective Ca solution for 5 min. Only one length of time for dipping was used due to time limitations. As such, 5 min was chosen, as it has been used previously by Hernández-Muñoz et al. (2006) in strawberry and Manganaris et al. (2007) in peach. Each treatment for each cultivar had 25 fruit: 10 treated fruit for postharvest inoculation and 15 for the assessment of postharvest quality of fruit during storage (see Section 6.2.7).

6.2.5 Postharvest calcium vacuum infiltration

An initial experiment was conducted to test if the Ca solution would infiltrate into fruit. Fruit immersed in Ca solution were placed in a glass desiccator and then a vacuum applied using a vacuum pump (BÜCHI Vac® V-500, Switzerland) for

times ranging between 10 sec and 5 min before holding the fruit in solution for 2 min and then releasing the vacuum. Holding fruit in solution for 2 min was necessary for Ca absorted into fruit tissue, particularly at short time of vacuum. Only one length of time for holding fruit in solution was used due to time limitations. Results showed that if the vacuum was applied for more than 10 sec, the fruit changed colour because of water soaking and had tissue damage while the solution remained inside the fruit after the vacuum was released. Therefore, it was decided fruit would be treated by applying the vacuum for 10 sec followed by holding the fruit for 2 min in solution before the vacuum was released.

Symptomless, uniform-sized red fruit were harvested from plants of capsicum cv. Aries and cv. Papri Queen and surface-sterilised before vacuum infiltration as per Section 6.2.4. Vacuum infiltration was applied as above. Twenty five fruit of each cultivar for one treatment were used: 10 treated fruit for postharvest inoculation and 15 for the assessment of postharvest quality of fruit during storage (see Section 6.2.7).

6.2.6 Effect of infiltration method on absorption of Ca into flesh of fruit

Visual assessment and tissue analysis of Ca concentration were used to examine whether Ca was absorbed into the flesh of fruit. Symptomless, uniform-sized fruit were harvested from plants of capsicum cv. Aries and cv. Papri Queen to examine whether Ca solution directly penetrated into the flesh. Three fruit for each cultivar were either dipped or vacuum infiltrated with Ca solution at a concentration of 8% w/v $CaCl_2.2H_2O$ amended with aniline blue 0.01% w/v as described earlier (Sections 6.2.4 and 6.2.5). Treated fruit were then cut in half and images were recorded using a digital camera (Canon D500, Japan). Fruit were then peeled with a knife to about 1 mm deep before collecting for nutrient analysis to ensure that Ca concentration was measured only in the flesh. Samples for ICP-OES were then prepared as per Section 4.2.3.

In a separate experiment, to determine whether Ca was absorbed into flesh after storage, three fruit of cv. Aries were dipped or vacuum infiltrated with Ca solution at a concentration of 8% w/v $CaCl_2.2H_2O$ as described earlier (Section 6.2.4 and 6.2.5) before storage at 10°C for 10 days. Fruit of cv. Papri Queen was not used

due to its thin flesh making it too soft to peel after 10 days of storage. RO water was used to treat fruit as a control. After 10 days of storage, peel and flesh were collected for nutrient analysis as per Section 4.2.3.

6.2.7 Postharvest inoculation and assessment of postharvest quality of fruit

Ten treated fruit (as per Section 6.2.4 and Section 6.2.5) were wounded on opposite sides and inoculated with *B. cinerea* as per Section 4.2.4.3. After inoculation, fruit were placed in plastic container lined with paper towel and stored at 10℃ and a relative humidity (RH) of >90%. Rot development was quantified daily by measuring lesion area on individual fruit as per Section 4.2.5.1.

To assess postharvest quality, fruit were assessed at harvest and then after storage for 5, 10, 15 or 23 days. Three fruit at each time-point per treatment (a total of 15 fruit) were used to assess shelf life, extractable colour, firmness, total soluble solid content (TSSC) and titratable acidity (TA) as per Section 4.2.5.1.

6.2.8 Statistical analysis and photography

Experiments were designed using the factors of cultivar and Ca concentration. Data were subjected to repeated measurements analysis of variance (ANOVA) using GenStat and means of the treatments compared using the LSD at a significance level of $P \leq 0.05$. Means and standard errors were determined using Microsoft Excel.

6.3 Results

6.3.1 Effect of calcium chloride on fungal growth of *B. cinerea*

The diameter of fungal colonies on PDA from day 2 to day 4 was significantly smaller with increasing Ca concentration from 4% to 8% w/v $CaCl_2.2H_2O$ (Fig 6.1A). The effect of 0% and 2% w/v $CaCl_2.2H_2O$ on fungal colonies was similar from day 1 to day 4. Calcium chloride initially inhibited the rate of hyphal growth from day 1 to day 2 or day 2 to day 3, but the rate of hyphal growth was not significantly different from day 3 to day 4 (Fig 6.1B).

Colony morphology showed that mycelial growth on PDA amended with calcium chloride concentration of 4, 6 or 8 % w/v $CaCl_2.2H_2O$ was woollier with white colour after 4 days, while mycelial growth on PDA amended with lower calcium chloride was almost smooth on the surface (Fig 6.1C).

6.3.2 Effect of postharvest calcium dips on Ca uptake and nutrient status of fruit

Nutrient analysis showed that Ca concentration in fruit was not significantly different among the four Ca concentrations tested for either cv. Aries or cv. Papri Queen (Table 6.1). After dipping in 8% w/v $CaCl_2.2H_2O$ amended with aniline blue, dye was observed on the skin but not in the flesh of fruit from either cultivar (Fig 6.2A, B). No significant differences in the concentration of other nutrients were evident and all nutrients were sufficient (Reuter and Robinson 1997) (Appendix A.9).

After 10 days of storage, Ca concentration in flesh from Ca-treated fruit was not statistically significant (Table 6.2). No significant differences in the concentration of other nutrients were evident and all nutrients were sufficient (Reuter and Robinson 1997) (Appendix A.10).

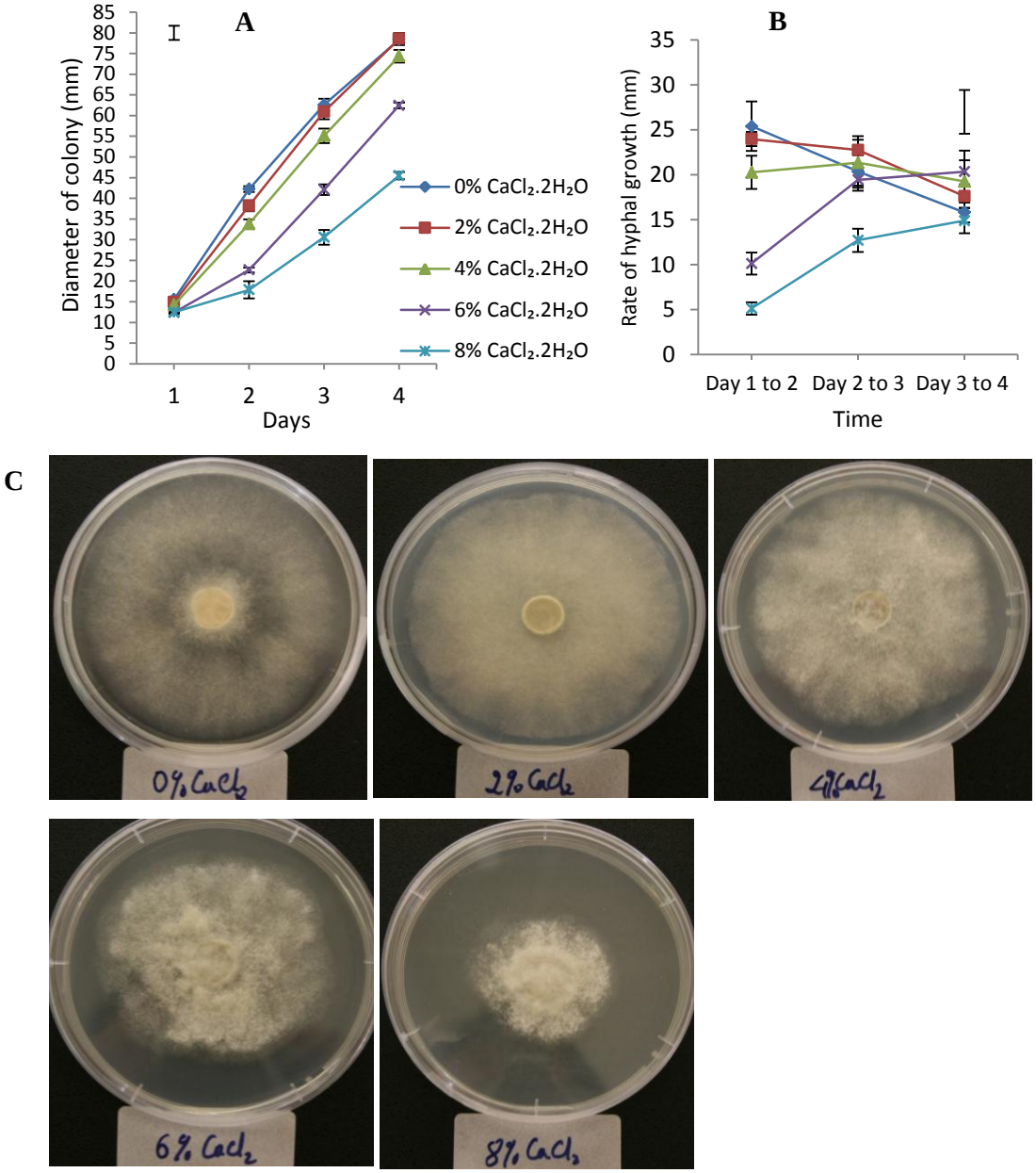


Fig. 6.1 Effect of $CaCl_2.2H_2O$ on mycelial growth of *B. cinerea*; (**A**) Diameter of colony on PDA; (**B**) Rate of hyphal growth on PDA. Data are means ± SE from n = 10 across two experiments. LSDs ($P \leq 0.05$) for among individual $CaCl_2.2H_2O$ concentrations for each time-point are shown. (C) Prepresentative image shown for fungal colonies on PDA amended with 0, 2, 4, 6 or 8% w/v $CaCl_2.2H_2O$ after 4 days

Table 6.1 Effect of postharvest Ca dips on Ca concentration in fruit tissues. Other nutrients are in a table in Appendix A.9. The nutrient concentrations were determined using ICP-OES analysis. Fruit for nutrient analysis were collected at 0 days after harvest and storage. Fruit were treated at four concentrations: 0, 2, 5 and 8% w/v $CaCl_2.2H_2O$. Dipping was conducted by placing fruit in the respective calcium solution for 5 min. Data are presented as means ± SE from n = 3.Calcium concentration in fruit tissue was not significantly different between Ca treatments (P values are shown)

Cultivar	**$CaCl_2.2H_2O$ concentration (%)**	**Ca concentration in fruit tissue (mg kg^{-1} DW)**
Aries	0	845.00 ± 185.00
	2	765.00 ± 135.00
	5	690.00 ± 160.00
	8	780.00 ± 20.00
	P value	*P = 0.89*
Papri Queen	0	915.00 ± 25.00
	2	1070.00 ± 460.00
	5	725.00 ± 155.00
	8	1020.00 ± 250.00
	P value	*P = 0.82*

Table 6.2 Effect of storage time and postharvest Ca dips on Ca concentration in fresh of fruit from cv. Aries. Other nutrients are in a table in Appendix A.10. Fruit were treated with 8% w/v $CaCl_2.2H_2O$ and analysed after 10 days of storage. Control fruit were treated with RO water and nutrient analysed at harvest. Dipping was conducted by placing fruit in calcium solution for 5 min. Fruit were peeled with a knife to about 1 mm deep to collect peel and flesh for nutrient analysis. Data are presented as means ± SE from n = 3. Means with the same letters in column were not significantly different as determined using the LSD ($P<0.05$)

Sample	**Fruit tissue**	**Ca concentration in fruit tissue (mg kg^{-1} DW)**
Control	Flesh	485.00 ± 25.00^{a}
Dip (8% w/v $CaCl_2.2H_2O$)	Flesh	800.00 ± 10.00^{a}
	Peel	1390.00 ± 190.00^{b}
	LSD (P value)	*498.60 (P = 0.023)*

Fig. 6.2 Effect of postharvest Ca dips on penetration of Ca solution into fruit. Capsicum fruit from cv. Aries (**A**) and cv. Papri Queen (**B**) were dipped in Ca solution at a concentration of 8.0% w/v $CaCl_2.2H_2O$ amended with 0.01% w/v aniline blue for 5 min. No stain in placenta was observed. Representative images are shown

6.3.3 Effect of postharvest calcium dips on postharvest quality and grey mould development

General appearance (GA) (9 = the best condition and 1 = the worst condition) (as in Section 4.2.5.2), regardless of cultivar or Ca treatment, decreased significantly during storage while GA was not significantly different among fruit treated with any of the four Ca concentrations (Fig 6.3A, B). Shelf life of fruit was not significantly different among fruit, regardless of Ca concentration applied or cultivar (Fig 6.3C, D).

Extractable colour generally appeared to increase during storage in fruit, regardless of Ca concentration applied and cultivar (Fig 6.4A, B). Extractable colour of fruit was significantly higher at 23 DAH than at harvest. However, there was no significant difference for extractable colour among fruit treated with any of the four Ca concentrations, regardless of cultivar.

Firmness of fruit from both cultivars was not significantly different among fruit treated with any of the four Ca concentrations (Fig 6.4C, D). For cv. Aries, regardless of Ca concentration, firmness of fruit was significantly lower at 5 DAH compared with those at harvest but then significantly increased at 10 DAH, with no change from 15 DAH to 23 DAH (Fig 6.4C). For cv. Papri Queen, firmness of fruit at harvest and at 5 DAH was not significantly different but then firmness significantly increased at 10 DAH (Fig 6.4D).

TSSC of fruit from both cultivars was not significantly different among fruit, regardless of Ca concentration applied and cultivar (Fig 6.4E, F). TSSC of fruit from cv. Aries and treated with any of the four Ca concentrations was not significantly different during storage from that observed at harvest (Fig 6.4E). For cv. Papri Queen, TSSC of fruit treated with four Ca concentrations was similar from harvest to 15 DAH and then significantly increased at 23 DAH (Fig 6.4F). TSSC in fruit from cv. Papri Queen was significantly higher than in fruit from cv. Aries, regardless of Ca concentration.

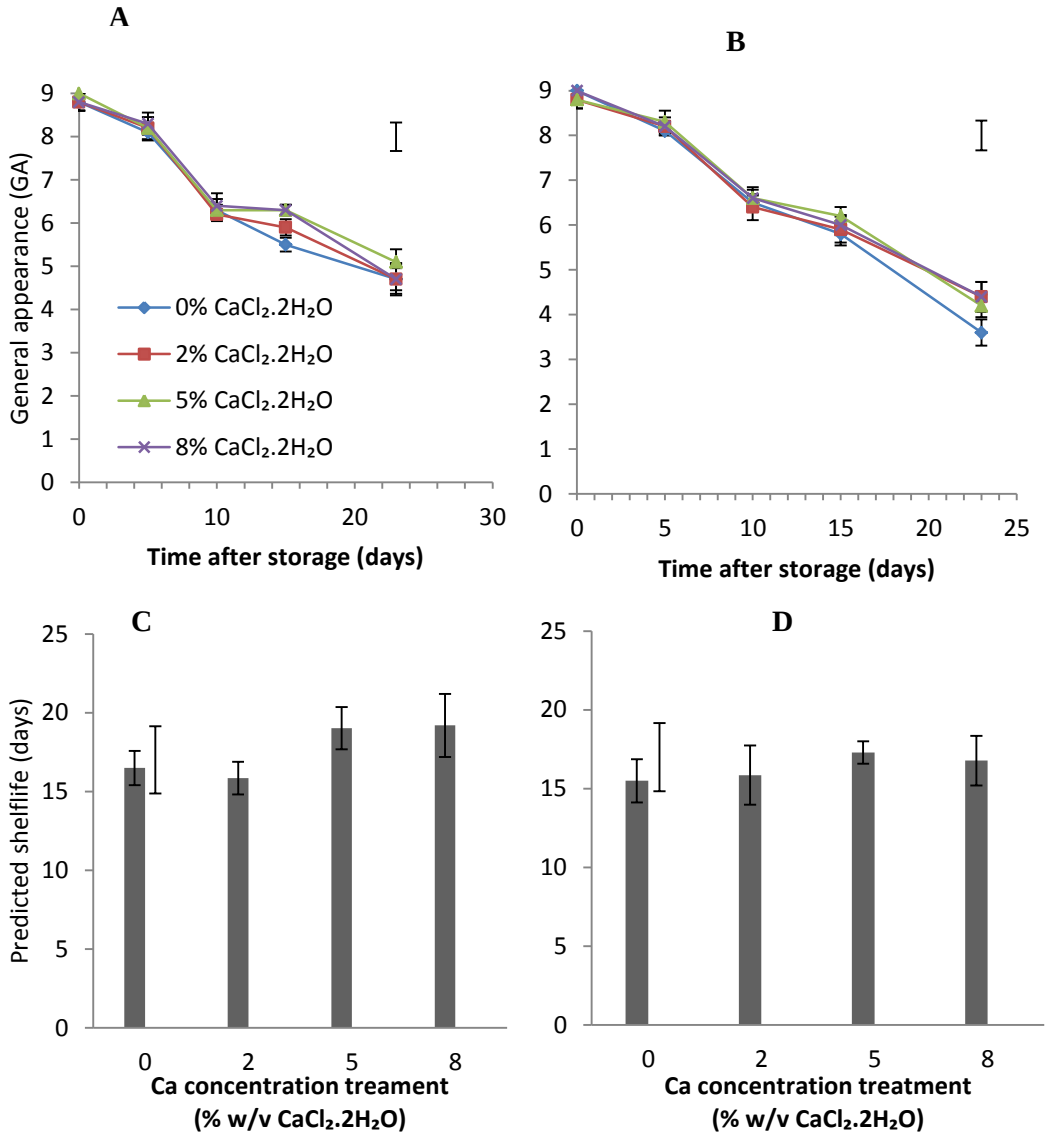


Fig. 6.3 Effect of postharvest calcium dips on general appearance (**A** and **B**) and shelf life (**C** and **D**) of fruit from cv. Aries and cv. Papri Queen, respectively during storage at 10°C and relative humidity of >90%. Fruit were treated at four concentrations: 0, 2, 5 or 8% w/v $CaCl_2.2H_2O$. Dipping was conducted by placing fruit in the respective calcium solution for 5 min. Shelf life of fruit was predicted at a general appearance (GA) of 5.5 by using a third degree polynomial in GenStat. Data are means ± SE from n = 3. LSDs ($P \leq 0.05$) for among individual calcium concentrations are shown

TA of fruit from cv. Aries was significantly different among fruit treated with different Ca concentrations (Fig 6.4G). Initial TA of fruit treated with 0% and 2% $CaCl_2.2H_2O$ was similar and significantly lower than for those treated with 5% and 8% $CaCl_2.2H_2O$. TA of fruit treated with the two higher Ca was significantly lower than those treated with two lower Ca concentrations at 15 DAH, but was the same at 23 DAH. For cv. Papri Queen, TA of fruit treated with any of the four Ca concentrations was not significantly different for all time points of storage (Fig 6.4H). TA in fruit from cv. Papri Queen was significantly higher than in fruit from cv. Aries, regardless of Ca concentration.

Grey mould developed significantly more slowly in fruit dipped in high Ca concentrations in cv. Aries (Fig 6.5A). Lesion area on fruit treated with 8% $CaCl_2.2H_2O$ was significantly smaller than on those treated with 0% or 2% w/v $CaCl_2.2H_2O$ at 10 DPI, whereas lesion area on fruit treated with 5% w/v $CaCl_2.2H_2O$ was not significantly different from fruit treated with 0%, 2% or 8% w/v $CaCl_2.2H_2O$. At 12 DPI, lesion area on fruit treated with 8% w/v $CaCl_2.2H_2O$ was significantly smaller than on those treated with the three lower concentrations, while lesions on fruit treated with 5% $CaCl_2.2H_2O$ was also significantly smaller than on fruit treated with 0% or 2% w/v $CaCl_2.2H_2O$. Lesion area was the same on fruit treated with 2% $CaCl_2.2H_2O$ and control fruit. For cv. Papri Queen, lesion area on fruit from cv. Papri Queen was significantly smaller than that on fruit from cv. Aries, regardless of Ca concentration, and lesion area was not affected by Ca concentrations (Fig 6.5B).

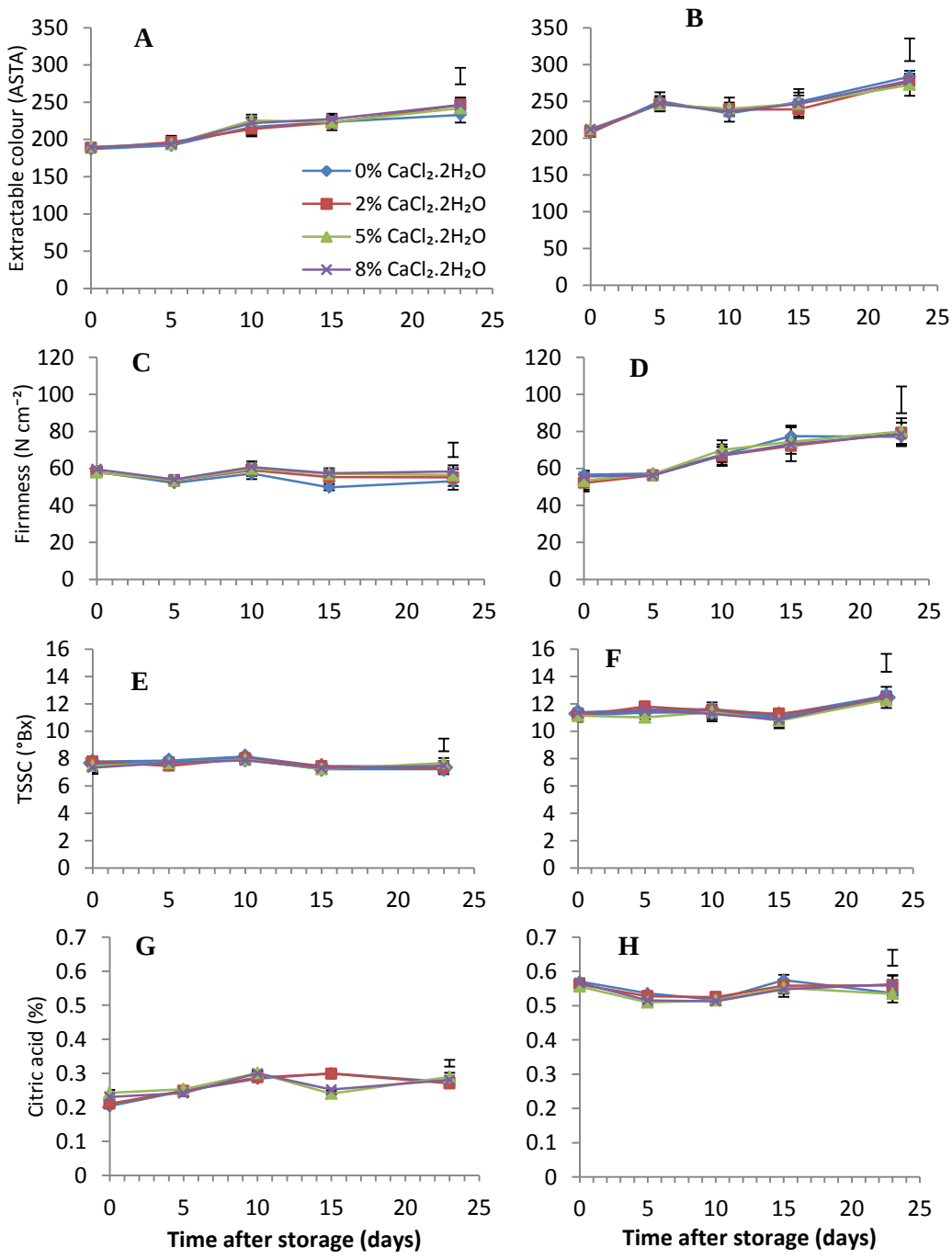


Fig. 6.4 Postharvest quality of capsicum fruit during storage at 10°C and relative humidity of >90%: extractable colour (**A** and **B**); firmness (**C** and **D**); TSSC (**E** and **F**) and acidity (**G** and **H**) for fruit from cv. Aries and fruit from cv. Papri Queen, respectively. Fruit were dipped in Ca solution at 0, 2, 5 or 8% w/v $CaCl_2.2H_2O$ for 5 min before storage. Data are means ± SE from n = 3. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point during storage

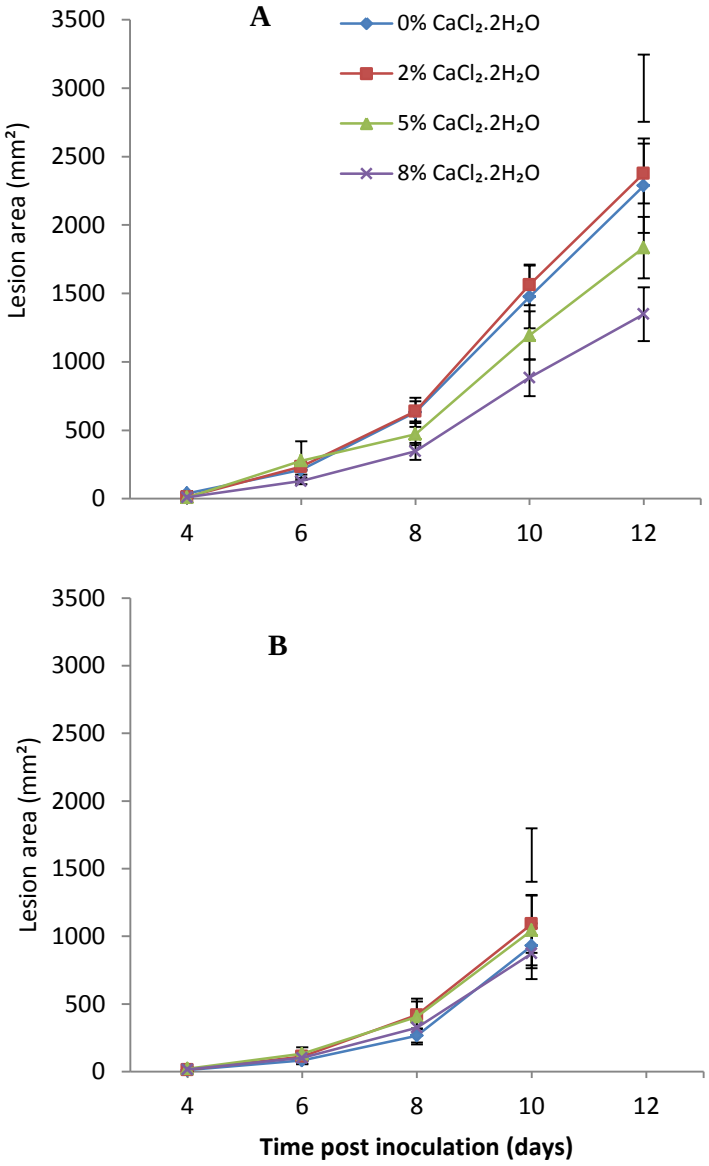


Fig. 6.5 Effect of Ca dips on grey mould development on postharvest-inoculated fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90%. Fruit were dipped in Ca solution at 0, 2, 5 or 8% w/v $CaCl_2.2H_2O$ for 5 min. Fruit were wounded on opposite sides and inoculated with 40 µL suspension of 10^5 conidia mL^{-1}. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point post inoculation

6.3.4 Effect of postharvest Ca vacuum infiltration on Ca uptake and nutrient status of fruit

Nutrient analysis showed that Ca concentration in fruit was not significantly different among the four Ca concentration treatments for either cv. Aries or cv. Papri Queen (Table 6.3). No significant differences in concentration of other nutrients were evident and all nutrients were sufficient (Reuter and Robinson 1997) (Appendix A.11). Dye was observed in the placenta of fruit from both cultivars, but not in flesh (Fig 6.6A and B).

After 10 days of storage, the Ca concentration in flesh from Ca-treated fruit was greater ($P = 0.009$) than that in control fruit (Table 6.4). Calcium concentration in flesh of fruit treated by using vacuum infiltration was significantly greater ($P = 0.009$) than that in flesh of fruit treated by using dips. No significant differences in concentration of other nutrients were evident and all nutrients were sufficient (Reuter and Robinson 1997) (Appendix A.12).

6.3.5 Effect of postharvest Ca vacuum infiltration on postharvest quality and grey mould development

GA, regardless of cultivar and Ca concentration applied, decreased significantly after storage but was not significantly different among fruit treated with any of the four Ca concentrations (Fig 6.7A, B). Shelf life of fruit was not significantly different among fruit, regardless of Ca concentration applied and cultivar (Fig 6.7C, D).

Extractable colour generally appeared to increase during storage of fruit, regardless of Ca concentration applied and cultivar (Fig 6.8A, B). Extractable colour of fruit was significantly higher at 23 DAH than at harvest. However, there was no significant difference for extractable colour among fruit treated with any of the four Ca concentrations, regardless of cultivar.

Table 6.3 Effect of postharvest Ca vacuum infiltration on Ca concentration in fruit tissues. Other nutrients are in a table in Appendix A.11. The nutrient concentrations were determined using ICP-OES analysis. Fruit for nutrient analysis were collected at harvest. Fruit were treated at four concentrations: 0, 2, 5 or 8% w/v $CaCl_2.2H_2O$. Vacuum infiltration was conducted by placing fruit in the respective calcium solution, drawing a vacuum for 10 sec and then holding fruit in solution for 2 min before vacuum release. Data are presented as means ± SE from n = 3. Calcium concentration in fruit tissue was not significantly different between Ca treatments (P values are shown)

Cultivar	**$CaCl_2.2H_2O$ concentration (%)**	**Ca concentration in fruit tissue (mg kg^{-1} DW)**
Aries	0	710.00 ± 160.00
	2	900.00 ± 160.00
	5	730.00 ± 160.00
	8	890.00 ± 300.00
	P value	*0.83*
Papri Queen	0	765.00 ± 95.00
	2	1715.00 ± 65.00
	5	845.00 ± 345.00
	8	825.00 ± 95.00
	P value	*0.06*

Table 6.4 Effect of storage time and postharvest Ca vacuum infiltration on Ca concentration in fruit from cv. Aries. Other nutrients are in a table in Appendix A.12. Fruit were vacuum infiltrated with 8% w/v $CaCl_2.2H_2O$ and nutrient analysed after 10 days of storage while control fruit were vacuum infiltrated with RO water and nutrient analysed at harvest. Vacuum infiltration was conducted by placing fruit in Ca solution, drawing a vacuum for 10 sec and then holding fruit in solution for 2 min before vacuum release. Fruit were peeled with a knife to about 1 mm deep to collect peel and flesh for nutrient analysis. The nutrient concentrations were determined using ICP-OES analysis. Data are presented as means ± SE from n = 3. Means with the same letters in column were not significantly different as determined using the LSD ($P<0.05$)

Sample	**Fruit tissue**	**Ca concentration in fruit tissue (mg kg^{-1} DW)**
Control	Flesh	485.00 ± 25.00^{a}
Vacuum infiltration (8% w/v $CaCl_2.2H_2O$)	Flesh	2075.00 ± 325.00^{b}
	Peel	3500.00 ± 600.00^{c}
	LSD(P value)	*1274.3(P = 0.028)*

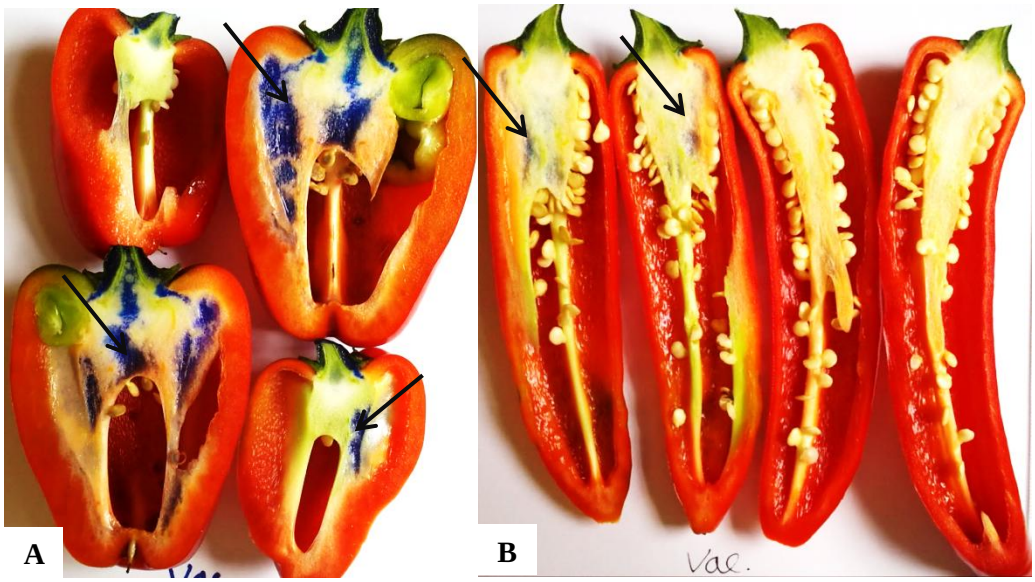


Fig. 6.6 Effect of postharvest Ca vacuum infiltration on penetration of Ca solution into fruit. Capsicum fruit from cv. Aries (**A**) and cv. Papri Queen (**B**) were placed in Ca solution at 8% w/v $CaCl_2.2H_2O$ amended with 0.01% w/v aniline blue, drawing a vacuum for 10 sec and then holding fruit in solution for 2 min before vacuum release. Representative images are shown. Arrows show whether the placenta tissues were stained

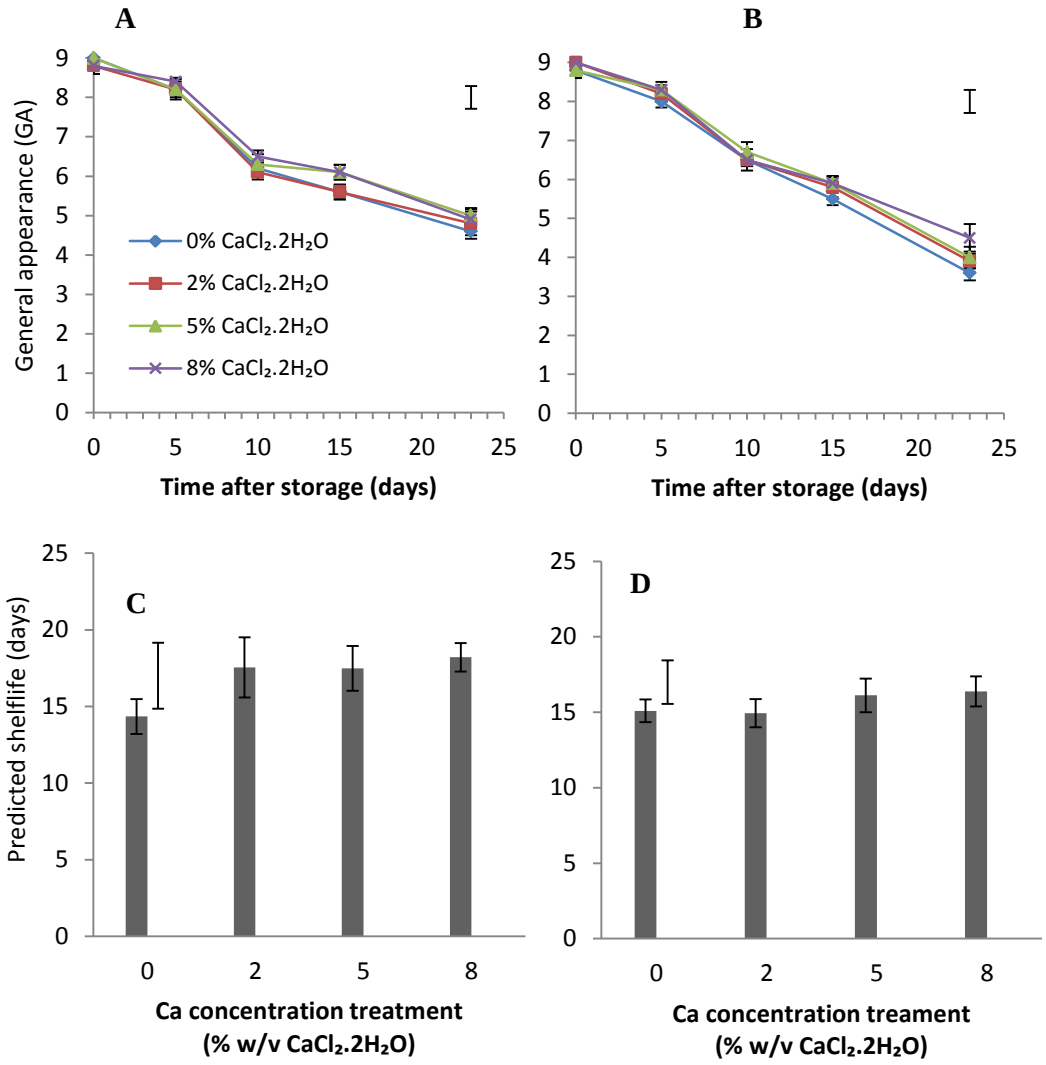


Fig. 6.7 Effect of postharvest calcium vacuum infiltration on general appearance (**A** and **B**) and shelf life (**C** and **D**) of fruit cv. Aries and fruit cv. Papri Queen, respectively during storage at 10°C and relative humidity of >90%. Shelf life was predicted for fruit treated at four concentrations: 0, 2, 5 or 8% w/v $CaCl_2.2H_2O$. Vacuum infiltration was conducted by placing fruit in Ca solution, drawing a vacuum for 10 sec and then holding fruit in solution for 2 min before vacuum release. Shelf life of fruit was predicted at a general appearance (GA) of 5.5 by using a third degree polynomial in GenStat. Data are means ± SE from n = 5. LSDs ($P \leq 0.05$) for among individual Ca concentrations are shown

Firmness of fruit from both cultivars was not significantly different among fruit treated with any of the four Ca concentrations (Fig 6.8C, D). For cv. Aries, firmness of fruit did not change from harvest to 10 DAH and then significantly declined at 15 DAH, but did not change at 23 DAH (Fig 6.8C). For cv. Papri Queen, firmness of fruit treated with any of the four Ca concentrations did not change at 5 DAH compared with those at harvest but then significantly increased at 10 DAH but did not change at 15 DAH and 23 DAH (Fig 6.8D).

TSSC of fruit was not significantly different among fruit, regardless of Ca concentration applied and cultivar (Fig 6.8E, F). TSSC of fruit from cv. Aries treated with any of the four Ca concentrations was not changed after harvest (Fig 6.8E). For cv. Papri Queen, TSSC of fruit treated with four Ca concentrations was similar from harvest to 15 DAH and then significantly increased at 23 DAH (Fig 6.8F). TSSC in fruit from cv. Papri Queen was significantly higher than in fruit from cv. Aries, regardless of Ca concentration.

TA of fruit from cv. Aries was significantly different among fruit vacuum infiltrated with different Ca concentrations (Fig 6.8G). Initial TA of fruit treated with 0% $CaCl_2.2H_2O$ was significantly lower than those treated with any of the three concentrations of $CaCl_2.2H_2O$. At10 DAH, regardless of Ca concentration, TA of treated fruit was significantly greater than at harvest. TA of fruit treated with the two higher Ca concentrations was significantly lower than that treated with the two lower Ca concentrations at 15 DAH, but the same at 23 DAH. TA in fruit from cv. Papri Queen was significantly higher than in fruit from cv. Aries, regardless of Ca concentration.

Lesion area on fruit from cv. Aries treated with 0% $CaCl_2.2H_2O$ was significantly greater than on those treated with any of the three Ca concentrations from 10 DPI to 12 DPI. However, there was no significant difference for lesion area on fruit treated with 2, 5 or 8% w/v $CaCl_2.2H_2O$ (Fig 6.9A). For cv. Papri Queen, lesion area was not significantly different among fruit treated with any of the four Ca concentrations (Fig 6.9B). Lesion area on fruit from cv. Papri Queen was significantly smaller than that on fruit from cv. Aries, regardless of Ca concentration.

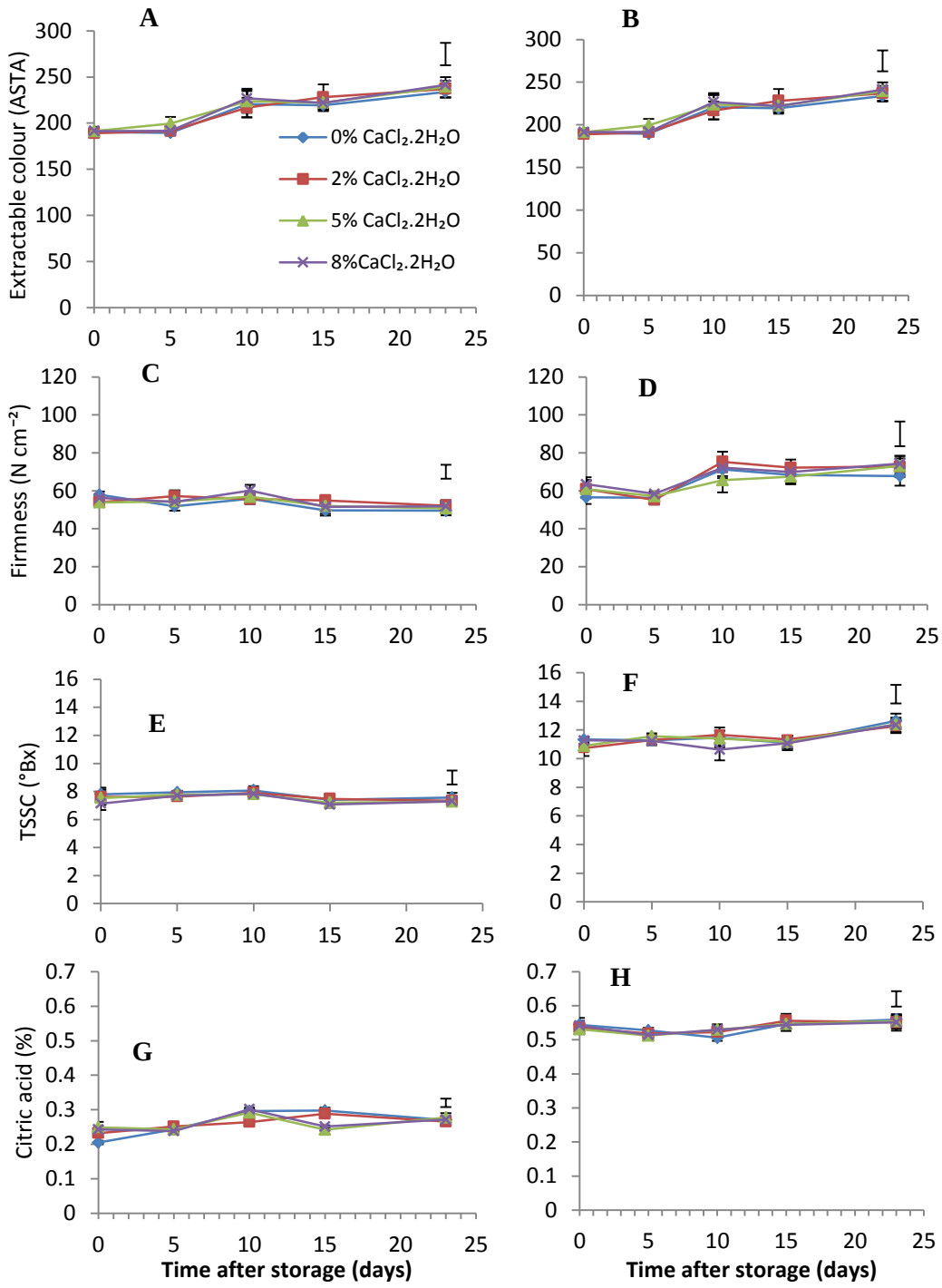


Fig. 6.8 Postharvest quality of fruit during storage at 10°C and relative humidity of >90%: extractable colour (**A** and **B**); firmness (**C** and **D**); TSSC (**E** and **F**) and acidity (**G** and **H**) for fruit cv. Aries and fruit cv. Papri Queen, respectively. Fruit were vacuum infiltrated by placing fruit in Ca solution at 0, 2, 5 or 8% w/v $CaCl_2.2H_2O$, drawing a vacuum for 10 sec and then holding fruit in solution for 2 min before vacuum release. Data are means ± SE from n = 3. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point during storage

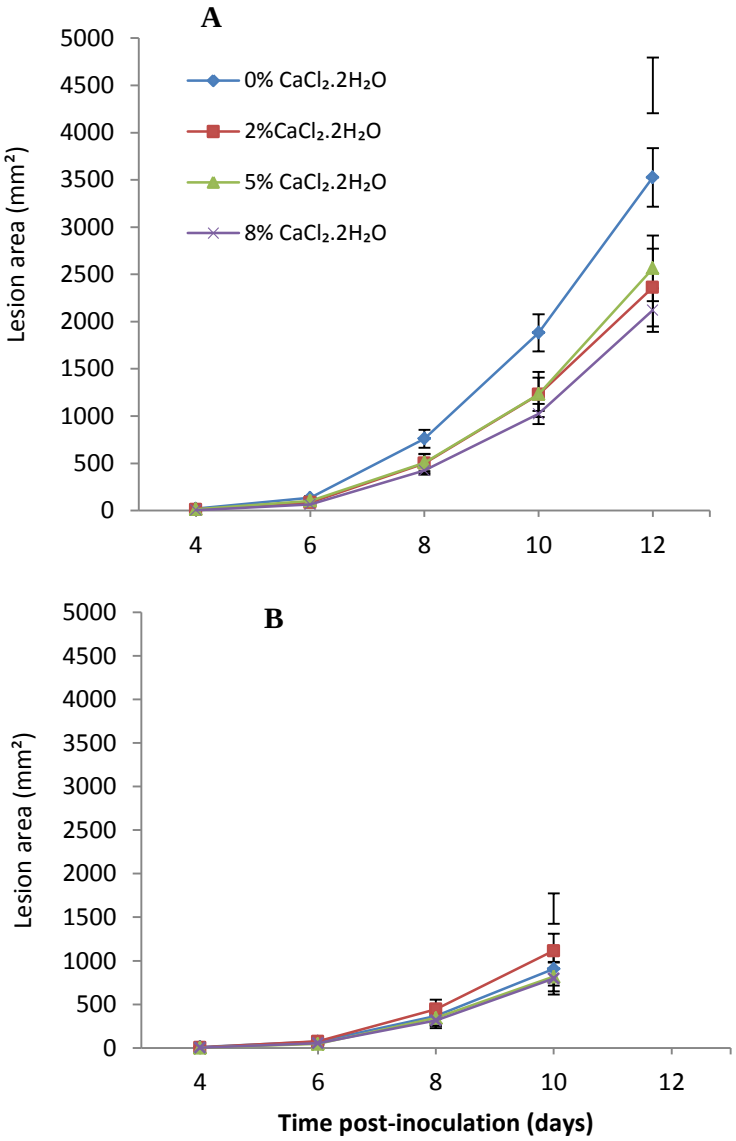


Fig. 6.9 Effect of Ca vacuum infiltration on grey mould development on postharvest-inoculated fruit of capsicum cv. Aries (**A**) and cv. Papri Queen (**B**) during storage at 10°C and relative humidity of >90%. Fruit were vacuum infiltrated by placing fruit in Ca solution at 0, 2, 5 or 8% w/v $CaCl_2.2H_2O$, drawing a vacuum for 10 sec and then holding fruit in solution for 2 min before vacuum release. Fruit were wounded opposite sides and inoculated with 40 µL suspension of 10^5 conidia mL^{-1}. Data are means ± SE from n = 10. LSDs ($P \leq 0.05$) are shown for among individual Ca concentrations for each time-point post inoculation

6.4 Discussion

Calcium concentration in fruit tissue was not significantly different among calcium treatments, regardless of application method. Postharvest application of Ca did not affect the shelf life or postharvest quality of capsicum fruit during storage. However, for cv. Aries, lesion area on Ca-treated fruit inoculated postharvest was significantly smaller than that on control fruit suggesting that postharvest Ca treatment may be a successful control method for grey mould of capsicum.

Postharvest Ca dips or vacuum infiltration, regardless of Ca concentration, did not increase Ca concentration in flesh of capsicum fruit significantly when sampling for nutrient analysis was immediately after Ca treatment. Calcium concentration in flesh of 'Golden Delicious' apples was reported to not increase significantly compared to a control when fruit were dipped with a $CaCl_2$ solution at concentrations of 0, 2, 4, 8 or 12%, but to increase significantly when fruit were vacuum infiltrated with the same Ca concentrations (Conway and Sams 1983). However, dipping peach fruit in Ca solution for 5 min increased flesh Ca concentration significantly (Manganaris et al. 2007). Flesh Ca concentration in strawberry was also higher compared to a control when fruit were dipped with 1% $CaCl_2$ for 5 min (Hernández-Muñoz et al. 2006). These observed differences of the ability of Ca to penetrate into different fruit may be explained by differences in the surface properties of fruit. The waxiness of capsicum (Govindarajan and Salzer 1985) could therefore possibly prevent Ca from being taken up easily. The surface properties of capsicum fruit may also be variable among cultivars with fruit from cv. Papri Queen appearing to be more waxy than fruit from cv. Aries. Lenticels are probably the primary pathway for Ca to enter fleshy fruit (Betts and Bramlage 1977) but Ca may also penetrate through cracks in the cuticle and epidermis (Glenn et al. 1985). Calcium may also penetrate through stomata given that there is a positive correlation between stomatal pore area with the rate of water uptake into fruit as reported in sweet cherry (Simon 2006). The lack of Ca penetration into capsicum flesh in the present research may therefore be due to a shortage of cracks and/or stomata and the waxiness of the capsicum peel. Further research is suggested to examine surface properties of capsicum fruit compared to

another fruit. Vacuum infiltration improved Ca uptake but Ca solution entered fruit through stem-end tissues and went straight to the placenta but not into the flesh (Fig 6.6A and B). This was probably due to vesicle structure of the placenta (Broderick and Cooke 2007), Ca was stored in vesicle and not easily transported to flesh. In order to confirm Ca was only in flesh, the placenta was removed when sampling for nutrient analysis.

Although Ca concentration was not increased initially by dipping or vacuum infiltration, the Ca concentration did increase significantly after 10 days of storage when fruit had been vacuum infiltrated. Calcium concentration increased by more than 300% in the flesh and 600% in the peel of vacuum-infiltrated fruit after 10 days storage (Table 6.4). The amount of Ca solution that was taken up into 'Golden Delicious' apples by using pressure infiltration was greater after 6 months of storage than after shorter storage intervals (Roy et al. 1999) and the authors revealed that cracks in the epicuticular wax layers and the cuticular layers of apple fruit extended during cold storage. Calcium in the peel of peach fruit decreased while there was a corresponding increase of Ca in the flesh after 5 days of cold storage (Manganaris et al. 2007). In the present research, increasing cracks or extension of cracks in the cuticular layers of capsicum during cool storage may create more openings for penetration of Ca. Further research is required to examine the cracks on the surface of capsicum fruit during cool storage. Moreover, Ca solution possibly penetrated into the flesh from the placenta during storage because of cell-wall breakdown in the flesh tissue due to ripening and senescence (Brady 1987).

The increased Ca concentration in the peel and flesh of fruit treated with Ca postharvest, has been accompanied by a maintained quality of fruit during storage in strawberry (García et al. 1996; Hernández-Muñoz et al. 2006), peach (Manganaris et al. 2007) and apple (Conway et al. 1994b). The common explanation is that the presence of Ca ion stabilises cell wall structure by forming cross bridges to link the pectin components and/or to other acidic polysaccharides (Demarty et al. 1984). In the present research, postharvest Ca application was expected to maintain the postharvest quality of capsicum fruit during storage. However, postharvest quality of capsicum fruit was not affected by postharvest

application of Ca, regardless of cultivar or Ca concentration. Calcium concentration in Ca-treated fruit may increase in flesh during storage, but was not sufficient to affect postharvest quality of fruit and the shelf life. However, there could be a potential economic impact as the shelf life of Ca-treated fruit may be significantly longer than the control if capsicum fruit were stored for a longer period. Symptoms of surface damage were not observed on Ca-treated fruit, but salt accumulation was observed on the surface of fruit treated with Ca concentrations of 5% or 8% w/v $CaCl_2.2H_2O$. This contributes to the penetration of Ca into the flesh during storage as noted above. A residual amount of $CaCl_2$ on the surface of fruit has been linked to detection of bitterness by consumers (Luna-Guzmán and Barrett 2000). However, dipping fruit with Ca concentration of 4% w/v $CaCl_2$ did not affect sensorial quality of strawberry fruit (García et al. 1996).

Lesion areas on Ca-treated fruit from cv. Aries were significantly smaller than those on the control fruit, regardless of whether Ca was applied as a dip or by vacuum infiltration. Postharvest treatment with Ca solution at 2% or 4% w/v $CaCl_2$ by using pressure infiltration reduced decay areas caused by *B. cinerea* in apples by 40% or 60%, respectively (Conway et al. 1994b). Postharvest treatment with Ca solution at the concentration of 2.0% or 3.0% w/w $CaCl_2$ was also reported to delay Botrytis rot on strawberry cv. Selva (Naradisorn 2008). Effect of increased Ca concentration in fruit on reducing the development of *B.cinerea* may be directly by inhibiting conidial germination and germ-tube elongation or indirectly by strengthening cell wall structure (Demarty et al. 1984; Conway et al. 1991). Direct inhibition of growth of *B. cinerea* seems most likely in this research given that adding $CaCl_2.2H_2O$ to PDA on which *B. cinerea* was grown resulted in reduced hyphal growth. This result was in agreement with research conducted by Wisniewski et al. (1995). The inhibitory effect of $CaCl_2$ or $MgCl_2$ on *B. cinerea* and *P. expansum in vitro* was examined with a range of concentrations from 0 to 200 mM. The research concluded that the Ca cation rather than chloride anion was responsible for the inhibition of conidial germination and germ-tube growth. In higher eukaryotes, high internal Ca concentration has been shown to cause inhibition of fungal growth and cell death (Carafoli 1987). In the present research, presence of Ca on the surface of Ca-treated fruit from cv. Aries may have caused

fewer conidia to germinate. Even though wounding fruit promotes *B. cinerea* growth, Ca concentration at wounded sites on Ca-treated fruit was most likely higher than those on the control fruit. Therefore, increasing Ca concentration on the surface of fruit may be a potential way to reduce decay in fruit during storage although an increase of Ca concentration in flesh did not occur. However, grey mould development on fruit from cv. Papri Queen was not significantly different among Ca treatments. Natural resistance of cv. Papri Queen to *B. cinerea* as described in Chapter 3 may overcome the positive effect of Ca in reducing the numbers of conidial germination on surface of fruit. Further research is required to examine conidial germination on wound sites of capsicum fruit treated with different Ca concentrations at a commercial scale.

If Ca is able to be taken up into fruit, the stability of the cell walls can be increased due to Ca binding with polygalacturonate chains, making the cell walls less accessible to enzymes that cause softening or to degrading enzymes produced by fungi (Conway et al. 1994b). High concentrations of Ca ions in tissues of tomato was thought to inhibit the activity of degrading enzymes, including pectinesterase (PE) and polygalacturonase (PG) (Wills and Rigney 1980), which were most important for *B. cinerea* to access more nutrients from cell-wall breakdown (Williamson et al. 2007). Decay caused by *P. expansum* on apple fruit reduced more than 50% when Ca tissue concentration was increased to 1200 - 1500 g Ca kg^{-1} DW, but a lower concentration may restrict progress of *B. cinerea* (Conway et al. 1991). In the present research, increasing Ca concentration reduced grey mould development significantly in cv. Aries, but was not always associated with higher levels of Ca in flesh as evidenced by the non-significant effect of firmness of fruit. Therefore, a direct effect of Ca on inhibition of the fungus itself than on cell-wall strengthen of fruit was indicated in this research.

6.5 Conclusion

Postharvest application of Ca could be recommended for capsicum cv. Aries fruit before storage or commercialisation to reduce grey mould growth on fruit during storage. However, the effect of different Ca concentrations on conidial germination on wound sites of capsicum fruit should be researched further. The vacuum infiltration method was more effective than dipping in increasing Ca

concentration in the flesh when capsicum fruit were subsequently stored in cool conditions. Therefore, Ca vacuum infiltration would be recommended for capsicum fruit which needs a long period of storage. However, the ideal length of time for vacuum infiltration needs to be determined in further research.

CHAPTER SEVEN

General discussion

7.1 Outcomes of study

Preharvest and postharvest losses of horticultural crops, including capsicum, due to grey mould disease caused by the fungus *Botrytis cinerea* are of significant concern to growers and produce marketers. A reduction of disease in fruit and improvement of fruit quality by using preharvest application of Ca and/or B via soil amendment or as a foliar spray has been reported in a wide range of horticultural crops, including strawberry (Chéour et al. 1990; Naradisorn et al. 2006), peach (Manganaris et al. 2005; Elmer et al. 2007), sweet cherry (Ippolito et al. 2005) and table grape (Amiri et al. 2009). However, how *B. cinerea* infects capsicum fruit has not been previously reported. In addition, knowledge of the effect of Ca or B on grey mould of capsicum and quality of fruit is limited. This research established the infection pathway of *B. cinerea* in capsicum and explored the effect of Ca or B application on grey mould of capsicum and quality of fruit.

Regardless of cultivar, when *B. cinerea* infected capsicum fruit preharvest, symptoms of grey mould were only apparent after harvest, while a greater concentration of inoculum caused more servere disease on fruit. Flowers were particularly sensitive to infection by *B. cinerea* and often died. The infection of flowers by *B. cinerea* followed by a latent period while fruit developed prior to disease symptoms forming has been reported for other fruit, including red raspberry (Dashwood and Fox 1988), strawberry (Jarvis and Borecka 1968), grape (Keller et al. 2003) and tomato (Lavy-Meir et al. 1989). This research confirmed the latency of *B. cinerea* in capsicum fruit, but infection of flowers during anthesis was probably not the only way for *B. cinerea* to infect fruit. Attached petals and shed pollen lodged on young fruit may supply moisture and nutrients for subsequent penetration of *B. cinerea* in fruit and this hypothesis requires further research.

Wounded fruit that were inoculated with *B. cinerea* after harvest showed a quicker development of grey mould than unwounded fruit when *B. cinerea* was present but latent before harvest. Wounding breaks the cuticle and epidermis which are

the primary defence to protect fruit from pathogen. Wounding is not desirable for marketability of fruit, but often occurs during harvest and postharvest handling of capsicum. *B. cinerea* requires a wound in the epidermis to enter susceptible tissue and initiate infection (Spotts et al. 1998). Wounding, therefore, probably caused failure of cuticle and epidermis resistance, leading to cell death and nutrient release which allowed *B. cinerea* to colonise tissue rapidly Also, the accumulation of aldehydic products at wounding sites contributed to cell death associated with preading lesion into healthy tissues (Deighton et al., 1999). The number of dead flowers and disease incidence on fruit after harvest was positively correlated with conidial concentration used to inoculate flowers or young developing fruit. Development of grey mould on wounded fruit inoculated postharvest was also positively correlated with conidial concentration of *B. cinerea*, regardless of ripening stage. Cultural practices, including ensuring an open canopy and removal of debris, to reduce moisture and inoculum could be applied to capsicum growing in glasshouses to minimise infection by *B. cinerea*. Careful and hygienic postharvest handling may help to reduce loss during subsequent storage and marketing of capsicum fruit.

Capsicum cv. Aries was more susceptible to infection by *B. cinerea* than capsicum cv. Papri Queen, suggesting the susceptibility of different cultivars to *B. cinerea* may be genetically controlled. A genotypic difference in the production of antifungal compounds has already been described for capsicum fruit (Howard et al. 2000; Deepa et al. 2007). A greater accumulation of capsidiol, an antifungal compound, was found a capsicum cultivar resistant to fungal pathogens (Egea at al., 1996). A long term might therefore be to use plant breeding strategies to create resistant cultivars. However, the basis of resistance in cv. Papri Queen in the present research is not known and therefore further research is needed.

Preharvest application of Ca or B to the soil or as a foliar application reduced grey mould development on fruit after harvest. In this study, Ca or B concentration in fruit tissues did not increase greatly in response to their application to plants, but did increase significantly in leaf tissues. There was a negative correlation between grey mould on fruit from cv. Aries and B concentration in leaves and fruit, while grey mould on fruit was not correlated with Ca concentration in leaves or fruit.

However, applying a higher concentration of Ca [4.0 mM $Ca(NO_3)_2$ via soil amendment or 1.0% w/v $Ca(NO_3)_2$ as a foliar spray] appeared to reduce disease incidence. The decrease of grey mould on fruit from cv. Aries that was derived from young developing fruit inoculated preharvest may be due to the role of Ca and B in the plant defence response, reducing cuticle degradation and maintaining cell wall structure (Benhamou 1996; Blevins and Lukaszewski 1998; Hansch and Mendel 2009), limiting latent infection of *B. cinerea* in fruit. Capsidiol, a phytoalexin, is an antifungal compound produced by capsicum, but its presence has not been reported specifically in response to Ca or B treatment in capsicum. However, Ca and B (0.05 or 0.1 mM B via soil amendment or 2.0 or 7.0 mM B as a foliar spray) were not important in reducing grey mould development on fruit from the less susceptible cv. Papri Queen. The encouraging results from this research suggest that preharvest application of Ca or B might be recommended to growers as a potential way to reduce grey mould on fruit from susceptible cultivars. However, preharvest application of Ca or B did not reduce grey mould development on wounded fruit inoculated with *B. cinerea* after harvest. In contrast, postharvest application of Ca, regardless of whether by dipping or vacuum infiltration, reduced grey mould development on wounded fruit. A direct inhibitory effect of Ca on conidial germination and germ-tube elongation on wounded fruit was probably responsible for any effect seen (Wisniewski et al. 1995). Postharvest application of Ca could therefore be used to reduce grey mould on fruit during storage and marketing in capsicum.

Quality parameters of fruit including extractable colour, firmness, total soluble solid content (TSSC) and titratable acidity (TA), from plants treated with Ca and B, regardless of whether by soil or foliar application did not improve compared to the control. This was probably because application of Ca and B did not change concentrations of nutrients in fruit due to natural immobility of Ca and B within the plant (Brown and Shelp 1997; White and Broadley 2003). Calcium and B increases cell wall strength and maintains structural integrity of the fruit membrane (Hewett 2006). Increasing Ca and B has been demonstrated to improve quality of fruit, such as apples (Khalifa et al. 2009), peach (Manganaris et al. 2005; Elmer et al. 2007), sweet cherry (Ippolito et al. 2005), strawberry

(Naradisorn et al. 2006) and table grape (Amiri et al. 2009). Therefore, if Ca and B concentration in fruit can be increased the the quality of capsicum fruit may also improve. Further research should therefore be focused on ways to increase Ca or B in fruit tissue.

When capsicum plants received the same amount of Ca or B, regardless of cultivar or application method, Ca or B concentration in the fruit was significantly lower than that in the leaves. Even though Ca or B increased significantly in the leaf tissue, there was no concomitant increase in the fruit tissue. These results present a challenge to the capsicum industry for increasing Ca or B concentration in the fruit. In addition, B concentration in the leaves and the fruit from plants that received a soil application appeared to be greater than those from plants that received a foliar spray. The natural waxiness of capsicum fruit (Govindarajan and Salzer 1985) may prevent Ca and B from penetrating directly into the flesh when applied as a foliar spray. Altering surface properties of capsicum fruit for Ca or B penetration may be necessary by adding surfactants in nutrient solution before spraying but this requires further research to determine appropriate levels of surfactants in the nutrient solution for increasing Ca and B in fruit. Calcium concentration in flesh after treatment also did not increase significantly when a Ca solution was applied directly to red fruit postharvest by using a dip or vacuum infiltration. This study only tested one duration of dipping and the effect of longer times for Ca penetration may need to be examined.

Using fertigation to supply plants with Ca and B is easier than foliar spray for growers because equipment use and labour can be saved. However, foliar application would be necessary to maintain Ca and B nutrition at adequate levels in fields without irrigation systems and/or when nutrient uptake by plants is low. Foliar sprays should not be applied close to harvest because the negative effect on taste of fruit may be detected by the customer due to residual salt on the surface of fruit even after washing (Wójcik and Lewandowski 2003). Therefore, which method is used depends on cultural practice and the desired quality of products. However, care needs to be taken with B application because of the narrow range between B deficiency and B toxicity and yield losses are possible when B is applied to the soil because of toxicity. Symptoms of B toxicity were observed on

leaves from capsicum plants that received a soil application at 0.1 mM H_3BO_3, but the yield and quality of fruit were not affected. Moreover, when plants had a larger shoot mass (at the time of the second harvest), the B uptake appeared to be decreased. Given that individual capsicum plants normally have more than two harvests of red fruit, if B is not supplied between harvests then B deficiency is very likely. Although foliar B spray was less effective than soil amendment with H_3BO_3 to increase B in the shoot by the first harvest, foliar sprays may be necessary to ensure B remains in the plant tissues at adequate levels throughout the lifecycle.

Boron or Ca concentration in leaves from cv. Aries was significantly greater than that in leaves from cv. Papri Queen. Boron or Ca uptake is dependent on environmental and genotypic factors. Higher relative humidity was reported to reduce transpiration rate in leaves leading to reduced Ca concentration in the leaves, but increased Ca concentration in fruit of tomato (Adams and Holder 1992). At higher temperatures and in moist soil conditions, the rate of Ca and B uptake by plants was significantly higher than that at lower temperatures and when soil was dry soil (Gupta 1979; Klein and Ferguson 1987). Capsicum plants in this research were grown in the greenhouse which differ in humidity and temperature compared to the field. The effect of these factors on B or Ca status in capsicum should be examined in further research. Moreover, the effect of genotypic factors on Ca and B efficiency has been previously reported. For example, maize inbred with the "Ca-efficient" genotype ('Oh43') only required one quarter of the level of Ca required by maize inbred with the "Ca-inefficient" (A251') to produce the same shoot biomass (Clárk 1983). A genotype-related difference in B uptake and distribution has also been reported in tomato (Bellaloui and Brown 1998) and wheat (Ahmed et al. 2007). Having Ca and B available in plant tissues may enable better plant defence responses against the pathogen (Benhamou 1996; Blevins and Lukaszewski 1998). Therefore, Ca and B efficiency in capsicum may provide better defence to pathogen attack. The effect of genotypic factors on Ca and B efficiency in capsicum plant requires more research.

7.2 Further research

From the findings of this research, there are a number of things that need further research.

1. *Determining the inoculum source for infection of* B. cinerea *preharvest.*
   Given the possible involvement of floral parts in infection of capsicum fruit by *B. cinerea*, further study is required to examine the role of petals and shed pollen in latent infection of developing fruit.

2. *Identifying the basis of resistance against B. cinerea in capsicum.*
   Characterisation of genes in relation to coding for pathogenesis-related proteins and defence responses may provide valuable information for breeding programs to create resistance cultivars for the future. In tomato, transgenic plants expressing polygalacturonase inhibitor protein (qPGIP) reduced growth of *B. cinerea* on fruit (Powell et al. 2000). Absence of the endo-β-1,4 glucanases Cel1 and Cel2 enhances resistance to *B. cinerea* (Flors et al. 2007).

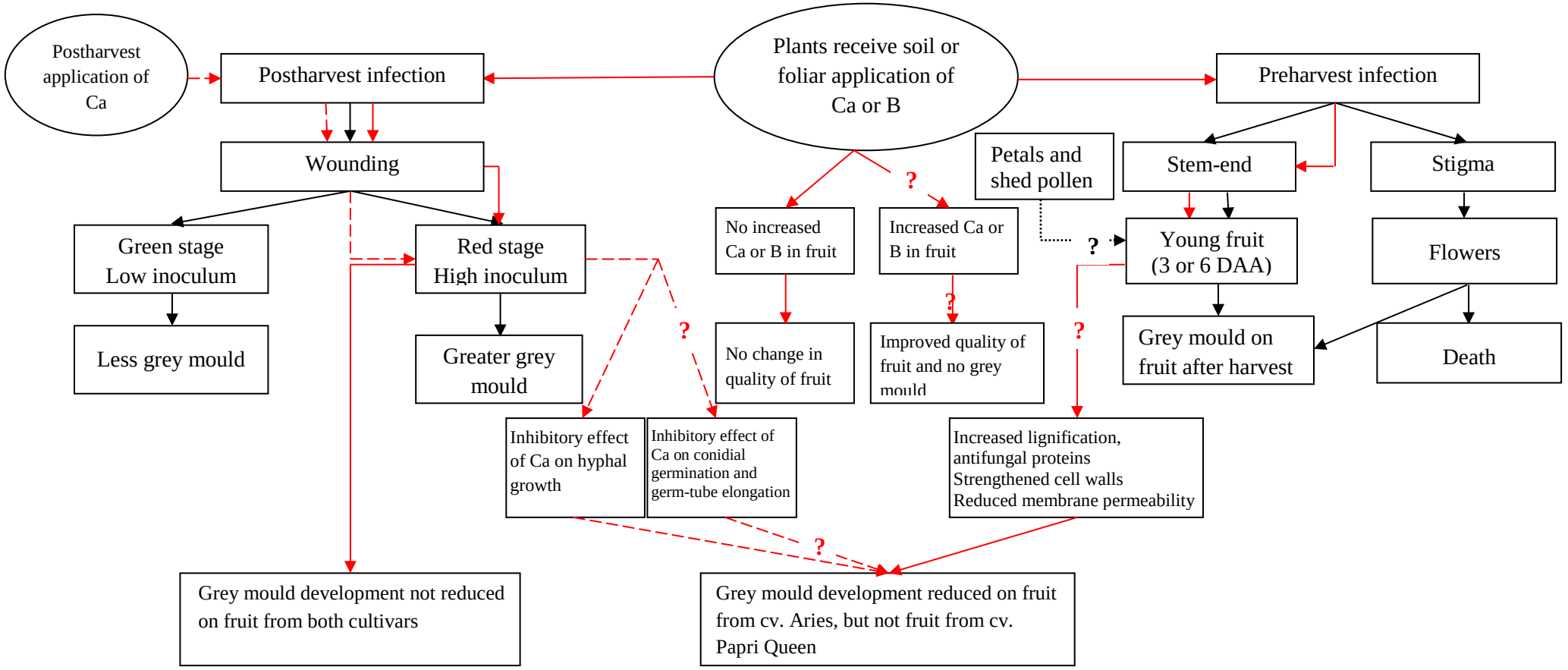
3. *Determining factors which influence Ca and B uptake into the fruit.*
   Further research on the effects of environmental factors on Ca and B uptake and distribution in the plant may provide better ways to increase Ca and B in the capsicum fruit. Moreover, in order to increase Ca or B penetration into fruit by using a foliar application, further studies are required to examine the role of surfactants in altering permeability of the cuticles in capsicum fruit. In addition, longer length of time for dipping fruit in Ca solution to provide more opportunity for Ca to penetrate into flesh is required in further research.

   Genetic engineering to increase Ca and B uptake and distribution in capsicum fruit are required in further research to create Ca and B efficient cultivars for long-term strategy.

7.3 Conclusions

Based on findings of this research and previously proposed questions (Chapter 2), a model of the infection pathway of *B. cinerea* in capsicum fruit and the effect of Ca or B nutrition on grey mould and quality of fruit has been established (Fig 7.1). This model may provide the basis for management of *B. cinerea* in the capsicum industry. Moreover, there are some existing questions which need further research so that better methods for controlling grey mould in capsicum may be developed in the future.

Fig. 7.1 Model of the infection pathway of *B. cinerea* in capsicum fruit and the effect of Ca or B on grey mould and quality of capsicum fruit



Infection pathway of *B. cinerea* in capsicum

The effect of preharvest application of Ca or B on grey mould development and quality of fruit

The effect of postharvest application of Ca on grey mould development on fruit

?? Unclear and further research is needed

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Appendix A.1

Statement of Authorship

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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Thong D. Le		
Contribution to the Paper	Conducted experiments, performed analysis on all samples, interpreted data and wrote manuscript		
Signature		Date	8/10/2013

Name of Co-Author	Amanda J. Able		
Contribution to the Paper	Experimental design, supervised development of work, helped in data interpretation, edited and corrected manucript		
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Contribution to the Paper	Suggested experimental design, helped to edit and correct the manuscript		
Signature		Date	18/10/13.

Appendix A.2

Effect of soil boron application on nutrient concentrations in leaf and fruit of capsicum. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that received 0, 0.05 or 0.1 mM H_3BO_3 in Hoagland's solution (200 mL per plant every 2 days). Data are presented as means ± SE from *n = 6*

Nutrient (mg kg^{-1} DW)	cv. Aries						cv. Papri Queen					
	0.00 mM H_3BO_3		0.05 mM H_3BO_3		0.1 mM H_3BO_3		0 mM H_3BO_3		0.05 mM H_3BO_3		0.1 mM H_3BO_3	
	Leaf tissues	Fruit tissues	Leaf tissues	Fruit tissues	Leaf tissues	Fruit tissues	Leaf tissues	Fruit tissues	Leaf tissues	Fruit tissues	Leaf tissues	Fruit tissues
Iron	82.92 ± 9.21	47.19 ± 3.57	104.39 ± 12.76	48.33 ± 3.09	88.95 ± 3.95	44.87 ± 1.70	84.64 ± 3.64	34.71 ± 0.66	84.65 ± 1.11	44.31 ± 1.78	89.17 ± 5.40	41.22 ± 1.88
Manganese	59.21 ± 11.68	9.29 ± 0.33	56.99 ± 6.20	10.85 ± 0.46	49.73 ± 7.99	11.93 ± 0.55	36.88 ± 2.09	8.71 ± 0.28	34.78 ± 5.11	8.51 ± 0.46	35.59 ± 3.61	9.15 ± 0.34
Copper	5.19 ± 0.57*	4.23 ± 0.24	4.72 ± 0.51*	3.91 ± 0.28	4.33 ± 0.27*	3.69 ± 0.16	2.74 ± 0.23*	3.27 ±0.05	2.19 ± 0.046*	2.57 ± 0.06	2.66 ± 0.18*	2.57 ± 0.12
Molybdenum	4.57 ± 1.08	1.05 ± 0.04	3.92 ± 0.72	1.28 ± 0.02	3.81 ± 0.15	1.18 ± 0.02	4.62 ± 0.49	< 1.00	4.54 ± 0.29	< 1.00	3.97 ± 0.22	< 1.00
Zinc	33.56 ± 2.46	17.77 ± 0.51	37.60 ± 0.35	18.79 ± 0.87	34.43 ± 0.99	19.74 ± 0.36	22.96 ± 1.25	19.65 ± 0.33	24.68 ± 0.92	19.24 ± 0.66	26.00 ± 0.87	20.32 ± 1.42
Calcium	33500.00 ± 3500.00	836.67 ± 23.94	29250.00 ± 4346.93	1013.33 ± 54.28	31000.00 ± 3511.88	928.33 ± 49.27	34750.00 ± 2015.56	1008.33 ± 42.02	33000.00 ± 2160.25	1141.67 ± 27.76	33750.00 ± 478.71	768.33 ± 25.63
Magnesium	4875.00 ± 221.27	1140.00 ± 39.82	4675.00 ± 385.95	1251.67 ± 36.09	4650.00 ± 322.75	1301.67 ± 53.72	5175.00 ± 85.39	1218.33 ± 34.62	5025.00 ± 118.15	1286.67± 30.92	5250.00 ± 107.78	1230.00 ± 42.73
Sodium	0.80 ± 0.00	103.74 ± 2.26	0.78 ± 0.03	97.99 ± 3.52	0.80 ± 0.00	72.99 ± 3.56	< 0.80	51.98 ± 3.31	< 0.90	29.25 ± 1.20	< 0.90	35.04 ± 1.70
Potassium	50500.00 ± 645.50	26166.67 ± 245.33	48000.00 ± 1683.25	25166.67 ± 245.33	49250.00 ± 1250.00	24666.67 ± 291.87	47000.00 ± 816.50	24000.00 ± 394.41	49250.00 ± 1547.85	21683.33 ± 486.53	45250.00 ± 750	25833.33 ± 702.51
Phosphorus	2475.00 ± 232.29	2733.33 ± 104.70	2800.00 ± 302.77	2850.00 ± 128.56	2525.00 ± 131.50	2681.67 ± 92.62	2225.00 ± 75.00	2000.00 ± 27.91	2375.00 ± 85.39	2178.33 ± 62.19	2350.00 ± 28.87	2015.00 ± 82.13
Sulphur	6250.00 ± 342.78	1941.67 ± 43.11	5650.00 ± 221.74	1951.67 ± 32.10	5700.00 ± 173.21	1911.67 ± 31.08	5025.00 ± 201.56	1591.67 ± 23.34	5100.00 ± 302.77	1588.33 ± 10.30	4675.00 ± 188.75	1623.33 ± 26.83
Aluminium	2.71 ± 0.63	2.49 ± 0.31	1.61 ± 0.43	1.85 ± 0.26	1.72 ± 0.23	0.81 ± 0.10	4.27 ± 1.39	1.43 ± 0.34	3.07 ± 0.43	0.49 ± 0.04	4.45 ± 1.84	0.57 ± 0.08

*deficient as stated by Reuter and Robinson (1997)

Appendix A.3

Effect of foliar boron application on nutrient concentrations in leaf and fruit tissues of cv. Aries. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that sprayed 0, 0.025 or 0.075 mM H_3BO_3 in the first trial and 0.0, 2.0 and 7.0 mM in the second trial from flowering to harvest red fruit. Plants were watered with Hoagland's solution without boron (200 mL per plant every 2 days). Data are presented as means ± SE from *n = 3*

Nutrient (mg kg^{-1} DW)	**The 1st trial**						**The 2nd trial**					
	0.00 mM H_3BO_3		**0.025 mM H_3BO_3**		**0.075 mM H_3BO_3**		**0.0 mM H_3BO_3**		**2.0 mM H_3BO_3**		**7.0 mM H_3BO_3**	
	Leaf tissues	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**
Iron	68.00 ± 0.23	40.56 ± 4.81	60.88 ± 0.4	41.14 ± 2.04	57.24 ± 0.32*	34.06 ± 3.07	58.39 ± 0.01*	41.80 ± 41.12	69.25 ± 0.01	50.13 ± 3.60	56.79 ± 0.19*	47.77 ± 6.41
Manganese	64.10 ± 0.13	12.60 ± 1.03	66.64 ± 0.65	11.74 ± 0.71	72.14 ± 0.3	10.15 ± 1.89	53.31 ± 0.37	13.72 ± 0.68	54.43 ± 1.00	10.45 ± 1.12	62.44 ± 0.75	9.69 ± 0.82
Copper	3.01 ± 0.04*	3.56 ± 0.19	2.86 ± 0.01*	3.34 ±0.14	2.68 ± 0.01*	3.33 ± 0.26	3.05 ± 0.03*	4.63 ± 1.03	3.65 ± 0.03*	2.65 ± 0.09	3.17 ± 0.05*	3.02 ± 0.28
Molybdenum	5.68 ± 0.11	1.56 ± 0.05	5.39 ± 1.12	1.32 ± 0.04	4.65 ± 0.02	1.25 ± 0.09	3.51 ± 0.06	3.57 ± 1.26	3.82 ± 0.00	< 1.00	3.76 ± 0.02	1.00
Zinc	40.05 ± 0.18	18.86 ± 1.14	38.13 ± 0.16	18.82 ± 0.72	38.21 ± 0.2	15.58 ± 1.39	29.31 ± 0.11	27.26 ± 4.00	29.80 ± 0.76	17.52 ± 1.01	29.11 ± 0.26	22.76 ± 2.52
Calcium	49000.00 ± 1000.00	973.33 ± 20.28	47000.00 ± 0.00	903.33 ± 65.66	48000.00 ± 0.00	946.67 ± 124.1	29500.00 ± 500.00	823.33 ± 46.31	28000.00 ± 0.00	776.6 ±69.36	34000.00 ± 0.00	816.67 ± 102.69
Magnesium	5300.00 ± 0.00	1223.33 ± 50.44	5100.00 ± 0.00	1193.33 ± 66.92	5650.00 ± 50.00	1090.00 ± 129.00	3650.00 ± 50.00	1120.00 ± 78.10	4100.00 ± 100.00	1093.33 ± 60.64	4250.00 ± 50.00	1060.00 ± 77.67
Sodium	5.01 ± 0.72	45.87 ± 1.75	2.92 ± 0.58	48.01 ± 6.26	6.96 ± 1.09	49.94 ± 6.73	44.22 ± 0.89	74.21 ± 6.71	24.45 ± 1.03	83.16 ± 10.83	20.49 ± 0.87	90.53 ± 4.70
Potassium	53500.00 ± 500.00	27666.67 ± 881.92	50000.00 ± 0.00	24000.00 ± 0.00	50500.00 ± 500.00	24000.00 ± 2000.00	47000.00 ± 0.00	18900.00 ± 1050.40	51500.00 ± 1500.00	21100.00 ± 900.00	48000.00 ± 0.00	20666.67 ± 333.33
Phosphorus	2700.00 ± 0.00	3033.33 ± 202.76	2700.00 ± 0.00	2866.67 ± 66.67	2500.00 ± 0.00	2550.00 ± 453.70	3650.00 ± 50.00	2633.33 ± 240.37	3600.00 ± 100.00	2800.00 ± 208.17	3400.00 ± 100.00	2566.67 ± 176.38
Sulphur	5700.00 ± 0.00	2133.33 ± 88.12	5100.00 ± 0.00	1936.67 ± 58.41	5000.00 ± 0.00	1800.00 ± 161.70	6000.00 ± 0.00	1820.00 ± 140.12	6300.00 ± 0.00	1866.67 ± 17.64	5900.00 ± 100.00	1736.67 ± 78.39
Aluminium	1.36 ± 0.01	< 0.40	1.61 ± 0.17	< 0.30	1.33 ±0.36	< 0.40	3.52 ± 0.15	10.11 ±4.65	3.32 ± 0.33	0.76 ± 0.12	2.88 ± 0.31	1.29 ± 0.60

*deficient as stated by Reuter and Robinson (1997)

Appendix A.4

Effect of foliar boron application on nutrient concentrations in leaf and fruit tissues of cv. Papri Queen. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that sprayed 0, 0.025 or 0.075 mM H_3BO_3 in the first trial and 0, 2 and 7.0 mM in the second trial from flowering to harvest red fruit. Plants were watered with Hoagland's solution without boron (200 mL per plant every 2 days). Data are presented as means ± SE from *n = 3*

Nutrient (mg kg^{-1} DW)	**The 1st trial**						**The 2nd trial**					
	0.00 mM H_3BO_3		**0.025 mM H_3BO_3**		**0.075 mM H_3BO_3**		**0.0 mM H_3BO_3**		**2.0 mM H_3BO_3**		**7.0 mM H_3BO_3**	
	Leaf tissues	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**
Iron	67.81± 0.97	36.01 ± 5.39	52.63 ± 1.64*	33.65 ± 2.13	57.14 ± 1.01*	43.26 ± 6.50	70.21 ± 0.89	42.42 ± 1.21	57.08 ± 0.48*	39.34 ± 5.86	57.69 ± 0.62*	35.39 ± 5.18
Manganese	52.38 ± 0.86	11.47 ± 0.50	45.79 ± 1.71	9.32 ± 0.48	58.87 ± 0.43	11.06 ± 1.10	46.28 ± 1.69	10.78 ± 0.26	46.49 ± 0.09	10.32 ± 1.05	48.17 ± 0.04	11.09 ± 0.39
Copper	3.69 ± 0.01*	3.39 ± 0.5	2.51 ± 0.05*	2.89 ± 1.34	2.73 ± 0.05*	3.86 ± 0.48	4.00 ± 0.11*	2.83 ± 0.03	2.87 ± 0.06*	3.35 ±0.19	3.94 ± 0.19*	3.0 ± 0.72
Molybdenum	5.04 ± 0.12	1.34 ± 0.20	5.65 ± 0.07	1.10 ± 0.06	4.78 ± 0.1	1.39 ± 0.31	4.17 ± 0.01	< 1.00	3.57 ± 0.15	< 1.00	3.62 ± 0.01	< 1.00
Zinc	47.04 ± 0.38	20.55 ± 1.93	42.70 ± 1.11	17.41 ± 1.18	40.19 ± 0.24	16.28 ± 1.17	31.53 ± 1.07	15.54 ± 0.56	36.11 ± 0.26	16.74 ± 1.47	30.37 ± 0.35	15.15 ± 1.89
Calcium	36000.00 ± 0.00	1730.00 ± 191.40	43000.00 ± 0.00	1073.33 ± 44.1	41500.00 ± 500.00	1583.33 ± 257.70	23500.00 ± 500.00	746.67 ± 93.51	27500.00 ± 500.00	793.33 ± 23.33	23500.00 ± 500.00	800.00 ± 174.74
Magnesium	5300.00 ± 100.00	1293.33 ± 23.33	5650.00 ± 50.00	1003.33 ± 33.83	4850.00 ± 50.00	1173.33 ± 121.97	4400.00 ± 100.00	990.00 ± 23.09	4800.00 ± 0.00	973.33 ± 89.69	4300.00 ± 0.00	1006.67 ± 38.44
Sodium	< 1.00	56.67 ± 7.47	17.91 ± 1.89	42.72 ± 3.78	< 1.00	51.08 ± 6.50	51.62 ± 1.94	95.24 ± 8.78	23.54 ± 1.20	99.54 ± 8.03	28.06 ± 0.37	75.53 ± 7.72
Potassium	62000.00 ± 0.00	26333.33 ± 881.92	54500.00 ± 500.00	26666.67 ± 333.33	54000.00 ± 0.00	25666.67 ± 333.33	49500.00 ± 1500.00	25666.67 ± 1333.33	43500.00 ± 500.00	24666.67 ± 2333.33	50500.00 ± 500.00	23000.00 ± 1527.53
Phosphorus	3300.00 ± 0.00	2600.00 ± 251.66	3400.00 ± 0.00	2733.33 ± 145.3	3500.00 ± 0.00	3066.67 ± 284.80	4200.00 ± 100.00	2700.00 ± 57.74	4400.00 ± 0.00	2966.67 ± 133.33	3950.00 ± 50.00	2566.67 ± 317.98
Sulphur	7450.00 ± 50.00	1966.67 ± 185.59	7100.00 ± 0.00	1923.33 ± 62.27	6000.00 ± 0.00	2026.67 ±126.67	7000.00 ± 0.00	1840.00 ± 85.05	6200.00 ± 0.00	1976.67 ± 75.35	6600.00 ± 0.00	1773.33 ± 78.81
Aluminium	4.19 ± 1.83	< 0.4	17.34 ± 13.52	< 0.3	3.00 ± 0.69	< 0.68	4.21 ± 0.44	2.71 ± 0.72	2.42 ± 0.10	3.50 ± 0.9	2.54 ± 0.23	0.82 ± 0.43

*deficient as stated by Reuter and Robinson (1997)

Appendix A.5

Effect of soil Ca application on nutrient status in capsicum plant tissues. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that received a soil application: 1.5, 4.0 or 8.0 mM Ca in Hoagland's solution (200 mL each plant every 2 days) from flowering to harvest of red fruit. Data are presented as means ± SE from *n = 3*

Nutrient (mg kg^{-1} DW)	**cv. Aries**						**cv. Papri Queen**					
	1.5 mM Ca		**4.0 mM Ca**		**8.0 mM Ca**		**1.5 mM Ca**		**4.0 mM Ca**		**8.0 mM Ca**	
	Leaf tissues	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**
Iron	85.50 ± 9.50	43.00 ± 1.53	113.00 ± 2.00	32.00 ± 3.00	93.00 ± 2.00	31.67 ± 1.86	84.50 ± 4.50	37.67 ± 3.76	127.50 ± 2.50	29.67 ± 2.85	104.00 ± 3.00	35.00 ± 2.00
Manganese	*4.65 ± 0.65	13.33 ± 1.86	*7.55 ± 0.65	9.70 ± 0.66	*8.70 ± 0.40	10.73 ± 0.82	*4.50 ± 0.70	13.67 ± 0.88	*5.30 ± 0.00	8.73 ± 0.52	*7.50 ± 0.00	9.67 ± 0.33
Boron	43.00 ± 3.00	12.67 ± 0.67	61.00 ± 1.00	12.67 ± 1.20	56.50 ± 2.50	12.67 ± 0.33	48.50 ± 1.50	12.67 ± 0.67	49.00 ± 1.00	12.33 ± 0.33	49.00 ± 0.00	15.67 ± 0.88
Copper	8.10 ± 0.80	6.03 ± 0.38	8.35 ± 0.25	5.47 ± 0.68	7.90 ± 0.20	5.33 ± 0.65	6.15 ± 0.15	3.77 ± 0.18	8.20 ± 0.20	3.50 ± 0.15	8.50 ± 0.10	3.90 ± 0.06
Molybdenum	33.00 ± 0.00	1.77 ± 0.18	67.00 ± 0.00	1.90 ± 0.15	17.00 ± 0.00	1.03 ± 0.03	34.00 ± 1.00	0.73 ± 0.00	44.50 ± 0.50	1.43 ± 0.13	18.00 ± 1.00	1.01 ± 0.01
Magnesium	7200.00 ± 200.00	1153.33 ± 91.35	6000.00 ± 200.00	1283.33 ± 115.66	6800.00 ± 100.00	1050.00 ± 45.09	6450.00 ± 250.00	1123.33 ± 63.60	7000.00 ± 0.00	1206.67 ± 37.56	5400.00 ± 100.00	1083.33 ± 8.82
Sodium	7.80 ± 1.00	183.00 ± 26.85	7.15 ± 0.95	96.00 ± 6.66	19.50 ± 7.50	142.00 ± 9.81	20.00 ± 15.00	170.00 ± 45.35	19.55 ± 11.45	68.67 ± 6.17	14.30 ± 3.00	96.33 ± 11.84
Potassium	82000.00 ± 1000.00	25333.33 ± 666.67	62500.00 ± 1500.00	25333.33 ± 1855.92	54000.00 ± 0.00	26333.33 ± 1333.33	75500.00 ± 2500.00	23333.33 ± 1452.97	68000.00 ± 2000.00	24000.00 ± 577.35	59000.00 ± 1000.00	24666.67 ± 1201.85
Phosphorus	7000.00 ± 100.00	3433.33 ± 120.19	5700.00 ± 100.00	2900.00 ±208.17	3300.00 ± 0.00	2033.33 ± 66.67	6000.00 ± 100.00	2966.67 ± 145.30	5400.00 ± 0.00	2533.33 ± 66.67	4100.00 ± 0.00	1776.67 ± 33.33
Sulphur	7550.00 ± 450.00	2013.33 ± 101.71	8250.00 ± 50.00	1970.00 ± 165.63	7850.00 ± 250.00	1840.00 ± 65.06	6850.00 ± 50.00	1536.67 ± 35.28	7100.00 ± 100.00	1423.33 ± 50.44	6600.00 ± 100.00	1556.67 ± 38.44
Selenium	< 9.5	< 8	< 7	< 8	< 7	< 8	< 8.00	< 8.00	< 7.50	< 8.00	< 7.5	< 8.00

*deficient range as stated by Reuter and Robinson (1997)

Appendix A.6

Effect of soil Ca application on nutrient status in capsicum plant tissues. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that received a soil application: 0.0, 1.5 or 4.0 mM Ca in Hoagland's solution (200 mL each plant every 2 days) from flowering to harvest of red fruit. Data are presented as means ± SE from *n = 3*

Nutrient (mg kg⁻¹ DW)	**cv. Aries**						**cv. Papri Queen**					
	0.0 mM Ca		**1.5 mM Ca**		**4.0 mM Ca**		**0.0 mM Ca**		**1.5 mM Ca**		**4.0 mM Ca**	
	Leaf tissues	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**
Iron	66.30 ± 0.54	47.82 ± 0.66	77.93 ± 2.83	52.03 ± 3.79	*40.94 ± 3.08	37.08 ± 0.18	78.92 ± 1.80	46.55 ± 6.93	79.50 ± 9.71	47.69 ± 4.32	66.40 ± 3.33	41.80 ± 5.02
Manganese	195.46 ± 14.54	10.27 ± 0.21	220.00 ± 0.00	10.37 ± 0.76	57.21 ± 0.41	8.04 ± 0.36	78.88 ± 2.09	13.14 ± 1.12	132.75 ± 8.31	12.08 ± 0.33	47.26 ± 2.32	8.04 ± 1.16
Boron	117.40 ± 6.79	13.05 ± 0.08	105.21 ± 4.64	11.98 ± 0.40	113.69 ± 1.88	11.56 ± 0.51	62.87 ± 7.32	12.06 ± 0.17	86.41 ± 2.36	9.77 ± 0.42	86.79 ± 1.65	12.13 ± 0.26
Copper	*3.91 ± 0.03	3.17 ± 0.17	*3.56 ± 0.23	3.54 ± 0.41	*2.57 ± 0.17	3.19 ± 0.10	*4.95 ± 1.05	3.08 ± 0.17	*2.94 ± 0.02	2.63 ± 0.03	*2.84 ± 0.19	2.60 ± 0.17
Molybdenum	2.65 ± 0.12	< 1.00	2.97 ± 0.03	1.12 ± 0.05	2.88 ± 0.21	1.14 ± 0.08	2.74 ± 0.09	1.03 ± 0.01	2.84 ± 0.07	< 1.00	3.49 ± 0.30	< 1.00
Magnesium	6200.00 ± 300.00	890.00 ± 30.55	5300.00 ± 200.00	826.67 ± 29.06	4100.00 ± 100.00	960.00 ± 45.83	4800.00 ± 100.00	1033.33 ± 83.53	4400.00 ± 300.00	840.00 ± 11.55	3700.00 ± 300.00	916.67 ± 143.45
Sodium	6.74 ± 0.62	184.28 ± 3.16	20.43 ± 0.38	172.77 ± 7.25	19.75 ± 3.66	137.62 ± 18.11	17.93 ± 1.26	224.36 ± 23.34	10.76 ± 0.19	204.05 ± 25.95	11.19 ± 2.20	223.12 ± 12.23
Potassium	44500.00 ± 500.00	21000.00 ± 577.35	33500.00 ± 500.00	19500.00 ± 776.75	35500.00 ± 1500.00	21466.67 ± 1348.25	42000.00 ± 1000.00	21333.33 ± 1201.85	39500.00 ± 1500.00	19400.00 ± 208.17	43000.00 ± 1000.00	20200.00 ± 416.33
Phosphorus	2100.00 ± 0.00	2166.67 ± 88.19	2550.00 ± 50.00	2666.67 ± 218.58	1975.00 ± 125.00	2600.00 ± 57.74	2850.00 ± 250.00	2600.00 ± 173.21	2850.00 ± 50.00	2566.67 ± 185.59	2700.00 ± 200.00	2396.67 ± 254.97
Sulphur	6300.00 ± 300.00	1823.33 ± 27.28	6200.00 ± 200.00	1950.00 ± 126.62	4750.00 ± 150.00	1846.67 ± 60.64	5800.00 ± 300.00	1876.67 ± 113.48	5550.00 ± 250.00	1833.33 ± 122.52	5300.00 ± 0.00	1736.67 ± 108.68
Aluminium	25.41 ± 0.07	3.08 ± 0.54	7.96 ± 0.11	3.62 ± 0.86	1.40 ± 0.21	1.84 ± 0.20	18.54 ± 2.41	4.90 ± 1.19	8.38 ± 0.74	3.33 ± 0.33	2.65 ± 0.31	4.69 ± 1.29

*deficient range as stated by Reuter and Robinson (1997)

Appendix A.7

Effect of foliar Ca application on nutrient status in capsicum plant tissues. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that received a foliar application: 0.5, 0.75 or 1.0% w/v $Ca(NO_3)_2$ from flowering to harvest of red fruit. Plants were watered with Hoagland's solution without Ca (200 mL each plant for every two days). Data are presented as means ± SE from *n = 3*

Nutrient (mg kg^{-1} DW)	**cv. Aries**						**cv. Papri Queen**					
	0.5% w/v $Ca(NO_3)_2$		**0.75% w/v $Ca(NO_3)_2$**		**1.0% w/v $Ca(NO_3)_2$**		**0.5% w/v $Ca(NO_3)_2$**		**0.75% w/v $Ca(NO_3)_2$**		**1.0% w/v $Ca(NO_3)_2$**	
	Leaf tissues	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**
Iron	75.50 ± 0.50	67.00 ± 12.34	72.00 ± 2.00	54.00 ± 1.53	62.50 ± 0.50	37.00 ± 1.53	83.00 ± 1.00	46.00 ± 2.31	74.00 ± 4.00	39.67 ± 0.88	71.00 ± 3.00	42.67 ± 3.71
Manganese	126.50 ± 12.50	18.33 ± 2.60	128.50 ± 7.50	15.33 ± 1.67	104.50 ± 8.50	13.00 ± 0.58	100.00 ± 4.00	16.33 ± 1.20	90.00 ± 3.00	14.33 ± 0.67	77.50 ± 5.50	15.00 ± 0.58
Boron	69.00 ± 7.00	10.63 ± 0.37	72.50 ± 8.50	10.60 ± 0.95	76.00 ± 8.00	9.87 ± 0.66	74.50 ± 2.50	11.00 ± 0.58	66.00 ± 4.00	12.00 ± 0.78	47.00 ± 2.00	12.67 ± 0.33
Copper	10.30 ± 0.70	9.27 ± 0.38	9.75 ± 0.05	8.80 ± 0.40	8.85 ± 0.35	6.97 ± 0.23	*6.65 ± 0.15	7.30 ± 0.12	*5.15 ± 0.15	7.13 ± 0.27	*4.30 ± 0.10	6.40 ± 0.35
Molybdenum	6.95 ± 0.05	1.80 ± 0.25	5.80 ± 0.20	1.77 ± 0.12	7.10 ± 0.35	1.33 ± 0.12	6.00 ± 0.50	1.17 ± 0.07	6.05 ± 0.05	1.00 ± 0.00	4.80 ± 0.00	0.83 ± 0.04
Magnesium	5250.00 ± 350.00	1440.00 ± 87.18	5250.00 ± 150.00	1283.33 ± 77.53	4250.00 ± 50.00	1103.33 ± 3.33	4000.00 ± 100.00	1263.33 ± 85.70	3750.00 ± 150.00	1330.00 ± 36.06	3250.00 ± 150.00	1240.00 ± 45.09
Sodium	7850.00 ± 250.00	118.33 ± 14.53	7550.00 ± 150.00	110.33 ± 12.12	6850.00 ± 200.00	80.33 ± 4.10	6600.00 ± 250.00	94.67 ± 14.31	6700.00 ± 650.00	69.67 ± 8.45	5750.00 ± 350.00	34.00 ± 5.51
Potassium	44000.00 ± 2000.00	23666.67 ± 333.33	47500.00 ± 1500.00	23333.33 ± 881.92	43500.00 ± 1500.00	20233.33 ± 392.99	48500.00 ± 500.00	25666.67 ± 881.92	42500.00 ± 500.00	24666.67 ± 666.67	39500.00 ± 1500.00	27666.67 ± 1666.70
Phosphorus	3050.00 ± 250.00	3500.00 ± 152.75	3050.00 ± 50.00	3500.00 ± 57.74	2850.00 ± 150.00	2933.33 ± 66.67	3050.00 ± 50.00	3566.67 ± 66.67	2450.00 ± 50.00	3466.67 ± 33.33	2300.00 ± 100.00	3500.00 ± 115.47
Sulphur	7850.00 ± 150.00	2133.33 ± 33.33	7550.00 ± 150.00	2103.33 ± 112.60	6850.00 ± 50.00	1766.67 ± 24.04	6600.00 ± 300.00	1700.00 ± 46.19	6700.00 ± 100.00	1550.00 ± 20.00	5750.00 ± 150.00	1583.33 ± 86.67
Aluminium	9.65 ± 1.35	6.03 ± 0.23	9.50 ± 1.50	4.50 ± 0.49	11.50 ± 1.500	2.47 ± 0.58	8.05 ± 0.15	3.80 ± 3.06	9.25 ± 0.75	1.77 ± 1.27	7.40 ± 0.2	0.90 ± 0.01

Appendix A.8

Effect of foliar Ca application on nutrient status in capsicum plant tissues. The nutrient concentrations were determined using ICP-OES analysis. Youngest mature leaves and red fruit for nutrient analysis were collected at harvest from plants that received a foliar application: 0.0, 0.5 or 1.0% w/v $Ca(NO_3)_2$ from flowering to harvest of red fruit. Plants were watered with Hoagland's solution without Ca (200 mL each plant for every two days). Data are presented as means ± SE from *n = 3*

Nutrient (mg kg^{-1} DW)	**cv. Aries**						**cv. Papri Queen**					
	0.0% w/v $Ca(NO_3)_2$		**0.5% w/v $Ca(NO_3)_2$**		**1.0% w/v $Ca(NO_3)_2$**		**0.0% w/v $Ca(NO_3)_2$**		**0.5% w/v $Ca(NO_3)_2$**		**1.0% w/v $Ca(NO_3)_2$**	
	Leaf tissues	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**	**Leaf tissues**	**Fruit tissues**
Iron	92.29 ± 1.23	49.83 ± 9.12	99.13 ± 0.06	38.41 ± 2.55	90.96 ± 0.66	44.46 ± 0.87	109.79 ± 0.64	44.13207 ± 0.18	121.40 ± 0.99	60.05 ± 3.10	113.88 ± 1.32	56.09 ± 4.14
Manganese	146.52 ± 1.67	17.21 ± 0.52	161.76 ± 1.49	15.14 ± 1.45	174.19 ± 1.27	14.24 ± 0.27	112.52 ± 0.25	15.57581 ± 0.53	115.76 ± 0.04	18.98 ± 0.63	120.45 ± 0.72	20.43 ± 4.74
Boron	330.00 ± 0.00	17.14 ± 1.49	285.00 ± 5.00	12.32 ± 0.22	250.00 ± 0.00	12.89 ± 0.52	280.00 ± 0.00	19.27215 ± 1.86	270.00 ± 0.00	18.83 ± 0.63	250.00 ± 0.00	20.37 ± 2.58
Copper	5.69 ± 0.98	4.45 ± 0.75	5.90 ± 0.72	3.93 ± 0.07	5.01 ± 0.04	3.97 ± 0.09	*3.94 ± 0.02	2.547399 ± 0.25	*4.64 ± 0.16	3.98 ± 0.54	*4.07 ± 0.03	3.67 ± 0.26
Molybdenum	2.22 ± 0.03	1.35 ± 0.02	2.13 ± 0.08	< 2.00	1.62 ± 0.01	< 2.00	2.79 ± 0.03	< 1.03 ± 0.03	3.38 ± 0.05	< 1.00	2.77 ± 0.05	< 1.50
Zinc	30.51 ± 0.79	27.72 ± 3.04	30.97 ± 0.08	21.95 ± 3.82	26.62 ± 0.23	20.37 ± 0.69	24.87 ± 0.06	17.29 ± 0.39	25.47 ± 0.03	26.68 ± 2.41	19.39 ± 0.31	23.58 ± 1.38
Magnesium	7100.00 ± 100.00	1660.00 ± 60.00	6300.00 ± 0.00	1340.00 ± 40.00	5900.00 ± 0.00	1285.00 ± 105.00	5550.00 ± 50.00	1515.00 ± 5.00	6450.00 ± 50.00	1810.00 ± 120.00	5200.00 ± 0.00	1900.00 ± 200.00
Sodium	44.50 ± 2.30	169.60 ± 16.64	66.32 ± 0.26	176.00 ± 15.78	45.80 ± 0.84	215.00 ± 5.00	29.63 ± 0.66	134.98 ± 21.61	32.43 ± 0.42	205.32 ± 34.68	60.16 ± 0.45	189.81 ± 20.19
Potassium	75000.00 ± 1000.00	27500.00 ± 1500.00	77500.00 ± 1500.00	25000.00 ± 0.00	72500.00 ± 500.00	26000.00 ± 1000.00	74000.00 ± 0.00	25500.00 ± 500.00	75000.00 ± 0.00	28000.00 ± 0.00	75500.00 ± 1500.00	32500.00 ± 3500.00
Phosphorus	3450.00 ± 50.00	3400.00 ± 400.00	4000.00 ± 0.00	3150.00 ± 150.00	3300.00 ± 0.00	3250.00 ± 150.00	4800.00 ± 0.00	3000.00 ± 100.00	4500.00 ± 0.00	3600.00 ± 0.00	4050.00 ± 50.00	3750.00 ± 350.00
Sulphur	9500.00 ± 100.00	2300.00 ± 200.00	9600.00 ± 0.00	2075.00 ± 125.00	8050.00 ± 50.00	2150.00 ± 50.00	8050.00 ± 50.00	1615.00 ± 55.00	8200.00 ± 0.08	1960.00 ± 140.00	7550.00 ± 0.00	1985.00 ± 15.00
Aluminium	12.66 ± 0.47	2.13 ± 0.09	13.29 ± 0.01	5.45 ± 0.34	9.84 ± 0.54	4.93 ± 1.76	11.43 ± 0.21	1.77 ± 1.20	7.02 ± 0.00	1.95 ± 1.10	8.29 ± 0.00	2.91 ± 1.04

*deficient range as stated by Reuter and Robinson (1997)

Appendix A.9

Effect of postharvest calcium dips on nutrient concentrations in fruit tissues. The nutrient concentrations were determined using ICP-OES analysis. Fruit for nutrient analysis were collected at 0 day after harvest and 0 day of storage. Fruit were treated at four concentrations: 0, 2, 5 and 8% w/v $CaCl_2.2H_2O$. Dipping was conducted by placing fruit in the respective calcium solution for 5 min. Data are presented as means ± SE from *n = 3*

Nutrient (mg kg^{-1} DW)	**cv. Aries**				**cv. Papri Queen**			
	0.0 % $CaCl_2.2H_2O$	**2.0 % $CaCl_2.2H_2O$**	**5.0 % $CaCl_2.2H_2O$**	**8.0 % $CaCl_2.2H_2O$**	**0.0 % $CaCl_2.2H_2O$**	**2.0 % $CaCl_2.2H_2O$**	**5.0 % $CaCl_2.2H_2O$**	**8.0 % $CaCl_2.2H_2O$**
Iron	49.05 ± 6.29	39.77 ± 3.12	45.85 ± 2.73	39.21 ± 2.13	37.01 ± 2.20	36.57 ± 2.42	37.28 ± 2.87	35.07 ± 1.18
Manganese	12.59 ± 0.85	13.21 ± 3.12	14.46 ± 1.93	16.53 ± 1.08	14.47 ± 1.15	16.92 ± 3.61	11.81 ± 0.72	16.78 ±0.36
Boron	14.86 ± 1.86	15.07 ± 1.56	16.80 ± 2.02	14.34 ± 0.02	16.89 ± 0.34	18.60 ± 1.85	17.11 ± 0.02	17.91 ± 0.07
Copper	7.85 ± 0.92	8.97 ± 0.67	13.15 ± 4.11	9.15 ± 0.80	8.10 ± 1.31	8.12 ± 1.77	7.39 ± 0.89	6.88 ± 0.96
Nickel	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
Zinc	30.17 ± 8.87	25.62 ± 0.59	21.33 ± 0.51	22.55 ± 1.88	29.56 ± 2.13	27.16 ± 0.48	22.02 ± 2.64	21.87 ± 0.89
Magnesium	970.00 ± 20.00	945.00 ± 175.00	1075.00 ± 45.00	950.00 ± 70.00	1090.00 ± 10.00	1075.00 ± 105.00	955.00 ± 85.00	1165.00 ± 95.00
Sodium	198.52 ± 41.48	193.93 ± 16.07	138.52 ± 0.28	130.41 ± 15.71	99.61 ± 14.79	123.70 ± 26.53	91.00 ± 3.03	90.62 ± 17.81
Potassium	20950.00 ± 1050.00	22000.00 ± 1000.00	21500.00 ± 500.00	21500.00 ± 500.00	24500.00 ± 1500.00	26000.00 ± 0.00	25000.00 ± 0.00	26000.00 ± 1000.00
Phosphorus	2950.00 ± 50.00	3000.00 ± 300.00	3350.00 ± 50.00	3100.00 ± 100.00	3300.00 ± 100.00	3150.00 ± 550.00	3000.00 ± 200.00	3150.00 ± 150.00
Sulphur	1825.00 ± 15	1840.00 ± 100.00	1955.00 ± 45.00	1735.00 ± 15.00	1940.00 ± 360.00	1910.00 ± 390.00	1730.00 ± 250.00	1860.00 ± 340.00
Aluminium	9.08 ± 4.18	5.96 ± 0.79	3.71 ± 1.58	5.52 ± 0.59	4.52 ± 2.97	4.77 ± 0.24	1.72 ± 0.89	3.75 ± 2.78

Appendix A.10

Effect of storage time and postharvest Ca dips on nutrient status of fruit from cv. Aries. The nutrient concentrations were determined using ICP-OES analysis. Fruit were treated with 8% w/v $CaCl_2.2H_2O$ and analysed after 10 days of storage. Control fruit were treated with RO water and nutrient analysed at harvest. Dipping was conducted by placing fruit in calcium solution for 5 min. Fruit were peeled with a knife to about 1 mm deep to collect peel and flesh for nutrient analysis. Data are presented as means ± SE from *n = 3*

Nutrient (mg kg^{-1}DW)	**Control (flesh)**	**Dip** (8% w/v $CaCl_2.2H_2O$)	
		Flesh	**Peel**
Iron	49.83 ± 9.12	36.74 ± 0.86	53.52 ± 1.59
Manganese	17.21 ± 0.52	13.15 ± 0.1	23.73 ± 0.65
Boron	17.14 ± 1.49	15.24 ± 0.9	16.76 ± 0.87
Copper	4.45 ± 0.75	3.65 ± 0.4	4.07 ± 0.00
Molybdenum	1.35 ± 0.02	< 2.00	< 1.00
Zinc	27.72 ± 3.04	17.08 ± 0.58	13.69 ± 1.67
Magnesium	1660.00 ± 60.00	1570.00 ± 70.00	1580.00 ± 180.00
Sodium	169.60 ± 16.64	191.00 ± 29.00	141.77 ± 14.19
Potassium	27500.00 ± 1500.00	24500.00 ± 500.00	18350.00 ± 1650.00
Phosphorus	3400.00 ± 400.00	2500.00 ± 100.00	2500.00 ± 400.00
Sulphur	2300.00 ± 200.00	1850.00 ± 80.00	2085.00 ± 115.00
Aluminium	2.13 ± 0.09	4.07 ± 0.67	0.92 ± 0.13

Appendix A.11

Effect of postharvest Ca vacuum infiltration on nutrient concentrations in fruit tissues. The nutrient concentrations were determined using ICP-OES analysis. Fruit for nutrient analysis were collected at harvest. Fruit were treated at four concentrations: 0, 2, 5 or 8% w/v $CaCl_2.2H_2O$. Vacuum infiltration was conducted by placing fruit in the respective calcium solution, drawing a vacuum for 10 sec and then holding fruit in solution for 2 min before vacuum release. Data are presented as means ± SE from *n = 3*

Nutrient (mg kg^{-1} DW)	**cv. Aries**				**cv. Papri Queen**			
	0.0 % $CaCl_2.2H_2O$	**2.0 % $CaCl_2.2H_2O$**	**5.0 % $CaCl_2.2H_2O$**	**8.0 % $CaCl_2.2H_2O$**	**0.0 % $CaCl_2.2H_2O$**	**2.0 % $CaCl_2.2H_2O$**	**5.0 % $CaCl_2.2H_2O$**	**8.0 % $CaCl_2.2H_2O$**
Iron	43.76 ± 6.50	46.31 ± 5.41	54.31 ± 7.69	51.98 ± 7.36	43.78 ± 2.42	53.94 ± 10.48	43.93 ± 8.19	36.76 ± 2.87
Manganese	13.35 ± 3.44	17.53 ±0.01	13.00 ± 1.08	17.94 ± 1.18	14.34 ± 0.66	19.40 ± 1.91	13.19 ± 4.08	20.35 ± 0.89
Boron	13.77 ± 2.52	15.43 ± 2.80	15.29 ±1.01	16.63 ± 0.53	15.69 ± 2.54	14.94 ± 0.32	13.02 ± 0.24	19.03 ± 2.27
Copper	8.56 ± 1.43	10.58 ± 1.81	9.65 ± 2.80	11.77 ± 1.54	8.89 ± 1.48	9.27 ± 1.05	9.40 ± 1.74	6.55 ± 0.30
Nickel	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.50	< 1.00	< 1.00
Zinc	28.88 ± 4.20	34.49 ± 13.21	42.91 ± 13.21	27.32 ± 6.32	30.94 ± 6.31	35.42 ± 5.92	25.68 ± 1.91	23.93 ± 1.11
Magnesium	1010.00 ± 150.00	1090.00 ± 30.00	995.00 ± 30.00	1205.00 ± 35.00	1085.00 ± 75.00	1515.00 ± 145.00	1115.00 ± 85.00	1285.00 ± 75.00
Sodium	195.38 ± 0.21	172.71 ± 27.29	206.74 ± 27.29	143.96 ± 3.63	82.46 ± 1.75	95.32 ± 6.96	147.49 ± 47.12	71.96 ± 15.88
Potassium	22300.00 ± 2700.00	24500.00 ± 4500.00	24500.00 ± 4500.00	28000.00 ± 1000.00	24000.00 ± 0.00	26000.00 ± 0.00	26500.00 ± 1500.00	28000.00 ± 3000.00
Phosphorus	3150.00 ± 250.00	3400.00 ± 300.00	3150.00 ± 300.00	3650.00 ± 50.00	3350.00 ± 150.00	3650.00 ± 50.00	3500.00 ± 300.00	3000.00 ± 100.00
Sulphur	1860.00 ± 240.00	2055.00 ± 245.00	1915.00 ± 245.00	2300.00 ± 0.00	1800.00 ± 300.00	2040.00 ± 60.00	2255.00 ± 345.00	1645.00 ± 95.00
Aluminium	4.82 ± 1.54	6.16 ± 3.14	7.72 ± 3.14	4.59 ± 3.38	1.58 ± 0.14	7.35 ± 5.50	2.08 ± 1.65	0.97 ± 0.12

Appendix A.12

Effect of storage time and postharvest Ca vacuum infiltration on nutrient status of fruit from cv. Aries. Fruit were vacuum infiltrated with 8% w/v $CaCl_2.2H_2O$ and nutrient analysed after 10 days of storage while control fruit were vacuum infiltrated with RO water and nutrient analysed at harvest. Vacuum infiltration was conducted by placing fruit in Ca solution, drawing a vacuum for 10 sec and then holding fruit in solution for 2 min before vacuum release. Fruit were peeled with a knife to about 1 mm deep to collect peel and flesh for nutrient analysis. The nutrient concentrations were determined using ICP-OES analysis. Data are presented as means ± SE from *n = 3*

Nutrient (mg kg^{-1}DW)	**Control (flesh)**	**Vacuum infiltration**	
		Flesh	**Peel**
Iron	49.83 ± 9.12	39.09 ± 0.18	59.78 ± 5.13
Manganese	17.21 ± 0.52	15.26 ± 0.32	28.86 ± 0.26
Boron	17.14 ± 1.49	13.16 ± 0.28	17.23 ± 0.68
Copper	4.45 ± 0.75	2.76 ± 0.45	4.07 ± 0.08
Molybdenum	1.35 ± 0.02	< 3.00	< 1.00
Zinc	27.72 ± 3.04	15.23 ± 0.56	19.08 ± 0.34
Magnesium	1660.00 ± 60.00	1725.00 ± 105.00	1580.00 ± 40.00
Sodium	169.60 ± 16.64	250.00 ± 20.00	176.09 ± 33.92
Potassium	27500.00 ± 1500.00	21000.00 ± 1000.00	20150.00 ± 1850.00
Phosphorus	3400.00 ± 400.00	2200.00 ± 0.00	2650.00 ± 250.00
Sulphur	2300.00 ± 200.00	1820.00 ± 0.00	2250.00 ± 150.00
Selenium	< 5.00	< 10.00	< 4.00