Strawberry powdery mildew:

epidemiology and the effect of host nutrition on disease

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Abstract

Knowledge of disease epidemiology and the impact of plant nutrient status on development of disease is fundamental in establishing effective management strategies for crop pathogens such as *Podosphaera aphanis* Br. (Braun *et al.*, 2002), the causal agent of powdery mildew on strawberries. The following study investigated the conditions conducive for powdery mildew in strawberry crops in South Australia, the effect of foliar concentration of potassium and calcium on yield and pathogen development on the strawberry cultivars Aromas (resistant to powdery mildew) and Selva (susceptible), the potential for use of foliar-applied potassium silicate to control disease and identification of genes differentially expressed during disease.

Meteorological conditions associated with establishment of powdery mildew were observed over three consecutive seasons in commercial strawberry crops grown in Woodside, South Australia. Conducive conditions appear to be >28 ºC, <55 % relative humidity (RH) with no rain during the day (for conidiation), followed by a night with >10 ºC, >90 % RH and no rain (for germination). Colony development was then promoted by days of >15 ºC and <70 % RH, with nights of >8 ºC, >80 % RH and less than 2 mm rain in every 24 hour period. These are consistent with epidemiological studies of this pathogen (Peries, 1962a; Jhooty and McKeen, 1964; Mukerji, 1968; Perera and Wheeler, 1975; Byrne *et al*, 2000; Miller *et al*, 2003; Blanco *et al*, 2004; Davik and Honne, 2005; Amsalem *et al*, 2006). This knowledge may facilitate prediction of times considered high risk for establishment of powdery mildew in strawberry crops. Subsequently, this may allow optimisation of fungicide application and improved management of this disease and reduced yield loss and management expenses.

P. aphanis developed at an increased rate on leaves of Selva with low calcium content compared with development on normally fertilised leaves. Increased numbers of conidia germinated successfully on leaves of Aromas with low calcium content compared with development on normally fertilised Aromas leaves, however, the germinated conidia still failed to develop into sporulating colonies. Potassium nutrition had no obvious effect on *P. aphanis* development. Foliar concentration found to be adequate for growth of cultivars, Selva and Aromas were 6.0 mg/g and 4.5 mg/g Calcium, dry weight and 11.0 mg/g and 12.5 mg/g potassium, dry weight (respectively).

Potassium silicate, buffered to pH 7.0 and applied as a foliar fertiliser, reduced the severity of powdery mildew below the economic threshold, though not below the disease severity on plants treated with the fungicide, Systhane® (Bayer CropScience). As potassium silicate can be produced organically this compound may provide a useful management tool for both organic and conventional strawberry growers.

Although the cultivar Aromas was not immune to disease under conducive conditions and high inoculum load in the field, inoculation of healthy Aromas plants with *P. aphanis* in the laboratory failed to produce disease. Conidia were seldom found attached to the leaf surface of healthy Aromas leaves. Germination and subsequent colony development were also not observed in inoculated samples. This suggests there is some mechanism of Aromas that inhibits development of this fungus. Preliminary investigation of differential expression in Aromas inoculated with *P. aphanis,* identified sequences with homology to a putative antimicrobial protein and photosynthesis-related genes.

The results of these studies should enable growers to increase both crop yields and control of powdery mildew, one of the major economic diseases in South Australia. The epidemiological knowledge attained will be valuable, and may provide the basis for

future forecast modelling for *P. aphanis* in strawberry crops in South Australia. Recommendations for calcium and potassium leaf content will allow growers to monitor their fertiliser regime for increased yield of these cultivars. Aromas was identified as a powdery mildew resistant cultivar potentially suitable for production in South Australia, and the genes associated with this resistance response may be used in future studies of *Fragaria* species and breeding for powdery mildew resistant cultivars.

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Chapter 1 Introduction

1.1 Introduction

Strawberry production in South Australia contributes a small (6.6 % of Australian production in 2005) though valuable part of the Australian fresh strawberry market (17 % Australian market value) [Australian Bureau of Statistics (ABS), 2005; 2006]. One of the greatest challenges faced by South Australian growers is the control of powdery mildew, which develops easily in the hot, dry climate of the region. Powdery mildew is caused by *Podosphaera aphanis* Braun (formerly, *Sphaerotheca macularis* (Wallr. ex Fr.) Jacz.), of the order Erysiphales (Braun *et al.*, 2002). Limitations on commercial use of effective pesticides make this disease difficult to control while the most productive cultivars under South Australian conditions are very susceptible to powdery mildew. This study investigated the epidemiology of powdery mildew in South Australian strawberry crops and whether or not refined nutrition of the fruiting plants limits the effect of powdery mildew on yield.

1.2 Strawberries

Strawberries are perennial, dicotyledonous, non-deciduous, herbaceous plants. The cultivated strawberry, *Fragaria* x *ananassa* (L.) Duch. (family Rosaceae) that dominates commercial production world-wide (Hokanson and Maas, 2001), resulted from a chance cross between the natural octoploids, male sterile *Fragaria chiloensis* (L.) Duch. and *Fragaria virginiana* Duch. (Hancock, 1999).

Strawberry plants produce leaves and flowering stems from a woody crown in a spiral arrangement (Figure 1.1). Leaves are pinnate and trifoliate, oval with coarsely-toothed edge and can be densely covered in hairs (Allardice, 1991; Hancock, 1999). Roots arise from the crown where the crown tissue comes into contact with the soil.

Branching occurs at 2 to 5 cm from the base of the crown and root growth continues to form a fibrous mass (Hancock, 1999).

The fruiting habit of *F.* x *ananassa* is unique to this species. The inflorescence is a cyme terminated in a primary flower. Symmetric branches from each flowering stem result in two secondary flowers, four tertiary flowers and, depending on the cultivar and time of season, eight quaternary flowers. Flowers have ten sepals, five petals, 20 to 30 stamens and 60 to 600 pistils, depending on flower order (the primary flower having more stamens and pistils than the secondary and so on) (Figure 1.2). Each pistil contains a single ovule that develops into an achene (Hancock, 1999; Darnell *et al.*, 2003). Development of fruit from pollination to ripeness takes from 20 to 60 days, depending on the cultivar and weather conditions (Strand, 1994).

Strawberry plants also produce stolons, known as runners. These runners produce roots when they come into contact with soil and then can be detached from the parent plant (Strand, 1994). Runners are produced in virus-free regions and shipped, bare-rooted, to strawberry farms for planting. In crop production, removal of runners is expensive and labour intensive, so cultivars that produce fewer runners are favoured.

Strawberry cultivars are classified as short-day, long-day and day-neutral according to their photoperiodic flowering response. Short-day cultivars (also known as June-bearing in the Northern hemisphere) are facultative, initiating buds when growing under short days (<14 hour days) or when temperatures are under 15 °C. Generally, a high level of chilling in these cultivars increases vigour, stimulates runner production, and concentrates ripening (Hancock, 1999). Long-day (or ever-bearing) cultivars are often considered to have a weak photoperiodic response and initiate flowers under short nights. Day-neutral cultivars are insensitive to day length with respect to flower

Figure 1.1 Diagram of a strawberry plant and daughter plant. The leaf blade and petiole, peduncle and the arrangement of flowers and fruit on the flowering bract are shown (adapted from Strand, 1994). Image reproduced with permission from the Statewide Integrated Pest Management Program. Copyright: The Regents of the University of California. BS 02/07.

Figure 1.2 Diagrams of a flower (**A**) and fruit (**B**) of strawberry. The arrangement of the reproductive tissue of the strawberry (achenes) embedded in the maternal receptacle tissue is shown (Strand, 1994). Fused sepals are called a calyx. Image reproduced with permission from the Statewide Integrated Pest Management Program. Copyright: The Regents of the University of California. BS 02/07.

induction (Darnell *et al.*, 2003) and rely on cooler temperatures to induce dormancy (Maas, 1984). The day-neutral character has been incorporated into many commercial strawberry cultivars, resulting in plants that fruit continuously throughout spring and summer in most regions.

1.2.1 Strawberry production

World wide, strawberry cultivation covers over 250,000 hectares [Food and Agricultural Organisation of the United Nations (FAO), 2006]. The most significant strawberry producers are the United States of America (USA) (1,004,000 tonnes in 2004), Spain (288,000 t), the Russian Federation (215,000 t), Korea (202,000 t), Japan (198,000 t), Poland (186,000 t), Italy (168,000 t), Turkey (155,000 t) and Mexico (150,000 t) (FAO, 2006).

The Australian strawberry market, worth over A$135m in 2005 (ABS, 2005), has been growing in recent years, with 24,000 t of strawberries produced in 2005 compared with 15,000 t in 2000 (ABS, 2006; FAO, 2006). Strawberries are grown in most regions of Australia (except the Northern Territory), with the majority of production occurring in Queensland (10,146 t in 2005), followed by Victoria (8,069 t), Western Australia (3,461 t) and South Australia (1,557 t) (ABS, 2006). The varying climates across Australia allow different regions to produce crops at different times of the year. The majority of Australian strawberries are consumed locally, and customers enjoy an almost all year round supply. Exports account for around 2,000 t annually (FAO, 2006).

Harvest in South Australia starts in October and continues until the middle of May. In 2005, 63 hectares of strawberries were sown in South Australia, producing 1,557 tonnes of strawberries, making up 6.6 % of the total Australian production (ABS, 2006). South Australian production accounts for the entire Australian fresh strawberry

market between the months of February and May increasing the unit value of the South Australian market (A$10.27 kg in 2004, compared with A$5.35 to $7.12 kg for other states) (ABS, 2005).

Australian strawberry production is primarily in fertigated hill production systems. Fertigation is the application of soluble fertilisers through drip-irrigation lines (Tagliavini *et al.*, 2005). Runners are obtained from virus-free propagators in the alpine regions of Victoria and Tasmania, and from Queensland (P. James, personal communication, 2003) (Lines *et al.*, 2004). Strawberries are harvested for one or two seasons before the plants are dug up and the field sown with pasture or organic mulch, such as corn, for a minimum of one season (P. Mason, personal communication, 2003; D. Parker, personal communication, 2005).

The South Australian strawberry industry is sufficiently valuable to warrant research and development into problems typically encountered in this region. The most pressing issues facing South Australian strawberry growers are the phase-out of methyl bromide and powdery mildew control. As the phase-out of methyl bromide is a worldwide venture towards ozone restoration, alternatives have been and continue to be the focus of many studies in strawberry (Porter *et al.*, 2004). The sustainability of the industry relies on the identification and adoption of an alternative that gives comparable disease control and equivalent yields (Mattner *et al.*, 2004; Medina *et al.*, 2004; Porter *et al.*, 2004). Registration of fungicides effective for reducing powdery mildew severity for use in South Australia would have the most immediate impact on control of this disease (P. Mason, personal communication, 2003). However, this is a relatively short term solution as *P. aphanis* has previously evolved fungicide resistance relatively quickly (Howard and Albregts, 1982) and more sustainable methods of control, such as cultivars with improved resistance, would be eagerly adopted by growers. The extensive

use of fungicides on strawberry crops is a growing concern for consumers and organic produce is gaining popularity, suggesting that more "ecologically friendly" methods of production and disease control also warrant investigation.

1.3 Powdery mildew of strawberries

P. aphanis is an obligately biotrophic fungus (Braun *et al.*, 2002) that occurs in all parts of the world where strawberries are grown (Maas, 1984). The asexual stage of *P. aphanis* was first reported on strawberry crops in the United Kingdom (UK) by Berkeley in 1854 (Corke and Jordan, 1978). Cleistothecia have also been reported although they are not commonly formed (Peries, 1962a; Corke and Jordan, 1978; Howard and Albregts, 1982). The causal agent of powdery mildew on strawberry was initially thought to be the hop pathogen *Podosphaera macularis* Braun (formerly *Sphaerotheca humuli* (DC) Burr) (Mukerji, 1968), but has since been shown to be physiologically restricted to the strawberry (Horn *et al.*, 1972; Corke and Jordan, 1978). However, there have been recent reports that *P. aphanis* can also infect Eucalypts (Cunnington *et al.*, 2003).

Powdery mildew symptoms comprise small patches of white mycelium on the underside of the leaf that coalesce to cover the entire lower surface and, in cases of severe infection, spread to the upper leaf surface, though this is uncommon in the field. Dark blotches appear in the lower leaf tissue beneath the mycelium. Severe foliar infection can result in defoliation or reduced photosynthesis in damaged leaves, partially due to the density of the mycelia. In some cases, leaf edges roll upwards exposing the white mycelium. Mycelial growth may extend to leaf petioles flowering stems, flowers and fruits (Corke and Jordan, 1978; Maas, 1984; Strand, 1994).

While photosynthetic stress due to powdery mildew on the leaves may cause a loss of potential yield, the majority of losses attributable to powdery mildew are the result of infection of flowers and fruit. Infected flowers may become enveloped in mycelium, deformed or may die. Pollen production or retention is reduced and results in poor fruit set (Gooding *et al.*, 1981; Maas, 1984; Strand, 1994). Infected immature fruits become hard and fail to ripen normally. Fruit on which there has been disease during the ripening process may develop raised achenes and have a dull unattractive appearance, or become misshapen and split, increasing vulnerability to infection by secondary pathogens. The fungus may sporulate on the fruit surface covering it in white conidiophores, rendering them unmarketable (Gooding *et al.*, 1981; Maas, 1984). Fruit that have powdery mildew when harvested tend to dehydrate and lose weight rapidly, reducing quality and shelf life (Corke and Jordan, 1978; Maas, 1984).

1.3.1 The life cycle of *Podosphaera aphanis*

P. aphanis usually occurs in the conidial state on leaves and stems and, when infection is severe, spreads to sepals and fruits (Mukerji, 1968) (Figure 1.3). Epidemics are attributed to conidia carried by wind to infect new growth (Wilhelm, 1961; Strand, 1994; Blanco *et al.*, 2004). Overwintering occurs as mycelium on infected leaves (Blanco *et al.*, 2004; Amsalem *et al.*, 2006) and contaminated transplants are thought to be the major source of primary inoculum in fruit production areas (Strand, 1994).

Conidia are smooth-walled and barrel-shaped, 25 to 38 μm long and 15 to 23 μm wide, with whorl-patterned ends (Mukerji, 1968; Braun *et al.*, 2002). Within 1 to 3 minutes (min) of contact with suitable host tissue, conidia of the Erysiphales excrete a proteinaceous material which degrades cutin, aiding adhesion to the leaf surface and allowing uptake of exogenous materials that stimulate germination (Kunoh *et al.*, 1990;

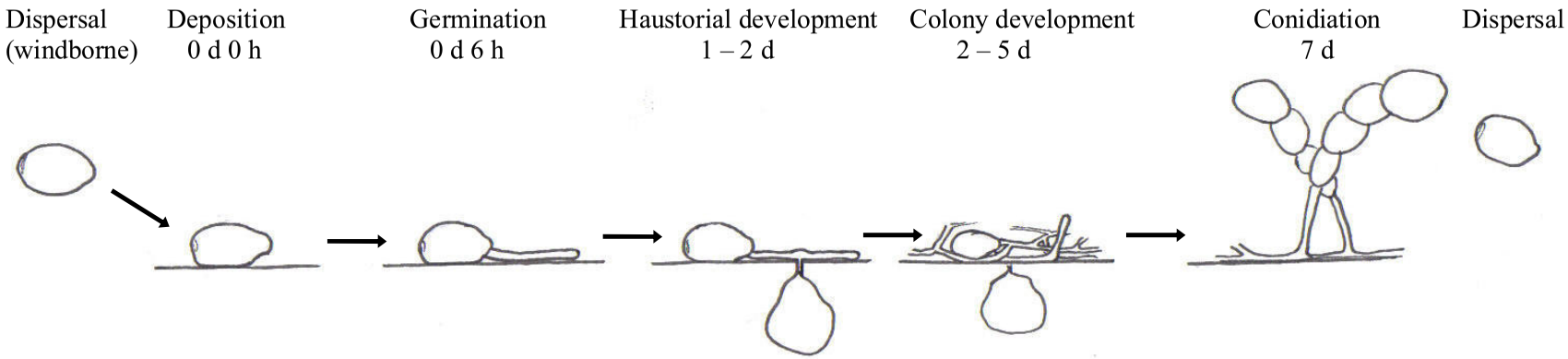


Figure 1.3 The asexual lifecycle of *Podosphaera aphanis*.

Pascholati *et al.*, 1992; Nicholson *et al.*, 1993; Mims *et al.*, 1995; Celio and Hausbeck, 1998; Pryce-Jones *et al.*, 1999; Nielsen *et al.*, 2000; Green *et al.*, 2002; Zhang *et al.*, 2004). Germination of *P. aphanis* conidia is stimulated by temperatures between 15 and 25 °C (Peries, 1962a; Amsalem *et al.*, 2006) when relative humidity (RH) is above 75 % (Jhooty and McKeen, 1964; Mukerji, 1968), but conidia have also been observed to germinate under a wide range of temperature and humidity conditions (Miller *et al.*, 2003; Amsalem *et al.*, 2006). However, germination and colony development are adversely affected by free water (Peries, 1962a; Perera and Wheeler, 1975; Sivapalan, 1993a).

Given optimum conditions, conidia on strawberry leaves germinate 4 to 6 hours after inoculation and form indistinct appressoria (Braun *et al.*, 2002) within 12 hours (Jhooty and McKeen, 1964). Germ tubes of *P. aphanis* do not usually terminate in appressoria, as is common in other species of the Erysiphales (notably *E. graminis*). Instead, the primary hyphae grows in line with the germtube, past the appressoria (Jhooty and McKeen, 1964). Primary haustoria are usually developed by 36 hours (Peries, 1962b; Jhooty and McKeen, 1964), then secondary and tertiary haustoria are produced at sites of hyphal branching (Green *et al.*, 2002). Conidiophores and conidia are produced after tertiary haustorial generation, developing between 3 to 5 days post inoculation (dpi) (Peries, 1962b; Jhooty and McKeen, 1964). Conidiation is visible to the naked eye from 6 dpi (Peries, 1962b).

Hyphae are hyaline, highly branched, and grow straight in any direction from the infection site (Mukerji, 1968). Mycelium is relatively persistent and can turn brown with age (Braun *et al.*, 2002). The optimum temperature for mycelial growth is between 18 and 22.5 °C. RH has no effect on the rate of fungal development provided it is between 12 and 100 % at any given temperature (Peries, 1962a; Schnathorst, 1965;

Miller *et al.*, 2003). The development of strawberry powdery mildew is promoted by the alternation between dry days and cool, humid nights (Davik and Honne, 2005). In favourable conditions, chains of dry, hyaline conidia are produced (Maas, 1984).

Conidial maturation is a photosensitive process. Conidia of *Oidium* sp., which cause powdery mildew on ponsettia, that are produced at night remain immature until exposed to light the following day (Byrne *et al.*, 2000). Conidial maturation is not only dependent on light, but is also affected by availability of metabolites during sunlight hours (Schnathorst, 1965). Conidia are most effectively dispersed by wind (Wilhelm, 1961; Schnathorst, 1965; Blanco *et al.*, 2004). The effect of RH, temperature and wind speed on the release and dispersal of conidia has been the focus of several studies of powdery mildew (Adams *et al.*, 1986; Byrne *et al.*, 2000; Blanco *et al.*, 2004). Adams *et al*. (1986) found high RH inhibited conidial release in *S. pannosa, E. pisi* and *E. graminis*. Mature conidia of *S. pannosa* were released in response to an abrupt decrease in RH, regardless of light or wind conditions, and increasing temperature stimulated conidial release further when RH was low. Adams *et al.* (1986) suggested that the effect of change in RH and temperature on the electrical charge on the leaf surface was sufficient to actively discharge conidia. The conidial release response to abrupt RH change was observed to continue for up to 40 min. Byrne *et al.* (2000) observed that sustained high temperatures (>25 °C) decreased release of conidia in poinsettia crops. Peries (1962a) was able to measure dispersal of *P. aphanis* conidia only up to 3.05 metres (m), with 90 % of conidia falling within 1.6 m of the infected plant in still conditions. Dispersal radius increases with wind speed (Jarvis *et al.*, 2002), and more conidia are disturbed during harvesting in strawberry crops (Blanco *et al.*, 2004). Spore dispersal is inhibited by rain, such that the rain reduces the number of conidia in the air (Peries, 1962a; Strand, 1994; Blanco *et al.*, 2004). Knowledge of the conditions

conducive to conidium dispersal, germination and colony development when associated with local weather conditions may provide the basis for epidemiological forecasting for powdery mildew in strawberry.

Cleistothecia are nearly spherical, 60 to 125 μm in diameter, dark brown and smooth with many hyphal appendages. They are scattered or clumped on the leaf surface and each cleistothecium contains an ascus with eight ascospores. Ascospores are hyaline, oval, 18 to 25 μm long and 12 to 18 μm wide (Mukerji, 1968). Cleistothecia are produced in low light intensity or short days, high humidity and relatively low temperatures during winter. However, some researchers have concluded that they do not play an essential part in the life history of *P. aphanis* (Peries, 1962a; Schnathorst, 1965; Maas, 1984). After cleistothecia were observed in crops for the first time in Florida (1981), sulfur and benomyl failed to control powdery mildew severity in subsequent seasons (Howard and Albregts, 1982). This implies *P. aphanis* has the ability to quickly become resistant to some types of fungicide when multiple mating types are present and selection pressure for fungicide resistance results from extensive use of a single mode of action fungicide. However, in experiments undertaken in the UK during the early 1960s, cleistothecia that were overwintered outdoors contained deformed asci with disintegrating ascospores, or no asci at all. Peries (1962a) was not able to infect plants with ascospores from overwintered cleistothecia, and observed no natural dehiscence.

1.3.2 Host response to powdery mildew

The pathogen and host interaction during powdery mildew disease has been studied extensively in cereals (Kunoh, 2002). The initial defence response of cereals is to form a halo - a circular area of autofluorescence in the host wall under the primary growth tube of the fungus. Haloes contain silicon (Carver *et al.*, 1987), proteins such as hydrolytic enzymes (Takahashi *et al.*, 1985) and phenolics (Kunoh *et al.*, 1985; Aist *et*

al., 1988). Haloes are well developed before the penetration peg is initiated. This is swiftly followed by cytoplasmic aggregation and potential accumulation of fungitoxic compounds (Aist and Bushnell, 1991).

This cytoplasmic aggregation contributes to the formation of a papilla after the emergence of the penetration peg. Papillae containing high concentrations of phenolic compounds are frequently implicated in resistance to penetration (Aist *et al.*, 1988). If the fungus avoids papilla-mediated resistance, the penetration peg enters the cell and produces a haustorium. This allows the fungus to compete with the plant for available metabolites, especially sugars (Gay and Manners, 1987). High levels of infection divert significant amounts of the host's resources into fungal biomass through haustoria, to an extent which can disrupt the host's capacity for growth (Clarke and Akhkha, 2002).

Koh *et al.* (2005) described the subcellular responses of a susceptible host cell associated with invasion of *Arabidopsis* by *Erysiphe cichoracearum.* These include vesicular-like structures forming near the penetration peg, and movement of peroxisomes, mitochondria and Golgi bodies to the site of penetration. They also observed that nuclei of infected cells were twice as large as normal.

Understanding the responses of both the resistant and susceptible host to invasion by *P. aphanis* provides potential to exploit any differences for novel means of powdery mildew control.

1.3.3 Powdery mildew control

To manage disease successfully one needs to know how to recognise disease symptoms, where pathogens originate, how they spread, how they infect their host and what environmental conditions favour their development (Strand, 1994). Control of disease centres around three basic concepts: genetic resistance, pathogen-free planting material and control of pathogen or vectors (Maas, 1984). The dominant cultivars under

production in South Australia are susceptible to powdery mildew (as per Section 1.2.2) and while genetic improvements may provide resistance, other forms of control are currently being used. Containerised strawberry plants, called plugs, and micropropagation of runners from meristematic tissue could provide cleaner planting material but is expensive and is therefore unlikely to be adopted for large scale plantings in the foreseeable future (Menzel and Waite, 2004; Moisander *et al.*, 2004). Field production of pathogen-free bare-rooted strawberry runners is complicated by vegetative production and the ability of *P. aphanis* mycelium to overwinter in the crowns (Jarvis *et al.*, 2002). In addition, the cropping period in South Australia extends past the time of planting, allowing for the infection of new runners from the time of planting. While cultural sanitisation can minimise the inoculum between seasons, the use of chemicals is the main avenue for control of powdery mildew during the fruiting season in strawberry crops. Many biological control agents have been investigated for control of powdery mildews in horticultural crops, though few have provided effective control in field conditions (Bélanger and Labbé, 2002).

1.3.3.1 Control using host resistance

Tolerance is the ability of the plant to grow and produce a yield in spite of the disease burden. In contrast, resistance describes the relative ability of the host to actively prevent the growth of the pathogen (Agrios, 1997). There are many preformed factors including constitutively expressed waxes, cell wall components, antimicrobial peptides, proteins and non-proteinaceous secondary metabolites that deter invasion of plants by fungal pathogens (Osbourn, 1996; Ringli *et al.*, 2001; Veronese *et al.*, 2003). Observations of *P. aphanis* on *F. ovalis* and *F. chiloensis* showed that cuticle thickness may play a role in penetration success of *P. aphanis* (Jhooty and McKeen, 1964). *F. ovalis* has an upper cuticle thickness between 0.32 to 0.98 μm. Powdery mildew

symptoms occurred with equal severity on upper and lower leaf surfaces in this species, whereas, in *F. chiloensis,* which has an upper cuticle thickness of 2.88 to 5.12 μm powdery mildew was rarely observed to develop on the upper surface. Similarly, greater volumes of cutin acids can be extracted from resistant cultivars of *F.* x *ananassa* suggesting that the cuticle layer of resistant leaves may provide a physical barrier to fungal penetration (Peries, 1962a; Jhooty and McKeen, 1964).

No race-specific, non-host or papillae-mediated resistance responses of *F.* x *ananassa* to *P. aphanis* have been identified. However, there are many described in other powdery mildew pathosystems (Braun *et al.*, 2002; Collinge *et al.*, 2002). Papilla-mediated resistance is non-race specific, with a phenolic antifungal compound that forms in papillae preventing fungal penetration (Lyngkjær *et al.*, 1997; Röpenack *et al.*, 1998; Collins *et al.*, 2002). Failed development of haustoria and subsequent cell collapse has also been observed in resistant cultivars of muskmelon (Cohen *et al.*, 1990). Race-specific resistance has been described in cereals (Collinge *et al.*, 2002; Collins *et al.*, 2002; Hsam and Zeller, 2002), cucurbits (Jahn *et al.*, 2002), hops (Seigner *et al.*, 2004) and *Arabidopsis* (Vogel and Somerville, 2002; Vogel *et al.*, 2002). Races of *P. macularis* are also known to exist (Seigner *et al.*, 2004). As yet, there have been no races or gene-for-gene resistance responses described for *P. aphanis*, although Davik and Honne (2005) suggest selection for virulence in the pathogen caused the breakdown of resistance in the Scandinavian cultivar Korona.

1.3.3.2 Biological and "soft" chemical control

The use of biological control agents and prophylactic treatments against powdery mildew fungi are reviewed by Bélanger and Labbé (2002) and Kiss (2003). The use of mycoparasites, such as *Ampelomyces quisqualis* Ces., *Lecanicillium lecanii* (Zimm.), and *Sporothrix flucculosa* Traquair and the mite *Orthotydeus lambi*, has been

the focus of many studies of glasshouse-grown crops such as cucumber (Dik *et al.*, 1998; Bélanger and Labbé, 2002). However, results are often inconsistent and contrary (Verhaar *et al.*, 1996; Dik *et al.*, 1998). For control of *P. aphanis* on strawberry, *L. lecanii* appeared most promising, with a good level of control attained in glasshouse trials where the humidity was high and relatively constant. However, field trials resulted in inconsistent powdery mildew control (Miller *et al.*, 2004). Bees have been used to effectively disseminate *Trichoderma harzianum* to control *B. cinerea* in field-grown strawberry (Kovach *et al.*, 2000) and may prove effective with mycoparasites of *P. aphanis* also.

Novel and more environmentally benign treatments that reduce the severity of powdery mildew on strawberry include a mixture of methionine and riboflavin (Wang and Tzeng, 1998), plant-derived chitinase (Karasuda *et al.*, 2003) and foliar applications of silicon (Wang, 2003). Karasuda *et al.* (2003) significantly reduced powdery mildew severity on leaves and strawberries with applications of chitinase E prepared from yam. Chitinases are pathogenesis related (PR) proteins that can induce systemic acquired resistance (SAR) in plants and also degrade fungal cell walls (Bélanger and Labbé, 2002). The use of foliar-applied potassium silicate for powdery mildew control has been investigated in a number of important horticultural crops including grape (Bowen *et al.*, 1992), cucumber (Menzies *et al.*, 1991; Dik *et al.*, 1998), melon (Menzies *et al.*, 1992) and strawberry (Wang, 2003). The mechanism by which these applications reduce powdery mildew are uncertain, though silicon absorbed into plant cells is thought to induce resistance (Bélanger *et al.*, 1995) and silicate deposits on the leaf surface have been shown to act as a physical barrier to appressorial penetration (Bowen *et al.*, 1992). Cohen *et al.* (1996) showed that a detergent (Zohar LQ-215) reduced disease severity in cucumber by inhibiting mycelial growth and sporulation.

1.3.3.3 Chemical and cultural control

Chemical control of powdery mildew is required throughout the vegetative and reproductive stages in the strawberry growth cycle. Sulfur-based fungicides are frequently used as protectants, and provide moderate disease suppression (Corke and Jordan, 1978; Maas, 1984; Strand, 1994). Foliar powdery mildew is controlled by applying systemic fungicides when disease symptoms first appear. Benomyl and other systemic fungicides have been used successfully to control powdery mildew in a glasshouse environment, however, resistant strains of the fungus usually develop (Maas, 1984; Hollomon and Wheeler, 2002). In recent trials in Queensland, Australia, Euparen Multi® (Bayer CropScience, active ingredient (AI): tolylfluanid), Flint® (Bayer CropScience, AI: trifloxystrobin) and Systhane® (Bayer CropScience, AI: myclobutanil) were effective in protecting strawberry plants and fruit against powdery mildew when applied at 7-day intervals from early in the season (Hutton and Gomez, 2005). Systhane® has been registered for use on strawberry crops in South Australia since 1999 (APVMA, 2006). Flint® was registered for use on strawberry crops in South Australia in 2005 (D. Parker, personal communication, 2005). Euparen Multi® is applied regularly for control of *Botrytis cinerea* (P. James, personal communication, 2003). Controlling foliar infection helps prevent fruit infection (Strand, 1994), however, controlling foliar infection in the field has not significantly increased yields even in highly susceptible cultivars (Maas, 1984).

Controlling powdery mildew on plants after harvest reduces the amount of disease that develops the following season (Strand, 1994). Defoliating over-wintering plants mechanically or using a herbicide is common practice to minimise primary inoculum (Corke and Jordan, 1978).

1.3.4 Assessment of disease severity

In trials pertaining to disease control it is important to measure disease severity to determine the extent to which the test treatments have controlled disease. Most field-based studies of powdery mildews have involved scales of symptom severity to analyse cultivar susceptibility, and efficacy of chemical, biological and prophylactic treatments (Nicot *et al.*, 2002). Disease severity scales for assessment of powdery mildew on strawberries, based on symptoms on foliage, are described by Darrow *et al.* (1954), Daubeny (1959), Peries (1962b), McNicol and Gooding (1979), Gooding *et al.* (1981), Davik and Honne (2005) and Brahm *et al.* (2005), however there are no studies that consider the loss of yield from infection on fruit.

1.4 Host nutrition

There have been many nutritional studies of strawberry both in conventional (Kirsch, 1958; Eaves and Leefe, 1962; Johanson and Walker, 1963; Bould, 1964; Boyce and Matlock, 1966; Voth *et al.*, 1967; Saxena and Locascio, 1968; John *et al.*, 1975; Haynes and Goh, 1987; May and Pritts, 1990; Chow *et al.*, 1992) and organic production (Daugaard, 2001; Wang and Lin, 2002; Berglund *et al.*, 2006). Strawberries are most productive when the availability of certain nutrients is limited, most notably nitrogen, as large amounts promote excessive foliage production and poor-flavoured fruit (Corke and Jordan, 1978). There may also be more than one optimal combination of nutrients for maximum yield in strawberries, depending on the desired end product, as individual yield components respond differently to nutrients applied. Genetic differentiation in uptake and utilization of nutrients appears extensive (May *et al.*, 1993)

When one or more of these essential nutrients is limited, growth is impaired and plants are at increased risk of environmental and pathogenic stress. Typically, plants suffering nutrient stress exhibit particular patterns of chlorosis and/or necrosis of the

leaf tissue. These nutrient deficiency symptoms are well documented for strawberry crops (Johanson and Walker, 1963; Ulrich *et al.*, 1980; Weir and Cresswell, 1993). However, precise fertilisation recommendations considering cultivar, soil type and climate in combination are uncommon (May and Pritts 1990).

Specific nutrient management practices are required for each cultivar that consider the climatic and edaphic conditions where the strawberries are grown to ensure large yields of quality fruit.

1.4.1 South Australian soils and climate

The soils of South Australia are predominantly calcareous with sandy loam texture and 10 to 20 % clay content [Australian Soil Resource Information System (ASRIS), 2006]. These soils have naturally low organic matter and poor structure, frequently with clay near the surface which restricts drainage and impedes root growth [Australian Natural Resource Atlas (ANRA), 2006]. The strawberry growing regions of South Australia are predominantly in the Adelaide Hills and Riverland regions. The soils in the Adelaide Hills are red-brown chromosols in the valleys and on slopes, where strawberry production occurs, developed from tillites, shales and impure limestones (Northcote, 1983; Kew *et al.*, 2004; ASRIS, 2006). Chromosols generally have adequate water retention, drainage, aeration, are low in phosphorus and nitrogen, and can be hardsetting where organic matter is low (ANRA, 2006). The soils in the Riverland are calcarosols (ASRIS, 2006). Calcarosols generally have low water retention, poor nutrient fertility, and are prone to wind erosion, salinity, alkalinity and sodicity (ANRA, 2006).

The strawberry growing regions of South Australia are temperate, with warm summers with low rainfall, and moderately warm, wet winters. Rainfall in the Adelaide Hills region averages 600 mm and the Riverland 500 mm annually (BOM, 2006).

1.4.2 Methods used for nutrition experiments

Not knowing which nutrients limit plant growth is a major difficulty often encountered in the field. Ideally, one would start with a situation where all nutrients are unlimited, except for those nutrients of interest, which are absent. This can be realised in sand and solution cultures where fertilisation can be manipulated (May *et al.*, 1993).

The use of sand culture has been successful in many nutrient investigations of strawberries (Bould, 1964; Johanson, 1980). The method used by Bould (1964) required application of nutrient solution one to three times per week, depending on growth rate, to pots of sand with good drainage. However hydroponic plant culture methods have been used extensively in strawberry nutrition studies (Chow *et al.*, 1992; Sarooshi and Cresswell, 1994; Cengiz *et al.*, 2003), particularly in studies of silicon (Miyake and Takahashi, 1986; Kanto *et al.*, 2004; Treder and Cieslinski, 2005). Chow *et al.* (1992) described nutrient requirements in a deep-flow hydroponic system. Calcium, magnesium, manganese and nitrate were under relatively high demand during flower and fruit formation, suggesting these may need to be monitored and adjusted, while levels of potassium, iron and copper needed to be maintained in order to maximise yield. Miyake and Takahashi (1986) applied potassium silicate in the nutrient solution of a hydroponic system to observe how silicate affected the growth and yield of strawberry plants. Kanto *et al.* (2004) used a similar system to determine the effect of silicate on powdery mildew development in strawberry plants (see Section 1.4.4.5)

Many field-based nutrient experiments with strawberry have involved treatments applied as basal fertilisers prior to planting. These have little meaning to fertigated production, which is commonly used in South Australia. Fertigated production allows different ratios of nutrients to be applied during the growing season to optimise

production. More recently, experiments have incorporated fertigation, to resemble industry practice more closely (Miner *et al.*, 1997).

Foliar application of nutrients has also been investigated in a number of studies (Eaves and Leefe, 1962; Wang and Galletta, 1998; Wang, 2003). These will be discussed in more detail in Section 1.4.4.

1.4.3 Tissue sampling methods

Inductively coupled plasma atomic emission spectrophotometer (ICPAES) analysis of foliar tissue samples is the most common method used for determining the nutrient status of a plant and provides a basis from which to compare results of different studies. Tissue nutrient levels in plants tend to be dynamic, and analysis of this tissue demonstrates which nutrients the plant absorbs at any given time. This has advantages over soil analysis as the latter may show elements that are not in forms readily mobilised by the plant. Calcium is an example of a nutrient that is often abundant in the soil, but not necessarily available to plants, as most calcium compounds other than $CaNO_3$ are very insoluble (Reuter and Robinson, 1997). Nutrient concentrations in strawberry tissues fluctuate widely depending on the time of sampling, plant part sampled, age of plant and cultivar. These factors must be considered when interpreting ICP analysis. Generally, the youngest open leaf (YOL), without petiole, sampled during the 6 weeks following harvest provides the best single plant tissue for determining both macro- and micro-nutrient status of strawberry plants (May and Pritts, 1990).

The large number of seeds on a strawberry fruit may cause it to be an especially strong sink, contributing to the inconsistency and general decline in foliar nutrient levels during harvest (John *et al.*, 1975). Although the fruiting period is generally associated with fluctuating nutrient levels and thus is avoided for leaf sampling and analysis, it is

likely that this is when nutrient limitations will have their most pronounced effects. Calcium, magnesium, manganese, boron and molybdenum concentrations in leaves have been observed to drop dramatically during harvest, while concentrations of potassium, iron, copper and zinc change little (May, 1994).

Without detailed response data for nutrient changes throughout the growth cycle of strawberry plants, it may not be possible to develop precise fertiliser recommendations from plant analyses. Currently, in strawberry, critical foliar levels have been set by experience and educated guesses, and not by a systematic approach. A systematic approach is therefore needed to advance our understanding of nutrition in strawberry, and to take advantage of nutrient application technology (May, 1994).

1.4.4 Nutrients related to disease resistance

In recent years, many studies have been directed towards the benefit of fertilisation on disease incidence. Some elements appear to affect disease severity through greater tolerance, for example phosphorus and sulfur, while others, including nitrogen and potassium, can alter specific plant resistance mechanisms (Graham, 1983). Many mineral nutrients are essential for normal plant development, and their absence can cause major interruptions of physiological processes, including those involved in plant defence. Furthermore, some nutrients in ample supply, including calcium, can benefit plants by enhancing growth habits that inhibit pathogen penetration (Marschner, 1995).

Aspects of response to host nutrition that need to be determined include whether fertilisation induces resistance mechanisms, causes change in plant anatomical structure or directly inhibits pathogen development (Ellingboe, 2001). The following nutrients

will be discussed in more detail with respect to strawberry production as they are of particular interest to this study: nitrogen, potassium, calcium and silicon.

1.4.4.1 Nitrogen

While nitrogen is essential for strawberry plants during early growth stages, bud differentiation and flowering, excessive applications can lead to poor quality fruit, late ripening and increased foliage (Voth *et al.*, 1967). Nitrogen accumulates mostly in the leaves and shoots of strawberry plants, though not in the fruit (Albregts and Howard, 1978; Chow *et al.*, 1992). Kirschbaum *et al.* (2004) found that fertigation with nitrogen in excess of 155 kg/ha in a season was unnecessary to maintain sufficient nitrogen concentrations of 2 to 2.8 mg/kg leaf tissue (May and Pritts, 1990). Nitrogen deficiency symptoms range from mild chlorosis to bright reddening of the leaf and calyx tissues. New leaves and fruit may be smaller, and root mass is reduced (Ulrich *et al.*, 1980).

High nitrogen supply may predispose plants to both disease and micronutrient deficiencies, which may themselves inhibit plant defence (Graham, 1983). High levels of nitrogen produce softer fruit with reduced flavour and that are more easily damaged increasing susceptibility to pathogen attack (Corke and Jordan, 1978; Miner *et al.*, 1997). Increased nitrogen may also affect leaf firmness and epidermal thickness by adversely affecting the concentration of foliar silicon (Marschner, 1995). The production of excess foliage, another symptom of excess nitrogen, is also thought to contribute to increasing disease pressure by reducing the air circulation in the canopy providing an environment which is suited to pathogen establishment.

1.4.4.2 Potassium

After nitrogen, potassium is the mineral element required in the largest volumes by plants. As the most abundant cation in the cytoplasm and with high mobility, K^+ ions

play a significant role in osmoregulation of all turgor-mediated processes including cell extension, stomatal function, charge balance, enzyme activation, protein synthesis, photosynthesis and long distance transport of photosynthate (Marschner, 1995; Mengal, 2007). Potassium deficient plants exhibit many changes in metabolism, including decreased starch content, increased oxidase activity and reduced cell size (Marschner, 1995).

Yield responses to potassium fertilisation have been varied and show high cultivar specificity (Bould, 1964; May, 1994; Haynes, 1987). Fertiliser conditions under which progeny are evaluated are thought to contribute to efficacy of resulting cultivars for nutrient uptake from the soil (Albregts *et al.*, 1991). For instance the Florida breeding program evaluates progeny under high potassium fertilisation whereas progeny of the Californian breeding program are evaluated in unaltered field conditions. In addition, field trials where base potassium in the soil has been adequate have shown no benefit from additional potassium fertilisation. In contrast, sand based pot experimental systems have allowed for elimination of potassium from the base nutrient supply. Characterisation of response to potassium can be measured in this manner.

Potassium levels are significantly affected by the stage of development at the time of sampling. Potassium normally accumulates in the petiole tissues (Chow *et al.*, 1992), but concentrations in leaf and petiole tissues decline during the period of flowering and fruiting (John *et al*., 1975). This high requirement during fruit development (Martinsson *et al*., 2004) is often coupled with potassium deficiency at the onset of fruit production (Strand, 1994). Strawberry fruit contain more potassium than any other mineral element (May and Pritts, 1990). This is attributed to the nutrients being supplied to the receptacle almost solely by the phloem and that the extent of cell expansion in this tissue makes it an especially strong sink for phloem mobile nutrients (Albregts and

Howard, 1978). Potassium fertilisation is generally thought to increase fruit firmness and size, though this appears to vary between cultivars (Saxena and Locascio, 1968; Albregts *et al.*, 1991). Albregts *et al.* (1991) observed increased fruit weight with increasing potassium in cultivar Chandler, and decreased fruit weight in cultivars Dover and Tufts. Potassium application to cultivar Red Gauntlet resulted in no measurable effects on amino nitrogen content, titratable acidity, sugar content, ascorbic acid content, and polyphenol content (Haynes and Goh, 1987).

Potassium deficiency causes the oldest leaves to develop chlorosis or reddish colouration (cultivar-dependent) from the tips inwards, while the next oldest leaves show darkening of the rachis which spreads upward to the midrib and downward to the petiole (Johanson and Walker, 1963; Johanson, 1980; Ulrich *et al.*, 1980; Strand, 1994; Lieten, 2004). Strawberries of potassium deficient plants fail to develop full colour and are tasteless (Strand, 1994), yet excessive potassium causes a disorder called albinism, where fruit are bitter, acid and have little or no red colour (Perkins-Veazie, 1995).

Plants suffering from any level of potassium deficiency show increased susceptibility to disease (Graham, 1983; Broadley, 1992). Fertilisation with potassium decreases infection of many plant species by foliar pathogens (Graham, 1983). While potassium fertilisation has been shown to increase strawberry yields (Corke and Jordan, 1978), this yield increase is likely partially attributed to reduced disease pressure (Marschner, 1995). It is plausible therefore that a greater yield response would be observed in susceptible than resistant cultivars.

1.4.4.3 Calcium

Calcium is essential for cell replication, elongation, structural integrity and enzymatic pathway signalling. The majority of calcium in plant cells is bound in the cell

walls and plasma membranes. Free Ca^{2+} ions are found in very low concentrations in the cytosol and primarily function as a messenger between environmental signals and plant growth responses (Marschner, 1995; Zeyen *et al.*, 2002; Pilbeam and Morley, 2007) and in perception of pathogen attack (Bush, 1995; Zeyen *et al.*, 2002).

Relatively few studies focus on the calcium requirements of strawberry plants for growth compared with the number of studies investigating the effect of calcium nutrition on strawberry quality (May and Pritts, 1990). Chiu and Bould (1976) state that a small but continuous supply of of calcium is required for normal development of strawberries and that the quantity of calcium fertilization required depends on the rate of plant growth. Kaya *et al.* (2002) did find that supplementation with calcium enhanced both growth and yield of strawberry plants grown under saline conditions. Lieten (2004) described no significant effect of restricted calcium application in the first cropping season of strawberry. However, in the subsequent season, symptoms of deficiency were observed and yield was reduced by 30 %.

Calcium is almost exclusively transported in the xylem tissues (Graham, 1983; Marschner, 1995). The receptacle tissue is predominantly supplied by phloem tissues and thus only receives a limited amount of calcium (Saure, 2005). However, studies of uptake of nutrients in a hydroponic system showed that strawberry flowers do appear to be a calcium sink and that calcium concentrations decrease rapidly as the strawberry develops (Chow *et al.*, 1992). The effect of increased calcium nutrition on strawberry quality is increased firmness, but often at the expense of good flavour (Eaves and Leefe, 1962; May and Pritts, 1990). Foliar applications of $CaCl_2$ may also slow ripening (Wójcik and Lewandowski, 2003).

Calcium deficiency is often due to factors such as low soil moisture and cool, cloudy, humid weather, rather than a lack of calcium in the soil (Hancock, 1999). The

uptake or translocation potential of a given cultivar also influences development of deficiency in adverse conditions (May and Pritts, 1990). Symptoms of calcium deficiency in strawberry include tip burn, small leaves that can be only partially expanded leaves, marginal leaf scorch and crinkled leaflets on younger leaves (Johanson and Walker, 1963; Chiu and Bould, 1976; Johanson, 1980; Ulrich *et al.*, 1980). Fruit are often small with dense achene covering towards the tip because of the inhibition of receptacle expansion (Chiu and Bould, 1976; Ulrich *et al.*, 1980; Lieten, 2004). In calcium deficient tissues, impairment of membrane integrity leads to increased respiration rate and eventual loss of compartmentalisation at a cellular level (Chiu and Bould, 1976; Hecht-Buchholz, 1979).

In addition to enhancing membrane integrity calcium is involved in perception of and subsequent response to pathogen attack. Calcium is intricately involved in the initial responses of host cells to attack by powdery mildew fungi. Studies of the response of cereals to *E. graminis* showed that adequate availability of calcium enhanced cytoplasmic aggregation (Kunoh *et al.*, 1983; 1985). As well as regulating the cytoplasmic events leading to papilla formation (Zeyen *et al.*, 2002), calcium is an integral component of this structure (Marshall *et al.*, 1985; Aist and Israel, 1986). Most parasitic fungi invade the apoplasm by releasing pectolytic enzymes, which dissolve the middle lamella and increase cytosolic Ca^{2+} (Bush, 1995). The activity of these enzymes is strongly inhibited by Ca^{2+} (Graham, 1983; Marschner, 1995).

Calcium nutrition significantly affects resistance to bacterial wilt in tomato (Yamazaki and Hoshina, 1995; Yamazaki *et al.*, 1996; 2000) and resistance of barley to *E. graminis* f.sp. *hordei* (Bayles and Aist, 1987; Bush, 1995). Disease resistance has also been correlated with high efficiency of calcium uptake in tomato cultivars (Yamazaki *et al.*, 1996), and anecdotal evidence suggests that this may also be the case

in some strawberry cultivars (e.g. cv. Kiewa) in relation to powdery mildew resistance (B. Morrison, personal communication, 2003). Research is required to substantiate whether calcium efficiency is correlated with resistance to *P. aphanis* in strawberry cultivars.

1.4.4.4 Silicon

While not widely regarded as a nutrient that is essential for plant growth, silicon is found abundantly in plants, soil and water. Even purified water is likely to contain silica (Werner and Roth, 1983). As it is very difficult to ensure silicon is not being supplied to the plant in some form (water or potting media) to investigate if growth is impaired due to a lack of silicon, the necessity for silicon is still controversial.

Strawberry plants fertilised with silicon produce more dry matter in roots and shoots (Wang and Galletta, 1998), and increased numbers of fruit of enhanced size and marketability (Miyake and Takahashi, 1986). This is thought to be due to increased chlorophyll content (Wang and Galletta, 1998) and enhanced pollen fertility (Miyake and Takahashi, 1986). Fruit quality traits influenced by silicate treatments are increased citric acid, malic acid and membrane bound glycol- and phospholipids, and decreased fructose, glucose, sucrose and myo-inositol content (Wang and Galletta, 1998). Miyake and Takahashi (1986) found that increased silicon in the leaves was positively correlated with calcium uptake, and negatively correlated with nitrogen and phosphorus uptake. Wang (1998) also demonstrated that silicon nutrition alters the partitioning of assimilates into the various tissues of strawberry plants.

Silicon is thought to affect fungal infection and development on plant tissues in two ways: as a physical barrier, and as a stimulator of disease resistance biochemical pathways in response to biotrophic fungi (Carver *et al.*, 1987; Samuels *et al.*, 1991; Bowen *et al.*, 1992; Chérif *et al.*, 1992a; Chérif *et al.*, 1992b; Menzies *et al.*, 1992;

Reynolds *et al.*, 1996; Blaich and Grundhöfer, 1998; Dik *et al.*, 1998; Prats *et al.*, 2006). Silicon is the most abundant element found in the haloes of penetration sites and is associated with callose deposition in papillae, adding to the structural integrity of challenged cells (Zeyen *et al.*, 2002). Silica transport proteins and various mRNAs induced by silicates have been implicated in plant defense (Epstein, 1999)

Silicate products are commonly used for powdery mildew control in glasshouse-grown cucumbers and roses (Bélanger *et al.*, 1995). Silicate treatments controlled grape powdery mildew (*Erysiphe necator*, formerly *Uncinula necator*) in seasons with low disease pressure, but failed to provide control when there was high inoculum load and the weather was conducive to infection (Reynolds *et al.*, 1996). *B. graminis* also did not readily infect oat plants, fertilised with silicon (Prats *et al.*, 2006). This is likely due to enhanced cell wall rigidity (Graham, 1983; Graham and Webb, 1991) and translocation of silicon and polyphenols to the site of penetration physically preventing fungal penetration of the epidermal tissue (Carver *et al.*, 1987; Bowen *et al.*, 1992; Chérif *et al.*, 1992b; Reynolds *et al.*, 1996). Cells adjacent to germinating conidia also show high concentrations of silicon and altered cell wall polysaccharides (Samuels *et al.*, 1991). Silicon supplementation alleviated yield loss caused by *Pythium aphanidermatum* in cucumber (Chérif *et al.*, 1994), while potassium silicate reduced powdery mildew severity in hydroponically grown strawberry (Kanto *et al.*, 2004), supporting unpublished observations of sand-cultured strawberry (Wang, 2003).

1.5 Breeding for powdery mildew resistance and nutrient efficiency in strawberry

Cultivars of the commercial strawberry, *F.* x *ananassa*, are generated by cross-hybridisation between lines that express defined and promising traits (Gidoni *et al.*, 1994). The heterozygous nature and vegetatively propagated growth habit of the

strawberry plant makes breeding less complicated than the breeding of many other plant species. The genus *Fragaria* comprises more than 20 species, with a basic chromosome number $x = 7$ representing four levels of ploidy: diploid, tetraploid, hexaploid and octoploid (Hancock, 1990). *F.* x *ananassa* has an octoploid genetic background ($2n = 8x = 56$) originating from two wild octoploid species, *F. chiloensis* and *F. virginiana*, which vary significantly for many agronomic and fruit quality traits (Bassil *et al.*, 2006). This high level of genetic variation has contributed to the development of over 500 strawberry cultivars (Galletta and Maas, 1990; Hancock, 1999). *F.* x *ananassa* is generally thought to be an allo-polyploid (Davik and Honne, 2005), that is a polyploid that follows disomic segregation, allowing Mendelian inheritance patterns to be used to determine the heritability of many traits (Galletta and Maas, 1990). However, uncertainty of the nature of inheritance in *F.* x *ananassa* has led to inconsistencies in interpretation of such studies (Lerceteau-Köhler *et al.*, 2003).

Inheritance of most horticulturally important traits in strawberry has generally been described as quantitative (Sjulin and Robbins, 1986; Hokanson and Maas, 2001; Kersey and Luo, 2003; Lerceteau-Köhler *et al.*, 2004; Sargent *et al.*, 2004b; Serçe and Hancock, 2005) though in some cases inheritance has been described using a combination of quantitative and Mendelian methods (Hsu *et al.*, 1969; Simpson, 1987). The quantitative method is based on continuous variation in phenotypic traits of the progeny, as opposed to the Mendelian method based on discrete variation in the progeny. Quantitative analysis admits that the phenotypic variation in a segregating population is the result of genotype interacting with environment, but the action of individual genes cannot be recognised. The genotypic portion includes the average effects of genes (additive variance), allelic interactions of genes (dominance variance) and non-allelic interactions of genes (epistatic variance) (Hsu *et al.*, 1969). Phenotypic

traits found to be quantitatively inherited, called quantitative trait loci (QTL), can only be mapped by following their co-segregation with other physiological or molecular markers. The prolific development of simple, reliable co-dominant molecular markers has made QTL mapping a common practice in plant breeding programs (Foulongne *et al.*, 2003; Kersey and Luo, 2003).

The constant desire for improved fruit quality, increased resistance to biotic and abiotic stress, expansion of environmental production range, and the evolution of cultural systems drive the need for production of improved strawberry cultivars. Present breeding objectives include resistance to diseases and insects, increased yield, day-length response for flower initiation, time of ripening, plant and flower cold hardiness, heat tolerance and fruit size and quality characteristics (Hokanson and Maas, 2001). Providing there is no compensation for yield, cultivars with improved disease resistance are favoured in regions where day-neutral cultivars are produced as the long fruiting season limits the types of fungicides that may be used to control disease.

There is growing interest in using wild germplasm in breeding programs (Clavero *et al.*, 2004) as not only do wild strawberry species show adaptations to an array of climates, they also vary in susceptibility to many diseases. Species such as *F. vesca, F. glauca, F. orientalis, F. moschata,* and *F. chiloensis* are resistant to powdery mildew and may provide resistance genes useful for breeding new cultivars (Miller and Waldo, 1957; Orchard and Adrichem, 1957; Hancock, 1999). Transgenic studies may also provide novel sources of resistance to powdery mildew in strawberries (Salmeron *et al.*, 2002; Dai *et al.*, 2004; Schestibratov and Dolgov, 2004).

1.5.1 Inheritance of powdery mildew resistance in *Fragaria*

Many studies have characterised the relative field resistance of strawberry cultivars to powdery mildew (Darrow *et al.*, 1954; Miller and Waldo, 1957; Daubeny,

1959;1961; Peries, 1962b; Hsu *et al.*, 1969; McNicol and Gooding, 1979; Gooding *et al.*, 1981; Maas, 1984; Adams *et al.*, 1986; Simpson, 1987; Nelson *et al.*, 1995;1996; Davik and Honne, 2005). Studies of the inheritance of resistance to *P. aphanis* in strawberry progeny have shown inheritance to be broad-spectrum, quantitatively inherited and likely to be subject to epistatic variance (Hsu *et al.*, 1969). Resistance appeared to be controlled by two additive dominant genes, and one epistatic gene for susceptibility. There is also some evidence of maternal inheritance of susceptibility (MacLaughlan, 1978), suggesting that even susceptible genotypes may carry genes for resistance to powdery mildew. Interpretation of inheritance studies has been exacerbated by the ploidy of the strawberry genome and use of bulked fungal isolates, which may mask race-specific resistance responses. Breeding has thus relied on developing cultivars with resistance based on the additive resistance of the parent cultivars (Davik and Honne, 2005).

Strawberry cultivars produced in Australia that are resistant to powdery mildew include Adena, Redlands Crimson, Earlisweet and Shasta (Broadley, 1992). However recently released Australian cultivars have been evaluated and cloned for production in regions where powdery mildew is seldom severe. These include the Victorian bred cultivars Kiewa and Adena, and Redlands Joy, and the Queensland bred cultivars, Rubygen, Brighteyes, Harmony and Sugarbaby. The phasing out of methyl bromide fumigation for soil pathogens has made breeding for resistance to *Colletotrichum*, *Fusarium*, *Verticillium* and *Phytophthora* spp. a higher priority than for *P. aphanis* (Herrington and Chandler, 2004). While a few of these cultivars are resistant to powdery mildew, they all have short-day and long-day flower initiation responses and are not economically viable for production in South Australia (R. Lewis, personal communication, 2003). However, the powdery mildew resistance of these cultivars may

provide valuable breeding material for incorporation into a suitable background for production in South Australian conditions. Breeding to incorporate disease resistance into strawberry cultivars is expensive, difficult and time-consuming, so practices that will maximise throughput of breeding programs and minimise field observation required, such as marker-assisted selection (MAS), will be invaluable to the strawberry industry.

1.5.2 Powdery mildew resistance status of strawberry cultivars in South Australian production

Currently, the strawberry cultivar Selva accounts for 98 % of production in South Australia. It was bred in California and is widely planted throughout the world, due to its relatively broad environmental tolerance (Hancock, 1999). Although Selva is very susceptible to powdery mildew, disease is usually controlled with fungicides. It grows relatively well in South Australian conditions (P. Mason, personal communication, 2003), and with suitable fertilisation produces high yields of large, firm fruit (Hancock, 1999). Other cultivars commonly grown in South Australia include Chandler and Camarosa (also of Californian origin), which are also susceptible to powdery mildew. Selva, Chandler and Camarosa are thought to be quite closely related as they share the maternal founding clone Middlefield (Dale and Sjulin, 1990). Davik and Honne (2005) showed the cultivars Selva, Chandler and Camarosa to have similar low breeding value for powdery mildew resistance. However, Chandler and Camarosa have Douglas, a powdery mildew resistant cultivar, also from the maternal founding clone Middlefield, as a primary parent (Dale and Sjulin, 1990). Douglas scored highly as a donor for powdery mildew resistance (Davik and Honne, 2005), and is thought to have inherited resistance from Tioga through its primary parent Tufts. (Simpson, 1987; Dale and Sjulin, 1990; Davik and Honne, 2005). In recent years, new cultivars released

from the University of California strawberry breeding program show potential for production in South Australia. These include Aromas (The University of Calfornia, Strawberry Licencing Program, 2001).

Aromas is a powdery mildew resistant, day-neutral cultivar. Aromas has a more erect growth habit than Selva, increasing air flow in the canopy and ease of harvesting. It is relatively resistant to anthracnose crown rot and spider mites, tolerant of the strawberry viruses typically encountered in California, and moderately susceptible to common leaf spot and Verticillium wilt. In trials in California, Aromas produced large fruit (24 to 26 g) similar in size though firmer than those of Selva (mean weight 23 g), with dark red flesh and superior appearance and flavour, adaptable to fresh or processing markets. Aromas also produced fewer small fruit than Selva, resulting in a much lower cull rate (The University of Calfornia, Strawberry Licencing Program, 2001). Aromas has a broader environmental tolerance than other day-neutral cultivars recently released by the University of California, namely Diamante, Gaviota and Pacific (The University of Calfornia, Strawberry Licencing Program, 2001). On the basis of performance in Californian trials and resistance to powdery mildew, Aromas was chosen for use in this research.

1.5.3 Molecular marker and gene mapping studies of *Fragaria*

The use of DNA-based markers allows researchers to mark genes or chromosome regions that are related to phenotypic traits such as disease resistance. Marker-tagged genes can be used in marker assistant selection (MAS) in breeding programs and linkage analysis can be used to determine genetic distances between traits, forming the basis of a genetic map (also known as a linkage map) (Mohan *et al.*, 1997; Hospital, 2003).

Various techniques have been used to produce molecular markers, with success based on reproducibility and ease of use. Initially, morphological markers and isozymes were used as markers. There has been rapid development of more precise markers based on polymorphism in the nucleotide sequence (Mohan *et al.*, 1997). These methods include restriction fragment length polymorphisms (RFLPs) (Williams *et al.*, 1991), random amplified polymorphic DNAs (RAPDs) (Williams *et al.*, 1990) and PCR-based DNA markers such as simple sequence repeats (SSRs) or microsatellites (Hearne *et al.*, 1992) and amplified fragment length polymorphic DNAs (AFLPs) (Vos *et al.*, 1995). To simplify data scoring markers identified by multilocus systems (RAPDs, SSRs and AFLPs) are frequently converted to locus-specific sequence characterised amplified region (SCAR) markers (Williams *et al.*, 1991; Albani *et al.*, 2004). These marker systems have been employed to study various traits in both diploid and octoploid *Fragaria* species (Table 1.1).

Major disadvantages of marker and linkage studies are the time and resources involved with producing test cross populations and evaluating them, in order to link the observed traits with molecular markers. Strawberries produce a large number of progeny to be screened, often making this approach impractical. However, once markers are defined, they can be used directly in breeding programs since they allow the researcher to predict phenotypes based on the presence or absence of the marker (MAS). A particular advantage of MAS is that polygenic traits can be analysed. However, the octoploid genomic constitution of *F.* x *ananassa* (2n = 8*x* = 56) has been a major impediment to genetic characterisation of the cultivated strawberry species (Davis and Yu, 1997). Comprehensive genomic maps of *F.* x *ananassa* and its relatives

Table 1.1 Molecular marker and mapping studies of *Fragaria*.

Marker	Species	Study purpose	Reference
Isozyme	*F.* x *ananassa*, *F. vesca*, *F. chiloensis*	Marker generation	Drawert *et al.*, 1974
	F. x *ananassa*	Cultivar characterisation	Bell and Simpson, 1994
	F. vesca	Mapping	Davis and Yu, 1997
		Isolated population characterisation	Arulsekar and Bringhurst, 1981
RAPD	*F.* x *ananassa*	Cultivar characterisation, relatedness studies	Gidoni *et al.*, 1994; Graham *et al.*, 1996; Landry *et al.*, 1997; Degani *et al.*, 1998; Congiu *et al.*, 2000; Harrison *et al.*, 2000
		Phytophthora resistance locus	Haymes *et al.*, 2000
	F. vesca	Marker generation	Davis *et al.*, 1995
RFLP	*F.* x *ananassa*	Cultivar identification	Kunihisa *et al.*, 2004
		Mapping	Viruel *et al.*, 2002
AFLP	*F.* x *ananassa*	Marker comparison studies	Degani *et al.*, 2001
		Mapping	Haymes *et al.*, 2000; Lerceteau-Köhler *et al.*, 2003
SSR	*F.* x *ananassa*	Relatedness studies and mapping	Viruel *et al.*, 2002; Folta *et al.*, 2005; Keniry *et al.*, 2006;
	F. x *ananassa*, *F. vesca, F. virginiana*, *F. chiloensis*	Marker generation and transfer	Ashley *et al.*, 2003; Bassil *et al.*, 2006
	F. vesca, *F. nubicola*	Mapping	Sargent *et al.*, 2004a; Sargent *et al.*, 2006
	F. vesca	Fruit ripening	Bonoli *et al.*, 2004
		Seasonal flowering locus	Cekic *et al.*, 2001; Albani *et al.*, 2004
		Relatedness studies and mapping	James *et al.*, 2003; Cipriani and Testolin, 2004; Hadonou *et al.*, 2004; Sargent *et al.*, 2004a

would allow MAS for incorporation of specific traits, maximising the opportunity to incorporate only the genes of interest without adverse agronomic traits (Hokanson and Maas, 2001).

Linkage maps of *Fragaria* have been constructed using isozymes (Davis and Yu, 1997) but these only allow a small number of loci to be characterised (Hokanson and Maas, 2001) as they are limited by the number of stans available (Kersey and Luo, 2003). Using RAPD markers, Davis and Yu (1997) constructed a linkage map of *F. vesca*, which is diploid with common ancestry to *F.* x *ananassa* (Hancock and Luby, 1993; Potter *et al.*, 2000). This species has a small genome of 164 Mbp (Bennett *et al.*, 2000). Significant progress has been made in constructing a saturated linkage map for *F. vesca* using expressed sequence tag simple sequence repeats (EST-SSRs) on an F_2 mapping population of *F. vesca* and *F. nubicola* (Sargent *et al.*, 2004a; Sargent *et al.*, 2006). This map now comprises 182 markers, spanning 424 cM over the seven linkage groups. Many of these markers have been shown to be transferable into cultivars of *F.* x *ananassa* providing a useful reference point for linkage mapping studies of the octoploid species (Sargent *et al.*, 2006).

Haymes *et al.* (2000) published a linkage map for *F.* x *ananassa*, based on seven RAPD, eleven AFLP and two SCAR markers used to map the location of three resistance genes to *Phytophthora fragariae* using bulked segregant analysis (BSA). Independently, Viruel *et al.* (2002) developed a linkage map for *F.* x *ananassa* with 123 RFLP and 177 SSR markers. Their map included 140 loci distributed in 17 linkage groups over a total distance of 627 cM. Lerceteau-Köhler *et al.* (2003) used ALFP markers for mapping studies in this species, however these markers are not readily transferable and may not be evenly spread throughout the genome of the octoploid species (Degani *et al.*, 2001). The wealth of EST-SSR markers presently being

generated from *F.* x *ananassa* will be valuable for future linkage mapping in this species and comparative mapping studies in the genus (Bassil *et al.*, 2006; Keniry *et al.*, 2006). Fourteen of the *F.* x *ananassa* EST-SSR markers identified by Bassil *et al.* (2006) were mapped to the F_2 population (*F. vesca* x *F. nubicola*) of Sargent *et al*. (2006). Research is now required to associate phenotypic traits, such as powdery mildew resistance, to the octoploid *Fragaria* maps for use in MAS breeding.

1.5.4 Candidate gene approach

Genes that are differentially expressed under certain conditions can be identified as candidate genes associated with the given event. Candidate genes have been successfully identified through changes in gene expression during ripening of grape (Davies and Robinson, 2000; Ficke *et al.*, 2004) and strawberry (Medina-Escobar *et al.*, 1997; Blanco-Portales *et al.*, 2002). Medina-Escobar *et al*. (1997) used a magnet-assisted subtraction technique combined by means of the polymerase chain reaction and Southern blot differential screening (MAST-PCR-SBDS) to isolate a large number of cDNAs from genes differentially expressed during the strawberry fruit ripening process, and proved this method to be sensitive, reliable and cost-effective. Using a candidate gene approach to identify genes associated with yellow fruit colour in *F. vesca*, Deng and Davis (2001) amplified segments of five regulatory genes from the anthocyanin biosynthetic pathway. When quantitatively analysed, a flavanone 3-hydroxylase (F3H) cosegregated with the locus for yellow fruit colour (recessive), suggesting this gene is necessary for red colour in strawberries of this species.

The primary advantage of methods for identification of genes using a candidate approach is that genes associated with specific physiological events can be determined without the use of QTL analysis, which requires evaluation of a mapping population segregating for the trait of interest. Construction and maintenance of such populations is

time and resource-consuming. In the absence of a suitable mapping population, a candidate gene approach was determined to be the most efficient way to identify putative genes associated with the resistance response of the cultivar Aromas to *P. aphanis*.

1.6 Summary and research aims

In summary, while there are many documented studies relating to the cultivated strawberry, *F.* x *ananassa*, and the causal agent of strawberry powdery mildew, *P. aphanis*, there is need for research that takes into account local production conditions and the cultivars grown in South Australia. Maximising potential host defence of existing cultivars through improved ferilisation and efficient use of fungicides to control outbreaks of *P. aphanis* would provide tools for management of powdery mildew in strawberry crops that could be implemented immediately. Identification of genetic sources of resistance relevant to South Australian production could benefit cultivar production for this region in the future.

Detailed epidemiological information and knowledge of the local weather conditions conducive to disease development could be correlated to provide warning of high risk conditions. This knowledge could allow strawberry growers to optimise control treatments, ultimately minimising fungicide use, benefiting the producer, the consumer and the environment. Similar information for the management of downy mildew is already being effectively disseminated and utilised by grape producers in South Australia via the state news weather forecast.

The use of nitrogen fertilisation in strawberry production is well understood and further investigation of how this nutrient affects foliar pathogens is not necessary. The importance of calcium and potassium nutrition in disease resistance and the interaction

of these with strawberry powdery mildew has been considered in relation to common field practice in South Australia as well as previous literature. The inconsistency of response to potassium fertilisation in different strawberry cultivars emphasises the necessity to optimise the potassium response for each commercially important cultivar. However, the effect of potassium on plant disease resistance is usually only seen where plants are deficient in potassium. Strawberry crops are regularly fertilised with potassium so it was felt that any benefit from further investigation would be minimal. Calcium, while it also is regularly used as fertiliser, is thought to be mobilised better by some cultivars than others and it has been suggested that calcium efficiency may be correlated with resistance to powdery mildew in strawberry (B. Morrison, personal communication, 2003). The apparent lack of comprehensive studies of pre-harvest calcium application also makes this nutrient a priority for characterisation of nutrient efficiency in commercial cultivars. Characterisation of the efficiency for calcium of the cultivars of interest to South Australia would be valuable to the local strawberry industry. Boron is also of interest, although it is felt that boron studies should be undertaken under optimal calcium nutrition, as these nutrients are known to interact, so this becomes a priority for investigation after calcium. Silicon fertilisation is not common in field-grown strawberry and hydroponic studies of Wang (2003) show that silica increased resistance of strawberries to powdery mildew, making this nutrient a potentially novel tool for powdery mildew management in strawberry crops.

Molecular and mapping studies of *Fragaria* are progressing rapidly and will be a useful starting point for inheritance studies in the future. The primary advantage of methods for identification of genes using a candidate approach is that genes associated with specific physiological events can be determined without the need for QTL analysis, which requires evaluation of a mapping population segregating for the trait of interest.

In the absence of a suitable mapping population, a preliminary investigation of potential candidate genes for powdery mildew resistance would be ideal.

The aims of this research therefore were to:

(1) Define the epidemiology of *P. aphanis* in the South Australian strawberry growing region;

(2) Define the efficiency of strawberry to uptake the nutrients calcium and potassium;

(3) Determine the way in which calcium, potassium and silicon affect powdery mildew disease and

(4) Identify potential candidate genes associated with powdery mildew resistance responses of the cultivar Aromas.

Chapter 2 General materials and methods

2.1 Plant material

The *F.* x *ananassa* cultivars Selva and Aromas were used in experimentation outlined in this thesis. Selva was chosen because it is the predominant cultivar in production in South Australia and is susceptible to powdery mildew. Aromas was chosen because it is agronomically similar to Selva but resistant to powdery mildew. The mechanism and inheritance of resistance in Aromas is unknown. Aromas is a recent release from the same breeding program as Selva (The University of Calfornia, Strawberry Licencing Program, 2001), and is likely to be derived from the same maternal clone (Davik and Honne, 2005).

Runners of both cultivars were obtained from the Toolangi Certified Strawberry Runner Growers' Cooperative Ltd, Victoria. These were freighted at 10 °C, bare-rooted, to the experimental sites and stored at 4 °C until planting. Roots were trimmed to approximately 15 cm and leaves and buds removed prior to planting.

2.2 Maintenance of plants

Runners were either planted into pots and kept in a glasshouse at the Waite Campus, The University of Adelaide (N-34° 58′ 13″ W-138° 37′ 51″) or planted on commercial strawberry farms in Woodside, South Australia (N-34° 57′ 9.9″ W-138° 52′ 45.5″) (Figure 2.1). Trials in the 2003/04 and 2004/05 seasons were on the property of Paul Mason, Mason Fresh Berries Inc., Gallas Rd, Woodside, South Australia (Figure 2.2). The 2005/06 trial was on the property of Don Parker, A. F. Parker and Sons Ltd., Willow View Rd, Woodside, South Australia (Figure 2.3).

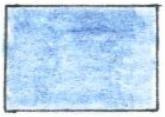
NOTE: This figure is included on page 43 of the print copy of the thesis held in the University of Adelaide Library.

Figure 2.1 Location of commercial strawberry plantations in Woodside, South Australia. **A** Location of P. Mason's property. **B** Location of D. Parker's property. Maps produced by Google Earth. Copyright: Europa Technologies and DigitalGlobe.

Legend for Figures 2.2 and 2.3

Strawberry plots. Red lines indicate row orientation

Pasture



Reservoirs

Buildings or road

Forest. Size of tree markings is relative to actual size.

Fence

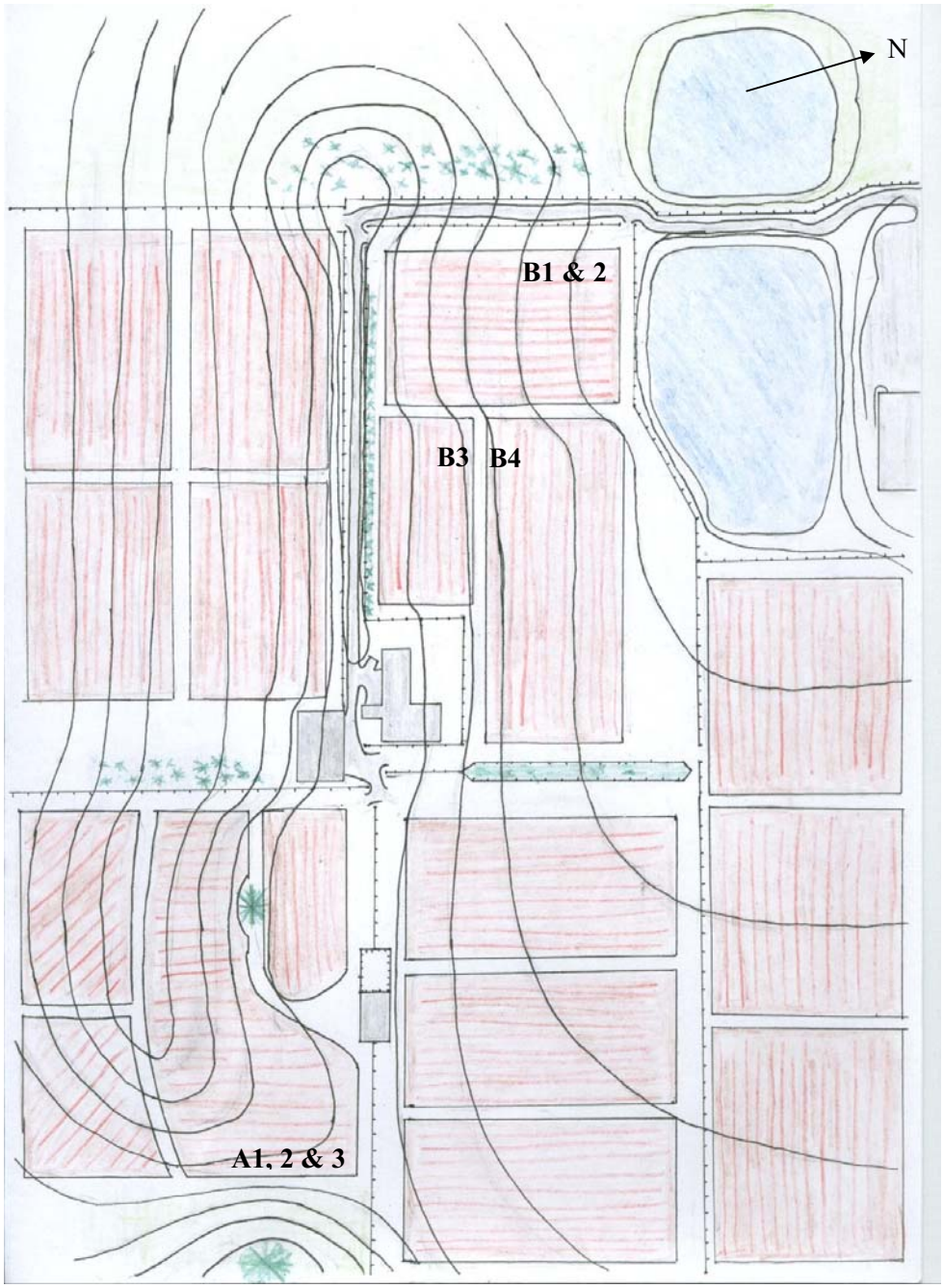


Figure 2.2 Map of P. Mason's property. Contour lines indicate approximate 5 m gradient, with highest point at the track to the farm house and the lowest point at the western-most reservoir. **A** indicates the location of the 2003/04 preliminary trial rows 1 to 3. **B** indicates the location of the 2004/05 trial rows 1 to 4.

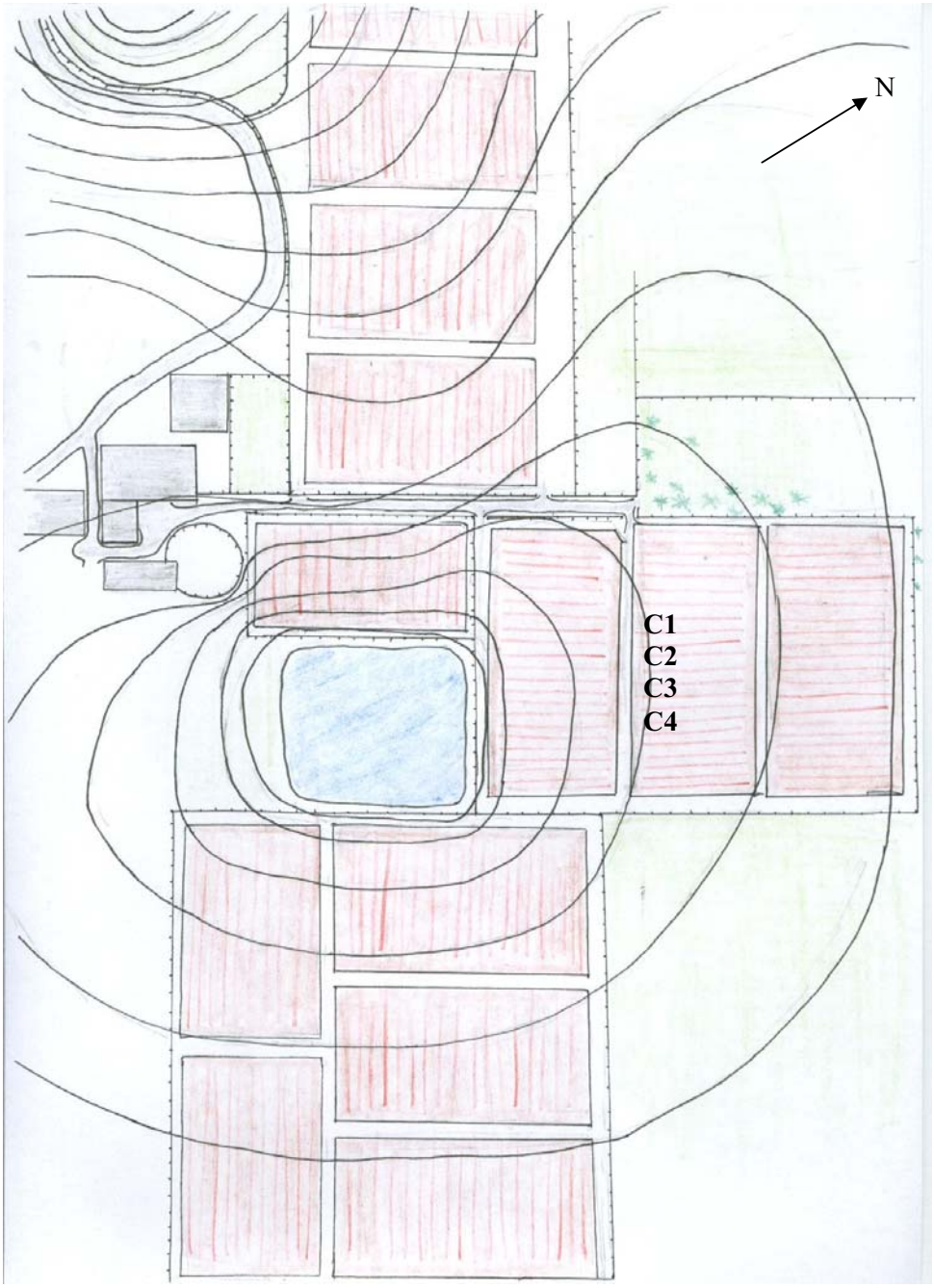


Figure 2.3 Map of D. Parker's property in Woodside, South Australia. Contour lines indicate approximate 5 m gradient, with the highest point at the western knoll and the lowest point at the reservoir. **C** indicates the location of the 2005/06 trial rows 1 to 4.

2.2.1 Glasshouse maintenance

Runners were planted into 20 cm diameter pots containing University of California (UC) potting mix (Baker, 1957). Pots were watered with reverse osmosis (RO) water to flow-through, generally with a hose. During summer frequent watering was required and an overhead mister irrigation system was used morning and night for 10 min to supplement watering by hose.

Insects in the glasshouse were monitored regularly, using sticky traps and by inspecting the leaves and flowers. Insects commonly found in the glasshouse were aphids (*Aphis* spp.), crown moths (*Pyralidae* spp.), fruit flies (*Drosophila* spp.), fungus gnats (*Bradysia* spp.), thrips (*Frankliniella* spp.), two-spotted spider mites (*Tetranychus urticae*) and white flies (*Trialeurodes vaporariorum*). Where possible, biological control agents were introduced to control these pests; *Stratiolaelaps hypoaspis Typhlodromips occidentalis*, *Phytoseiulus persimilis* and *Encarsia formosa* to control fungus gnat, thrips, two-spotted mite and whitefly (respectively). *E. formosa* pupae were sourced from Biological Services Pty Ltd. (South Australia), and cards were hung in shady positions (usually under leaves) at the distribution recommended by the supplier. *S. hypoaspis* and *T. occidentalis* were supplied (Biological Services Pty Ltd, South Australia) in mulch which was mixed to distribute insects and arthropods evenly and a small amount added to the surface of the soil of each pot. *P. persimilis* were supplied on *Phaseolis vulgaris* leaves (Beneficial Bug Company, New South Wales), which were distributed evenly amongst the canopy of strawberry plants. Occasional sprays were necessary to reduce pest populations in order for biological control to be effective. Chemicals applied to glasshouse experiments when necessary were Gremlin® [Sipcam Pacific Australia Pty Ltd, Australia, active ingredient (AI): Abamectin] for two-spotted mite, Rogor® (Sipcam Pacific Australia Pty Ltd, Australia, AI: Dimethoate)

for fungus gnat and crown moth larvae, and Confidor® (Bayer CropScience, Australia, AI: Imidachloprid) for aphids. *Drosophila* spp. were controlled by timely removal and disposal of ripe strawberries.

2.2.2 Field maintenance

Runners were planted in fertigated hills with black polyethylene mulch, prepared by the grower. Experimental plots were generally planted on the edge of commercial blocks for ease of maintenance. Planting occurred in May of each year. The fruiting season began in October and continued to April/May of the following year.

Watering, fertigation and management of pests and pathogens, except *P. aphanis*, were the responsibility of the grower. Soils in the Woodside area are chromosols. Prior to preparation for planting, fields were planted with maize which was ploughed in to increase the organic matter content of the soil and improve soil texture. Soil was then fumigated with methyl bromide to eradicate *Phytophthora* spp., *Verticillium dahliae*, *Pythium* spp., *Rhizoctonia* spp., *Cylindrocarpon* spp. and *Fusarium* spp. Two-spotted mite, the most common pest encountered in the field, was usually controlled with *P. persimilis* and pesticide applications when severe (*P. persimilis* was not used in the 2005/06 season). During harvest, fungicide was regularly applied (Euparen Multi®, Bayer CropScience, up to three times weekly) to control *Botrytis cinerea*. Trial plots received no fungicide for treatment of powdery mildew in the 2003/04 season and were treated as normal commercial plots in the 2004/05 and 2005/06 seasons.

2.3 Weather data collection

Meteorological data from the 3 years of trials were obtained from the Commonwealth of Australia, Bureau of Meteorology (BOM), and used to assess the conditions conducive to incidence and severity of powdery mildew in field trials. Data

were collected from the nearest BOM weather station at Mt Barker (station number 23733), 35°03'50" S 138°51'03" E, approximately 16 km from the trial sites, at an elevation of 360.0 m (Figure 2.4). Growers in the Woodside area use this publically available weather data as an indication of prevailing weather conditions (P. Mason and D. Parker, personal communication, 2003). The weather station consisted of a Stevenson Screen (C0545) containing a humidity and temperature sensor and a rain gauge (200 mm capacity). Temperature and humidity observations were recorded at 6 am and 3 pm, and the rain gauge measured and reset at 3 pm daily. Temperatures and RH observations spanning the trial periods were provided as a data file on request.

2.4 Fungal material

P. aphanis was cultured in the laboratory for use in controlled inoculations of whole plants and detached leaves for destructive sampling. Inoculum was initially obtained from the property of P. Mason in March 2003. *P. aphanis* was maintained on potted plants in a clear plastic container at 22 to 24 °C in natural light with a fan to circulate air. Water was applied every 1 to 3 days to cover the base of the container to water plants and maintain high humidity. Healthy leaf tissue was inoculated with *P. aphanis* by rubbing the underside of an infected leaf onto the underside of an up-turned recipient leaf or by using a camel-hair brush to dislodge conidia onto the new tissue. To culture *P. aphanis,* inoculated plants were retained until mycelium started to appear on the upper surface of the leaves, just prior to senescence, at which time new plants would be inoculated.

2.4.1 Detached leaf inoculations

Detached leaves were inoculated to observe development of *P. aphanis* on Aromas and Selva, and also to assess the effect of foliar nutrient content on fungal

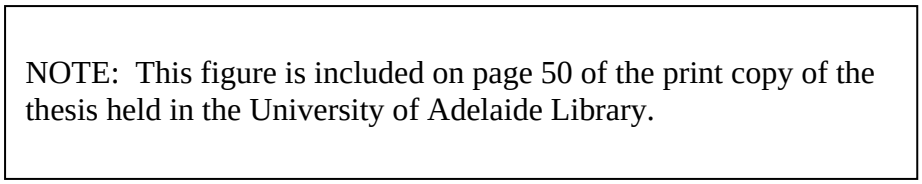


Figure 2.4 Map showing the location of the BOM weather station (**C**) in relation to the field sites (**A** and **B**).

development. This method was more efficient than glasshouse-based methods and allowed for staggered, replicated studies of a manageable number of samples.

Healthy, youngest open leaves (YOL) with long petioles were selected for inoculation. Detached leaves were initially maintained according to the protocol of Evans *et al.* (1996) for grape leaves. This method required the leaves to be surface sterilised with 5 % sodium hypochlorite and air-dried before inoculation. The leaf was placed on sterilised toothpicks on 1 % water agar plus 1 % streptomycin with the petiole inserted into the agar. These plates were sealed with Parafilm™ and kept at 24 °C in natural light, however, strawberry leaves only survived a few days under these conditions. Subsequently, the method used by Miller *et al.* (2003) in epidemiological studies of *P. aphanis* which involved detached strawberry leaves, was used. Leaves were placed with petioles (minimum 4 cm in length) in vials of RO water held in a high humidity chamber (Figure 2.5). The chamber was a 25 cm high, 18 cm diameter clear food-grade plastic cylinder with a 15 cm Petridish™ containing 250 mL RO water in the base and a 3 V fan in the lid to increase air circulation. The chamber was kept in natural light in an air-conditioned room at 22 to 24 °C. These conditions were expected to maintain RH over 85 %, as per the American Society for Testing Materials standards (1995).

2.5 Microscopy

Light microscopy was used to compare the leaf structure of Selva and Aromas and the development of *P. aphanis* on both detached leaves and leaf tissue from whole plants. The important developmental stages of germination, hyphal extension and conidiophore maturation, were observed using light microscopy. Observations of developmental stages were enhanced by use of Scanning Electron Microscopy (SEM) in addition to light microscopy. SEM also allowed discovery of penetration sites, and more

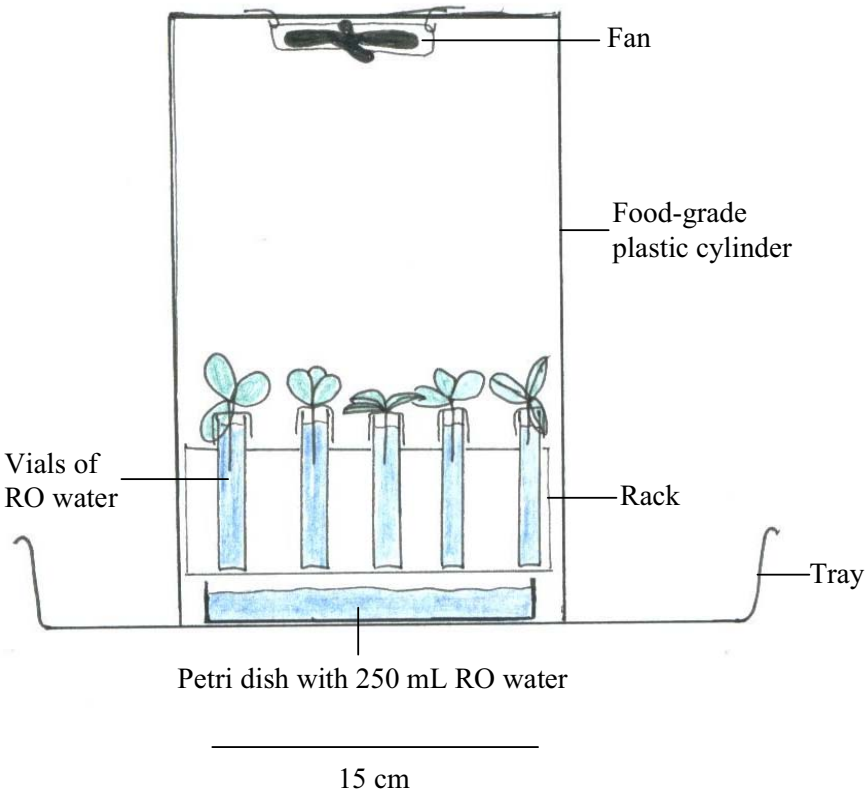


Figure 2.5 Sketch of the detached leaf setup as detailed in Section 2.4.1.

comprehensive understanding of specimens observed under light microscopy. Backscatter emission microscopy and X-ray analysis were used to identify patches of silicate on the leaf surface.

2.5.1 Light microscopy

Leaves were prepared for light microscopy by one of two methods, depending on the sectioning required for observation. For observation of fungal development on the leaf surface, leaf pieces (approximately 1 x 1 cm) were cleared in a 1:1:4 lactic acid: glycerol: ethanol solution for 1 to 7 days at room temperature (approximately 24 °C). Tissues were stained with 0.5 mM aniline blue in lactophenol solution for 10 min in a 50 °C water bath, washed with ethanol, then vacuum-infiltrated with fresh clearing solution before being mounted in 50 % glycerol on slides.

Thin sections were prepared for light microscopy with guidance from M. Wallwork, Adelaide Microscopy. Leaf pieces were cut twice in fixative (4 % paraformaldehyde/ 1.25 % glutaraldehyde in phosphate buffered saline (PBS), with 4 % sucrose, pH 7.2), and left at 4 °C in fixative overnight. Specimens were washed twice in PBS buffer, with 4 % sucrose for 10 min, before being post-fixed in 2 % osmium tetroxide (OsO_4) for 1 hour on a rotor at the minimum speed setting. Specimens were then dehydrated using a four-step ethanol concentration series (three changes of 20 min with each of 70, 90, 95 and 100 % ethanol) and a 30 min application of propylene oxide. Any specimens that remained floating at this stage were discarded. Resin infiltration of specimens was undertaken using a Procure/Araldite embedding kit (ProScitech, Australia). Resin was 3:5:11 DDDSA: Procure: Araldite, with 0.28 µL DMP30 catalyst added once the main components were well mixed. Resin was inverted on a rotor at a slow speed for at least 1 hour before use, and resin was only used for 48 hour after preparation. Infiltration was attained by an overnight application of 1:1 resin:

propylene oxide, followed by six changes of resin, morning and evening for 3 days. Specimens were kept on a rotor at a slow speed in a fume hood for the infiltration process. Following infiltration, specimens were embedded in fresh resin and baked at 70 °C overnight. Sections were cut on a Diatome diamond knife using a Reichert Ultracut E ultramicrotome by the Adelaide Microscopy staff. Ultrathin sections (approximately 70 nm thick) were picked up on copper grids and stained with uranyl acetate and lead citrate. Staining was achieved by drying the sections onto a slide at 80 °C, covering it with toluidine blue stain (2 % borax and 1 % toluidine blue in water) and heating the slide for 20 to 30 s. The stain was then washed off, and the slide dried, mounted in DPX™ (ProScitech, Australia) and a cover slip was added. Specimens were observed using a Zeiss Axioscop compound microscope with AnalySIS Life Science Research® software (Soft Imaging Systems).

2.5.2 Scanning electron microscopy, x-ray and backscatter emission analysis

SEM was undertaken at Adelaide Microscopy under the guidance of L. Waterhouse. Samples were examined in a Phillips XL30 Field Emission Scanning Electron Microscope, equipped with an Oxford Instruments CT1500 HF Cryotransfer Stage. Samples were attached to the holder using Tissue-Tek OCT compound mixed with carbon dag, frozen in semi-solid nitrogen and transferred under vacuum to the preparation chamber. In this chamber the temperature of the sample was raised from below -150 °C to -92 °C and held there for approximately 3 min to allow ice on the sample surface to sublime. The temperature was then reduced to -110 °C (at which sublimation ceases) and the sample coated with approximately 2 nm of platinum to make it electrically conductive. The sample was then loaded onto the microscope stage (held below -150 °C) and examined.

2.6 Assessment of disease severity

Descriptions of symptoms of powdery mildew on strawberry fruit in the literature and anecdotal evidence given by farmers were inconsistent. In the preliminary trial (2003/04 season) fruit abnormalities were observed and associated with foliar powdery mildew severity in order to create a scale of powdery mildew severity on fruit. This was compared with the foliar scale in the 2004/05 and 2005/06 seasons.

2.6.1 Powdery mildew severity on plants

A scale for assessing foliar powdery mildew was adapted from descriptions of foliar symptoms detailed in epidemiological studies undertaken by Peries (1962a). This subjective 0 to 6 visual scale (Table 2.1) was used in the preliminary field trial conducted in the 2003/04 season. Upon discussion with growers after the first year of trials, it was decided that the variation in class score 3 was too great and that separating this class into two classes would give a better indication of an economic threshold. The scale was therefore adapted so that symptoms that were previously classed as a severity of 3 "Small and/or coalescing patches on the lower surface of leaves" were differentiated into "Small and/or coalescing patches on the lower surface of less than 25 % of leaves on a plant" and "…on equal to or more than 25 % leaves on a plant". The expanded version (0 to 7) was used in the subsequent trials in the 2004/05 and 2005/06 seasons (Table 2.2).

The scales were applied to whole plants *in situ* in the field when plants were dry. The whole plant was observed for upward curling of leaves (Figure 2.6) or obvious mycelial growth, and then the underside of each leaf was systematically inspected for mycelium (Figure 2.7) and the observations combined to give the overall plant score. Shading the plant during inspection made the symptoms easier to see.

Table 2.1 The preliminary visual scale for assessing the severity of powdery mildew on strawberry plants in the 2003/04 season.

Score	Description
0	No visible mycelium
1	Few small patches of mycelium on underside of one or two leaves
2	Small patches on three or more leaves, or coalescing patches underside of one leaf
3	Small and/or coalescing patches on underside of leaves
4	Mycelium covering underside of one or more leaves, upward curling of leaf edges
5	Mycelium in the crown, on petioles, peduncles or topside of leaves, generally covering underside of leaves
6	Dead

Table 2.2 The visual scale for assessing the severity of powdery mildew on strawberry plants in the 2004/05 and 2005/06 seasons.

Score	Description
0	No visible mycelium
1	Few small patches of mycelium on underside of one or two leaves
2	Small patches on three or more leaves, or coalescing patches underside of one leaf
3	Small and/or coalescing patches on underside of <25 % of leaves
4	Small and/or coalescing patches on underside of ≥25 % of leaves
5	Mycelium covering underside of one or more leaves, upward curling of leaf edges
6	Mycelium in the crown, on petioles, peduncles or topside of leaves, generally covering underside of leaves
7	Dead

A **B**

Figure 2.6 Symptoms of powdery mildew on plants. **A**. Row of healthy strawberry plants. **B**. Row of plants severely affected by powdery mildew showing upward curling of leaves.

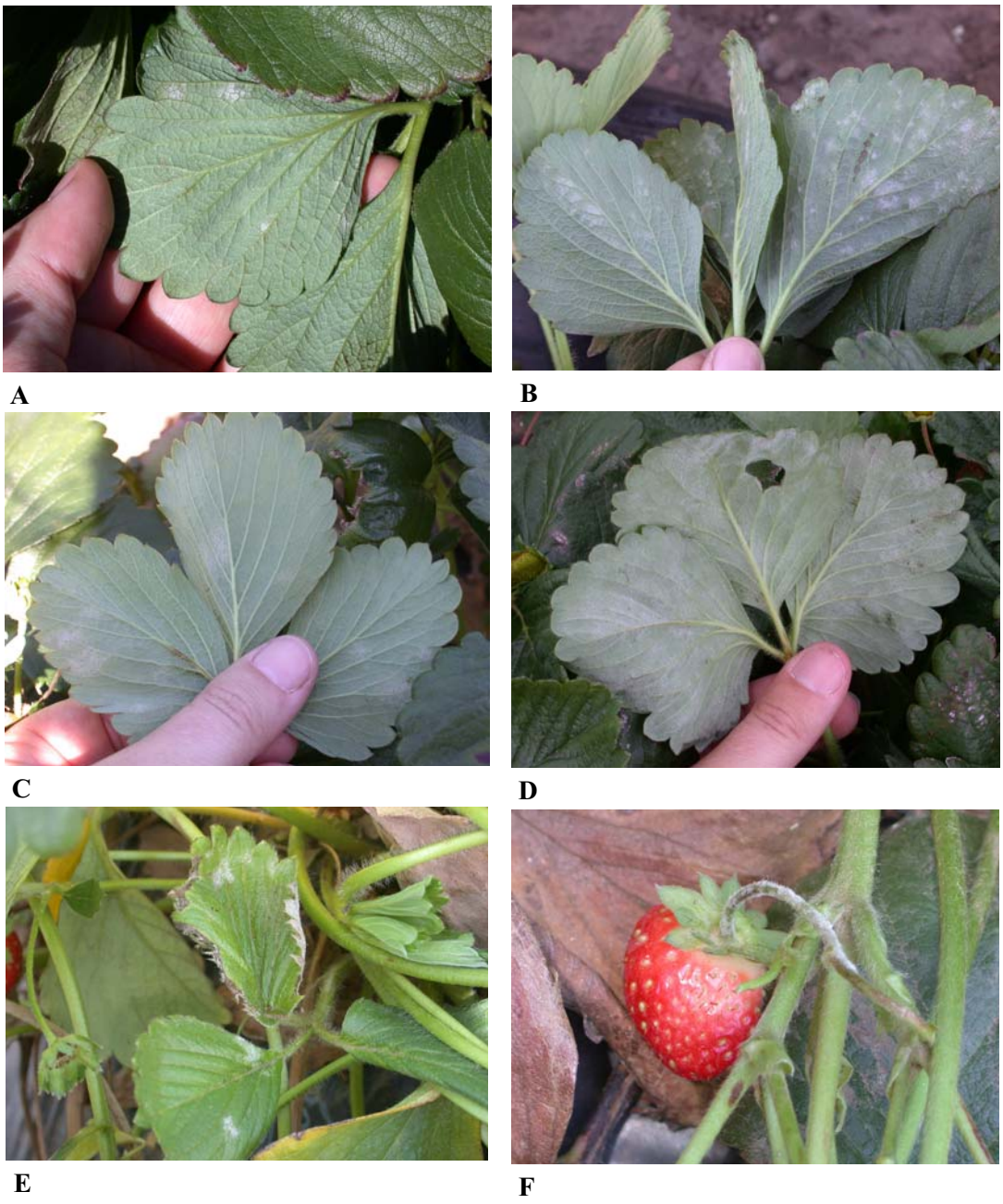


Figure 2.7 Foliar symptoms of powdery mildew on strawberry plants, with increasing severity. **A**. Abaxial surface with few small patches of mycelium (score 1-2). **B**. Many small patches (1-4). **C**. Coalescing patches of mycelium (2-4). **D**. Mycelium covering (5). **E**. Patches of mycelium on the upper surface of leaf. **F**. Mycelium on peduncle (or petiole) (6).

2.6.2 Powdery mildew severity on fruit

A subjective visual scale (Table 2.3) was created following the observation of symptoms associated with powdery mildew in the 2003/04 and was used to assess infection on the harvested strawberries in the 2004/05 and 2005/06 seasons. During sorting, the strawberries were examined for overall appearance. Stunting and malformation, caused when powdery mildew develops on immature fruit, is obvious by the uneven distribution of the achenes over the strawberry. The surface of each strawberry was closely examined, on all sides, for mycelium or epidermal cracking. These symptoms were usually found around the calyx, and in severe cases on the base and sides of the strawberry. The scale ranged from no visible mycelium (0) to mycelium covering the surface of the strawberry, with cracking of the epidermis and malformation (7) (Figure 2.8).

2.7 Harvesting

Strawberries were picked from the plant by gently holding the strawberry between index finger at the base and thumb at the calyx, and snapping the petiole at the base of the calyx by bending the fruit away from the petiole.

Harvested strawberries were sorted into marketable and unmarketable fruit. Marketable fruit was sorted into size classes then counted and weighed. Size class estimates (Figure 2.9) were based on the number of strawberries that fitted into a 250 g punnet (small >20, medium = 13 to 20, large = 9 to 12, extra large 5 to 8 and extra extra large = 4 or less). Approximate weights were calculated from these industry classes. In the 2003/04 season fruit were observed, and the following abnormalities were tallied: powdery mildew (Figure 2.8), cracking, misshapen, other fungi, cankers, loss of achenes, other (including avian, insect, rodent and bovine damage) (Figure 2.10). In the

Table 2.3 The visual scale used for assessing the severity of powdery mildew on strawberries. Economic threshold is a fruit with a score less than 3.

Score	Description	Marketable
0	No superficial mycelium on fruit surface	Yes
1	< 25 % fruit surface with mycelium	Yes
2	25 - 50 % fruit surface with mycelium	Yes
3	51 - 75 % fruit surface with mycelium	Sorter dependent
4	76 - 100 % fruit surface with mycelium	No
5	Mycelium on surface and epidermal cracking	No
6	Powdery, cracked and malformed	No (not normally picked)

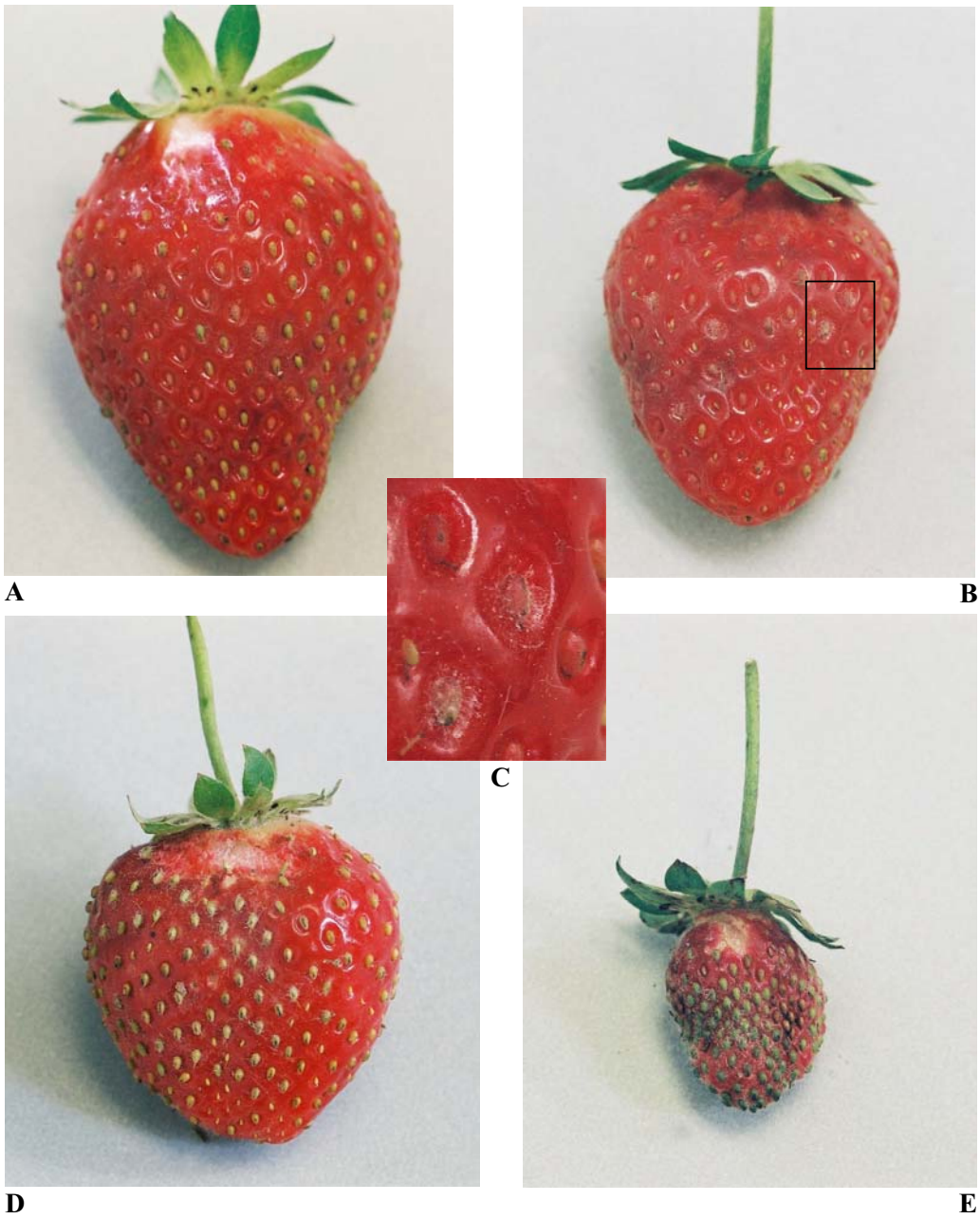


Figure 2.8 Symptoms of powdery mildew on strawberries. **A**. Mycelium barely visible around only a few achenes (score 1). **B**. Mycelium visible around some achenes (3). **C**. Enlarged image of *Podosphaera aphanis* sporulating around achenes (from Figure B). **D**. Mycelium visible around most achenes and on receptacle surface. The epidermis is cracked under the calyx (5). **E**. Mycelium visible over entire fruit, epidermal cracking and severe malformation (6).

Figure 2.9 Size classes of fruit. **A.** small, **B.** medium, **C.** large, **D.** extra large and **E.** extra extra large. Photos taken in 750 g punnets.

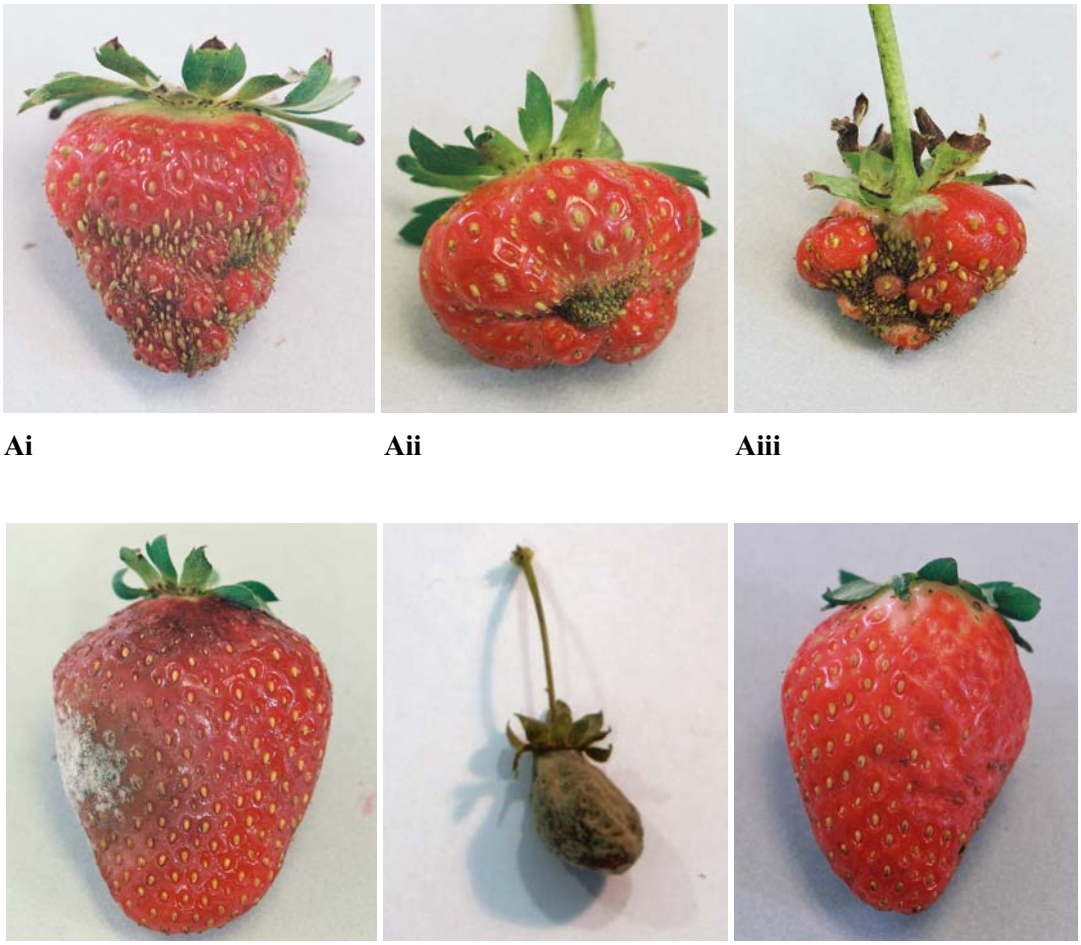


Figure 2.10 Unmarketable strawberries. Symptoms not caused by powdery mildew. **Ai-iii**. Misshapen. Causes: frost, mites, poor pollination (López-Medina *et al.*, 2004). **Bi-ii**. Fungal damage other than powdery mildew (picture shown is grey mould). **C**. Loss of achenes. Cause unknown.

2004/05 and 2005/06 seasons the scale for assessing powdery mildew severity on harvested strawberries was applied at this point.

2.8 Analysis of data

Analysis of variance (ANOVA) was undertaken using Genstat® version 8.0 (Rothamsted, U.K.) and correlation analysis was performed using Microsoft Excel®. Least Significant Differences (LSDs) between means and the probability of significance (f prob) were calculated using Genstat®. Other analyses are described in appropriate chapters.

2.9 Photography

Photographs of the field experiments were taken using a Nikon Coolpix 995 SLR digital camera. Images of fruit were taken using a Minolta SRT101 SLR Camera with a 100 mm macroscopic bellows lens and developed commercially. Microscopic images were captured using a Leica DC 300F camera and Leica IM1000 Image Manager software (Imagic Bildverabeitung AG). Blemishes not associated with the content of the image (eg. scratches on the film) were removed using Adobe Photoshop® 8.0 software (Adobe Systems Inc.).

Chapter 3 Epidemiology of *P. aphanis* on strawberry in South Australia

3.1 Introduction

Powdery mildew is of economic importance to South Australian strawberry producers as it affects yield and fruit quality. Historically, producers have used susceptible cultivars as they have a long fruiting season (October to May) and fruit relatively well in the South Australian environment. The limited availability of effective fungicides (particularly those with short withholding periods) and local environmental conditions that favour powdery mildew development have added to difficulties in effectively managing this disease (P. James, personal communication, 2003).

The susceptible strawberry cultivar Selva accounted for over 95 % of South Australian production between 2003 and 2006 (P. James, personal communication, 2006), however, to date no other cultivar has yielded equal to or better than Selva in South Australian conditions. Other day-neutral cultivars grown in South Australia to supplement harvest when Selva yields are low are Camino Real and Aromas (D. Parker, personal communication, 2005). These cultivars were only recently released from the University of California Strawberry Licensing Program and show many advantageous agronomic qualities including powdery mildew resistance (The University of Calfornia, Strawberry Licencing Program, 2001). However, Selva remains popular amongst South Australian growers because it fruits for longer than other cultivars (Don Parker, personal communication, 2005). These late season harvests attract premium purchase prices for fruit between late February and May (B. Bishop, personal communication, 2003). Fungicides are therefore required to manage powdery mildew in Selva crops.

Foliar symptoms of powdery mildew mainly consist of visible white mycelial growth and conidiophores. Mycelial colonies are usually found on the underside of the

leaf, and appear as small and defined in the early stages of disease, coalescing to cover the leaf surface (Section 1.2). In severe cases the leaf edges curl upwards or mycelium spreads to the upper surface of the leaf, petioles, peduncles and fruit (Maas, 1984). Incidence and severity of powdery mildew are generally assessed by examining leaves (Peries, 1962b; Gooding *et al.*, 1981; Miller *et al.*, 2003). Symptoms on fruit are reported to range from mycelial growth barely visible to the naked eye, to sporulating colonies over the entire surface, rendering fruit unmarketable (Gooding *et al.*, 1981). Deformities such as cracking and stunted growth are also attributed to this disease, however, the severity of powdery mildew on the basis of symptoms on fruit alone, has not been documented.

The main economic loss caused by powdery mildew is the amount of affected fruit discarded, followed by the expense of pesticides (Maas, 1984). Management of powdery mildew relies predominantly on preventative fungicide applications to prevent fungal growth on leaves, thus reducing the source of inoculum. It is assumed that runners imported from Victoria and Queensland are the primary source of inoculum as they are not disinfested before planting (P. Mason, personal communication, 2004). Alternatively, *P. aphanis* may overwinter as mycelium in existing crops, providing inoculum for new plants via wind-borne conidia from neighbouring fields.

Environmental conditions conducive to powdery mildew epidemics can be defined according to different stages of fungal development (Engelhard, 2004), including conidial dispersal, germination and colony development. The number of conidia released for dispersal increases with temperature and decreases with RH and rainfall, while dispersal is increased with wind velocity and peaks in mid-afternoon (Blanco *et al.*, 2004). Germination occurs within 12 hours of inoculation (Peries, 1962b) and is inhibited by free water (Sivapalan, 1993a). Optimal conditions for germination

are 15 to 25 °C (Peries, 1962a) and 95 % RH (Jhooty and McKeen, 1964; Mukerji, 1968). Colony development is optimal in temperatures ranging from 22 to 27 °C irrespective of RH (Peries, 1962a; Miller *et al.*, 2003). Incidence of powdery mildew decreases as rainfall increases (Boughey, 1949). The warm, dry conditions that favour powdery mildew development are common in the South Australian summer.

A secondary objective of this study was to compare powdery mildew development on the leaves and fruit of the susceptible cultivar Selva with that on the resistant cultivar Aromas, in South Australian field conditions. To understand development of *P. aphanis* on strawberry, microscopic observation of inoculated plant material was performed.

3.2 Materials and methods

General materials and methods common to other chapters have already been described (Chapter 2). Details particular to this chapter are outlined below. Selva was grown over the three seasons, however, Aromas was only grown in the 2003/04 and 2005/06 seasons.

3.2.1 Foliar powdery mildew severity assessment

Severity scales were used to assess powdery mildew on field-grown Selva in the fruiting seasons between May 2003 and May 2006, as described in Section 2.8.1. Assessment was also made of Aromas in the 2003/04 and 2005/06 seasons, but not in the 2004/05 season. Foliar observations of powdery mildew severity were made once or twice per week from December 14 2003 to March 14 2004 using the scale outlined in Table 2.1, and fortnightly from November 1 2004 to April 21 2005 and October 2005 to May 2006 using the scale outlined in Table 2.2. Only data collected from untreated Selva and Aromas plants will be considered in this chapter (for details of further treatments see Chapters 4 and 5). In the 2003/04 season three replicate plots of four

plants were observed and in the second year there were four replicates of four plants. These plots relied on natural inoculum.

3.2.2 Fruit powdery mildew assessment

Fruit was harvested twice weekly in the 2003/04 season (4 plants per plot) and three times per week in the 2004/05 and 2005/06 seasons (10 plants per plot). Harvested strawberries were sorted for unmarketable qualities and in the 2003/04 season the number with each abnormality tallied (Figures 2.7 and 2.9).

3.2.3 Yield assessment

Fruit were harvested and assessed as per Section 2.7. In the 2004/05 and 2005/06 seasons total marketable fruit were weighed in contrast to total weight (including culled fruit) as was measured in the 2003/04 season.

3.2.4 Microscopic observations of *P. aphanis* on Selva and Aromas

Light and scanning electron microscopy were used to observe the development of *P. aphanis* on leaves of Selva and Aromas. Detached leaves were inoculated and maintained as per Section 2.4.1. Samples were taken at 2, 4, 6 and 8 dpi by randomly selecting and removing half of a trifoliate leaflet (without the midrib) with a scalpel. Development of *P. aphanis* was observed using light microscopy as per Section 2.7.1. Small samples (2 x 2 mm) were randomly selected from inoculated tissue for SEM.

3.2.5 Analysis of data

The approximate affected leaf area for each disease score class was calculated based on the average plant having 300 to 600 cm^2 surface leaf area and the average fungal colony covering 0.25 cm^2 when first visible to the naked eye. Disease scores were then transformed to affected leaf area using the median percentage leaf area relative to the disease score. Repeated measures ANOVA (as per Section 2.8) were used

to analyse the effect of cultivar on the affected leaf area and percentage harvest culled due to powdery mildew. The relationship between fruit abnormality and foliar disease severity was analysed using Pearson's correlation test.

3.3 Results

3.3.1 Incidence and severity of powdery mildew in the field in South Australia

Powdery mildew was severe in the 2003/04 and 2004/05 seasons (Figures 3.1 and 3.2, respectively). Foliar powdery mildew for cultivar Selva reached 32.5 % leaf area in the 2003/04 season and 28.3 % leaf area in the 2004/05 season. Little or no disease was observed for most of the 2005/06 season (Figure 3.3) where powdery mildew on Selva only reached 0.3 % leaf area. Disease was most severe in February 2004 and January to February 2005.

From the 2003/04 season data, no significant correlations were found between foliar powdery mildew severity and the number of cracked, misshapen or powdery fruit (correlation coefficient = -0.022, 0.316 and 0.196, respectively). Of the abnormal fruit symptoms observed in the 2003/04 season, cracking and deformity were most frequently associated with powdery mildew affected fruit. However, there appeared to be other factors that caused these symptoms as well. The way in which the data were collected prevented separation of these for analysis.

In the 2004/05 season the percentage of harvest culled due to powdery mildew symptoms showed trends similar to the severity of foliar powdery mildew (Figure 3.2). Symptoms on fruit were observed 30 to 60 days after the most severe symptoms on leaves. Mycelial growth on strawberry fruit was observed from February to the end of May 2005. Few strawberries were culled due to powdery mildew damage in the 2005/06 season, coinciding with minimal foliar disease (Figure 3.3).

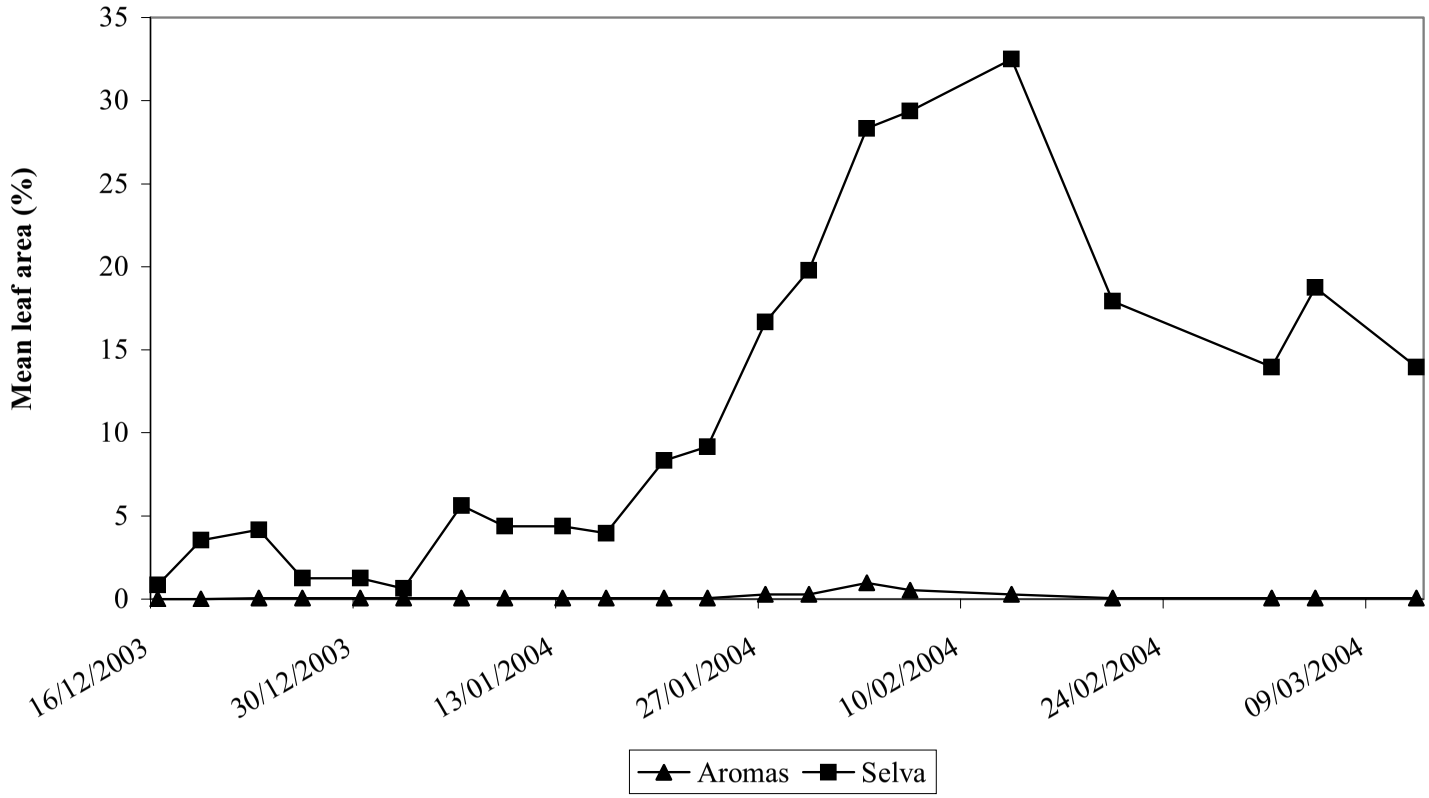


Figure 3.1 Powdery mildew severity on field-grown Selva and Aromas plants in the 2003/04 season. Foliar disease was measured one or two times weekly from 16/12/2003 to 14/03/2004 (Table 2.1). Data shown in mean ± SE for each time point, n = 24 from 3 replicate rows of plants, LSD = 11.79.

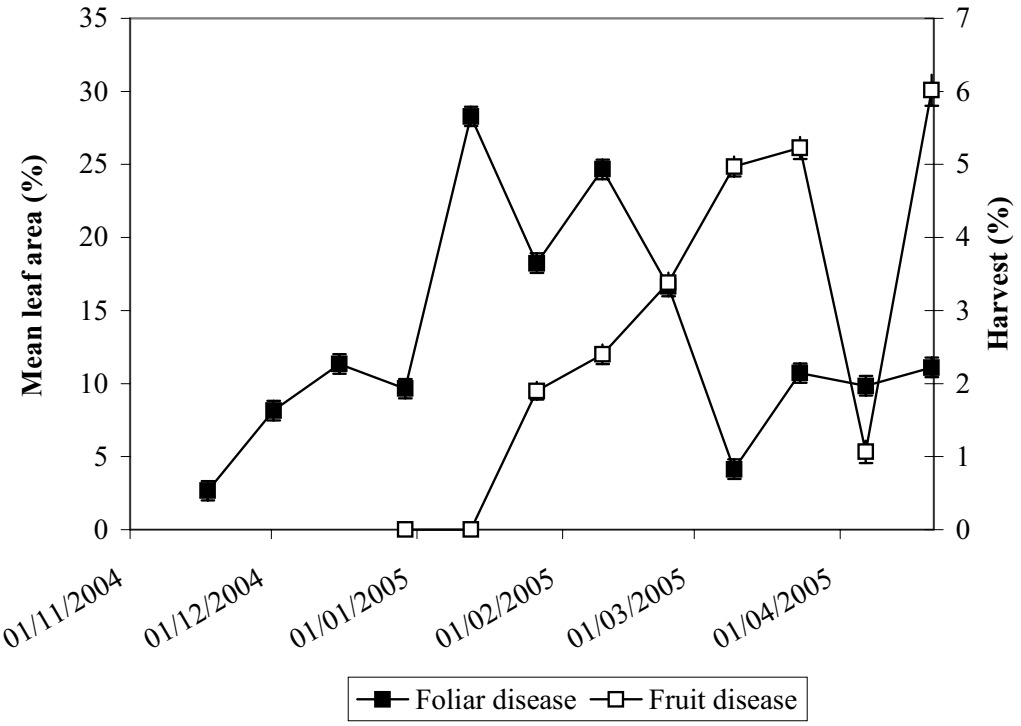


Figure 3.2 Powdery mildew severity on leaves of field-grown Selva plants and the percentage of harvest culled due to powdery mildew in the 2004/05 season. Foliar disease was measured fortnightly, from 14/11/2004 to 16/04/2005 (n = 16, over 4 replicates) (Table 2.2). Fruit disease was assessed when harvested, two or three times weekly, from 14/11/2004 to 16/04/2005. Percent harvest culled was calculated fortnightly (n = 40, over 4 replicates). No powdery mildew was observed on fruit prior to 07/03/2005. Data shown are means ± SE.

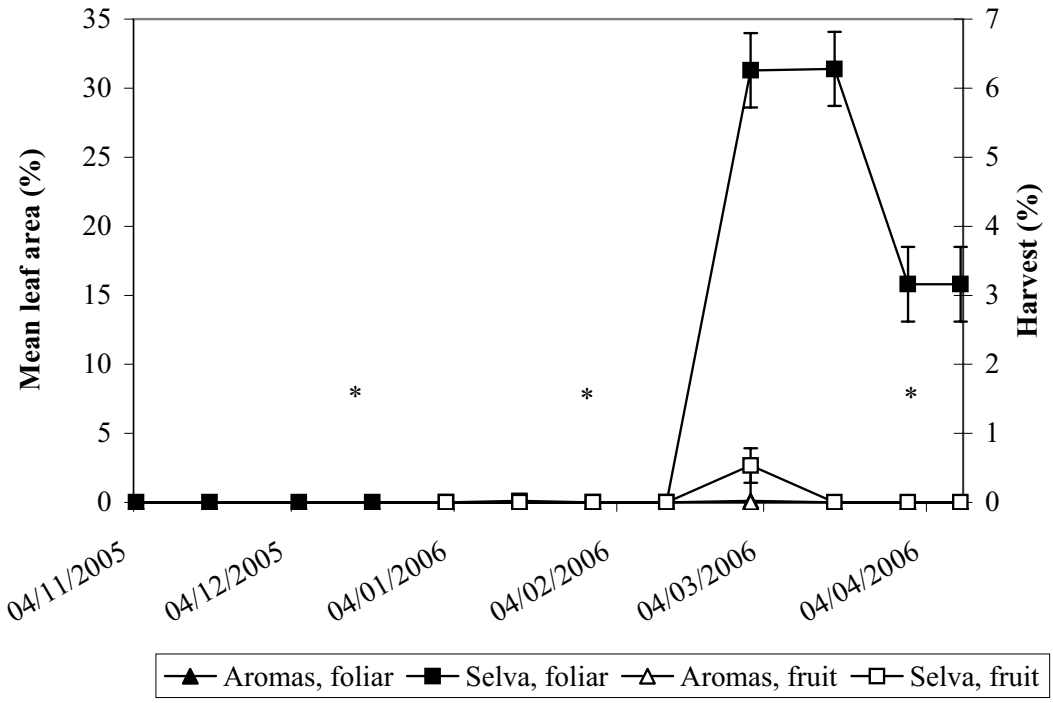


Figure 3.3 Powdery mildew severity on leaves of field-grown Selva and Aromas plants and the percentage of harvest culled due to powdery mildew in the 2005/06 season. Foliar disease was measured fortnightly, from 04/11/2004 to 14/04/2005 (n = 16, over 4 replicates) (Table 2.2). LSD of mean foliar disease score between cultivars = 0.2470. Fruit disease was assessed when harvested, two or three times weekly, from 02/01/2006 to 14/04/2006. Percent harvest culled was calculated fortnightly. Data shown are means ± SE. * indicates application of Flint® (300 g/ha) by the grower.

There was significantly less powdery mildew (measured by the leaf area) on cultivar Aromas than on Selva regardless of season. In the 2003/04 season, the mean affected leaf area for Aromas plots was never greater than 1 %, well below the economic threshold of 5 %. Incidence of powdery mildew on Aromas was 67 % less than on Selva and no individual plant had more than 5 % affected leaf area throughout the season. In the 2005/06 season, powdery mildew was only found on Aromas on 29th February 2006 (Figure 3.3), and only two individual plants were scored 1 for severity (equivalent of <0.01 % affected leaf area per plant).

The number of strawberries from Aromas with mycelial growth on the receptacle was 60 % less than those from Selva in the 2003/04 season. In the 2005/06 season, no strawberries harvested from Aromas were found to have mycelium of *P. aphanis* (Figure 3.3). The number and weight of harvests from Aromas and Selva were similar although significantly fewer fruit from Aromas were culled (Table 3.1).

3.3.2 Association of incidence and severity of powdery mildew on field-grown strawberry plants with temperature, rainfall and humidity

Visual associations were made between the weather data and the trends of foliar powdery mildew severity. In the 2003/04 season, severity increased with warm days (>20 °C) and warm, humid nights (>10 °C, 80 % RH) (Figure 3.4). Severity appeared to decrease when night RH was below 30 %. Rainfall events exceeding 5 mm appeared to conincide with reduced powdery mildew severity. In the 2003/04 season, plants were 'overhead-irrigated' on days exceeding 32ºC to minimise heat damage to the strawberries. Thus, during the two hot periods towards the end of the season (the beginning of February and the beginning of March), the plants would have been irrigated for approximately 6 hours every day.

In the 2004/05 season (Figure 3.5), disease was also more severe when nights

Table 3.1 Yield comparison of Aromas and Selva in the 2005/06 season at Woodside, South Australia. Fruit were harvested two or three times weekly between 1/01/2006 and 28/04/2006. Means given are for each plant.

Cultivar	Total weight (g)	Number marketable	Mean fruit weight (g)	Percent Cull
Selva	5865[a]	441[a]	13.30[a]	18.45[a]
Aromas	6871[a]	516[a]	13.28[a]	12.18[b]

Superscript denotes statistical difference (F prob < 0.05).

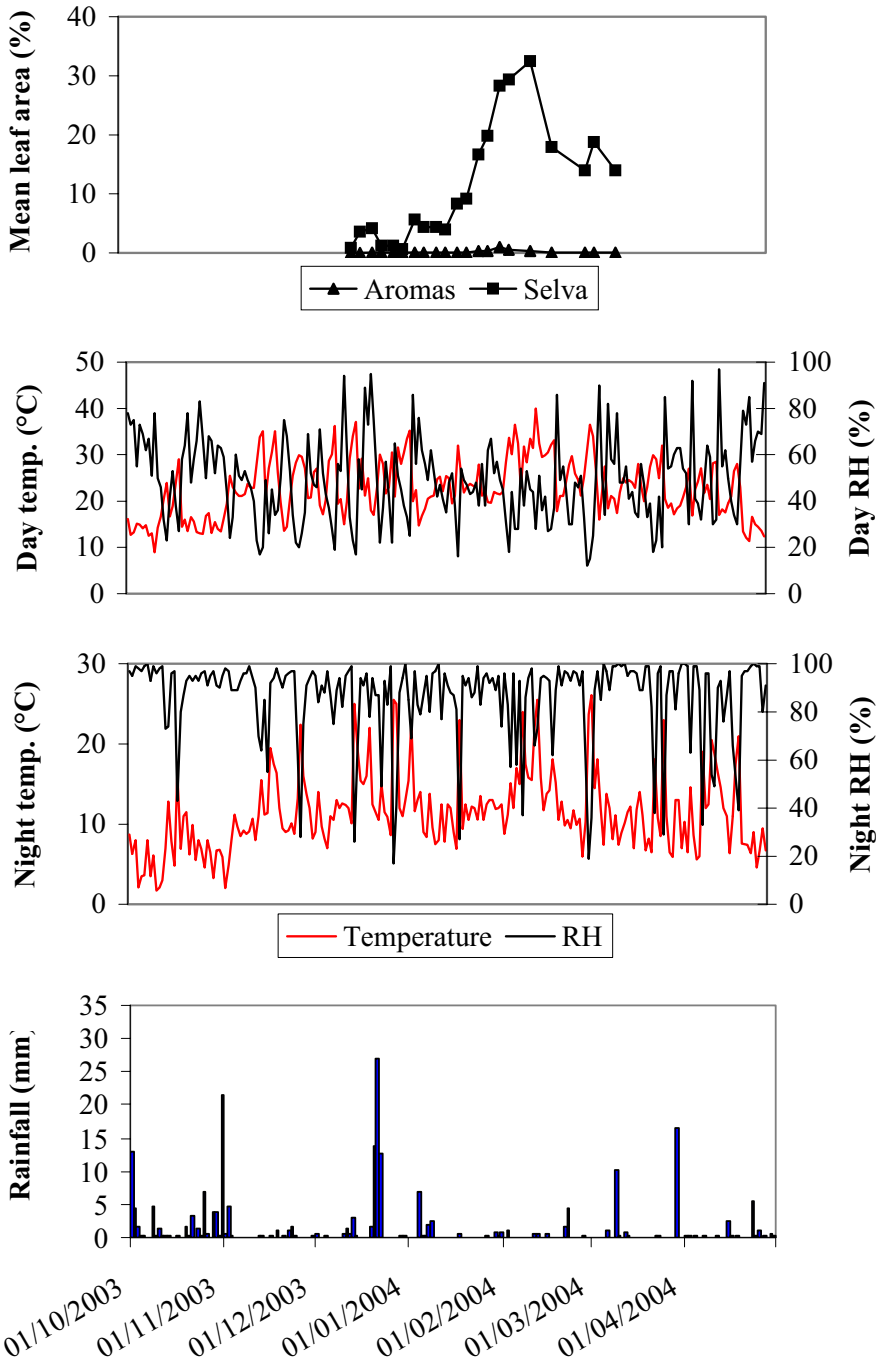


Figure 3.4 Powdery mildew severity and environmental factors in the 2003/04 season. Leaf area affected by powdery mildew (repeated from Figure 3.1 for comparison), day temperature (°C) and RH (%) (taken at 3 pm), night temperature (°C) and RH (%) (taken at 6 am the following morning) and daily rainfall (mm) (taken at 3 pm).

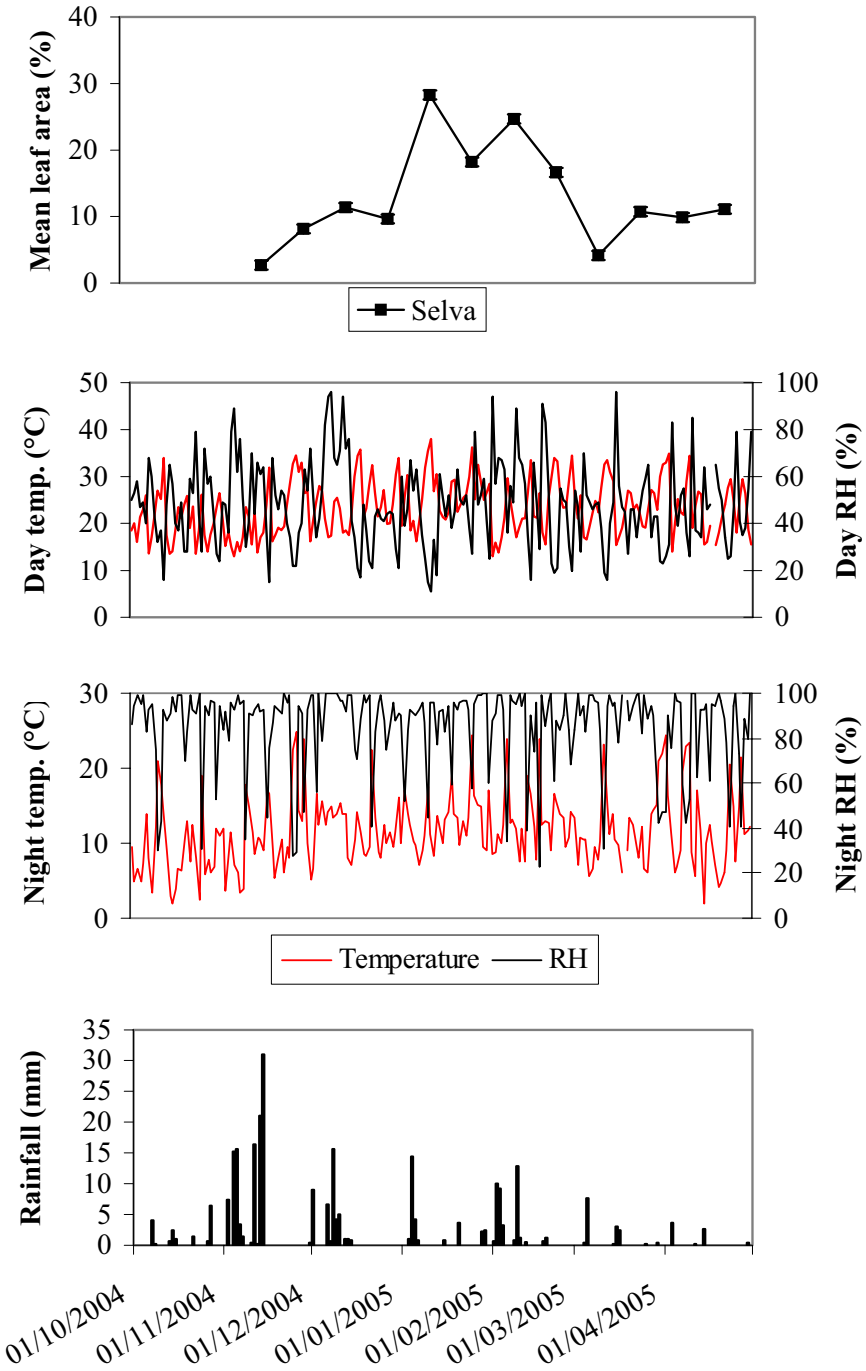


Figure 3.5 Powdery mildew severity and environmental factors in the 2004/05 season. Leaf area affected by powdery mildew (repeated from Figure 3.2 for comparison), day temperature (°C) and RH (%) (taken at 3 pm), night temperature (°C) and RH (%) (taken at 6 am the following morning) and daily rainfall (mm) (taken at 3 pm).

were warm (>10 °C) and humidity was consistently high (>85 %). There were fewer dry nights in the 2004/05 season. However, the night of 17/02/2005 registered 23 % RH, and the following assessment showed disease was significantly less severe. The weather was relatively wet early in the 2004/05 season (Figure 3.5), with three heavy (>15 mm per day) rainfall events between November and mid-December 2004. There was no overhead irrigation in this season. As for the 2003/04 season, rainfall events greater than 5 mm generally coincided with reduction in disease severity.

The 2005/06 season was the only season when disease assessment began prior to the appearance of powdery mildew in the commercial plots where the trials were planted. When infection did become established in the trial plot of Selva towards the end of February 2006, severity increased after a period of warm nights. However, the increase in severity was not significant (Figure 3.6). While association between weather and disease severity was inconclusive during this season, associations between weather conditions and the incidence of powdery mildew were observed. A hot, dry day (>28 °C, <55 % RH, no rain) with a warm, very humid night (>10 °C, >85 %, no rain), followed by 3 or more days of moderate day temperature (18 to 30 °C) and warm humid night conditions (>10 °C, >75 % RH) appeared to occur just before powdery mildew appeared in the trial plots (Table 3.2).

3.3.3 Development of *P. aphanis* on susceptible and resistant leaf tissue

On susceptible tissue, germinating conidia were found, with hyphal growth extending across as many as four epidermal cells from the site of penetration from 2 days post inoculation (dpi) (Figure 3.7). Appressoria could not be discerned using light microscopy, but were visible using SEM (Figure 3.8). During observation using light microscopy it was assumed that penetration and haustorium formation had occurred

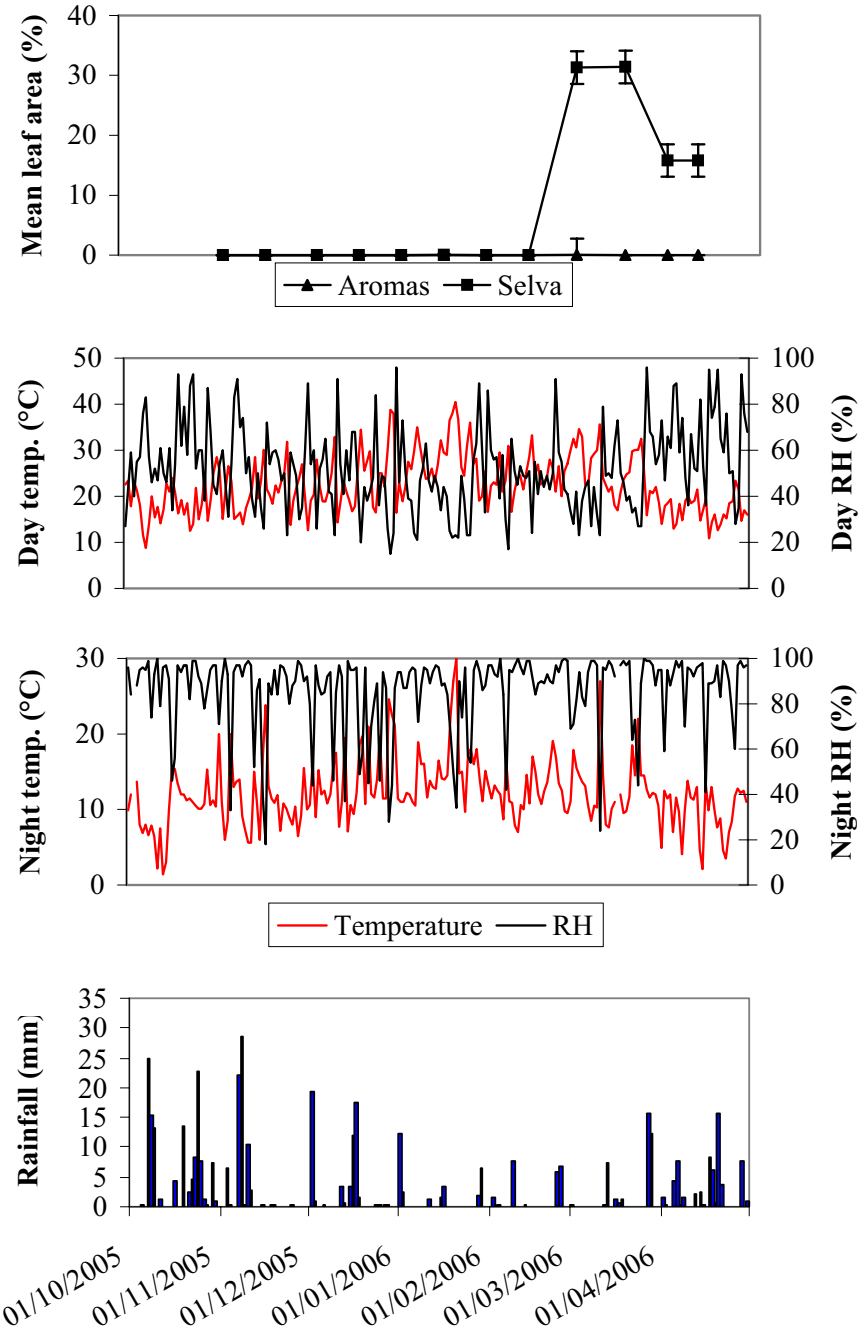


Figure 3.6 Powdery mildew severity and environmental factors in the 2005/06 season. Leaf area affected by powdery mildew (repeated from Figure 3.3 for comparison), day temperature (°C) and RH (%) (taken at 3 pm), night temperature (°C) and RH (%) (taken at 6 am the following morning) and daily rainfall (mm) (taken at 3 pm).

Table 3.2 Weather and foliar powdery mildew of Selva and Aromas for the 2005/06 season. Disease data collected from Woodside, and weather data collected from Mount Barker, South Australia (Sections 2.2 and 2.3). Predicted high risk periods (cells) and increased disease (text) are higlighted.

Nov	Day (1500)		Rain	Night (0600)		Leaf Area (%)		Dec	Day (1500)		Rain	Night (0600)		Leaf Area (%)		Jan	Day (1500)		Rain	Night (0600)		Leaf Area (%)	
	T (°C)	RH (%)	(mm)	T (°C)	RH (%)	Aromas	Selva		T (°C)	RH (%)	(mm)	T (°C)	RH (%)	Aromas	Selva		T (°C)	RH (%)	(mm)	T (°C)	RH (%)	Aromas	Selva
1	28.5	41	0	20	71			1	19.4	61	0	10	92			1	16.4	96	12.4	11.5	94		
2	25.5	54	0	11	90			2	12.6	89	19.4	10.6	80			2	22.9	46	2.4	11	94	0	0
3	15.1	60	6.6	6	100			3	19.1	54	0.8	16.2	44			3	18.9	73	0	11	87		
4	21.4	45	0.2	8.5	93	0	0	4	20.4	60	0	9	97			4	22	51	0	12.2	87		
5	26.5	31	0	20	33			5	28	26	0	15.2	88	0	0	5	27.5	39	0	12	94		
6	23.2	54	0	13	94			6	22.1	52	0.4	12	84			6	25.9	38	0	11	96		
7	15.1	83	22.2	13.7	97			7	18.9	56	0	12.5	85			7	29.7	24	0	10.5	95		
8	15.7	91	28.4	14	97			8	18.8	65	0	10.8	92			8	35	21	0	18.9	72		
9	16.5	70	0.4	9.1	92			9	21.6	42	0	12	94			9	31	47	0	16	88		
10	13.9	74	10.4	7.3	97			10	25	41	0	15.5	46			10	27.2	52	0	16.1	96		
11	17.5	50	2.8	5.6	99			11	32.9	23	0	17.5	85			11	23.8	63	1.2	11.6	95		
12	19.3	57	0	5.6	97			12	14.3	91	3.4	7.7	97			12	24	47	0	13.8	89		
13	21.7	39	0	15	52			13	18.5	54	0.6	11	92			13	26	42	0	13	94		
14	28.5	31	0	10.9	86			14	23.4	41	0	19.5	37			14	23	49	0	12.7	97		
15	19.5	50	0.2	6	91			15	21.1	60	3.4	7.1	99			15	26.8	44	1.6	16.5	96		
16	23.2	35	0	17	39			16	19	47	12	10.6	95			16	32.2	34	3.4	14.1	88	0	0.1
17	30.1	26	0	23.8	18			17	16.7	68	17.6	9.4	95			17	29.5	44	0	14	89		
18	21.5	72	0.4	13.1	89	0	0	18	17.8	68	1.4	12.1	96			18	29	40	0	14.5	84		
19	20.3	54	0.4	11.5	84			19	26.5	43	0	18.6	49	0	0	19	36.5	25	0	22.3	67		
20	18.4	59	0	10.9	95			20	34.5	20	0	19.7	58			20	38	22	0	27	50		
21	22.4	60	0	11.9	84			21	25.6	44	0	10.7	96			21	40.5	23	0.1	30	34		
22	20.8	56	0	7.2	97			22	27.6	38	0	21	45			22	36.7	22	0	14.8	90		
23	23.4	47	0	10.8	96			23	29.8	42	0	12.1	70			23	26.3	49	0	15	74		
24	25.1	50	0	10.1	92			24	17.6	48	0.4	11.6	81			24	24.4	39	0	9.7	96		
25	31.8	23	0.2	9	80			25	16.5	84	0.4	14	89			25	30.9	23	0	17	57		
26	13.8	59	0	8	88			26	25	36	0	19.7	46			26	36	23	0	18	54		
27	19	54	0	10.4	90			27	25	49	0.4	11.5	94			27	27	55	0	16	95		
28	21.7	49	0	6.5	99			28	22	48	0.2	11.5	87			28	28.2	63	1.8	18	99		
29	24	30	0	9.2	97			29	30	26	0	24.6	28			29	19	89	6.4	14	94		
30	27	35	0	15.5	90			30	38.8	15	0	22.5	44			30	19.9	62	0	11.1	86	0	0
								31	38	24	0	21.2	87			31	25.4	33	0	15.1	88		

Table 3.2 Continued.

Feb	Day (1500)		Rain	Night (0600)		Leaf Area (%)		Mar	Day (1500)		Rain	Night (0600)		Leaf Area (%)		Apr	Day (1500)		Rain	Night (0600)		Leaf Area (%)	
	T (°C)	RH (%)	(mm)	T (°C)	RH (%)	Aromas	Selva		T (°C)	RH (%)	(mm)	T (°C)	RH (%)	Aromas	Selva		T (°C)	RH (%)	(mm)	T (°C)	RH (%)	Aromas	Selva
1	16.6	86	0	13.2	97			1	29.8	34	0.2	11.1	69			1	14	73	1.6	4.9	95		
2	22.3	60	1.6	11.5	97			2	32.5	28	0	17.9	71			2	17.9	47	0.2	12.5	59		
3	23	56	0	13.2	93			3	30.6	42	0	15.5	82			3	18.5	66	0	11.5	95		
4	22.5	57	0.2	12.5	92			4	34.6	23	0	14.5	94			4	19.4	61	0	12	88		
5	29.5	39	0	12	100			5	33	38	0	13.7	83			5	13	88	4.2	7	93		
6	21	58	0	8.7	84			6	22.5	44	0	13.1	79			6	14	89	7.8	11.6	99		
7	23.5	30	0	16.5	42			7	22.7	47	0	10.6	94			7	18.4	59	0.2	9.8	96		
8	31	17	0	11.1	95			8	28.3	27	0	8.5	99			8	14.8	74	1.4	4.1	99		
9	16.6	65	7.6	10.9	94			9	29.2	44	0	10.5	99			9	19	48	0	9.9	70		
10	21.2	51	0	7.9	97			10	30	32	0	10.3	97			10	20	36	0	13.8	96	0	15.8
11	23.9	45	0	7	100			11	35.6	23	0	27	24			11	18.5	67	0	11.6	95		
12	24.6	53	0	10.6	96			12	24	79	0.4	14.7	96			12	19	52	2.2	11.3	92		
13	21.5	49	0.4	10	93	0	0	13	22.5	49	7.4	8	95			13	21.5	51	0	13	96		
14	25	48	0	14.6	99			14	21	50	0	7.6	99			14	14.7	82	2.6	4.7	97		
15	28.5	51	0	10.8	99			15	22.1	48	0	10.1	97			15	17.1	53	0.2	2.1	98		
16	33.3	24	0	17	91			16	18	62	1.2	11	92			16	19.4	36	0	13.5	41		
17	24.5	55	0	15	84			17	17	73	0.6			0	31.4	17	10.9	95	8.4	9.9	89		
18	26.9	41	0	12	89			18	21	50	1.2	12	97			18	14.4	74	6.2	13	89		
19	22.6	51	0	10.7	90			19	23.6	46	0	9.5	99			19	16	79	0.6	10	90		
20	23	44	0	12.4	89			20	24.8	38	0	9.8	97			20	12.6	95	15.8	7.6	97		
21	24.1	49	0	13.6	93			21	25.2	40	0	11.7	99			21	13.9	65	3.6	8.8	83		
22	28	43	0	16	90			22	29.8	33	0	18.5	64			22	16.1	59	0	4.6	99		
23	26	50	0	19.1	89			23	30.1	35	0	14.5	73			23	15.2	76	0	3.5	97		
24	21	91	5.8	16.9	97			24	30.1	27	0	22	44			24	18.5	50	0	7	90		
25	26.6	59	6.8	13.5	94			25	32.5	27	0	14.5	89			25	18.9	51	0	8.4	77		
26	20	55	0	12.5	99			26	25.5	58	0	14.5	100			26	23.4	28	0	11.8	60		
27	25.4	43	0	9.8	100			27	15.9	96	15.6	12.6	99			27	21.5	35	0	12.8	97		
28	27.1	41	0	9.5	99	0.1	31.3	28	21.1	68	12.4	11.6	99			28	14.6	93	7.8	12.2	99		
								29	20.7	66	0	12.2	97			29	16.9	76	0	12.5	96		
								30	22	54	0	12	88			30	16	68	0.8	11	97		
								31	18.6	58	0	10.5	95	0	15.8								

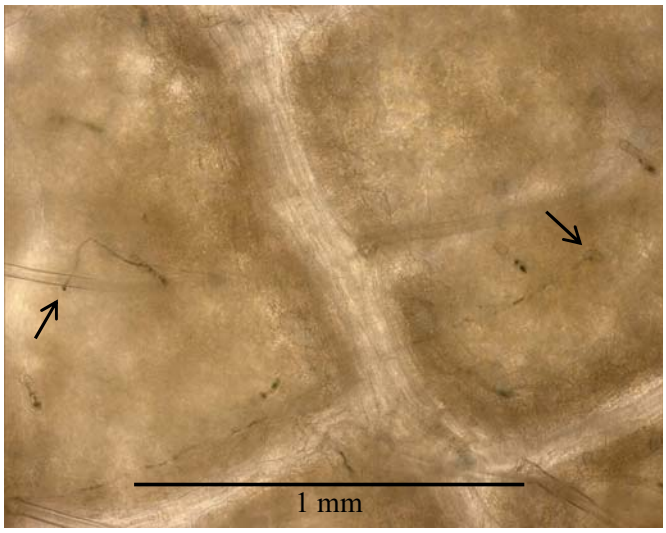


Figure 3.7 Germinating conidium of *Podosphaera aphanis* (indicated by right arrow) on detached Selva leaf, 2 dpi, observed using light microscopy. Hyphal growth extends over epidermal cells adjacent to the site of penetration. Immature conidiophores are evident (left arrow).

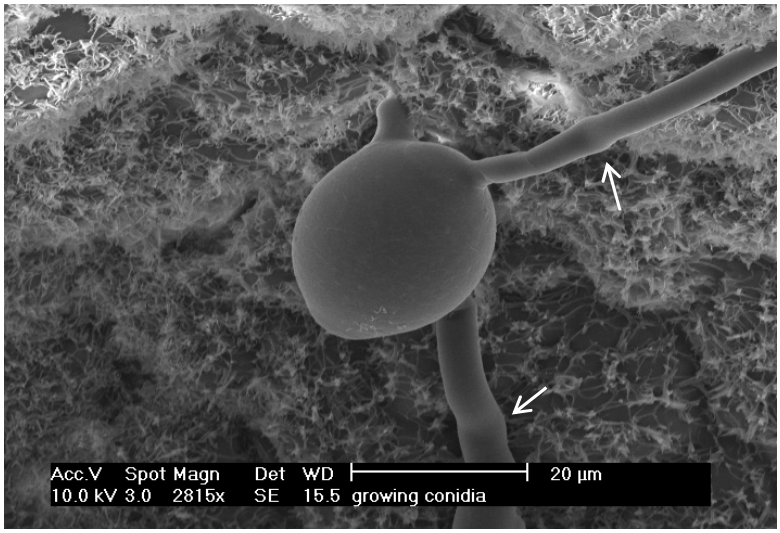


Figure 3.8 SEM of *Podosphaera aphanis*, showing conidium, hypha and appressoria, on Selva leaf tissue. Appressoria are indicated by arrows.

where hyphae had spread over such a distance. By 2 dpi conidiophores had started to develop and by 4 dpi these bore chains of hyaline conidia (Figure 3.9). After 7 dpi powdery mildew on the leaf surface was discernable with the naked eye (Figure 3.10).

Careful inspection of detached Aromas leaves inoculated with *P. aphanis* revealed no conidia attached to specimen leaf tissue after the clearing and staining procedure (data not shown).

3.4 Discussion

Successful management of disease relies on a sound understanding of both crop and pathogen life cycles and how they interact. These studies involved observation of powdery mildew in strawberry crops located in South Australia over three seasons to determine conditions that might be associated with epidemics and how this information could be used to minimise the impact of this disease.

Both the 2003/04 and 2004/05 seasons were considered severe for powdery mildew in the Adelaide Hills region (P. Mason, personal communication, 2005). The onset of disease was earlier in the 2004/05 season than the 2003/04 season. An epidemic was already established before harvesting commenced in October 2004. In contrast, in the 2005/06 season little powdery mildew was recorded. While it is believed that environmental conditions were the main reason for the lack of disease, comparison was confounded as this experiment was carried out at a different location with different cultural practices, such as double compared with single row plantings, and also coincided with the release of Flint® for use on strawberry crops in South Australia. The higher planting density of double row plantings used in the 2005/06 season, resulted in smaller plants and reduced density of the canopy. This would increase air circulation, restricting fungal development and increasing efficacy of fungicide application. As the

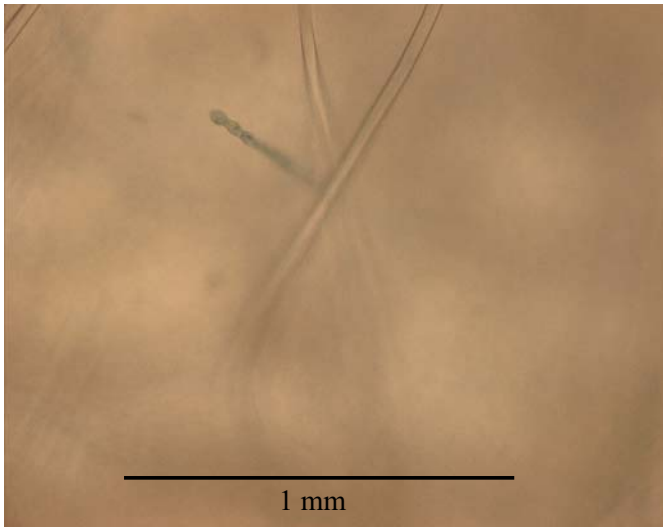


Figure 3.9 Chain of hyaline *Podosphaera aphanis* conidia still attached to conidiophore. Observed at 4 dpi on Selva using light microscopy.

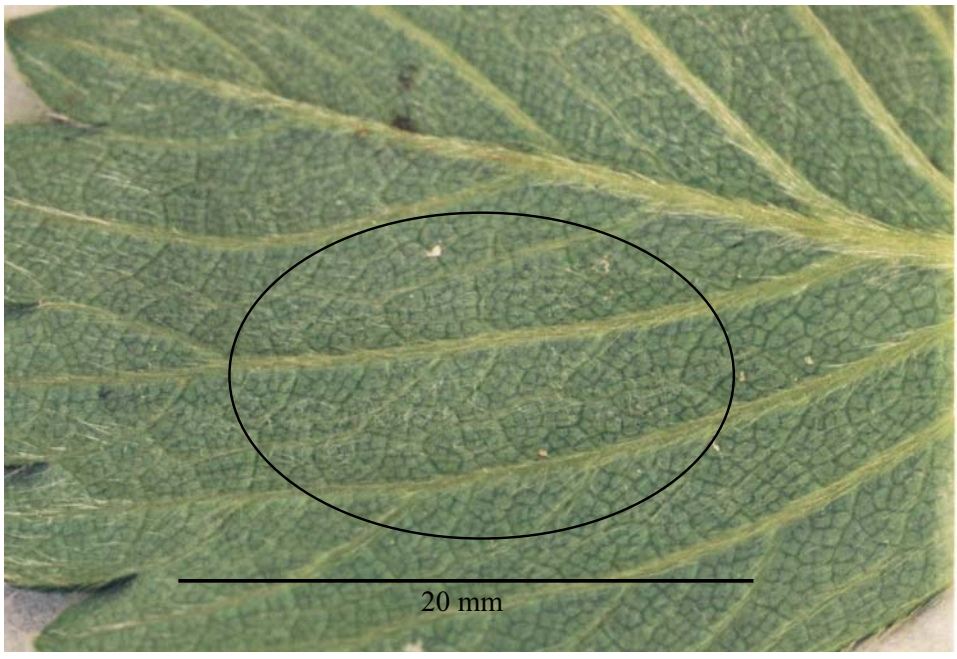


Figure 3.10 Powdery mildew on Selva leaf visible with the naked eye at 8 dpi.

grower was instructed to tend the trial plots as for commercial plots, Flint® was applied regularly during this trial. However, as a result of the curative effect of Flint® applied during this season, onset of new infections, and the environmental conditions that may have been conducive to this, were easier to observe.

There have been many studies of the effect of environment on the development of *P. aphanis* on strawberry, in both controlled and field conditions. However, no reports have been found of association between environmental conditions and powdery mildew severity on strawberry plants grown in commercial production conditions, as there have been for other pathosystems. Following studies of the closely related *P. macularis* on hop, Engelhard (2004) suggested that certain weather conditions appeared necessary to result in powdery mildew. These were a night of >10 °C with >1 mm rain followed by two days of >10 °C with <4000 Wh/m^2 wind speed and 1 mm rain per day or >5 mm in the days before. However, these suggestions were based on the assumption that ascospores were the primary inoculum, whereas primary inoculum of *P. aphanis* is thought to be conidia produced from mycelium that has persisted on foliage between seasons. In interpreting the data collected during the fruiting seasons between 2003 and 2006, disease development was defined by three stages, each requiring different specific environmental conditions. These stages were conidial dispersal, germination and colony development. Conditions conducive to each developmental stage were determined from the literature and compared with the weather and disease information recorded in these studies. The weather conditions deduced to be conducive for powdery mildew epidemics are summarised in Table 3.3 and detailed below. The presence of primary inoculum generated from overwintered mycelium on strawberry plants or *Eucalyptus* (Cunnington *et al.*, 2003), of which there were stands nearby all trial plots, was assumed.

Table 3.3 Weather conditions associated with onset of powdery mildew epidemics in strawberry crops in South Australia.

First day:	>28 °C, <55 % RH, no rain
First night:	>10 °C, >90 % RH, no rain
Following days:	>15 °C, <70 % RH (day time) with >8 °C, >80 % RH (night time), <2 mm rain per day

Conidiation of *P. aphanis* under field conditions is known to be increased by elevated temperatures when humidity is low (Blanco *et al.*, 2004) so the number of conidia in the air would be greatest during hot, dry conditions. The range of dispersal is also significantly affected by wind speed (Blanco *et al.*, 2004), however, wind speed data were not available for this study.

Germination of *P. aphanis* conidia in controlled conditions has been studied extensively (Miller *et al.*, 2003). The optimum temperature range for germination of *P. aphanis* conidia is 15 to 25 °C (Peries, 1962a; Amsalem *et al.*, 2006) but can successfully germinate in temperatures between 4 and 36 °C (Miller *et al.*, 2003). Conidia also germinate best above 75 % RH, but can germinate at as low as 8 % RH (Jhooty and McKeen, 1964; Schnathorst, 1965; Mukerji, 1968; Amsalem *et al.*, 2006). Unlike many other plant pathogenic fungi, conidia of powdery mildew fungi contain enough water for germination which facilitates germination in unfavourable conditions (Schnathorst, 1965). Free water has a lethal effect on conidia and no germination occurs on wet leaf surfaces (Peries, 1962a; Schnathorst, 1965; Perera and Wheeler, 1975). This concurs with the lack of disease increase observed after temperatures suited to conidiation and germination when there has been rain.

The time interval between conidial dispersal and germination was also considered. Studies of Byrne *et al.* (2000) and Blanco *et al.* (2004) have shown that concentrations of conidia follow a diurnal pattern and are at their highest between 1 and 3 pm. The weather reading at 3 pm would represent conditions that would influence conidial dispersal. Given suitable conditions, germination can be expected to occur within 6 hours (Peries, 1962a), so the weather conditions on the night following those conducive to conidial dispersal would therefore affect the number of successful

infections established. The weather data collected at 6 am were used to indicate night conditions and associated germination requirements.

Rainfall adversely affects all stages of strawberry powdery mildew (Peries, 1962a; Blanco *et al.*, 2004). Rain is detrimental to conidial dispersal as it acts to wash conidia from the air (Blanco *et al.*, 2004). Once impacted on the leaf surface, conidia can easily be washed off by rain drops before germination and attachment to the leaf tissue, however, the ability of conidia to withstand rainfall increases with time (Sivapalan, 1993b). In studies of *P. pannosa* on rose, Sivapalan (1993b) also found that mycelial growth and the number of conidiophores produced were reduced up to 70 % by simulated rainfall events occurring 24 to 60 hpi. This concurs with studies of Perera and Wheeler (1975) who measured significant reduction in colony growth if leaves were kept wet for 4 or more hours after inoculation. Colonies on the lower leaf surface and sheltering by other leaves reduces the impact of rain on mildew development. From this it was determined that little or no rain (defined in this study as <2 mm in a 24 hour period) would increase risk of powdery mildew infection, as observed when regular or heavy rain reduced disease (Table 3.2).

The conditions conducive to colony development are less specific and more variable between studies, however, they can be summarised as warm dry days, with warm humid nights (Miller *et al.*, 2003; Blanco *et al.*, 2004; Davik and Honne, 2005). From the literature, day temperatures above 15 °C and night conditions of above 8 °C and >80 % RH were considered conducive to colony development. Irradiation can have a detrimental effect on powdery mildew in strawberry crops, with cloudy conditions and shade facilitating colony development and production of conidia (Amsalem *et al.*, 2006). Leaf surface temperatures in bright sunlight are often 10 °C above ambient and RH is often 20 % above ambient (Schnathorst, 1965). Measurements of wind speed and

direction, duration of leaf wetness and intensity and length of irradiation may be important to consider in future studies of this nature (Hardwick, 2006).

The conditions identified as likely to be conducive for onset of an epidemic, as outlined in Table 3.2, were associated closely with observations of powdery mildew incidence in the 2005/06 season when disease was controlled by applications of Flint® (see Figure 3.3). While there were no obvious periods of conditions favourable to the onset of an epidemic leading into the 2003/04 and 2004/05 seasons, there are many warm days and nights that would have been favourable for colony development. High inoculum potential on nearby plants in both of these seasons would provide ample inoculum without conditions optimal for conidiation and dispersal. These findings support anecdotal evidence (P. Mason, personal communication, 2003) that minimising inoculum in the field is an important factor in controlling powdery mildew in strawberry crops under South Australian conditions.

Further observations over future seasons are required to consolidate information on environmental conditions necessary for disease so that effective forecasting can be achieved. Ideally, weather stations need to be located on future trial sites, and be capable of measuring wind speed, direction and leaf wetness as well as temperature, RH and rainfall. Furthermore, sensors within the canopy of the crop could be used to measure microclimate. This could also account for the effect of leaf hairs, which appear to be denser on young tissues. This study was limited to meteorological data collected approximately 10 km from the trial sites, however, local industry representatives concur that the conditions recorded by this station are usually a good indication of local conditions (P. Mason and P. James, personal communication, 2003, I. Daly, personal communication, 2006). Forecasting periods which are high risk for onset of powdery mildew epidemics would allow growers to optimise fungicide applications, such as the

UC Davis powdery mildew risk index used in the USA for grape farming. This index has now been adapted for use in grape (Gubler *et al.*, 1999), cherry (Grove *et al.*, 2000) and hop crops (Mahaffee *et al.*, 2003). A similar prediction system (Magarey *et al.*, 1991) is used for strategic treatment of grapevine downy mildew in South Australia with warnings of conducive periods for disease announced on local televised weather bulletins (Magarey *et al*., 2005). Reduced fungicide application would minimise selection for fungicide resistant mutants in the fungal population, as well as catering to growing consumer preference for food produced with minimal chemicals. Furthermore, preventative fungicide application schedules are frequently disrupted when the ground becomes too wet for the grower to negotiate with a tractor. Given that the conditions predicted to promote conidial dispersal and germination are warm days and nights without rain, it is unlikely that preventative fungicide applications would be missed due to such access problems if this forecasting system was in use. It is also plausible that overhead irrigation applied during the night after a hot day could wash many conidia from the leaves prior to germination and minimise the number of colonies that develop, as if simulating a rain event. Providing there are no adverse affects on fruit quality, this strategy may provide a useful management tool in both conventional and organic production of strawberries.

While *P. aphanis* can develop on leaves and fruit of all ages (Jarvis *et al.*, 2002), enhanced infection is observed on younger leaves (Amsalem *et al.*, 2006) and symptoms are easier to observe on these tissues. In contrast to the disease scale applied in this study, Brahm *et al.* (2005) examined percentage leaf coverage of the first three YOLs only. This may provide a more manageable sample size and minimise variation between operators and sampling times while still giving a good estimation of disease severity should future field studies of this nature be undertaken.

Although no significant correlations were observed between foliar powdery mildew severity and the number of fruit with the symptoms of cracking, malformation or powdery mildew in the preliminary season (Section 3.3.1). Causes such as heat and chemical application likely contributed to these symptoms. Some fruit showed more than one of these symptoms (e.g. powdery and cracked) but the manner in which these data were collected prevents this being taken into consideration in the analyses.

The severity of infection in the fruit appeared to lag behind that of the leaves (Figure 3.2). Pollination to maturity takes 20 to 60 days depending on weather and cultivar (Strand, 1994). This may suggest that the flower is inoculated as it emerges from the crown. It is possible that the ample inoculum on leaves in the 2003/04 and 2004/05 seasons resulted in severe powdery mildew scores on fruit. The microclimate of fruit can be expected to differ from that of leaves. Generally the flowering stem grows higher than the petioles of leaves and, as the fruit mature, is weighed down to rest on the polyethylene mulch. Air circulation and exposure to sun may be greater than for leaves. Also the transpiration rate of fruit is significantly less than that of leaf tissue, limiting the fruit's capacity to regulate temperature via transpiration (Schnathorst, 1965). This would result in increased temperature and reduced RH on the fruit surface. It is logical therefore that germination of conidia would be most successful beneath the calyx where air circulation would be less and RH increased, supporting observations of mycelium most commonly found beneath the calyx. Strawberries with mycelium on a large surface area were frequently harvested from within the canopy of the plant, where air circulation would also be low and the RH relatively high. Plants or cultivars that produce long flowering bracts that fall clear of the plant canopy would therefore be expected to be less vulnerable to powdery mildew, and yield fewer unmarketable fruit.

Powdery mildew was found on Aromas plants, though only when symptoms on Selva were very severe. This concurs with studies of relative powdery mildew resistance by Brahm *et al.* (2005). Even when powdery mildew was most severe on Aromas, no individual Aromas plant displayed symptoms above a disease class of 3, which was deemed an economically unacceptable level. This was consistent even in the 2003/04 season when no fungicide for powdery mildew control was applied to the trial plots. The habit of Aromas was taller, more erect and less dense than Selva. This concurs with reports from the University of California, Strawberry Licensing Program (The University of Calfornia, Strawberry Licencing Program, 2001). Improved air circulation and possibly irradiation and temperature at the leaf surface would be expected, resulting in conditions less favourable for powdery mildew development.

Assessment of powdery mildew severity on harvested Aromas fruit was made only in the 2005/06 season, when severity was low. During this season, no Aromas fruit were found to have any powdery mildew. The majority of Aromas fruit harvested were clear of the plant canopy, resting on the polyethylene mulch. This supports the hypothesis that the fruiting habit of Aromas allows this cultivar to escape the conditions conducive for powdery mildew development on the developing fruit. However, further field observations of Aromas in seasons with higher inoculum pressure would be required to determine the relative resistance of Aromas fruit in South Australian conditions.

Fungal structures, such as appressoria and haustoria, were difficult to observe under light microscopy. Other staining methods that differentially stain fungal and plant material, such as aniline blue-calcofluor double staining used by Cohen *et al.* (1990) to observe fungal structures (fluoresced blue) and host surface perforation (fluoresced yellow) in the infection of muskmelon leaves by *P. fuliginea*, may allow for improved

observation of *P. aphanis* development. However, staining can disturb fungal structures, so techniques such as SEM following cryo-preservation of fresh tissue (Cook *et al.*, 1997) and TEM, that allows visualisation of transverse sections of leaf material and fungal structures such as haustoria (Jhooty and McKeen, 1964), would be valuable for further observation of *P. aphanis* development.

In contrast to Selva, colony development was not observed on Aromas leaf tissue and germinating conidia were seldom present. Cell discolouration and necrosis, as would be expected of a hypersensitive response to *P. aphanis*, were not observed on Aromas (data not shown). However, the lack of development on healthy Aromas leaves suggests there is some mechanism preventing attachment, germination and establishment of the fungus in this cultivar. Constitutive plant defence mechanisms such as waxes (Jhooty and McKeen, 1964; Veronese *et al.*, 2003) and the presence of lignin-like materials in cell walls and cytoplasm (Cohen, 1990) may be responsible. However, histochemical comparisons between cultivars were not undertaken in these studies and further research is required to elucidate the mechanism of resistance of Aromas to *P. aphanis*.

In summary, initial observations of the weather conditions associated with powdery mildew epidemics provide the basis for future forecasting models for this disease. Also, Aromas possesses field resistance to powdery mildew and has potential as an alternative cultivar for production in South Australia, or as valuable parent material for powdery mildew resistance breeding.

Chapter 4 Calcium, potassium and silicon nutrition of strawberry plants and the effect of nutrition on development of *P. aphanis*

4.1 Introduction

Mineral nutrient deficiencies and broad nutrient requirement recommendations are well documented for strawberry plants (Johanson and Walker, 1963; Ulrich *et al.*, 1980; May and Pritts, 1990; Weir and Cresswell, 1993), however, studies have shown differences in nutrient requirement between cultivars (Albregts and Howard, 1978; Albregts *et al.*, 1991; Makus and Morris, 1998) and guidelines for fertilisation of strawberry crops are based on studies of cultivars and fertilisation methods that are rarely used in present day production (Boyce and Matlock, 1966; May and Pritts, 1990). Studies of the nutrient requirements of modern strawberry cultivars incorporating modern culturing techniques are therefore required to maximise yield and quality.

Mineral elements function as constituents of organic structure, activators of enzymatic reactions, charge carriers and osmoregulators (Marschner, 1995). As a result of their wide range in function, mineral elements play important roles in all metabolic activities including resistance against pathogen attack. A plant with balanced nutrition can better withstand disease. The nutrients potassium (K), calcium (Ca) and silicon (Si) have been implicated in disease resistance in many plant species (see Section 1.4.4).

Potassium plays a significant role in many metabolic reactions of plants (see Section 1.4.4.2). Deficiency symptoms occur first in the older leaves, as chlorosis or reddening of the leaf and petiole tissues (Johanson and Walker, 1963; Ulrich *et al.*, 1980). The recommended foliar potassium concentration range for strawberry plants is between 5 and 60 mg/g dry mass (Bould, 1964; Ulrich *et al.*, 1980; Mengal, 2007). However, yield responses to potassium fertilisation have been observed to differ greatly between cultivars (Albregts *et al.*, 1991).

Calcium is essential for cell replication, elongation and structure (see Section 1.4.4.3). The majority of calcium in plant cells is bound in the cell wall and at the plasma membrane, predominantly as calcium pectate and oxalate, providing rigidity in the cell wall and by bridging phospholipids and proteins, to stabilise membranes. The proportion of calcium pectate in cell walls influences the susceptibility of the tissue to fungal and bacterial infection (Marschner, 1995). Free Ca^{2+} ions are involved in signalling. Symptoms of calcium deficiency often include necrosis at the growing point of the plant, or impaired cell elongation at these points. This often presents as dead leaf tips on emerging leaves, crumpling of the leaf blade, and smaller leaves in general (Johanson and Walker, 1963; Chiu and Bould, 1967). The optimal range of foliar calcium concentration for strawberry plants is between 0.4 and 2.7 mg/g, dry mass (Ulrich *et al.*, 1980) and above 0.6 mg/g in receptacle tissue (Chiu and Bould, 1967).

It is assumed that silicon is not essential for the growth of most plants, though it has been impossible to prove this due to the abundance of silicon in soil and water (Werner and Roth, 1983). Wang (1998) showed that foliar applications of silicon had beneficial effects on growth of strawberry plants and increased levels of phospholipids, thought to act in signalling in plant defence responses (Laxalt and Munnik, 2002). Using microscopy, Cherif *et al.* (1992b) discovered high phenolic material in the cell walls of silicon-treated leaves from cucumber plants and suggested this material may be an antifungal compound. As silicate products can be generated organically, the effect of silicon in the control of fungal infection could be particularly useful to organic growers.

The following experiments were designed to determine the optimal foliar concentrations of potassium and calcium for maximum yield of fruit in strawberry cultivars, Selva and Aromas. Silicon was not included in optimal fertilisation studies because at commencement of these studies there was no evidence that this element

would significantly affect yield. However, as both silicon and calcium have been associated with disease defence against biotrophic pathogens in other plant species, and the knowledge of the fertilisation of these nutrients in strawberry is uncertain, these studies also investigated the effect of calcium and silicon on the development of *P. aphanis* on leaf tissue.

4.2 Materials and methods

4.2.1 Studies of potassium and calcium critical nutrition

To determine the requirement of the strawberry cultivars Selva and Aromas for the nutrients potassium and calcium, plants were maintained in free-draining pots with low nutrient sand and fertigated with a measured volume of nutrient solution (first planted in March 2005, then in May and November 2005). Prior to determining a suitable experimental system for these experiments a series of experiments using different systems was performed (See Appendix 3).

4.2.1.1 Glasshouse-based nutrition experimental conditions

Experiments were conducted in a glasshouse at the Waite Campus, The University of Adelaide (N34° 58′ 13″ W138° 37′ 51″). All experiments used low nutrient sands sourced from Mount Compass, Golden Grove and Waikerie and 20 cm diameter pots. Glasshouse-based experiments were maintained as per Section 2.2.1. Generally, plants were watered every second day with reverse osmosis (RO) water with a hose to flow-through, unless otherwise specified.

4.2.1.2 Free-draining measured volume fertigation system

In all of the controlled volume fertigated experiments treatments were based on 0.25 Hoagland's complete solution, or 0 % potassium and 0 % calcium solutions with added amounts of K_2SO_4 and $CaSO_4$, respectively, as required.

The following experiments were planted in free-draining pots into Waikerie sand. This sand was similar in texture and soil analysis to Golden Grove sand, used in the automatically fertigated experiment, which was not available.

Firstly, a preliminary experiment with 0 % potassium, 0 % calcium and 100 % potassium and calcium solution treatments was conducted to ascertain the suitability of this system for further use, and to determine the length of time required, using this system, for deficiency symptoms to develop, in cultivars, Selva and Aromas. The initial rate experiment had six concentrations of potassium and calcium, ranging from 0 % to 100 % solutions. Pots in both experiments were filled to 1.5 cm from the rim with sand and watered with RO water via a hose every second day. In addition, 250 mL of treatment solution was added to the sand, by hand, once per week.

In the follow-up experiment, each pot was placed in a 150 mm diameter Petri dish (with a volume of approximately 125 mL) to catch the flow-through. Treatment solution (125 mL) was applied to the soil of each pot three times per week. When the weather was hot they were also watered with 125 mL of RO water on the alternate days.

To determine critical potassium curves for cultivars, Selva and Aromas, using the controlled volume fertigated system, several experiments were performed. As a control, a treatment comprising complete 0.25 Hoagland's solution was included (234.6 mg/L potassium) in both experiments. In the initial rate experiment the potassium treatments were 0, 5, 10, 25 and 50 % the concentration of potassium in 0.25 Hoagland's solution (Table 4.1 A) (0, 11.73, 23.46, 58.65 and 117.3 mg/L potassium, respectively). In the follow-up experiment the potassium treatments were 0, 10, 20, 30 and 50 % the concentration of potassium in 0.25 Hoagland's solution (Table 4.1 A) (0, 23.46, 26.92, 70.38 and 117.3 mg/L potassium, respectively). This experiment was planted on the 24/11/2005 and harvested on the 19/01/2006.

Table 4.1 The composition of fertigation solutions used in the controlled volume fertigated experiments. The effect of **A**. potassium and **B**. calcium treatment on strawberry cultivars, Selva and Aromas were examined. Solutions used were 1 M (unless specified) and volumes were in mL added to make up 20 L of 0.25 Hoagland's solution.

A

	0 %	5 %	10 %	20 %	25 %	30 %	50 %	100 %
KH_2PO_4	0	0	0	0	0	0	0	5
$Ca(NO_3)_2$	25	25	25	25	25	25	25	25
KNO_3	0	0	0	0	0	0	0	25
NH_4NO_3	12.5	12.5	12.5	12.5	12.5	12.5	12.5	0
$Ca(H_2PO_4)_2$ (0.05M)	50	50	50	50	50	50	50	0
K_2SO_4 (0.5M)	0	15	30	60	75	90	150	0

B

	0 %	5 %	10 %	20 %	25 %	30 %	50 %	100 %
KH_2PO_4	5	5	5	5	5	5	5	5
$Ca(NO_3)_2$	0	0	0	0	0	0	0	25
KNO_3	25	25	25	25	25	25	25	25
NH_4NO_3	25	25	25	25	25	25	25	0
$CaSO_4$ (0.2M)	0	97.25	194.5	389	486.25	583.5	972.5	0

To determine critical calcium curves for cultivars, Selva and Aromas, using the controlled volume fertigated system, several experiments were performed. As a control, a treatment comprising complete 0.25 Hoagland's solution was included (240.5 mg/L calcium) in both experiments. In the initial rate experiment the calcium treatments were 0, 5, 10, 25 and 50 % the concentration of calcium in 0.25 Hoagland's solution (Table 4.1 B) (0, 12.03, 24.05, 60.13 and 120.25 mg/L calcium, respectively). In the follow-up experiment the calcium treatments were 0, 10, 20, 30 and 50 % the concentration of calcium in 0.25 Hoagland's solution (Table 4.1 B) (0, 24.05, 48.1, 72.15 and 120.25 mg/L calcium, respectively). This experiment was planted on 24/11/2005 and harvested on 29/06/2006.

4.2.1.3 Data collected in studies of potassium and calcium nutrition

Three types of data were collected during these experiments: visual observations and images of deficiency symptoms, measurements of growth and yield, and nutrient analysis of plant tissue (see Section 2.1.6). Symptoms observed on whole plants and leaves were photographed as per Section 2.2.5. Yield was measured in multiple ways. These were; fresh weight of plant measured before and after the experiments, the number of fruit per plant and weight of each fruit (recorded weekly) and the number of runners produced per plant and length of each runner, measured at the conclusion of the experiment.

4.2.1.4 Nutrient analysis

In the automatically fertigated experiment the youngest open leaf (YOL) blades were sampled monthly from 4 weeks after planting for nutrient analysis. In the initial manually fertigated experiment, YOLs and petioles were sampled monthly from 4 weeks after planting for nutrient analysis. When the majority of plants were bearing fruit, a single sample of the peduncles, receptacles and achenes was also taken and

bulked for analysis. In the follow-up manually fertigated experiment, plants were sampled once, after the plant that received the smallest concentration of the nutrient in question presented foliar deficiency symptoms.

Leaf, petiole and peduncle samples were placed into envelopes and dried in an 80 °C oven. The fruit (receptacle and achenes) were semi-dried in an Ezidry® Home Food Dryer at 55 °C overnight. The achenes were removed with tweezers and placed in a 50 mL Falcon tube (Greiner, Australia). The remaining receptacle was placed in an envelope and dried completely at 85 °C, before being ground with a mortar and pestle. Leaf samples were also ground, while all other plant parts were digested whole due to their small size. Nutrient analysis of plant material was carried out by the Waite Analytical Services using Radial CIROS ICPAES. Lucerne hay was used as a standard leaf material to calibrate ICPAES analysis. Silicon content was analysed by the Bureau of Sugar Experimental Stations (BSES) as per Ostatek-Boczynski and Haysom (2003).

4.2.2 Studies of the effect of foliar calcium and silicon on development of *P. aphanis*

Plants were grown with and without calcium and silicon using the free-draining manually fertigated system (Section 4.2.1.4), and leaves were removed, inoculated with *P. aphanis* and maintained in a high humidity chamber as per Miller *et al.* (2003) (Section 2.4). Development was compared using light microscopy, as per Section 2.5.1. Kasil2040® (PQ Corporation) was used as the silicate source. Plants were watered with 0.25 Hoagland's solution supplemented with 1000 mM K_2SiO_4 using the manually fertigated free draining with catchment system.

4.2.3 Microscopy of cellular structure of calcium deficient leaf tissue

Leaf samples from Selva and Aromas plants grown with and without Calcium (as per Section 4.2.1.4) were prepared for resin infiltration as per Section 2.5.1. The

samples were taken 3 to 6 mm from the tip of a leaflet, avoiding the midrib. Samples of tipburn affected leaves were taken avoiding the immediately affected area.

4.2.4 Statistical analysis

Analysis of Variance (ANOVA) was undertaken using Genstat® version 8.0 and correlation analysis was conducted using Microsoft Excel® (Section 2.8). Curves of best fit were fitted to graphs of foliar concentrations that gave 90 % of total yield (mean of a treatment) were deemed critical.

4.3 Results

4.3.1 Use of manually fertigated systems to determine nutrition requirements of strawberry plants

4.3.1.1 The effect of potassium treatment on deficiency symptom development, foliar potassium concentration over time and distribution to plant parts

In the preliminary experiment, symptoms of potassium deficiency were first observed after 7 weeks of treatment. These symptoms were dark margins on older leaves in the 0 % potassium treatments. ICP-OES analysis (Table 4.2) showed that the 0 % potassium treatment significantly reduced the foliar concentrations of potassium in both cultivars (f probs = <0.001). These were near or below the critical value of 10 mg/g potassium, as defined by Ulrich *et al*. (1980). Selva plants treated with 0 % potassium had significantly higher foliar concentration (10.5 mg/g, dry weight) when compared with Aromas (7.05 mg/g, dry weight). Calcium concentrations in the 0 % potassium treatments were significantly higher than those of the 100 % potassium treatments (f prob = 0.027). There were no significant effects of 0 % potassium treatment on boron, magnesium or phosphorus concentrations. However, magnesium was higher in Aromas leaves compared with Selva (Table 4.2).

Table 4.2 The effect of 0 % potassium treatment on the foliar concentrations of selected nutrients. Levels of boron, calcium, potassium, magnesium and phosphorus in the cultivars Selva and Aromas were measured using ICPAES analysis after 7 weeks of treatment with 100 % or 0 % of the potassium in 0.25 Hoagland's solution, applied manually (250 mL) to free-draining pots once per week. The critical concentration for potassium as defined by Ulrich *et al.* (1980) is 10 mg/g with a deficient range of 1.0 to 5.0 mg/g dry weight.

Element (mg/g, unless indicated)	Aromas		Selva	
	100 % potassium	0 % potassium	100 % potassium	0 % potassium
Calcium	8.85[a]	13.25[b]	8.55[a]	11.80[b]
Phosphorus	2.90[a]	2.60[a]	2.60[a]	2.70[a]
Potassium	18.30[a]	7.05[c]	19.70[a]	10.50[b]
Magnesium	5.15[a]	5.35[a]	4.20[b]	4.35[b]
Boron (mg/kg)	45.5[a]	52.0[a]	46.5[a]	48[a]

Statistical difference between treatments, within elements, for each cultivar is indicated by superscripts.

In the initial rate controlled volume fertigation experiment, the first symptoms of potassium deficiency in the 0 % potassium treatment, eight weeks after planting and the treatment was commenced. By 21 weeks into the experiment, most plants treated with the 0 and 5 % potassium solutions and some treated with 10 % potassium solutions displayed deficiency symptoms. Symptoms observed were marginal chlorosis and necrosis in the older leaves (Figure 4.1). Symptoms of potassium deficiency differed between cultivars with Selva displaying more necrosis in contrast to Aromas where chlorosis was more common. There was also some dark flecking on the younger leaves of the lower treatments.

The potassium treatments in the controlled volume experiment had a noticeable effect on foliar potassium concentrations of both Selva and Aromas (Figure 4.2). Selva plants treated with 25, 50 and 100 % potassium had higher foliar potassium than Aromas plants of the same treatment. By the last sampling date, foliar samples from the 0, 5 and 10 % potassium treatments recorded concentrations of less that 5.0 mg/g potassium the level predicted for potassium deficiency in strawberries (Ulrich *et al.*, 1980). This is consistent with the symptoms of foliar deficiency seen in these treatments at the time of harvesting.

There was concern that the foliar potassium concentrations decreased over time in the higher potassium treatments also. This was attributed to the free-draining nature of this system. In future experiments it was decided to place a tray beneath each pot to retain the treatment solution for plant growth, this would also allow for calculation of the total amount of potassium available to the plant in each treatment, as the flow-through would be retained.

After 20 weeks of treatment, the concentration of potassium appeared to be

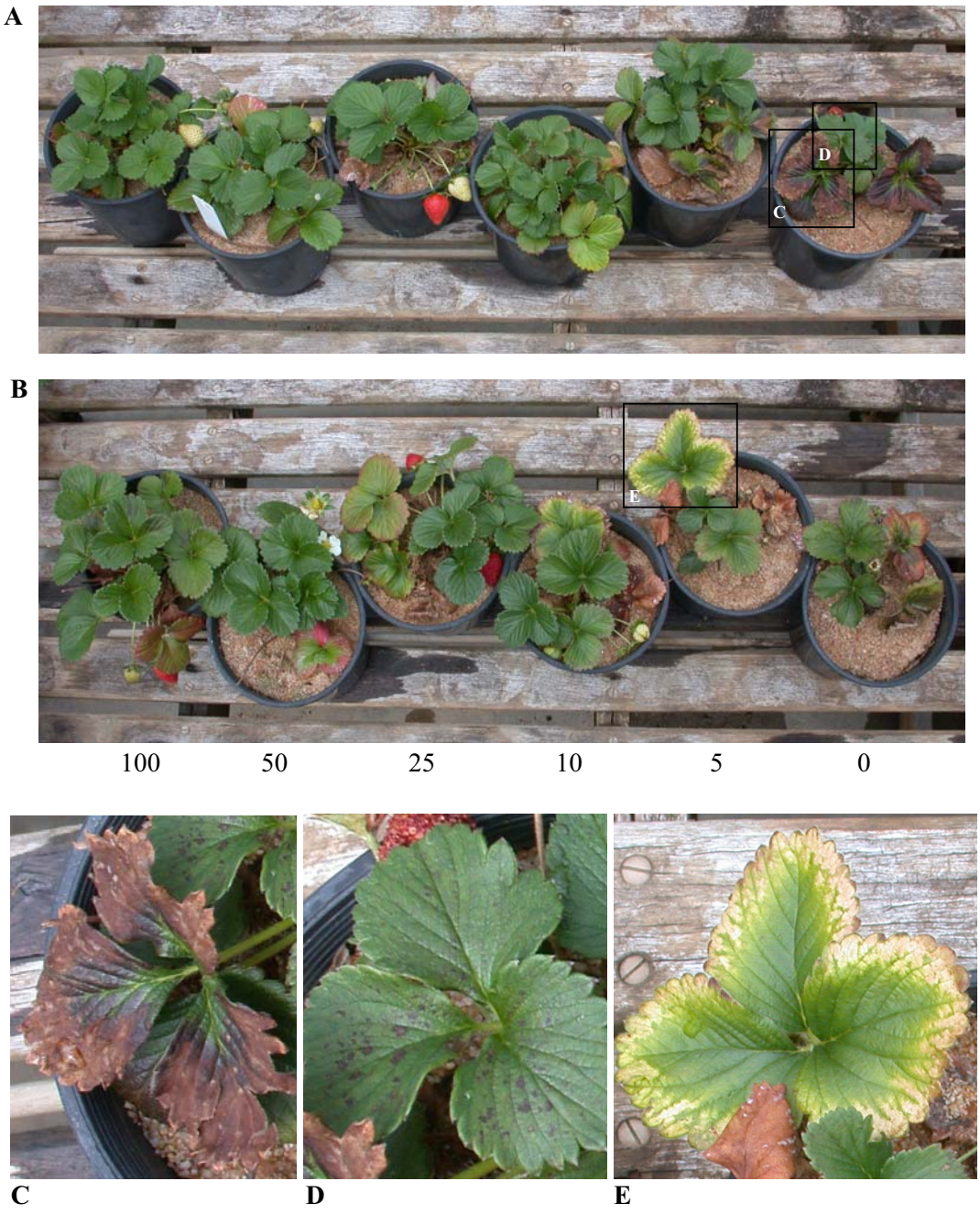


Figure 4.1 Foliar responses of cultivars **A**. Selva and **B**. Aromas to potassium treatments. Photos were taken after 21 weeks of treatment with 0 to 100 % potassium of 0.25 Hoagland's solution (as indicated above images) using the free-draining, manually fertigated system. Insets show marginal necrosis of old leaves (**C**) and flecking of young leaves (**D**) observed in Selva and marginal chlorosis of old leaves (**E**) observed in Aromas.

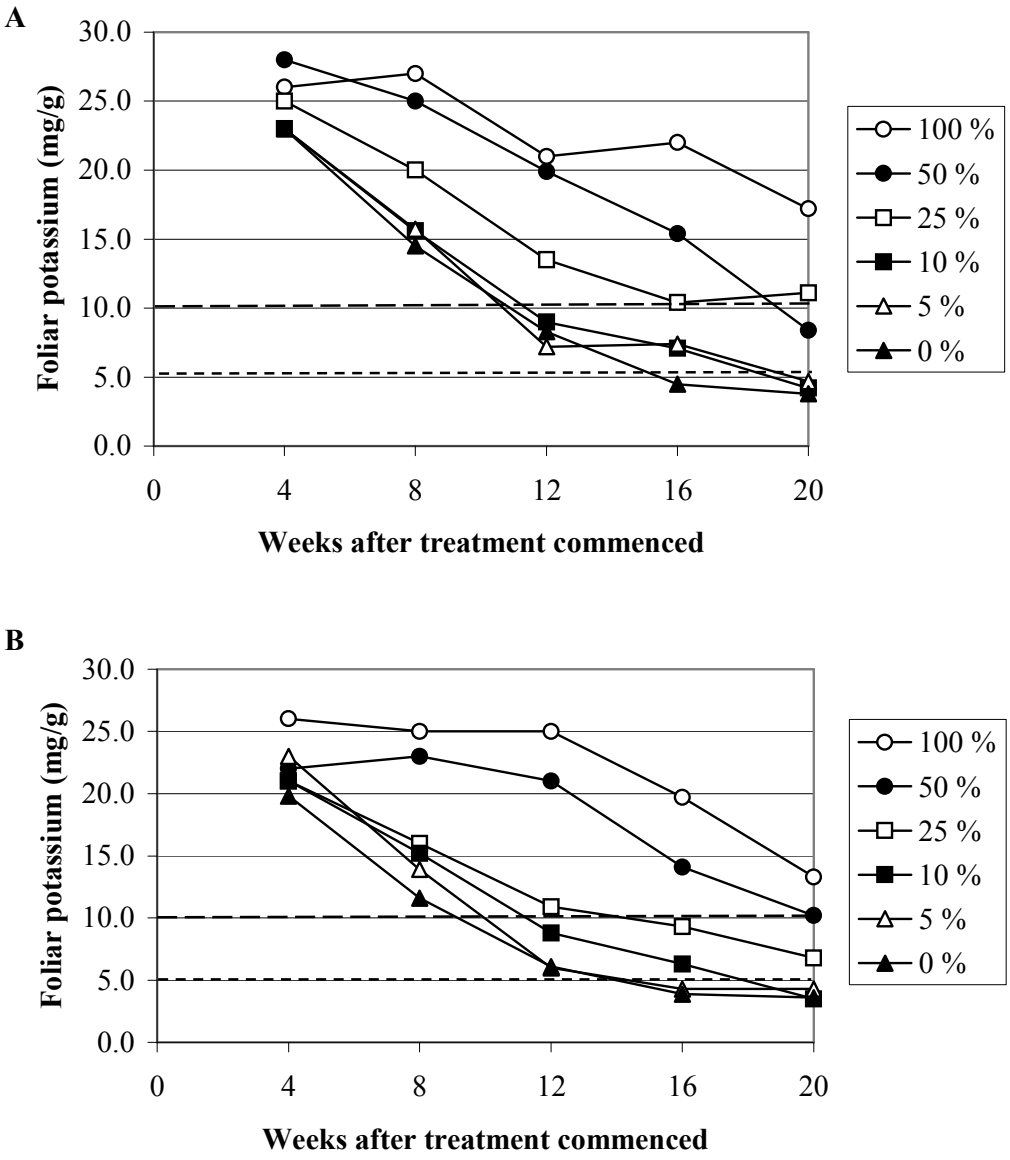


Figure 4.2 The effect of potassium treatment on foliar potassium concentration (dry weight) of cultivars **A**. Selva and **B**. Aromas, over time. Plants were treated with solutions containing 0 to 100 % potassium in 0.25 Hoagland's solution, using the free-draining, manually fertigated system. YOLs from 3 plants at each sample time were homogenised for ICPAES analysis, n = 1. Dashed lines indicate marginal (large dash) and deficient (small dash) potassium concentrations as defined by Ulrich *et al.* (1980).

highest in the peduncles in all treatments except Selva plants treated with 100 % potassium, where that concentration of potassium in the petiole was slightly higher, and the 0 % treatment for both cultivars where the concentration in the receptacle tissue was higher (Figure 4.3). There was an obvious effect of potassium treatment on concentrations of potassium in the petiole, leaf and peduncle tissues. However, in the receptacle and achene tissues the 100 % treated plants had higher concentrations than the other treatments, but the stepwise decrease with treatment was not present as with the other tissues. The potassium concentrations in the petiole and peduncle tissue of the 100 % potassium Aromas treatment were higher than those of Selva.

The bulk sampling strategy used in this trial in order to obtain sufficient material for ICPAES analysis (for example, the petioles of the YOLs from 3 plants sampled together) did not allow for ANOVA of these data.

4.3.1.2 Effect of potassium treatments on yield and nutrient efficiency

The potassium treatments had a significant effect on the concentration of potassium detected in the samples of YOLs taken at the conclusion of this experiment, 2 months after planting (Figure 4.4). The concentration of potassium was significantly higher in leaves of Selva in the 10, 30 and 50 % potassium treatments compared with Aromas.

The potassium treatments also had significant effects on growth. The fresh weight of plants at harvest was significantly affected by potassium treatment (f prob =<0.001) with the greatest mass produced in Aromas when treated with 30 and 50 % potassium and Selva treated with 50 % potassium (Figure 4.5 A). Shoot dry weight of Selva showed a near linear increase with treatments from 10 to 100 % potassium (Figure 4.5 B). Aromas produced the greatest dry shoot mass when treated with solutions of over 25 % potassium. In order to determine a foliar concentration of

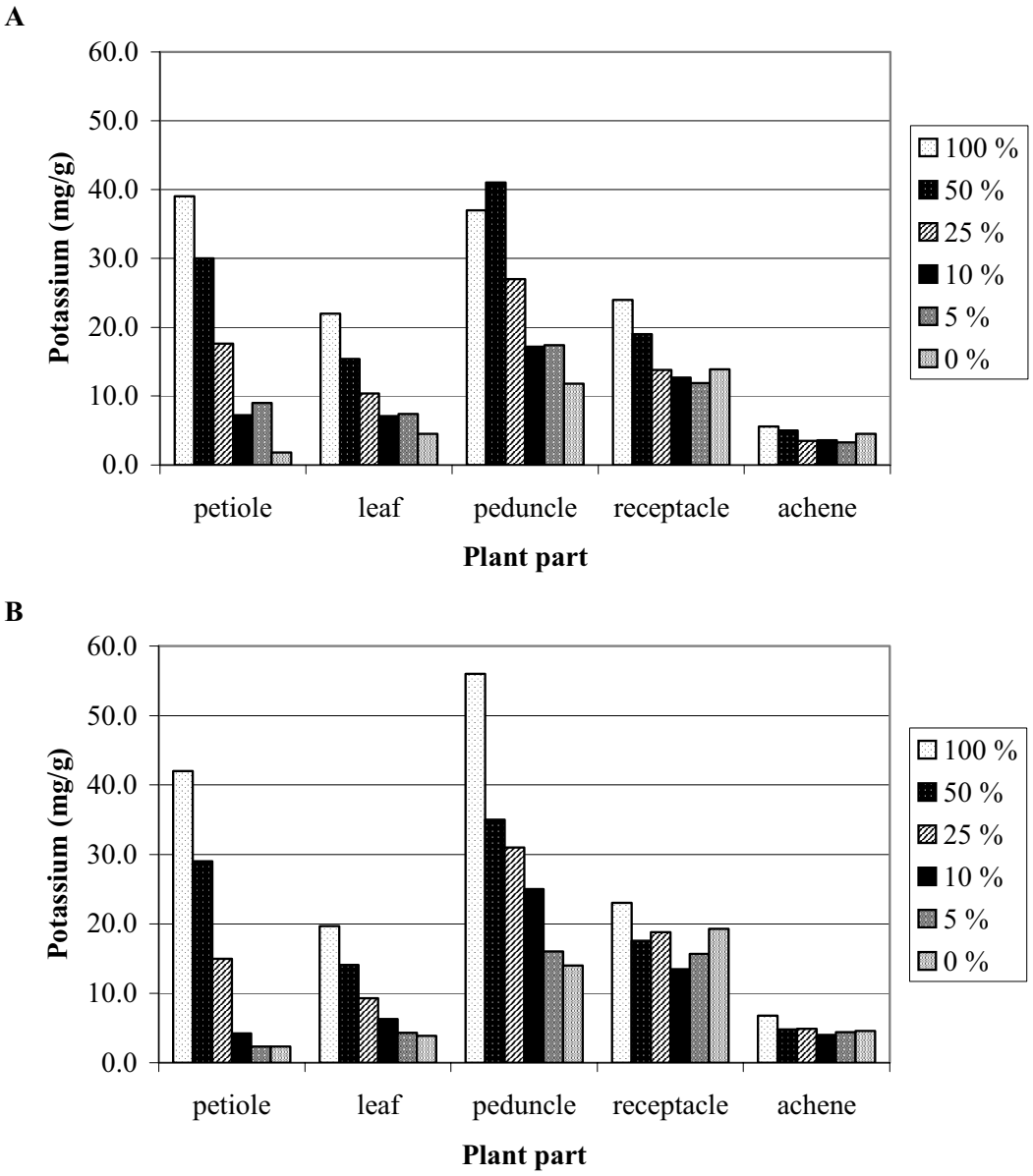


Figure 4.3 The effect of potassium treatment on the potassium concentration of petiole, leaf, peduncle, receptacle and achene tissues of cultivars, **A**. Selva and **B**. Aromas. Photos were taken after 20 weeks of treatment with 0 to 100 % potassium in 0.25 Hoagland's solution using the free-draining, manually fertigated system. Samples of each plant part from 3 or 4 plants were homogenised for ICPAES analysis, n = 1.

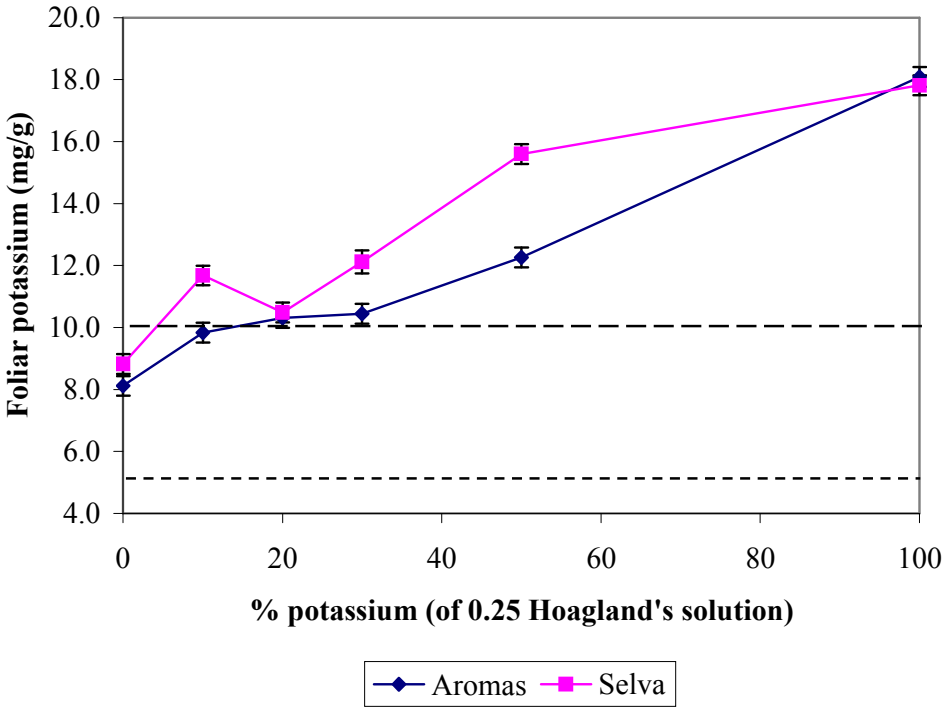


Figure 4.4 The effect of potassium treatment on foliar potassium concentration strawberry cultivars, Aromas and Selva. ICPAES analysis was undertaken after 8 weeks of treatment with 0 to 100 % potassium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Data shown is mean, ± SE (n = 8). Dashed lines indicate marginal (large dash) and deficient (small dash) potassium concentrations as defined by Ulrich *et al.* (1980).

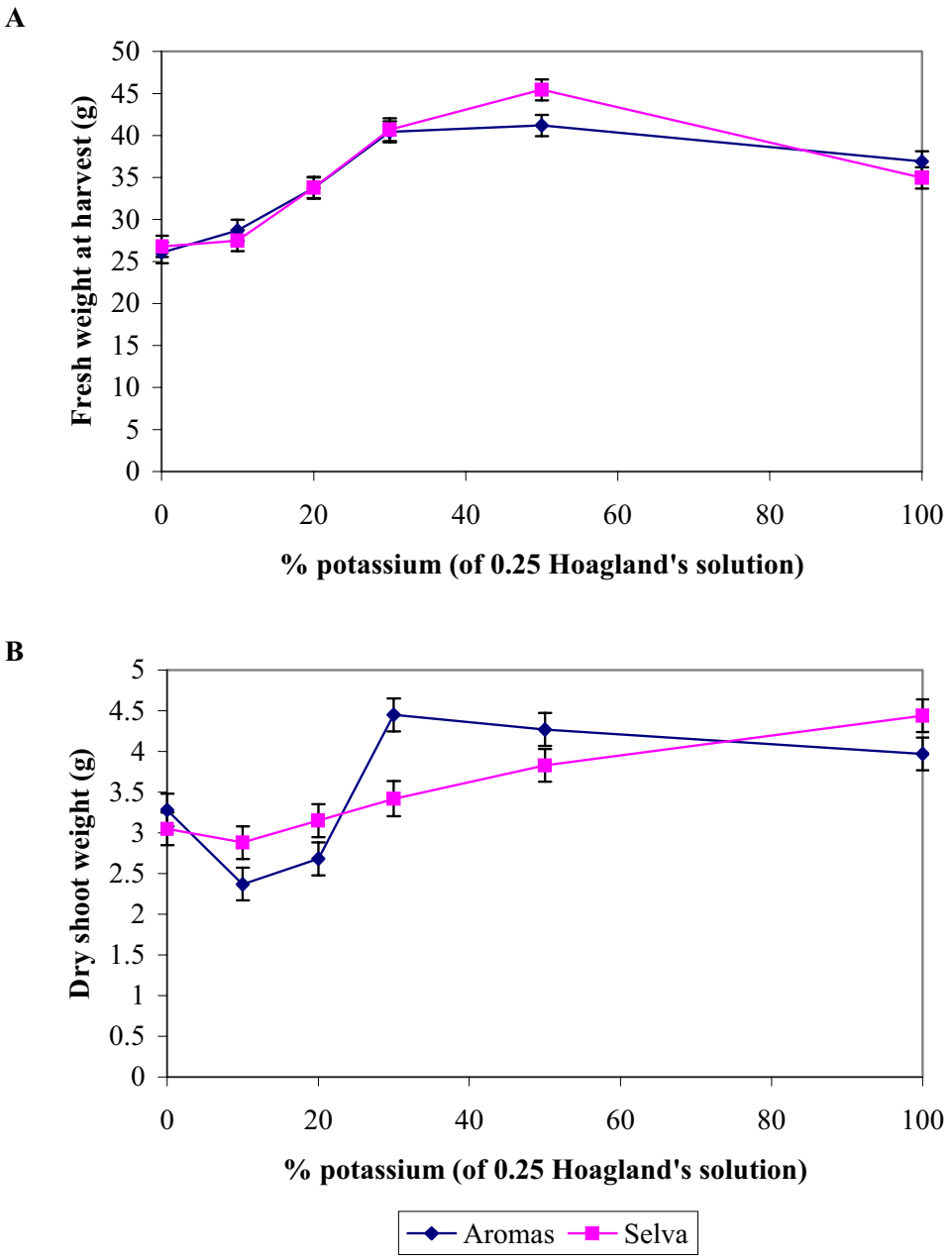


Figure 4.5 The effect of potassium treatment on **A**. fresh plant weight at harvest and **B**. dry shoot weight of strawberry cultivars, Selva and Aromas. Analysis was undertaken after 8 weeks of treatment with 0 to 100 % potassium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Fresh plant weight included shoots, runners and roots produced by plants, but excluded fruit. Shoots were removed at soil level and weighed after 24 hours at 80 °C. Data shown is mean, ± SE (n = 8).

potassium for optimal plant mass, the foliar potassium concentration was plotted against fresh weight of plants at harvest for Aromas and Selva (Figure 4.6) and the concentration of potassium that yielded 90 % of the total plant mass indicated. The optimal foliar concentration for Selva was between 11.0 and 15.0 mg/g dry weight, with the critical level being approximately 10.0 mg/g dry weight. The optimal foliar potassium concentation for Aromas was between 12.5 and 16.0 mg/g dry weight, with the critical level being approximately 12.0 mg/g dry weight.

The number of runners produced by each plant and the mean length of runners differed significantly between cultivars (both with f prob = <0.001). In general, Aromas yielded the most runners when treated with the 30 and 50 % potassium treatments than the other treatments. The mean length of runners produced by Aromas plants was also longest with these treatments. The number and length of runners was significantly less when treated with 100 % potassium treatments than when treated with 30 or 50 % potassium. Selva produced significantly fewer and shorter (Figure 4.7) runners than Aromas. The most runners produced by Selva were from 30 % potassium treated plants, however, the mean length was longest in the 100 % potassium treated plants.

Potassium treatment had no significant effect on the number of fruit produced per plant in either cultivar (Figure 4.8 A, f prob = 0.474) but did affect the mean weight of fruit harvested (Figure 4.8 B, f prob = 0.003). Significantly larger strawberries were produced by Aromas plants treated with 100 % potassium, than with any other treatment. Fewer berries were produced by Selva plants treated with 30 and 100 % potassium compared with Aromas plants treated with the same. The mean size of berries harvested was also greater from Aromas than Selva when treated with all except the 30 % potassium treatment. In order to determine a foliar concentration of potassium for optimal plant mass, the foliar potassium concentration was plotted against mean fruit

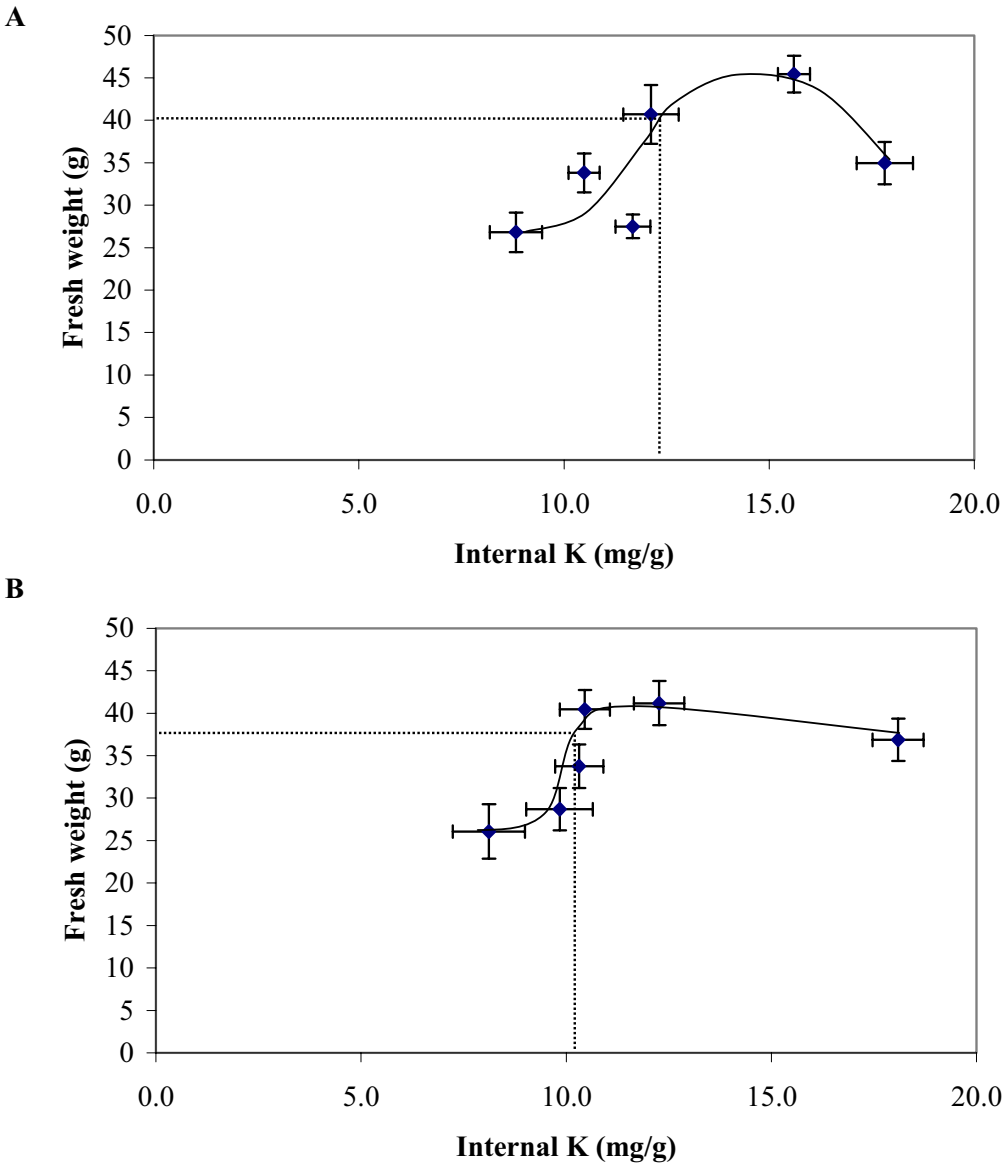


Figure 4.6 Fresh weight at harvest plotted against foliar potassium concentration (dry weight) of strawberry cultivars, **A**. Selva and **B**. Aromas. Analysis was undertaken after 8 weeks of treatment with 0 to 100 % potassium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Fresh plant weight included shoots, runners and roots produced by plants, but excluded fruit. Data shown is mean, ± SE (n = 8).

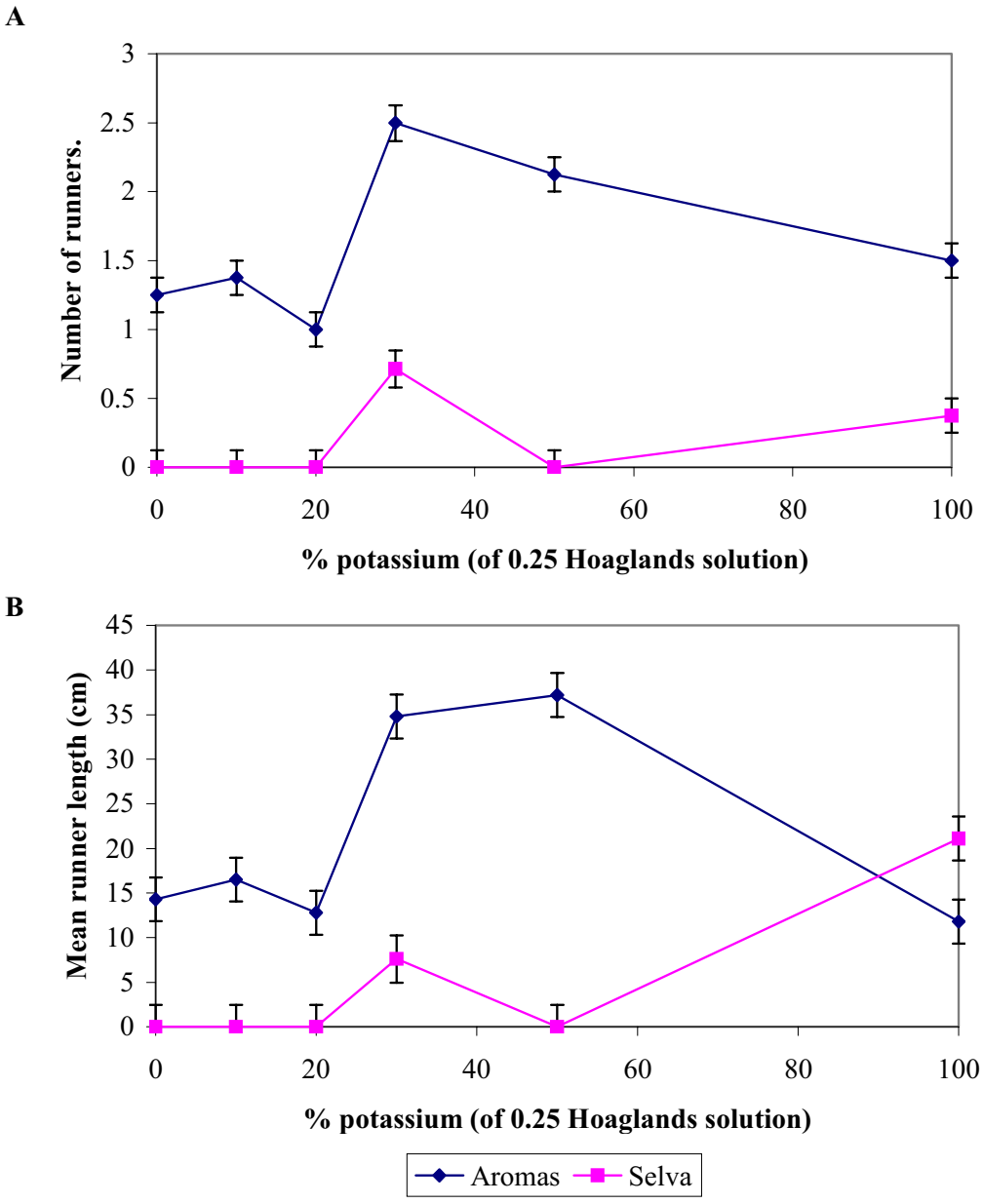


Figure 4.7 The effect of potassium treatment on **A**. the number of runners produced by each plant, and **B**. the mean runner length of strawberry cultivars, Selva and Aromas. Runners were measured, after 8 weeks of treatment with 0 to 100 % potassium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Data shown is mean, ± SE (n = 8).

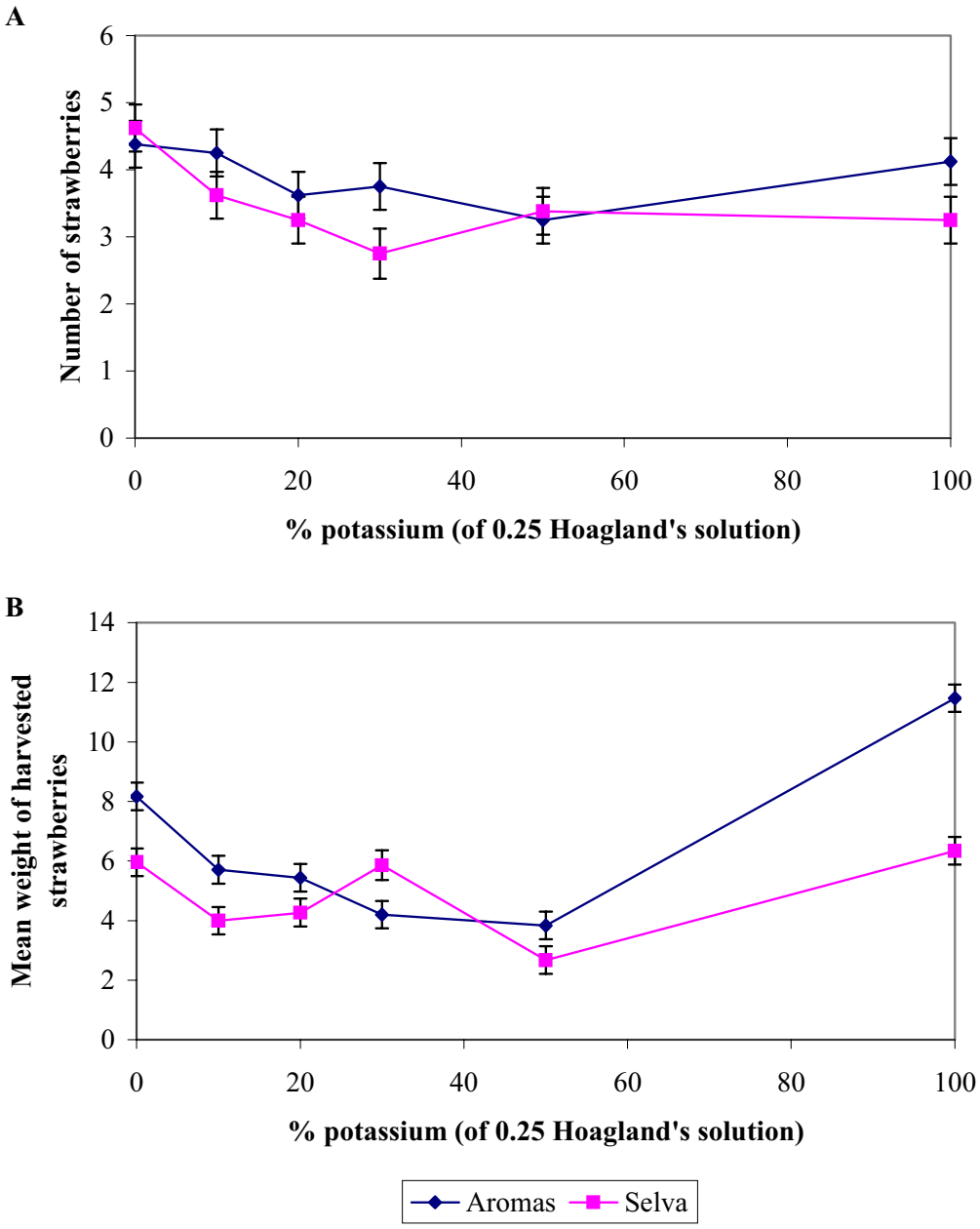


Figure 4.8 The effect of potassium treatment on **A**. the total number and **B**. mean weight of strawberries per plant of cultivars, Selva and Aromas. Fruit were measured weekly during 8 weeks of treatment with 0 to 100 % potassium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Data shown is mean, ± SE (n = 8).

weight for Aromas and Selva (Figure 4.9) and the concentration of potassium that yielded 90 % of the mean fruit weight indicated. The optimal foliar potassium range for Aromas for consistent production of larger fruit was between 11.0 and 15.0 mg/g dry leaf weight, and for Selva, was between 12.5 and 16.0 mg/g dry leaf weight.

There was no significant difference in foliar calcium due to potassium treatment (f prob = 0.176, Figure 4.10). Significant reductions in mean foliar boron, magnesium and phosphorus concentrations (f prob = 0.054, <0.001 and <0.001, respectively) were associated with increasing potassium treatment, however none of these was significantly correlated (correlation coefficient = -0.432, -0.568 and -0.15, respectively) with foliar potassium concentration (Figure 4.10).

4.3.2 Use of manual fertigation systems for assessment of calcium requirements of strawberry plants

4.3.2.1 The effect of calcium treatment on deficiency symptom development, foliar calcium concentration over time and distribution to plant parts

Symptoms of calcium deficiency took 12 weeks to develop to a point where crumpling of the young leaves could be observed in the 0 % calcium treatment in both cultivars (Figure 4.11), with symptoms appearing in Aromas before Selva. However, the newly opened leaves of both cultivars were smaller in the 0 % calcium treatment than the 100 % calcium in 0.25 Hoagland's solution control after 6 weeks.

The foliar concentration of calcium was significantly less in plants that received the 0 % calcium treatment than in plants that received the 100 % calcium treatment (Table 4.3, f prob = 0.015). However, the foliar concentration of the 0 % calcium treated Selva and Aromas plants after 12 weeks were 7.10 and 7.35 mg/g dry weight, respectively, which is above the critical value of 3.0 mg/g dry weight defined by

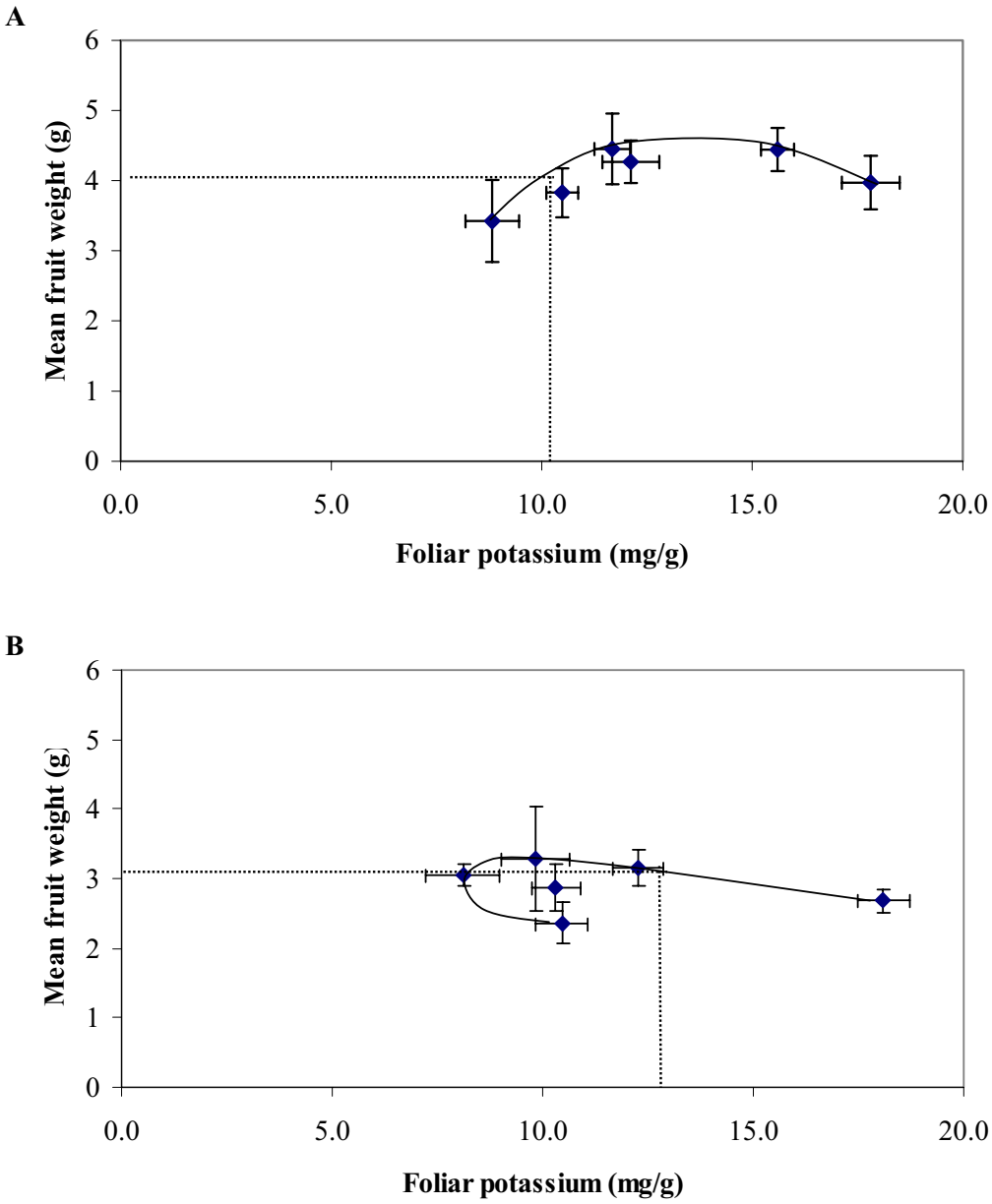


Figure 4.9 The mean fruit weight plotted against foliar potassium concentration (dry weight) of strawberry cultivars, **A**. Selva and **B**. Aromas. Analysis was undertaken after 8 weeks of treatment with 0 to 100 % potassium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Strawberries were harvest weekly. Data shown is mean, ± SE (n = 8).

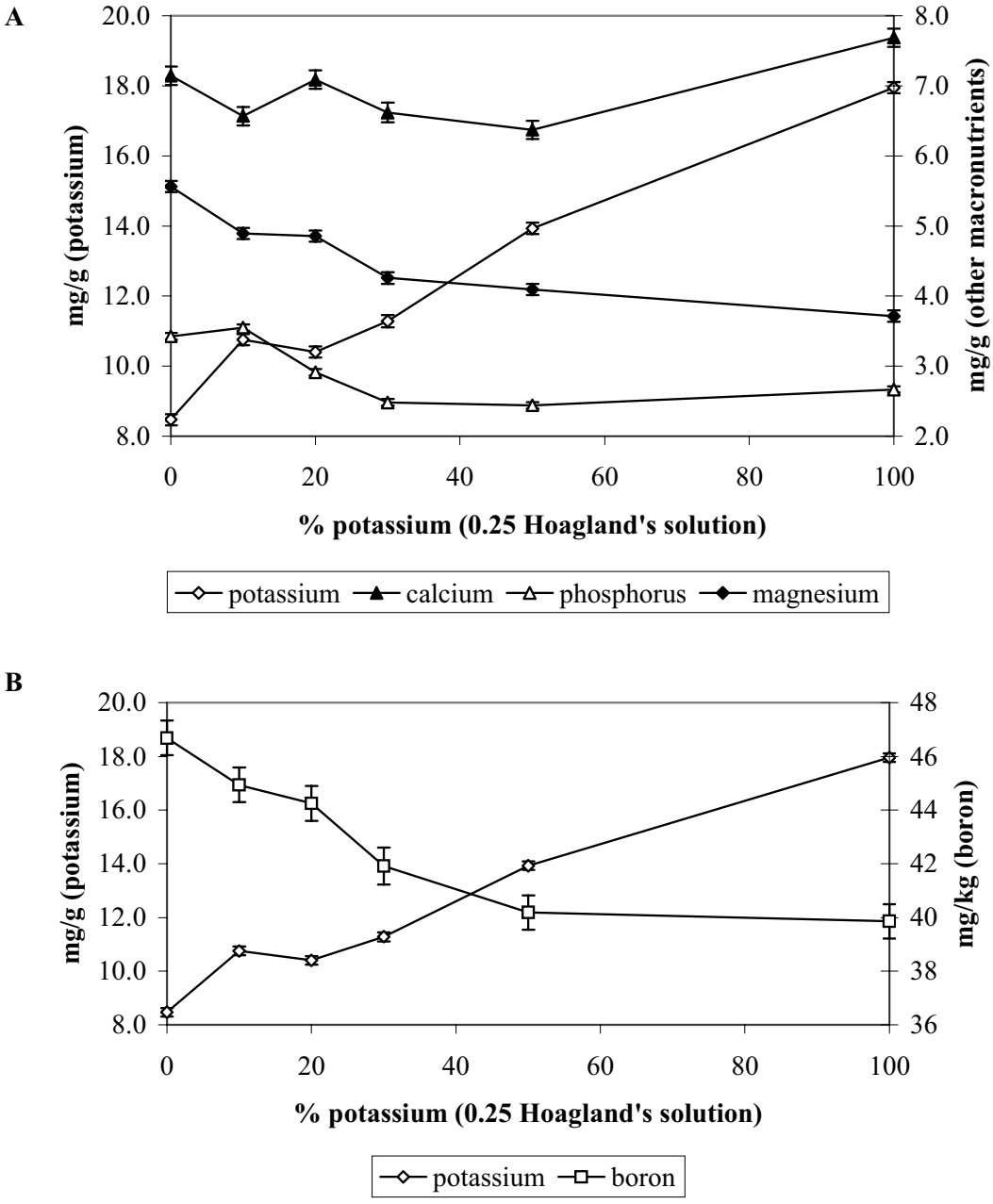


Figure 4.10 The effect of potassium treatment on foliar concentrations of **A.** potassium, calcium, phosphorus and magnesium and **B.** boron. ICPAES analysis was undertaken after 8 weeks of treatment with 0 to 100 % potassium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Data shown is mean (cultivars combined), ± SE (n = 16).

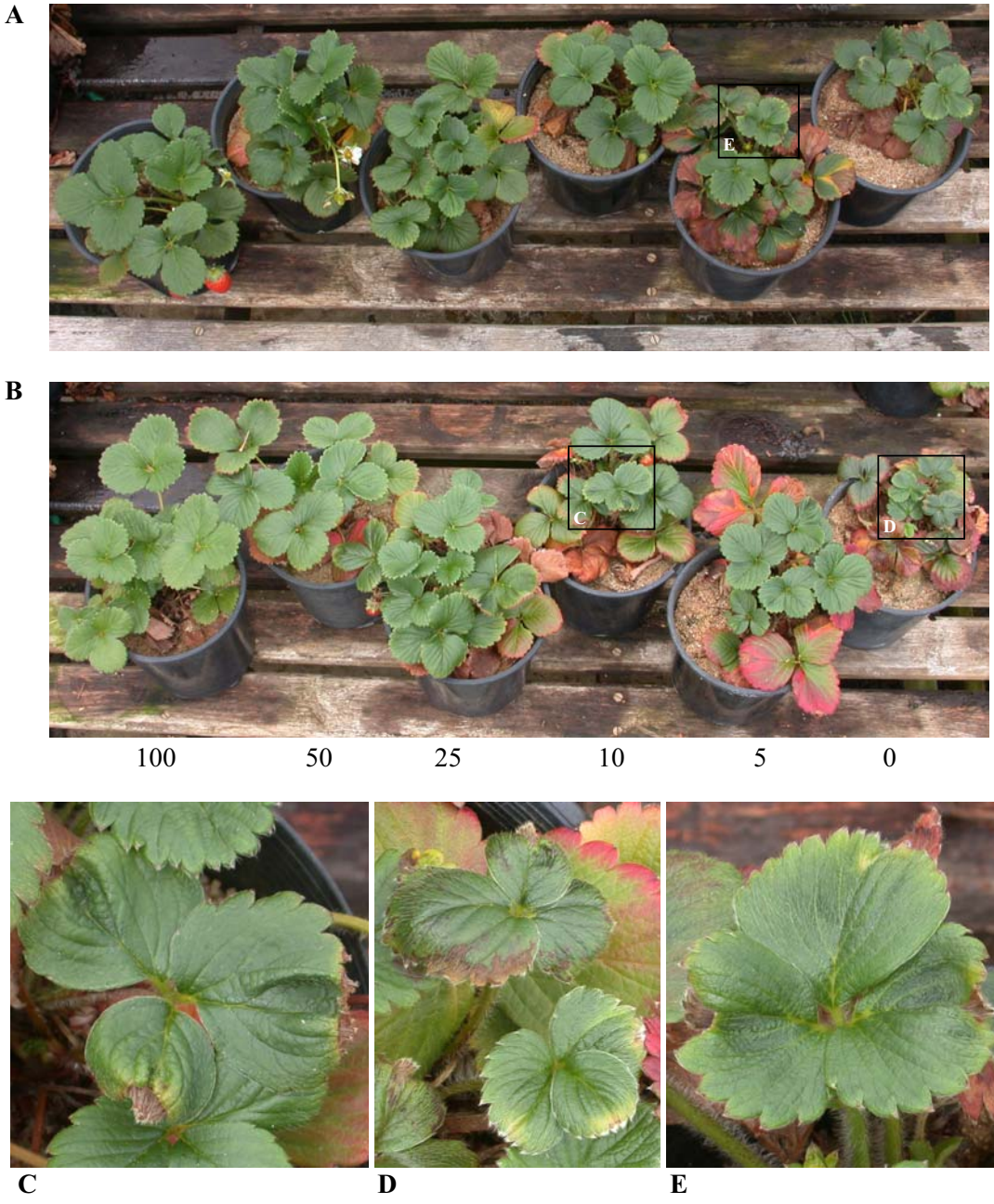


Figure 4.11 Foliar responses of cultivars **A.** Selva and **B.** Aromas to potassium treatments. Photos were taken after 21 weeks of treatment with 0 to 100 % potassium of 0.25 Hoagland's solution (as indicated above images) using the free-draining, manually fertigated system. Insets **C**, **D** and **E** show deformation of the growing point of young leaves, called tipburn. **C.** also shows crumpling of interveinal tissue.

Table 4.3 The effect of 0 % calcium treatment on foliar concentration of selected nutrients. The foliar concentrations of boron, calcium, potassium, magnesium and phosphorus were determined using ICPAES analysis. Leaf from cultivars Selva and Aromas after 7 weeks of treatment with 100 % and 0 % of the potassium in 0.25 Hoagland's solution, applied manually (250 mL) to free-draining pots once per week. The critical concentration for calcium as defined by Ulrich *et al.* (1980) is 3000 mg/kg with a deficient range of 0.8 to 2.0 mg/g dry weight.

Element (mg/g, unless indicated)	Aromas		Selva	
	100 % calcium	0 % calcium	100 % calcium	0 % calcium
Calcium	8.85[a]	7.35[b]	8.55[a]	7.10[b]
Phosphorus	2.90[a]	2.70[a]	2.60[a]	2.85[a]
Potassium	18.30[a]	16.80[b]	19.70[a]	16.85[b]
Magnesium	5.15[a]	5.30[a]	4.20[b]	4.70[b]
Boron (mg/kg)	45.5[a]	47.0[a]	46.5[a]	43.5[a]

Statistical difference between treatments, within elements, for each cultivar is indicated by superscripts.

Ulrich *et al.* (1980). Plants that received the 0 % calcium treatment also showed significantly reduced concentrations of foliar potassium (f prob = 0.066). A significant difference between cultivars was observed for magnesium (f prob = 0.028), however this element was not significantly affected by the 0 % calcium treatment. There was no significant effect of calcium deprivation on foliar boron and phosphorus concentrations in either cultivar.

The foliar calcium concentration fluctuated, then decreased after 8 weeks in all treatments except the 50 and 100 % calcium treatments, where calcium content peaked at the onset of fruiting before declining (Figures 4.12). There was little difference in effect of treatments containing 25 % calcium or less on the foliar calcium concentration of Selva. After 20 weeks of treatment, the foliar concentrations of Selva plants treated with 25 % calcium or less were equal to or below the critical value of 3.0 mg/g dry weight as defined by Ulrich *et al.* (1980). Only the 0 % calcium treated Selva plants had foliar concentrations of calcium below 2.0 mg/g, the upper limit of the deficient range defined by Ulrich *et al.* (1980). Between the 16 and 20 week observations there was also notable decline in the foliar calcium concentrations of 50 % and 100 % treated Selva plants. There was also little difference in foliar calcium concentration of Aromas plants treatments with 0 % and 5 % calcium treatments. After 20 weeks of treatment, the foliar calcium concentration of Aromas plants treated with 10 % calcium was 3.0 mg/g, equal to the critical level defined by Ulrich *et al.* (1980) and 2.0 mg/g when treated with the 0 and 5 % treatments, equal to the upper limit of the deficient range. However, the foliar calcium concentration of the 25 and 50 % calcium treated Aromas plants were maintained for 16 weeks, when fruiting had finished.

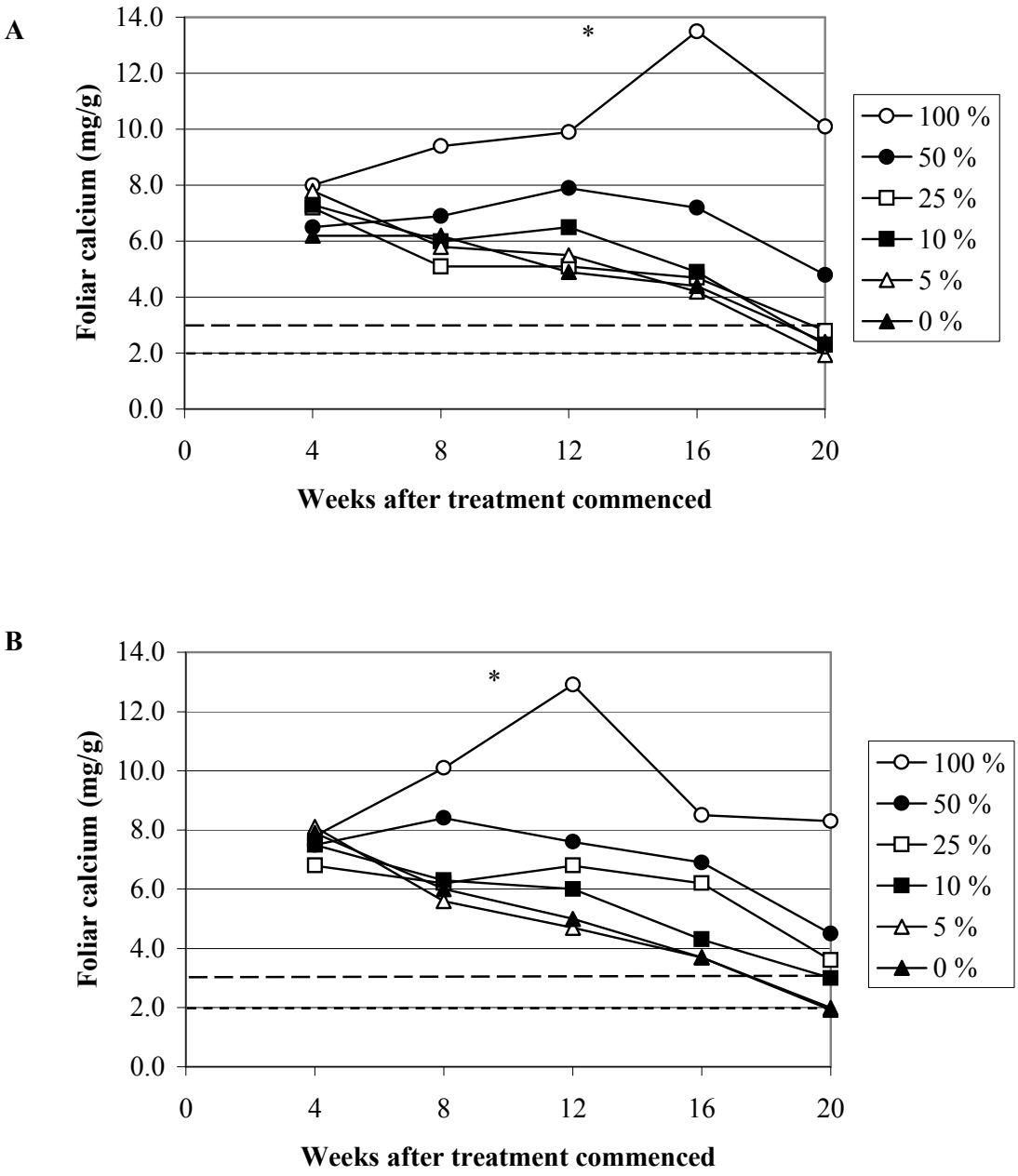


Figure 4.12 The effect of calcium treatment on the foliar calcium concentration (dry weight) of cultivars **A**. Selva and **B**. Aromas, over time. Plants were treated with solutions containing 0 to 100 % calcium in 0.25 Hoagland's solution, using the free-draining, manually fertigated system. YOLs from 3 plants at each sample time were homogenised for ICPAES analysis, n = 1. * indicates onset of fruiting. Dashed lines indicate marginal (large dash) and deficient (small dash) potassium concentrations as defined by Ulrich *et al.* (1980).

The receptacle tissue received the lowest proportion of calcium amongst the plant tissues (Figure 4.13). Generally, the concentrations of calcium in each plant part were lower with all treatments below 100 %. Calcium concentrations of receptacle tissue sampled from 100 % and 0 % treated plants were similar to those observed by Chiu and Bould (1976) from normal and deficient plants (2.1 and 0.5 mg/g, dry weight). The calcium concentration of achene tissue from the 100 and 10 % treated Selva and 100, 50 and 25 % treated Aromas plants was within the range of 3.1 to 7.2 mg/g, dry weight for normal plants observed by Makus and Morris (1998). Concentrations of calcium in the tissues between cultivars were similar, except in the 100 % calcium treatment where Selva had more calcium in the petioles and leaves at the sampling date, after 20 weeks of treatment.

4.3.2.2 Effect of calcium treatments on yield and nutrient efficiency

The calcium treatments significantly affected the foliar concentration of calcium recovered from the YOLs sampled at the conclusion of this experiment, 7 months after planting (Figure 4.14). Treated with 50 % calcium and below, Aromas plants had a significantly higher concentration of calcium in the leaf tissue compared with Selva. The foliar concentrations of Aromas appeared to stabilise between 4.5 and 6.0 mg/g calcium when treated with 20 % of the calcium in 0.25 Hoagland's solution or greater, whereas the foliar concentrations of Selva stabilised between 5.5 and 7.0 mg/g when treated with 50 or 100 % calcium in 0.25 Hoagland's solution.

This experiment was to be harvested when calcium deficiency symptoms appeared in the 0 % calcium treatment. However, these plants were severely damaged by strawberry crown moth larvae for up to an estimate of 4 weeks prior to correct identification. These larvae were found in the crown, between the petiole bracts.

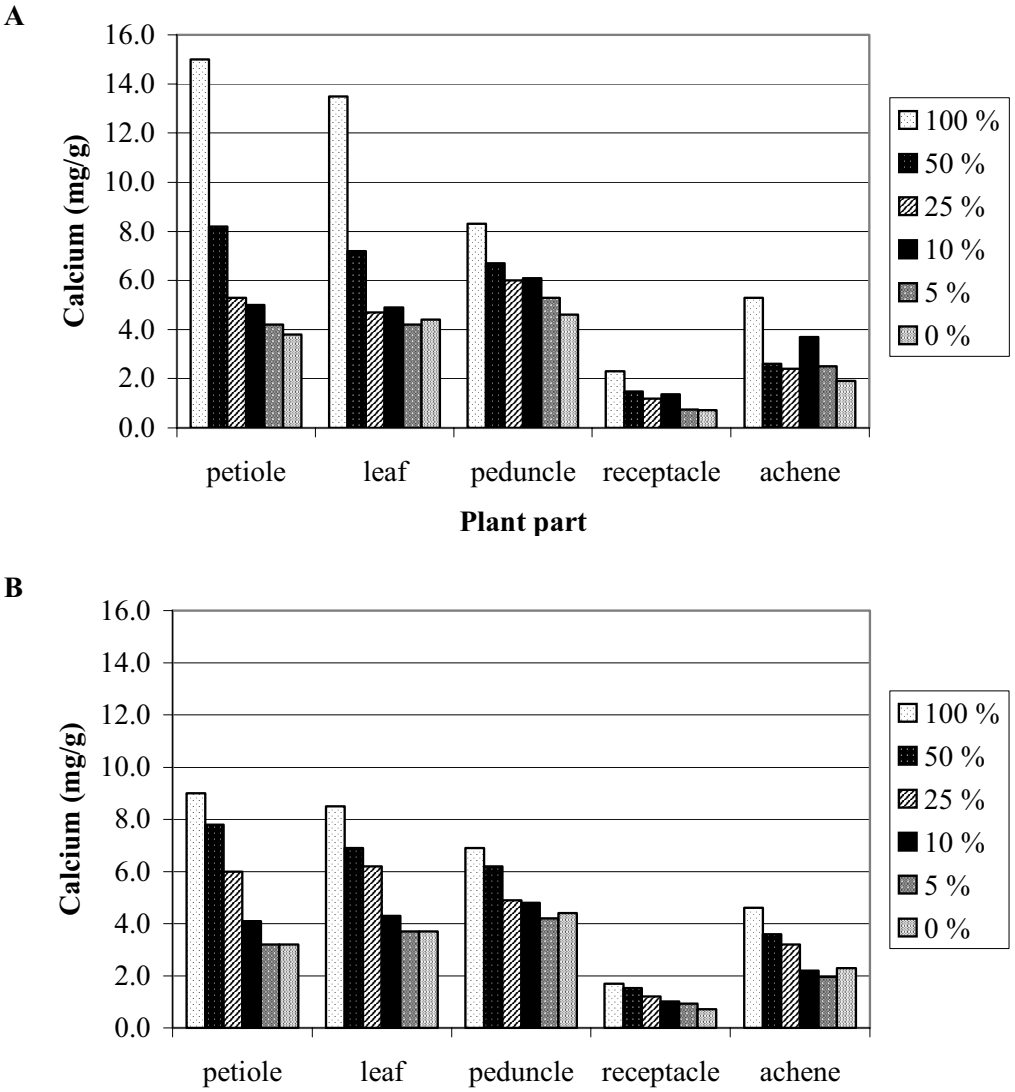


Figure 4.13 The effect of calcium treatment on the distribution of calcium to the petioles, leaves, peduncles, receptacles and achenes of cultivars **A**. Selva and **B**. Aromas. ICPAES analysis was undertaken after 20 weeks of treatment with 0 to 100 % calcium in 0.25 Hoagland's solution using the free-draining, manually fertigated system. Samples of each plant part from 3 or 4 plants were homogenised for ICPAES analysis, n = 1.

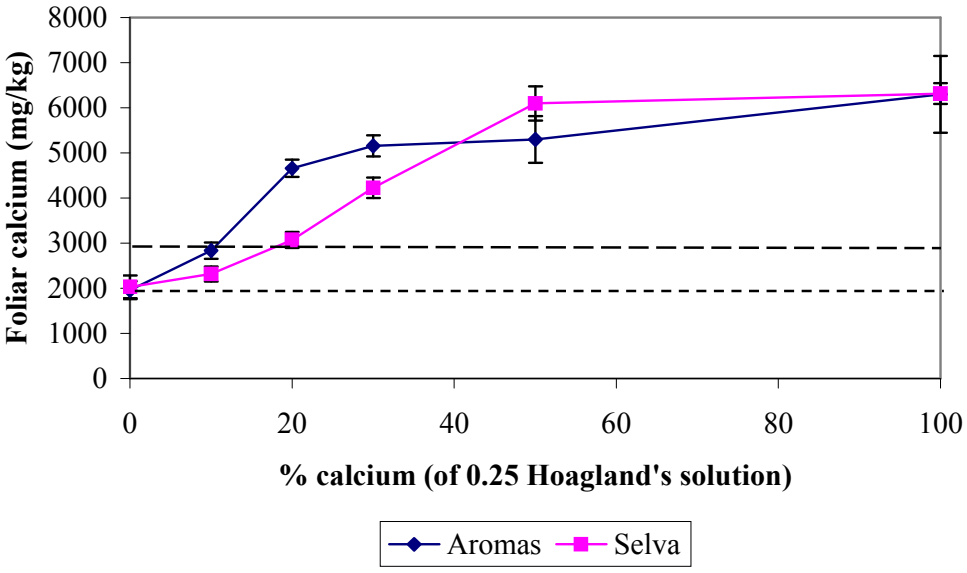


Figure 4.14 The effect of calcium treatment on foliar calcium concentration (dry weight) of strawberry cultivars, Aromas and Selva. ICPAES analysis was undertaken after 8 weeks of treatment with 0 to 100 % potassium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Data shown is mean, ± SE (n = 8). Dashed lines indicate marginal (large dash) and deficient (small dash) potassium concentrations as defined by Ulrich *et al.* (1980).

Damage included severed stems and roots, boring of the crown and decreased plant weight, which ultimately lead to death. Due to unsuccessful pesticide treatment for these larvae the experiment was terminated when deficiency symptoms were only evident on some plants, treated with the 0 and 5 % treatments.

There was a significant effect of calcium treatment on fresh weight of plants at harvest (Figure 4.15, f prob = <0.001). In Selva plants, the mass was greatest when treated with 0, 5, 10 and 100 % calcium and in Aromas, the mass was greatest when treated with 0 and 5% calcium (Figure 4.15 A). Fresh weight at harvest was similar between cultivars. The dry shoot mass (Figure 4.15 B) showed trends similar to the fresh weight. There were no significant differences between dry shoot weight of Selva, however, the dry shoot weight of Aromas plants treated with 0 and 5 % calcium had greater mass (f prob = <0.001). The association between foliar calcium concentration and fresh weight of plants at harvest for cultivars Aromas and Selva are shown in Figure 4.16. Optimal calcium concentration for maximised plant weight could not be determined from these data due to the detrimental effect crown moth larvae had on plant mass.

There was no significant effect of calcium treatment on the number of runners produced by plants, regardless of cultivar (Figure 4.17). Aromas produced significantly more runners than Selva (f prob = <0.001). Selva plants produced no runners when treated with 30 % calcium or greater. The number and mean length of runners produced by Aromas plants were not significantly affected by calcium treatment (f probs = 0.446 and 0.670, respectively).

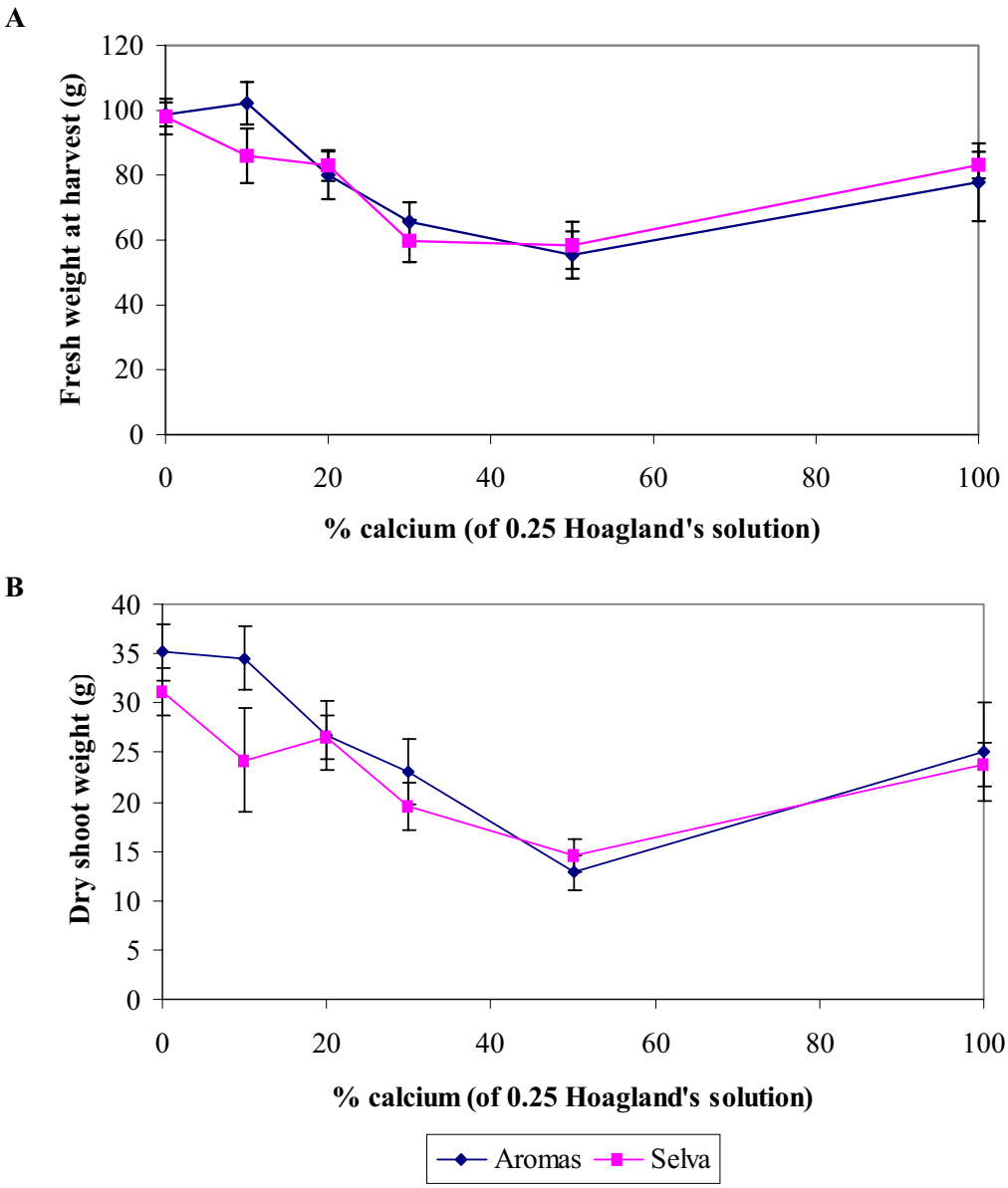


Figure 4.15 The effect of calcium treatment on **A**. fresh plant weight at harvest and **B**. dry shoot weight of strawberry cultivars, Selva and Aromas. Analysis was undertaken after 8 weeks of treatment with 0 to 100 % calcium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Fresh plant weight included shoots, runners and roots produced by plants, but excluded fruit. Shoots were removed at soil level and weighed after 24 hours at 80 °C. Data shown is mean, ± SE (n = 8).

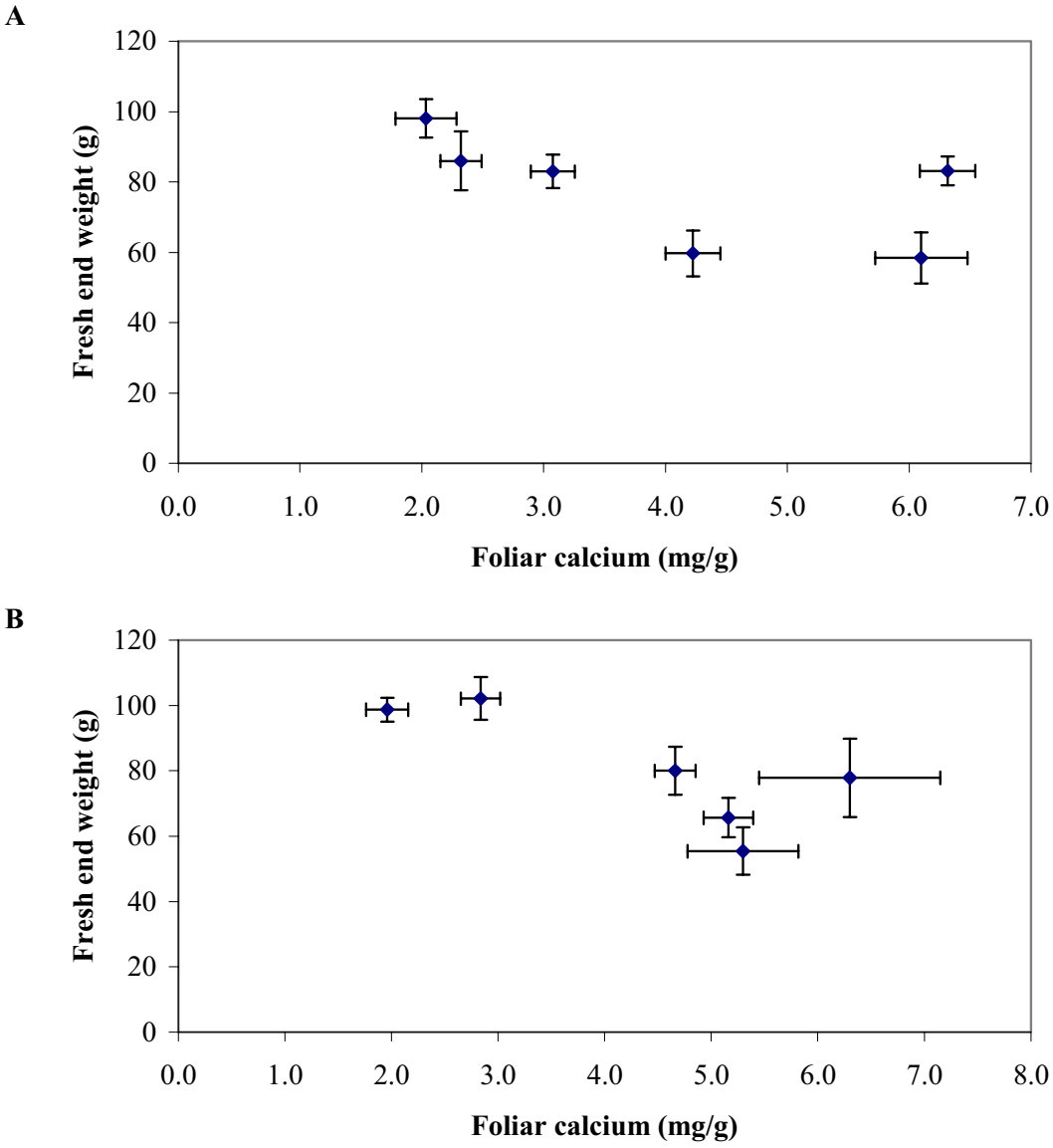


Figure 4.16 Fresh weight at harvest plotted against foliar calcium concentration (dry weight) of strawberry cultivars, **A**. Selva and **B**. Aromas. Analysis was undertaken after 8 weeks of treatment with 0 to 100 % calcium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Fresh plant weight included shoots, runners and roots produced by plants, but excluded fruit. Data shown is mean, ± SE (n = 8).

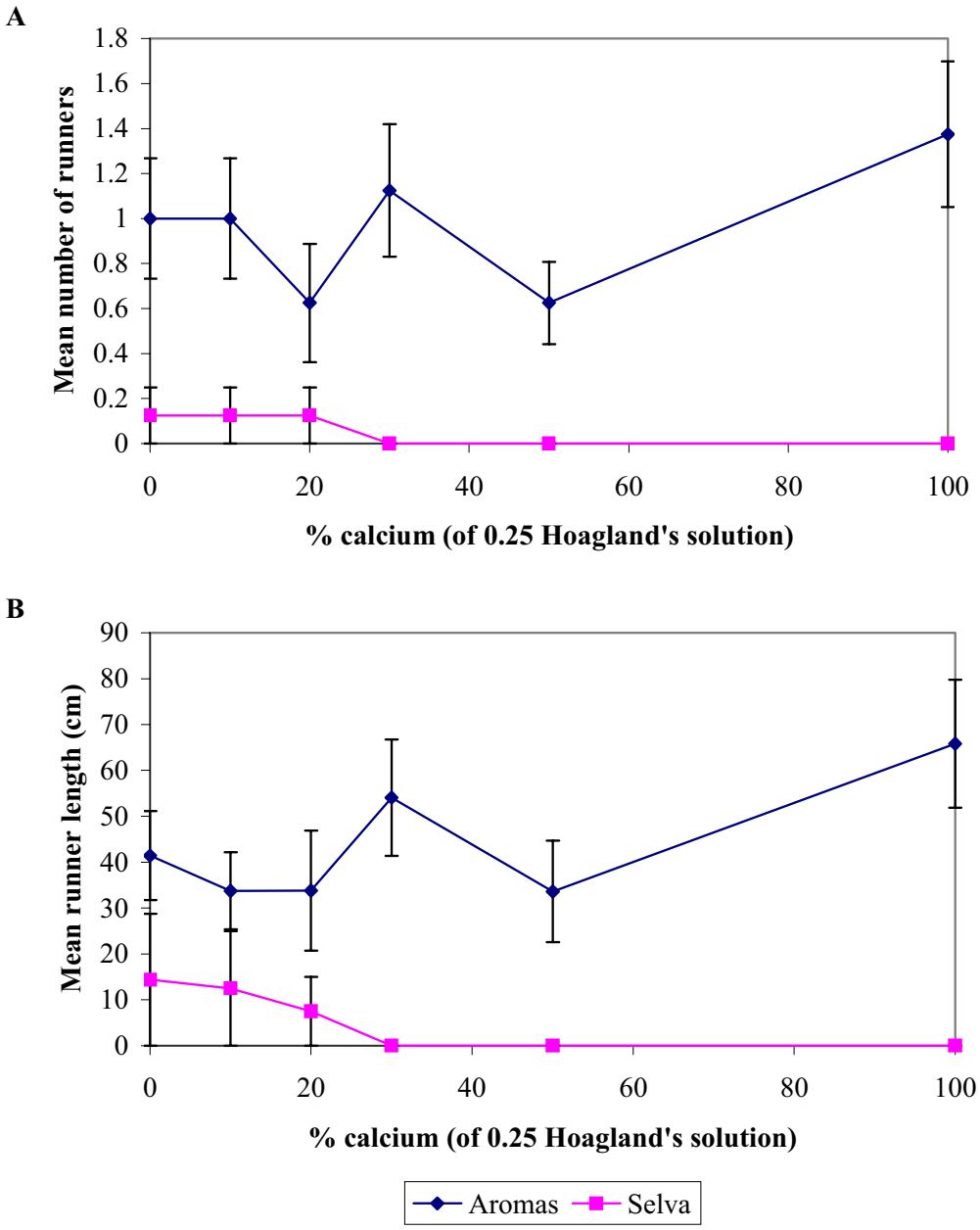


Figure 4.17 The effect of calcium treatment on **A**. the number of runners produced by each plant, and **B**. mean runner length of strawberry cultivars, Selva and Aromas. Analysis was undertaken after 8 weeks of treatment with 0 to 100 % calcium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Data shown is mean, ± SE (n = 8).

There was no significant effect of calcium treatment on the number of strawberries produced by each plant (Figure 4.18, f prob = 0.060). While there was considerable variation between the yields of plants within treatments the trend was for increased number and size of strawberries with increasing calcium treatment. Selva produced more strawberries than Selva over all the calcium treatments. Strawberries produced by Selva were generally larger than Aromas (f prob = <0.001). The increase in number of fruit produced by Selva was near linear, and Aromas plants treated with 20 % calcium and above produced significantly more fruit than when treated with 0 and 10 % calcium treatments. Mean fruit weight of Selva appeared to increase with increasing calcium treatment, however, there was no effect of calcium on mean fruit weight of Aromas (Figure 4.18 B). In order to determine a foliar concentration of calcium for optimal plant mass, the foliar calcium concentration was plotted against mean fruit weight for Aromas and Selva (Figure 4.19) and the concentration of potassium that yielded 90 % of the mean fruit weight indicated (Figure 4.20). The foliar calcium concentration range for maximum fruit weight for Aromas is above 3.8 mg/g calcium, dry weight. For Selva, the range is above 6.0 mg/g calcium, dry weight.

Increased foliar calcium concentration significantly reduced the magnesium concentration, but had no effect on potassium, phosphorus (Figure 4.20 A) or boron (Figure 4.20 B).

4.3.3 Effect of calcium on development of powdery mildew

Observations of the development of *P. aphanis* on detached leaves of Selva and Aromas grown with and without calcium are summarised in Table 4.4. *P. aphanis* developed faster on Selva leaves grown without calcium, and after 30 days colonies had coalesced to cover the abaxial surface and conidiation was prolific on the limited

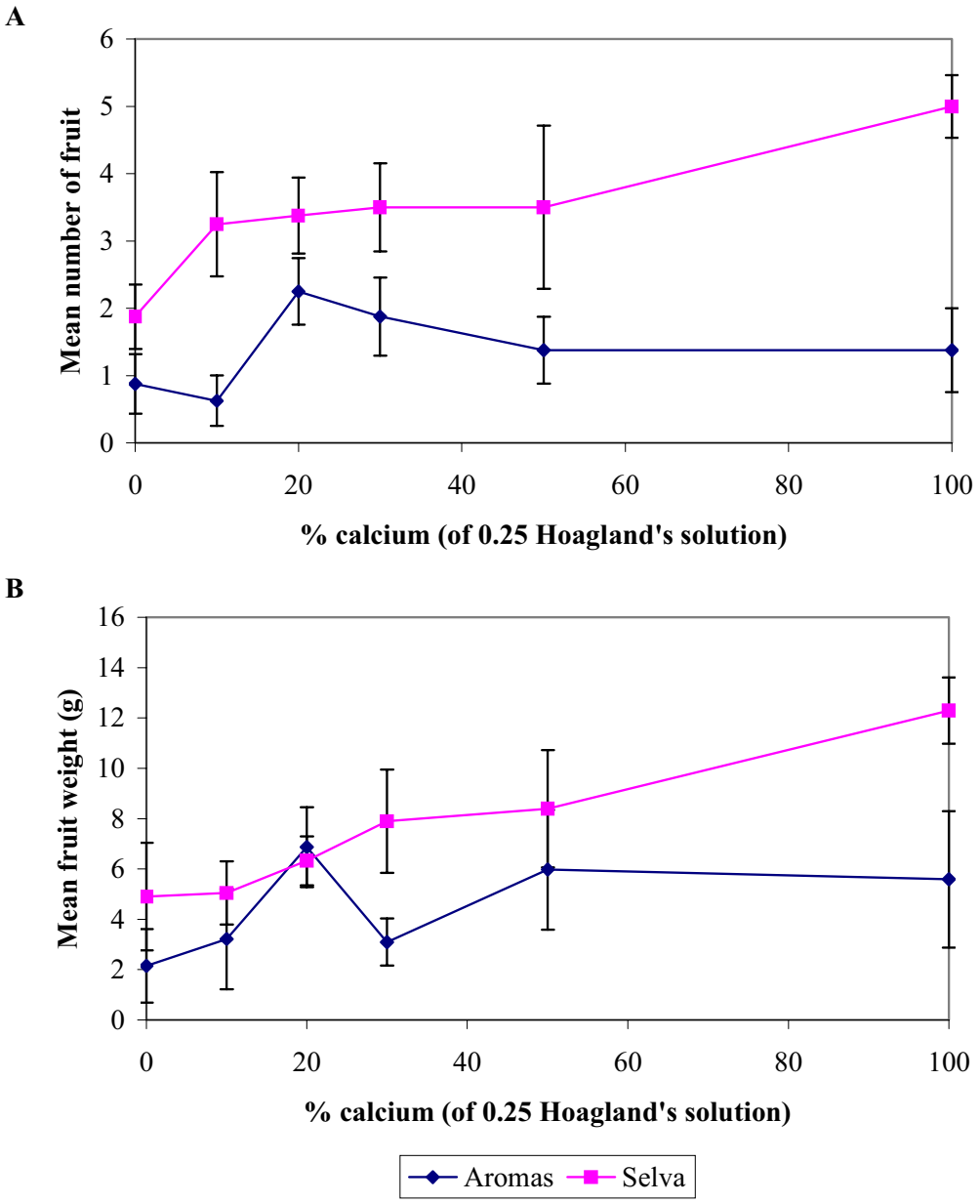


Figure 4.18 The effect of calcium treatment on A. the total number of strawberries harvested from each plant and B. mean weight of strawberries of cultivars, Selva and Aromas, during 8 weeks treated with 0 to 100 % calcium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Strawberries were harvested weekly. Data shown is mean, ± SE (n = 8).

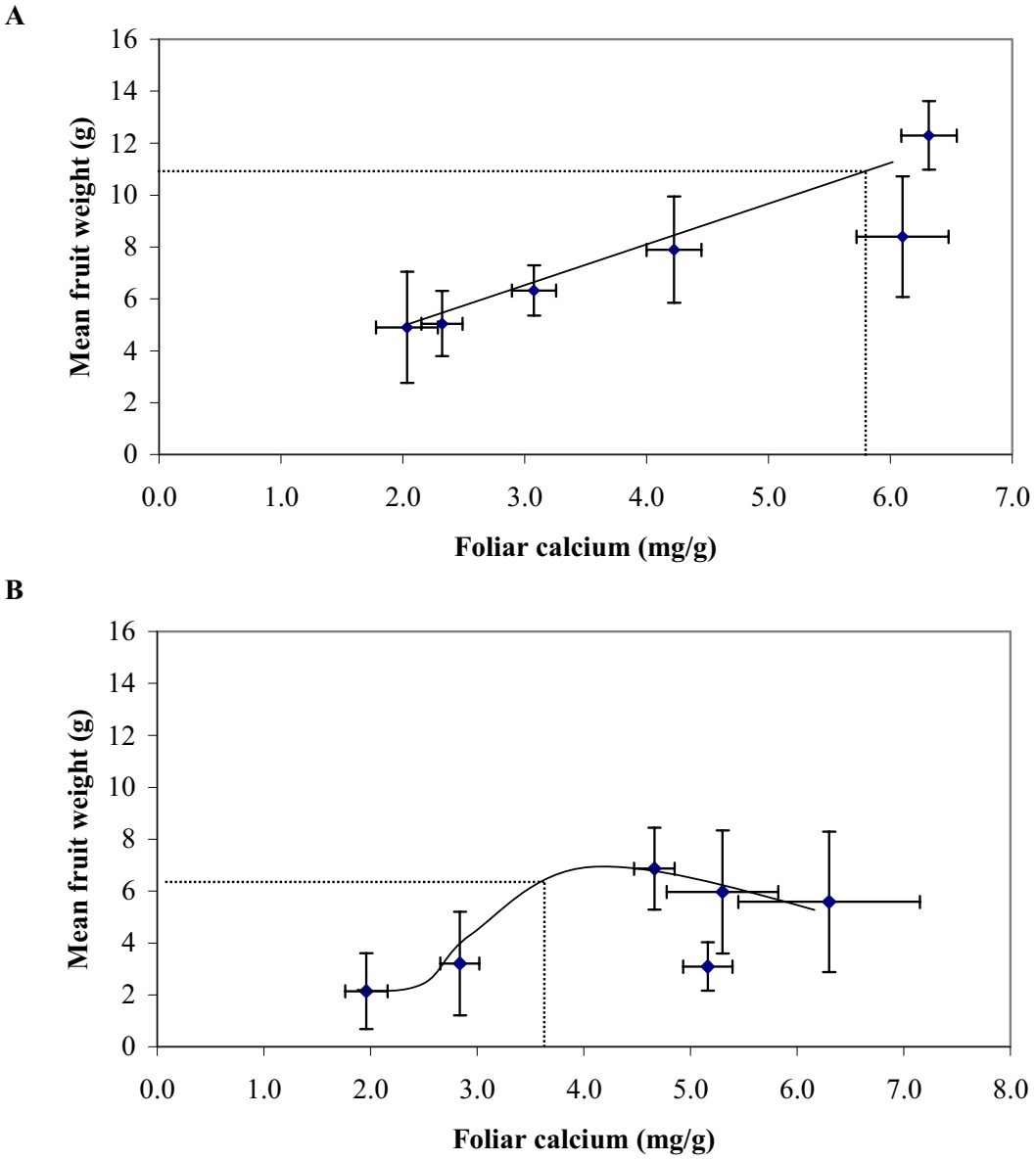


Figure 4.19 The mean fruit weight plotted against foliar calcium concentration (dry weight) of strawberry cultivars, **A**. Selva and **B**. Aromas. Analysis was undertaken after 8 weeks of treatment with 0 to 100 % calcium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Strawberries were harvest weekly. Data shown is mean, ± SE (n = 8).

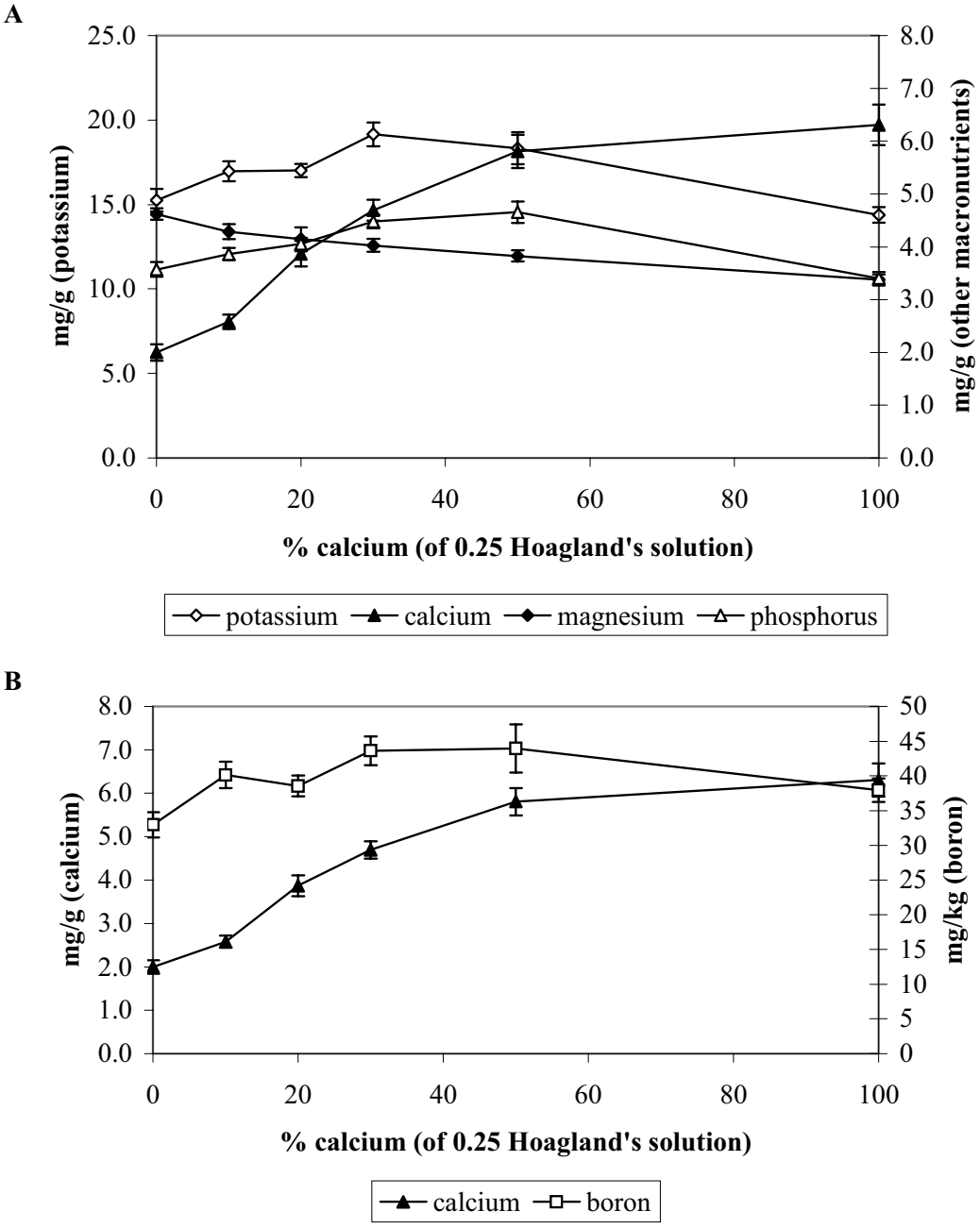


Figure 4.20 The effect of calcium treatment on foliar concentrations of **A.** potassium, calcium, phosphorus and magnesium and **B.** boron. ICPAES analysis was undertaken after 8 weeks of treatment with 0 to 100 % potassium in 0.25 Hoagland's solution using the free-draining with catchment, manually fertigated system. Data shown is mean (cultivars combined), ± SE (n = 16).

Table 4.4 Summary of observations of *P. aphanis* development on detached Selva and Aromas leaves, grown with and without calcium.

Cultivar	Calcium treatment	dpi	Germination	Growth of hyphae over one or more neighbouring cells	Conidiophores	Conidiation	Colonies distinct or coalesced
Selva	0 % calcium	2	✓	✓	×	×	Distinct
		4	✓	✓	✓	×	Distinct
		6	✓	✓	✓	✓	Distinct
		8	✓	✓	✓	✓	Coalesced
	100 % calcium	2	✓	✓	×	×	Distinct
		4	✓	✓	×	×	Distinct
		6	✓	✓	×	×	Distinct
		8	✓	✓	✓	×	Distinct
Aromas	0 % calcium	2	×	×	×	×	No colonies
		8	✓	×	×	×	No colonies
	100 % calcium	8	×	×	×	×	No colonies

✓ indicates development stage observed, × indicates development stage not observed.

calcium tissue (Figure 4.21). In comparison, colonies on leaves of Selva were distinct, up to 1 cm in diameter and conidiation was only just visible with the naked eye.

Germinated conidia were not found on Aromas leaves grown with 100 % calcium in 0.25 Hoagland's solution. On Aromas leaves grown in 0 % calcium many germinated conidia were found from 6 dpi (Figure 4.22), however, none produced hyphae and developed into colonies.

Microscopy of transverse sections of Selva and Aromas leaf tissue grown with and without calcium showed no consistent variation in cellular structure or cuticle thickness (Figure 4.23).

4.3.4 Effect of silicon on plant growth and development of *P. aphanis*

Plants that were watered with silicate supplemented 0.25 Hoagland's solution developed interveinal chlorosis (Figure 4.24), were smaller in size and yielded fewer fruit. No difference in the development of powdery mildew was observed over 40 days on these leaves when inoculated with *P. aphanis*, compared with leaves grown in unsupplemented 0.25 Hoagland's solution (data not shown).

4.4 Discussion

Plant nutrition is integral for maximising crop yields and maintaining plant health. Balanced fertilisation can maximise the plants ability to withstand infection by biotrophic pathogens such as *P. aphanis*. While it is well known that strawberry cultivars differ in requirement for nutrients, cultivar specific fertilisation requirements remain uncommon (May and Pritts, 1990). The purpose of the above studies was to determine the foliar concentration for optimal growth and yield produced by cultivars, Selva and Aromas, and the effect of limited nutrient supply on development of *P. aphanis* on leaves of these cultivars. Optimal ranges of potassium and calcium for yield have been determined and limited calcium was found to influence *P. aphanis*

0 % calcium 100 % calcium

Selva

Figure 4.21 Representative samples of powdery mildew on the abaxial surface of Selva leaves grown with and without calcium. Leaves were detached, inoculated with *P. aphanis* and maintained in the detached leaf chamber for 30 days as per Section 2.4.1.

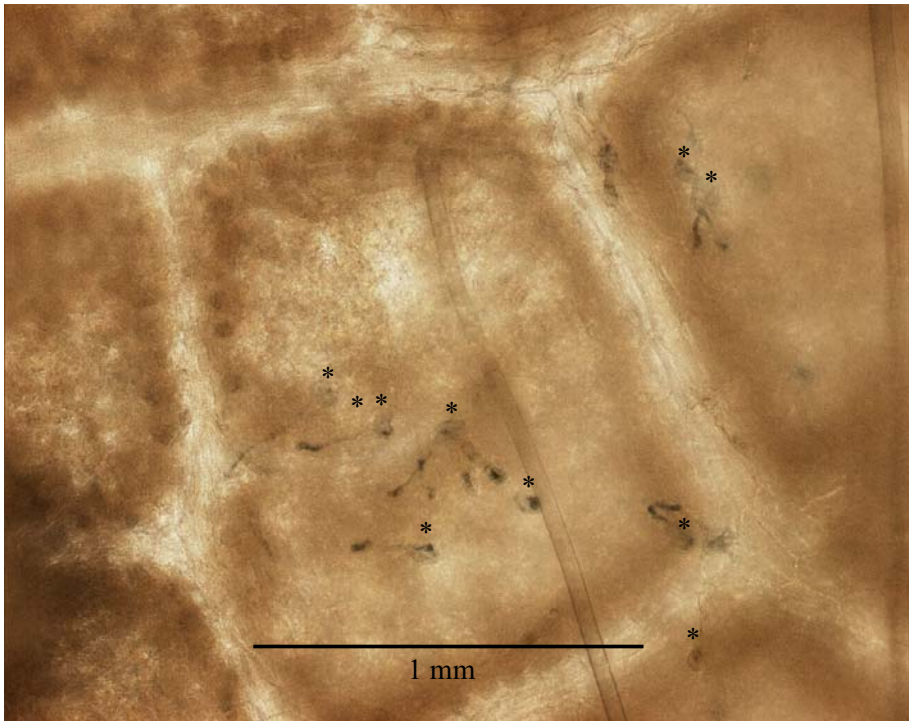


Figure 4.22 Germinated conidia on the abaxial surface of an Aromas leaf 8 dpi with *P. aphanis*. Conidia are indicated beneath asterisks.

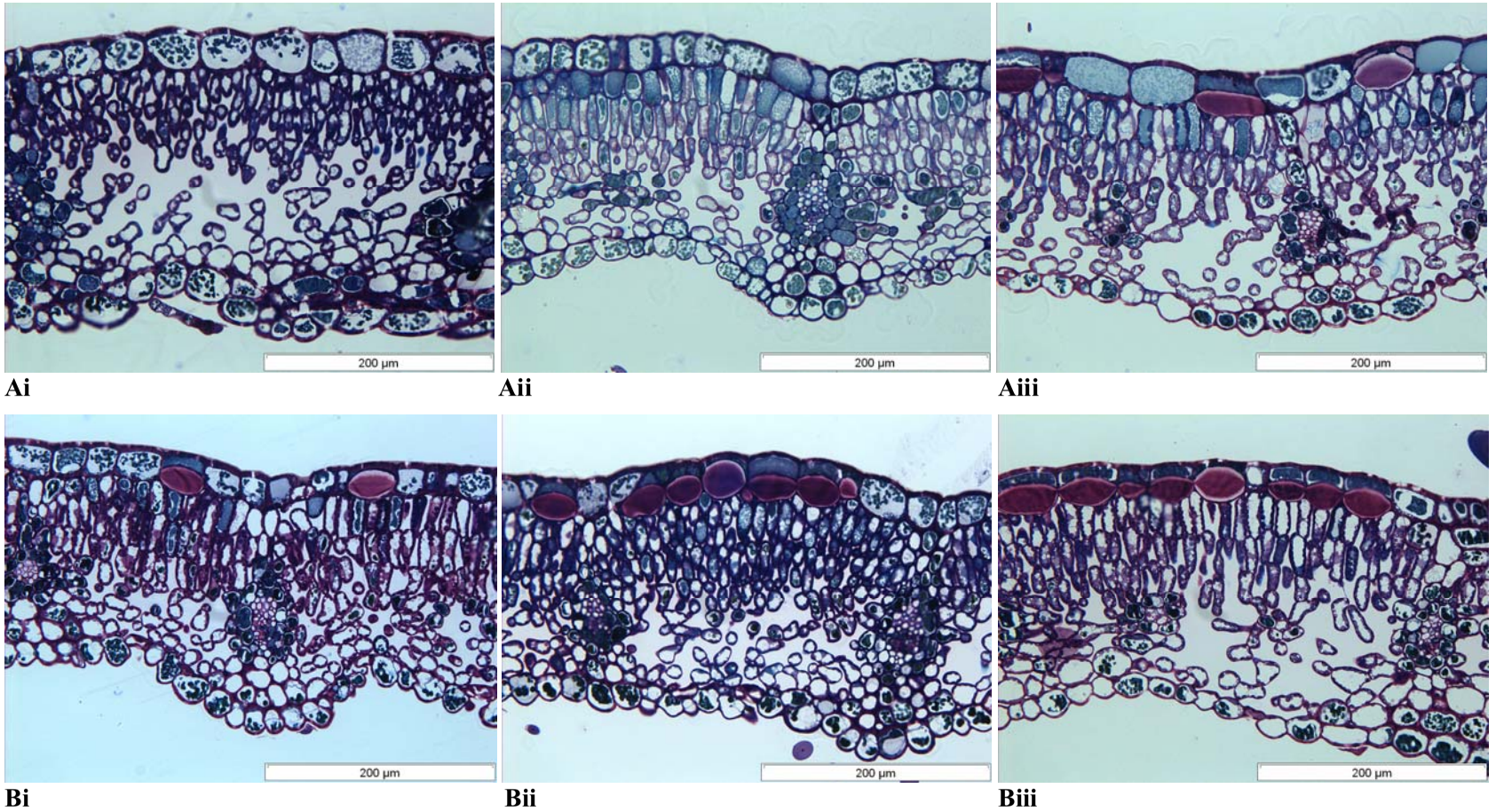


Figure 4.23 Light micrographs of tranverse sections from leaves grown with (A) and without (B) calcium. Samples were taken from Selva (i and ii) and Aromas (iii) and prepared using resin infiltration. Images were taken at random and were not representative of whole samples.

Normal Silicate-supplemented

Figure 4.24 The effect of silicate-supplemented Hoagland's solution on strawberry plants after 2 months of treatment. Symptoms shown are representative of those observed on both Selva and Aromas cultivars.

development on leaves of both the susceptible cultivar, Selva, and the resistant cultivar, Aromas.

Manipulation of the nutrient solutions of Hoagland and Arnon (1938) have ensured that all treatment solutions contained the same concentration of nitrogen, phosphorus and all micro-nutrient concentrations between treatments as would be in a complete treatment. This is also true for the nutrient not in question, i.e. the calcium concentration in the limited potassium treatments was normal, and vice versa. The importance of maintaining the levels of nitrogen was stressed as it has been shown to have significant effects on the ratio of vegetative to fruit production in strawberry crops (Haynes and Goh, 1987). Nitrogen levels of the original 0 % potassium and calcium solutions of Hoagland and Arnon (1938) were less than the complete solution. It is well known that $CaNO_3$ is one of few calcium compounds that is readily soluble and is commonly used in strawberry production settings to deliver calcium. It was decided against using this to change the rates of calcium in treatment solutions as it would add nitrogen at the same rate of calcium. $CaSO_4$ was used instead, although this required much larger volumes of solution as it is only soluble to 0.2 M. The small trial using 0 % potassium and calcium proved that they could be utilised as base solutions for treatments in more comprehensive trials.

The foliar concentrations of a number of nutrients often decline during fruit production, depending on requirements of fruit for given nutrients and the number and size of fruit (John *et al.*, 1975; Marschner, 1995). Where potassium or calcium was provided at limited rates, foliar concentrations declined, but in 100 % calcium treated plants, the calcium increased steadily until fruiting commenced, when the foliar concentration dropped rapidly. This concurs with results of May *et al.* (1994) and Chow *et al.* (1992) however contradicts findings of Haynes and Goh (1987).

The length of time required for the onset of calcium deficiency symptoms suggests that the root system of strawberry runners contain a high level of calcium prior to planting. It appears that this internal supply is not depleted for approximately 3 months of growth. This is in contrast to most crop plants which are planted from seed and thus contain a relatively limited internal supply. Furthermore, a large amount of calcium is required for events of cell elongation such as sprouting. Strawberry runners are planted with an established crown so the energy required for plant establishment would be comparatively less than if the same plant was to grow from a seed.

The optimal range for maximisation of plant mass and yield was determined for strawberry cultivars, Selva and Aromas. The optimal ranges for potassium for both cultivars were similar between plant mass and yield. The plant mass of the calcium treated plants was influenced by the damage cause by the strawberry crown moth larvae, however, the yield data, which was collected over the entire 7 months of the experiment was not significantly influenced by this damage, and thus this was used to determine the optimal range for calcium. Yield data has been used in most studies of strawberry fertilisation (Voth, *et al*., 1967; Chiu and Bould, 1976; Haynes and Goh, 1987; Albregts, *et al*., 1991; Miner, *et al*., 1997; Cengiz, *et al*., 2003; Kirschbaum, *et al*., 2004). Haynes and Goh (1987) measured the height of plants and counted the number of leaves, crowns and runners produced by each plant as well as yield. Chow *et al*. (1992) and Lieten (2004) measured fresh weight of plant at harvest (roots included) in addition to yield. Berglund (2006) appears to be the only other study that determined fertilisation recommendations solely on the dry shoot weight.

As defined by this study, the optimal foliar concentrations for Selva was above 10.0 mg/g potassium and 6.0 mg/g calcium, dry weight. The optimal range for Aromas was above 12.0 mg/g potassium and 3.8 mg/g calcium, dry weight. These are within the ranges defined by Ulrich *et al.* (2000).

May and Pritts (1990) describe the most suitable time to sample for determination of plant health status as YOLs taken 6 weeks after harvest. However, particularly in day-neutral strawberry cultivars that fruit for up to 6 months, yield can be limited where fertilisation has not been well managed before this sampling time. Thus it is suggested that determination of dynamic nutrient level for maximised yield during fruiting is an important area for future research.

The potassium and calcium treatments significantly affected concentrations of other nutrients in leaves. Potassium application has been observed to cause reduced levels of calcium and magnesium in leaf tissues (Chiu and Bould, 1976; Haynes and Goh, 1987; Peñalosa *et al.*, 1994). Peñalosa *et al.* (1994) also showed a negative effect of calcium treatment on the potassium to calcium ratio in leaf tissue. In addition to the effect of calcium application on potassium levels, it has also been observed to increase the levels of nitrogen in fruit tissues.

Aromas was able to produce plant mass and yield not significantly different from the 100 % calcium control treatment with 70 % less calcium applied. The mass and yield of Selva was significantly reduced by all restricted calcium treatments. This is an important finding for strawberry production where the availability of calcium from fertigation is of concern, especially in colder conditions (P. Mason, personnal communication, 2003) when calcium is less available from the soil.

The difference in *P. aphanis* development on calcium deficient leaf tissue compared with healthy leaf tissue of both cultivars may be explained by the effect deficiency has on the leaf structure and physiology (Chiu and Bould, 1976). Calcium is important for cell integrity, and thus it can be postulated that the fungus would meet less physical resistance to penetration in calcium deficient tissues. Also, the concentration of amino acids and sugars in the apoplasm and at the leaf surface increases considerably with calcium deficiency, which causes increased membrane permeability. The

concentration of the soluble assimilates in the apoplasm of host tissues (particularly of epidermal cells) can stimulate penetration and post-infection stages of fungal infection (Marschner, 1995). Selva, the susceptible cultivar, showed a higher requirement for calcium, supporting anecdotal evidence of the involvement of calcium efficiency in resistance of strawberry cultivars to powdery mildew.

In summary, recommendations of potassium and calcium foliar concentration requirement for optimal yields of Aromas and Selva plants can now be made from glasshouse-based pot experiments. The amount and method of application need further investigation for use in current fertigated cropping systems. In addition, the calcium status of strawberry tissue does affect development of the powdery mildew pathogen, *P. aphanis* supporting anecdotal evidence from farmers and strawberry breeders, however, potassium and silicon status appear to have less of an effect.

Chapter 5 Effect of foliar application of calcium chloride, potassium silicate and Systhane® on powdery mildew severity

5.1 Introduction

South Australian strawberry growers regularly suffer yield losses attributable to powdery mildew due to the failure of infected flowers to mature normally and fruit having an unmarketable shape, cracking or superficial mycelium. At the commencement of these studies, Systhane® (Bayer Corporation), the only product registered for control of powdery mildew on strawberries in South Australia, was not considered effective (P. James, personal communication, 2003). The susceptibility of the dominant cultivar in production, Selva, and the lack of a suitable resistant alternative added to the inability to effectively control this disease.

Foliar application of nutrients can reduce severity of fungal diseases by supplementing the nutrition of the plant, increasing natural foliar defence mechanisms, and in some cases adversely affecting fungal development (reviewed in Section 1.4.4). Calcium and silicon appear to have primary roles in plant defence through maintenance of membranes and cell walls (Marschner, 1995), and enhancement of cell wall rigidity (Graham, 1983; Datnoff *et al.*, 2001), respectively. Circumstantial evidence suggests that foliar calcium levels are often low in areas where powdery mildew is severe (P. Mason, personal communication, 2004), and that cultivars susceptible to this disease may not mobilise calcium from the soil as efficiently as others (B. Morrison, personal communication, 2003). Preliminary experiments showed calcium to be poorly mobilised in the susceptible cultivar Selva (Dunn, 2003; Dunn and Able, 2004). Potassium silicate products have previously been shown to control powdery mildew on grape, cucumber and muskmelon (Bowen et al., 1992; Menzies *et al.*, 1992). The objective of this study was to investigate the effect of foliar-applied calcium and silicate, and the interaction of

these products with current cropping practices, on powdery mildew severity and yield of strawberry crops with respect to disease management in the field. The efficacy of the fungicide Systhane® for powdery mildew management, and its effects on yield, were also considered in these experiments. As the local industry standard, this gave an indication of disease management currently attainable by strawberry farmers in South Australia.

5.2 Materials and Methods

Three field trials were undertaken between May 2003 and May 2006 (Table 5.1) as per Section 2.3. These were a preliminary trial in the 2003/04 season to investigate the effect and interactions of Systhane®, Stopit® and Kasil 2040® on the severity of powdery mildew, and two trials, over the 2004/05 and 2005/06 harvesting seasons, to evaluate the use of Systhane® and Kasil 2040® treatments for controlling powdery mildew in South Australian strawberry crops. All treatments were made in addition to the farmer's standard practice. Trial locations are described in Section 2.2.

5.2.1 Preliminary trial

Runners were planted in a completely randomised block design with three replicated blocks comprising two cultivars, Aromas and Selva, and three foliar treatments. Genstat version 6.0 (Rothamsted, UK) was used to optimise randomisation of the experimental layout. Four plants were planted in each plot, with one plant left untreated as a buffer between plots.

5.2.1.1 Treatments and application

The three foliar treatments applied to the trial were Systhane® (Bayer Corporation), Stopit® (Phosyn Plc) and Kasil 2040® (PQ Corporation). Systhane® is a group C, wettable fungicide with AI, myclobutanil. Stopit® is $CaCl_2$ and was chosen as the calcium foliar application as it was necessary to avoid $CaNO_3$ products (although

Table 5.1 Summary of field trials from 2003 to 2006. Treatments were applied fortnightly unless specified, at rates as per Section 5.2.1.1.

	2003/04 season	2004/05 season	2005/06 season
Property	P. Mason	P. Mason	D. Parker
Plantings	Single row	Single row	Double row
Grower-applied fungicide	None	Systhane®	Systhane® Flint®
Cultivars	Selva Aromas	Selva	Selva
Treatments	 Systhane® K_2SO_4 (nil silicate control) Kasil 2040® Kasil 2040® + Systhane® Stopit® Kasil 2040® + Stopit® Kasil 2040® + Systhane® + Stopit®	Water (control) Systhane® K_2SO_4 (nil silicate control) Kasil 2040® Kasil 2040® + Systhane®* Kasil 2040® buffered to pH 7 Kasil 2040® buffered + surfactant	Water (control) Systhane® K_2SO_4 (nil silicate control) Kasil 2040® + Systhane® Kasil 2040® buffered + surfactant
Applicator	Yates pump sprayer	Solo motorised mister	Solo motorised mister

* indicates applied fortnightly, monthly and fortnightly when the severity was above the economic threshold of 2.

these are commonly used in strawberry production due to the high solubility of $CaNO_3$) to avoid excessive foliar growth induced by nitrogen fertilisation. Kasil 2040® is a soluble K_2SiO_4 product. A K_2SO_4 control with the same concentration of potassium as the Kasil 2040® treatment was used for comparison to assess the effect of SiO_4 on disease severity.

Treatments were applied at the rates recommended by the manufacturers (1.2 g/L Systhane®, 4 mL/L Stopit® and 11.1 mL/L Kasil 2040®, applied so that each square metre received approximately 0.5 mL of treatment) using a 5L Yates Maxi Pressure Sprayer. Interactions between treatments were also investigated and these plots received multiple applications with each treatment as necessary. The application of treatments was fortnightly from 16/12/2003 until 27/01/2004. Spraying was conducted on a Tuesday unless the temperature was over 28 °C, it was raining or the wind was strong enough to blow treatments onto neighbouring rows. In such cases, spraying was postponed to the Wednesday or Thursday of the same week.

5.2.1.2 Powdery mildew assessment

The subjective 0 to 6 visual foliar disease scale (Table 2.1) was used for assessment of disease severity in the 2003/04 season. Disease was assessed twice weekly (on Tuesdays and Fridays). Observations were made in the morning after the dew had dried, which was usually around 10 am.

5.2.1.3 Harvest data collection

Fruit was picked twice weekly, on Tuesdays and Fridays. Fruit harvested was counted, weighed then sorted into marketable and unmarketable fruit. Marketable fruit was sorted into size classes (small, medium, large, extra large and extra extra large, see Section 2.7) and then observed for defects as described in Section 2.7. The number of fruit in each class was recorded.

5.2.2 Trials for assessing use of potassium silicate for management of powdery mildew

In the 2004/05 and 2005/06 trials, blocks were selected from existing plots that had been planted and maintained by the growers. In the 2004/05 season, plantings were spaced approximately 25 cm apart in a single row arrangement, whereas the 2005/06 plantings were spaced approximately 25 cm in a staggered double row arrangement (Section 2.3). Plots of ten plants with buffer plots of four plants were marked out in August of each year. Buffer rows were left untreated between each treated row. Sample size and the number of replicates were as recommended for statistical significance by T. Hancock, BiometricsSA. Genstat version 8.0 was used to allocate treatments applied to each plot in a random block design.

5.2.2.1 Treatments and application

The spray coverage achieved using the Yates® pump pack in the preliminary trial was estimated using water sensitive papers (Norvitis Crop Protection AG, Switzerland), which showed that fungicide was deposited mainly on the abaxial side of leaves to run off (Figure 5.1, left). A Solo® 444 motorised mister pack was employed in the subsequent trials to give a more evenly distributed application of treatment on both the upper and lower side of leaves in finer droplets to prevent runoff (Figure 5.1, right). Application using valve 2 at full throttle was calculated to apply between 10 to 20 mL to each plant. Treatments were applied along one side of the row then along the opposite side at a slow walking pace. The airflow from the mister pack was directed to disrupt the majority of leaves to maximise application to adaxial and abaxial leaf surfaces.

Nine treatments were applied in the first trial (2004/05 season). These were: 1)

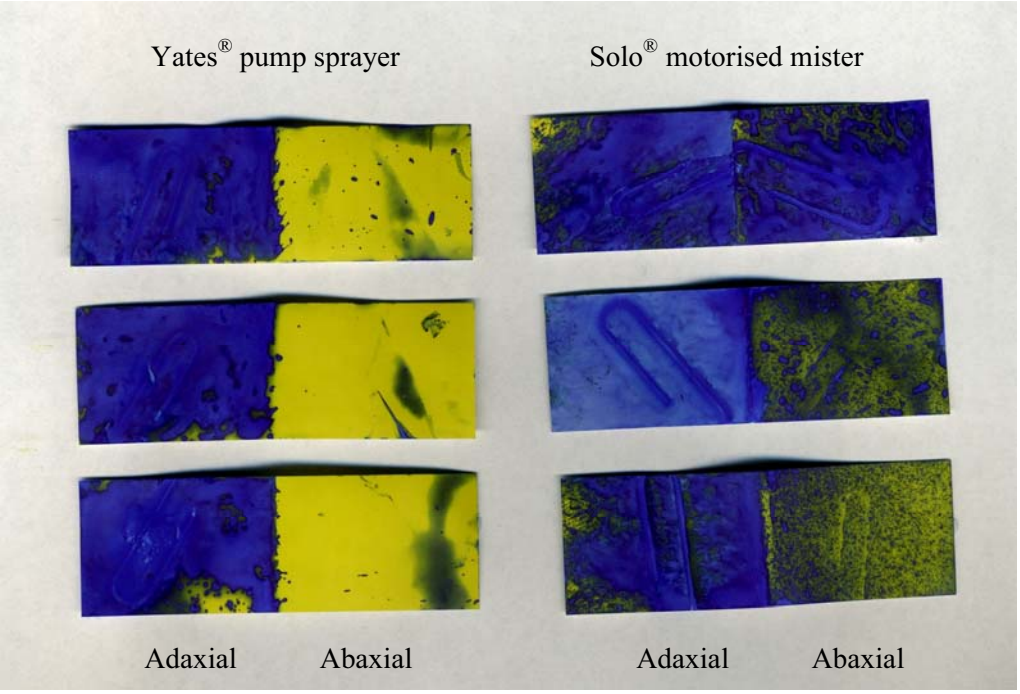


Figure 5.1 Comparison of application efficiency of the Yates® pump sprayer used in the 2003/04 season and the Solo® motorised mister used in the 2004/05 and 2005/06 seasons. The air-flow from the Solo® motorised mister disrupted leaf position and effectively applied treatment to the adaxial and abaxial leaf surfaces.

water control, 2) a no silicate control (2.73 g/L K_2SO_4), 3) Kasil 2040® (11.1mL/L), 4) Systhane® (1.2 g/L), 5) Systhane® and Kasil 2040® (applied fortnightly), 6) Systhane® and Kasil 2040® (applied monthly), 7) Systhane® and Kasil 2040® (applied when the mean powdery mildew score was 3 or more), 8) Kasil 2040® buffered to pH 7.0 and 9) Kasil 2040® buffered to pH 7.0 with Tween 20® added as a surfactant. In the following trial (2005/06 season) the number of treatments was reduced due to time constraints, so that treatments 1, 2, 4, 5 and 9 were repeated.

Treatments were applied fortnightly, generally on a Monday (weather permitting) or following the disease assessment (see Section 5.2.2.2), from the beginning of October to the end of April in both seasons.

5.2.2.2 Powdery mildew assessment

The 0 to 7 foliar disease scale (Table 2.2) was applied to the same four plants in each plot of ten on each occassion. Assessments were made fortnightly on Mondays, unless raining, in which case assessment was postponed to the Wednesday of the same week. The fruit disease scale (Table 2.3) was applied to harvested fruit as they were sorted.

5.2.2.3 Harvest data collection

Fruit was picked and sorted into cull and size classes and the number in each class was tallied as previously described (Section 2.7). The marketable fruit was weighed. This was considered to be more a more valuable measure of yield than the total weight (inclusive of culled fruit) used in the 2003/04 season.

5.2.3 Data analysis

Statistical analyses were carried out using Genstat version 8.0 software (see Section 2.8). The effect of treatments on harvest was determined using repeated

measures analysis of variance (ANOVA). Least significant differences (LSDs) between means were determined where P = <0.05. Disease score data were treated in two ways within the analyses of this chapter, using ordinal regression analysis and repeated measures ANOVA as outlined below.

5.2.3.1 Ordinal regression analysis of foliar powdery mildew

Ordinal regression analysis was performed on disease score data from each observation date using nested analyses to test the significance of between the novel treatments and their respective controls on powdery mildew severity. Where the counts for all plots in a given disease score were 0 this score was omitted from the analysis for this date.

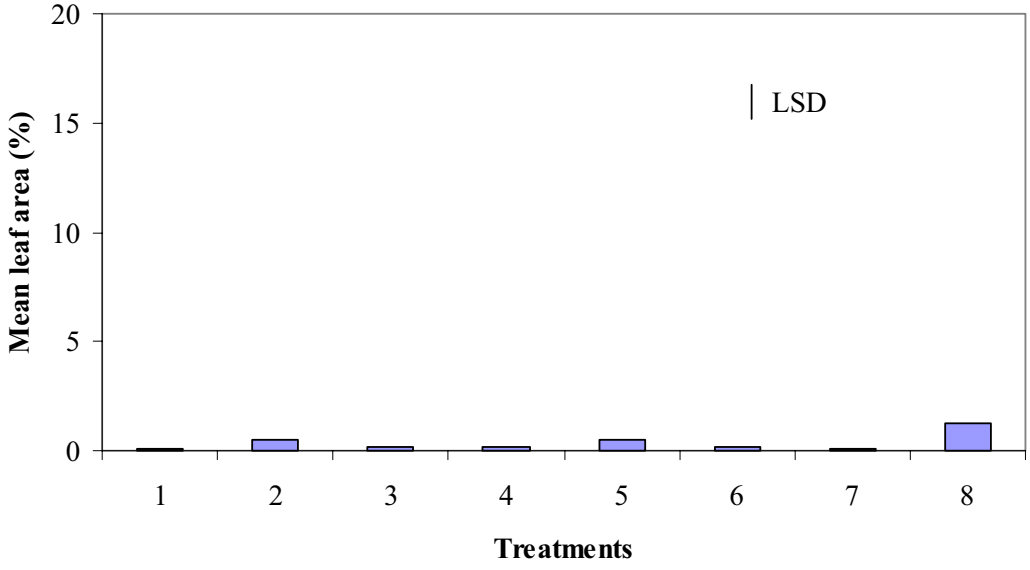
5.2.3.2 Repeated measures ANOVA of foliar powdery mildew

Disease score data was transformed to affect leaf area as per Section 3.2.5 and effects of treatments over the observation dates was analysed using the repeated measures ANOVA analysis in Genstat version 8.0.

5.3 Results

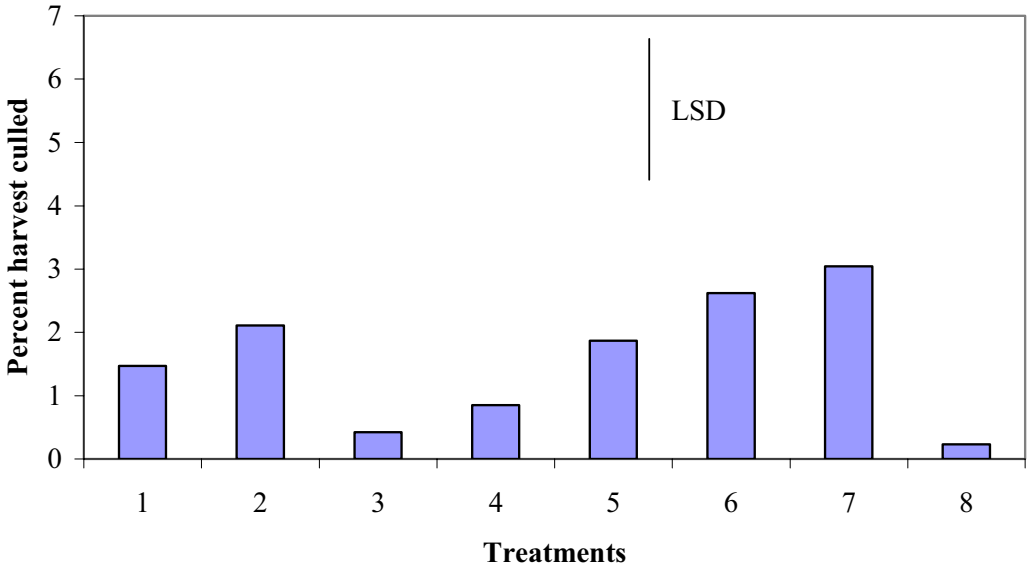
5.3.1 Preliminary trial (2003/04 season)

Powdery mildew was significantly less severe on the resistant cultivar Aromas than Selva regardless of treatment (mean affected leaf area = 0.4 and 9.93 % respectively, F prob = <0.001, see Chapter 3). The highest mean severity of foliar powdery mildew on Aromas was 1.25 % affected leaf area, regardless of foliar treatment (Figure 5.2). Treatment did not affect the percent harvest culled due to powdery mildew symptoms (Figure 5.3) nor the mean weight of fruit harvested from Aromas plots (Table 5.2). However, the number of marketable strawberries produced



1.	K_2SO_4 (-Si control)	5.	Stopit®
2.	Systhane®	6.	Stopit®+ Systhane®
3.	Kasil 2040®	7.	Stopit®+ Kasil 2040®
4.	Kasil 2040® + Systhane®	8.	Kasil 2040® + Systhane®+ Stopit®

Figure 5.2 Effect of Systhane®, Stopit® and Kasil 2040® treatments on the severity of powdery mildew on strawberry cultivar Aromas in the 2003/04 season. Affected leaf area was measured on 21 dates from 16/12/2003 to 27/01/2004 and the means calculated for each treatment over these dates. n = 1008, from 3 replicate plots of 4 plants. LSD = 0.4185



1.	K_2SO_4 (-Si control)	5.	Stopit®
2.	Systhane®	6.	Stopit®+ Systhane®
3.	Kasil 2040®	7.	Stopit®+ Kasil 2040®
4.	Kasil 2040® + Systhane®	8.	Kasil 2040® + Systhane®+ Stopit®

Figure 5.3 Effect of Systhane®, Stopit® and Kasil 2040® treatments on the percentage of strawberries harvested from Aromas culled due to powdery mildew mycelium on the receptacle. Fruit harvested from each plot was assessed twice weekly from 09/12/2003 to 23/03/2004. Mean values calculated from 3 replicates of 4 plots (n = 12) at each of the 31 harvest dates. LSD = 2.252.

Table 5.2 The effect of Systhane®, Stopit® and Kasil 2040® treatments on yield of cultivar Aromas in the 2003/04 season. Mean weight of total yield (g), the number of marketable fruit and the mean fruit weight (of all fruit harvested) are shown. Fruit was harvested from 48 plants (n) between 09/12/2003 and 23/03/2004. $LSD_{Total\ weight} = 878.1$, $LSD_{Number\ marketable} = 89.91$, $LSD_{Mean\ fruit\ weight} = 0.8413$.

Treatment	Total weight (g)	Number marketable	Mean fruit weight (g)
K_2SO_4 (-Si control)	2727^{ab}	258^{ab}	10.58^{a}
Systhane®	2277^{a}	198^{a}	11.39^{a}
Kasil 2040®	3328^{b}	306^{b}	10.84^{a}
Kasil 2040® + Systhane®	3011^{ab}	238^{ab}	11.11^{a}
Stopit®	3027^{ab}	273^{ab}	11.08^{a}
Stopit® + Systhane®	2909^{ab}	264^{ab}	11.10^{a}
Stopit® + Kasil 2040®	2278^{a}	205^{a}	11.20^{a}
Kasil 2040® + Stopit® + Systhane®	2646^{ab}	278^{ab}	11.19^{a}

Superscripts denote statistical difference at $P<0.05$.

and total yield weight were higher where treated with Kasil 2040® than with Systhane® or the combination of Systhane® and Kasil 2040® (Table 5.2).

In the ordinal regression analysis of the 2003/04 season Selva disease score data the Kasil 2040® and Systhane® treatments showed significant interaction to reduce powdery mildew severity over the majority of the observations dates (Table 5.3). This ameliorative effect was evident from the second application of this combination of treatments to the end of the observation period in March 2004. In contrast, a consistent beneficial effect of the Systhane® treatment alone was not observed until 3rd February 2004, after the fourth application on the 27th Janruary.

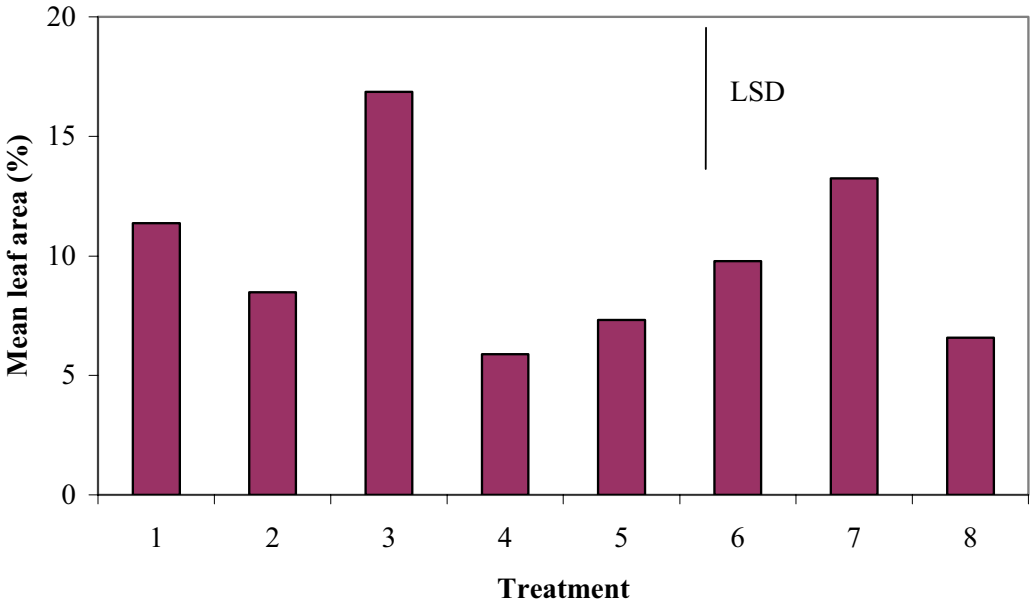
ANOVA of the disease scores transformed into estimates of leaf area showed similar treatment effects. The powdery mildew on Selva plants treated with both Kasil 2040® and Systhane® had severity significantly below that of the K_2SO_4 control (Figure 5.4). The Kasil 2040® treatment alone appeared to increase the severity of powdery mildew on Selva. The interaction between Kasil 2040® and Systhane® was significant (Fprob = 0.008) as the plants treated with both had a significantly lower mean powdery mildew score than the plants treated with Kasil 2040® alone (Figure 5.4). This trend was consistent from January 2004 to the conclusion of the trial when the powdery mildew was most severe (Figure 5.5).

On Selva, the mean affected leaf area for all plots treated with Systhane® was significantly lower (7.69 %) than plots not treated with fungicide (12.19 %) (Fprob = 0.006) (Figure 5.5). However, while the powdery mildew on Systhane®-treated plants was less severe than untreated plants throughout the season, the mean powdery mildew severity over time was not maintained below the economic threshold of 5 % affected leaf area from 20/01/2004 to the conclusion of this trial on 12/03/2004 (Figure 5.5).

Table 5.3 Summary of ordinal regression analysis of 2003/04 season disease score data of Selva. Disease was scored weekly on a scale of 0 to 6, where 0 represented no symptoms and 6 the plant was deceased. At each observation time the same four plants in each of three replicate plots were examined (n = 12). Superscripted numbers following dates denote the treatment application instance. Estimates consistent over multiple dates are shown in italics.

Treatments / **Date**	Systhane® vs. no fungicide	Stopit® vs. no calcium	Kasil 2040® (unbuffered) treatment vs. K_2SO_4 (-Si control)	Systhane® and Stopit® interaction	Systhane® and Kasil 2040® interaction	Stopit® and Kasil 2040® interaction	Systhane®, Kasil 2040® and Stopit® vs. no treatment
16/12/2003[1]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
19/12/2003	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
23/12/2003	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
26/12/2003	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
30/12/2003[2]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
02/01/2004	-1.022**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
06/01/2004	n.s.	-2.116*	n.s.	2.48**	n.s.	n.s.	n.s.
09/01/2004	n.s.	n.s.	n.s.	n.s.	*-2.25**	n.s.	n.s.
12/01/2004[3]	n.s.	n.s.	n.s.	n.s.	*-2.43**	n.s.	n.s.
16/01/2004	n.s.	n.s.	n.s.	n.s.	*-2.75**	n.s.	n.s.
20/01/2004	n.s.	-2.897*	n.s.	1.24*	*-4.63****	1.38**	n.s.
23/01/2004	n.s.	n.s.	n.s.	1.08**	*-5.56****	n.s.	n.s.
27/01/2004[4]	-0.39***	n.s.	n.s.	n.s.	*-4.18****	n.s.	n.s.
30/01/2004	0.43**	n.s.	n.s.	n.s.	*-5.16****	n.s.	n.s.
03/02/2004	*-2.314***	n.s.	n.s.	4.06**	*-1.37***	n.s.	n.s.
06/02/2004	*-1.903****	n.s.	n.s.	3.38*	*-3.25****	n.s.	n.s.
13/02/2004[5]	*-1.731****	n.s.	n.s.	n.s.	*-3.41****	n.s.	n.s.
14/02/2004	*-1.623**	n.s.	1.33*	3.34*	*-1.41***	n.s.	n.s.
02/03/2004	*-3.362****	n.s.	n.s.	2.91*	*-1.73**	n.s.	n.s.
05/03/2004	*-2.55****	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
12/03/2004	*-1.43***	-2.46*	n.s.	1.38*	*-11.2**	n.s.	n.s.

Superscripts denote approximate chi probability, where * is 0.011 to 0.05, ** is 0.010 to 0.001, *** is <0.001. n.s. denotes an insignificant result. n.d. denotes no disease present on the given date and therefore analysis was not possible.



1.	K_2SO_4 (-Si control)	5.	Stopit®
2.	Systhane®	6.	Stopit®+ Systhane®
3.	Kasil 2040®	7.	Stopit®+ Kasil 2040®
4.	Kasil 2040® + Systhane®	8.	Kasil 2040® + Systhane®+ Stopit®

Figure 5.4 Effect of Systhane®, Stopit® and Kasil 2040® treatments on the severity of powdery mildew on strawberry cultivar Selva in the 2003/04 season. Affected leaf area was measured on 21 dates from 16/12/2003 to 27/01/2004 and the means calculated for each treatment over these dates. n = 1008, from 3 replicate plots of 4 plants. LSD = 6.019.

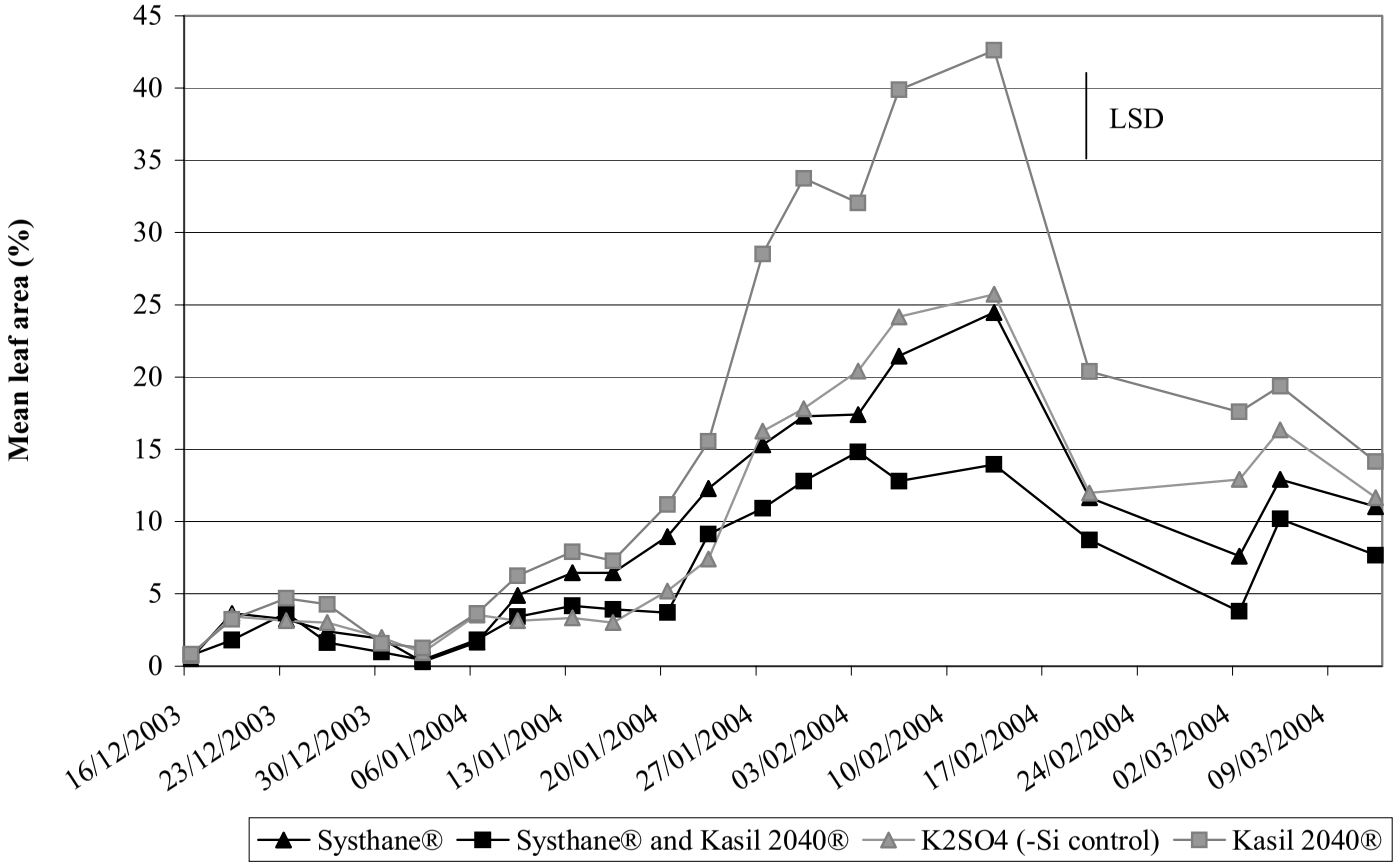


Figure 5.5 The effect of Systhane® applied fortnightly as a foliar spray on powdery mildew severity on cultivar Selva over the 2003 to 2004 season in the field. Affected leaf area was measured once or twice weekly. Fprob = <0.001, n = 12, LSD = 7.178.

The mean effect of Stopit® treatment was insignificant (Fprob = 0.329), as was the effect on powdery mildew severity over time compared with untreated plants (Fprob = 0.810) (Figure 5.6). Stopit® application significantly reduced severity of powdery mildew on six of the 21 observation dates. These were 06/01, 09/01, 20/01 and 30/01, 13/02 and 12/03/2004 (Figure 5.6). There was a significant interaction observed between the Systhane® and Stopit® treatments over time (Fprob = 0.007). However, after 20/01/2004 the combined treatment did not maintain the severity of powdery mildew below the economic threshold of 5 % affected leaf area.

None of the treatments significantly affected yield of Selva (Table 5.4) and only the combination of Stopit® and Kasil 2040® resulted in a higher percentage of strawberries culled due to powdery mildew mycelium on the receptacle than the Systhane® treatment (Figure 5.7).

5.3.2 Trials for assessing use of potassium silicate for management of powdery mildew (2004 to 2006 seasons)

Generally, powdery mildew was severe in the 2004/05 season (mean affected leaf area on Selva was 12.95 %) but not in the 2005/06 season (mean affected leaf area on Selva was 0.08 %. See also, Chapter 3).

In the 2004/05 season, ordinal regression analysis showed the severity of both foliar and fruit powdery mildew on Selva was significantly reduced at most of the observation dates by the fortnightly treatment with Systhane® alone compared with the water control treatment (Table 5.5). However, there was no significant effect of Systhane® treatment in the 2005/06 season (Table 5.6). The severity of foliar and fruit powdery mildew of plants treated with Systhane® and Kasil 2040® combined, were generally not significantly different from the Systhane® alone in the 2004/05 season. Foliar severity

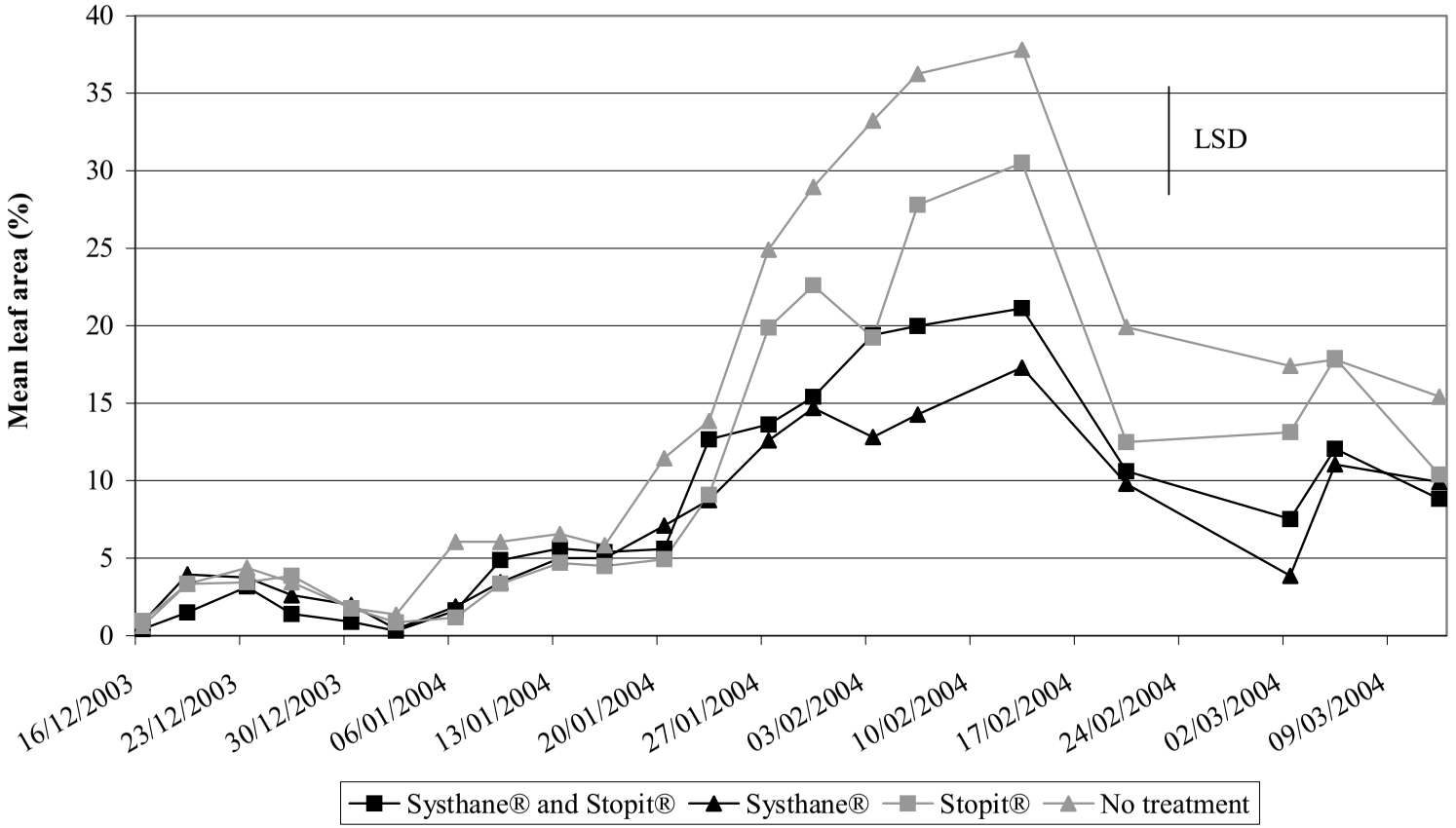
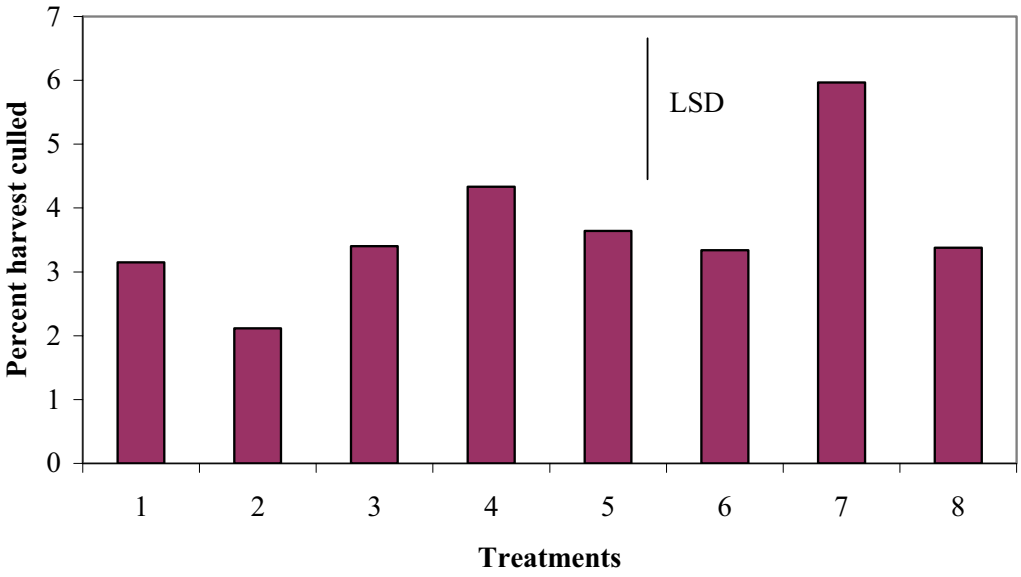


Figure 5.6 The effects of Stopit® and Systhane®, applied fortnightly alone and in combination, on powdery mildew severity on cultivar Selva in field conditions over the 2003/04 season. Affected leaf area was measured once or twice weekly. Data from Selva plants with no treatment applied also shown in Figure 3.1. Fprob = 0.007, n = 12, LSD = 7.178.

Table 5.4 The effect of Systhane®, Stopit® and Kasil 2040® treatments on yield of cultivar Selva in the 2003/04 season. Mean weight of total yield (g), the number of marketable fruit and the mean fruit weight is shown. Fruit was harvested from 48 plants (n) between 09/12/2003 and 23/03/2004. $LSD_{Total\ weight}$ = 878.1, $LSD_{Number\ marketable}$ = 89.91, $LSD_{Mean\ fruit\ weight}$ = 0.8413.

Treatment	Total weight (g)	Number marketable	Mean fruit weight (g)
K_2SO_4 (-Si control)	2567[a]	246[a]	10.48[a]
Systhane®	2779[a]	264[a]	10.7[a]
Kasil 2040®	3177[a]	316[a]	10.12[a]
Kasil 2040® + Systhane®	2933[a]	287[a]	10.38[a]
Stopit®	3157[a]	307[a]	10.35[a]
Stopit® + Systhane®	2826[a]	287[a]	9.84[a]
Stopit® + Kasil 2040®	2473[a]	236[a]	10.53[a]
Kasil 2040® + Stopit® + Systhane®	2433[a]	230[a]	10.54[a]

Superscripts denote statistical difference at P<0.05.



1.	K_2SO_4 (-Si control)	5.	Stopit®
2.	Systhane®	6.	Stopit®+ Systhane®
3.	Kasil 2040®	7.	Stopit®+ Kasil 2040®
4.	Kasil 2040® + Systhane®	8.	Kasil 2040® + Systhane®+ Stopit®

Figure 5.7 Effect of Systhane®, Stopit® and Kasil 2040® treatments on the percentage of strawberries harvested from Selva culled due to powdery mildew mycelium on the receptacle. Harvest from each plot was assessed twice weekly from 09/12/2003 to 23/03/2004. Fruit were deemed unmarketable if smaller than 5 g, were misshapen or had blemishes. Mean values were calculated from 3 replicates of 4 plots (n = 12) at each of the 31 harvest dates. LSD = 2.252.

Table 5.5 Summary of ordinal regression analysis of 2004/05 season disease score data of Selva. Disease was scored weekly on a scale of 0 to 6, where 0 represented no symptoms and 6 the plant was deceased. At each observation time the same four plants in each of three replicate plots were examined (n = 12). Superscripted numbers following dates denote the treatment application instance. Estimates consistent over multiple dates are shown in italics.

Treatments	K_2SO_4 (-Si control) vs. water control		Systhane® vs. water control		Systhane® and Kasil 2040® treatment vs. Systhane® control		Systhane® and Kasil 2040® treatment, applied fortnightly and as necessary vs. monthly				Kasil 2040® treatments vs. Systhane® control						Kasil 2040® treatments vs. K_2SO_4 (-Si control)						Kasil 2040® buffered treatments vs. unbuffered (pH11.4)			
							Fortnightly application		Application as necessary		pH 11.4		pH 7.0		pH 7.0 + Tween20®		pH 11.4		pH 7.0		pH 7.0 + Tween20®		pH 7.0		pH 7.0 + Tween20®	
Date	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit
17/11/2004[4]	n.s.		n.s.		-2.3**		n.s.		2.1**		n.s.		n.s.		n.s.		2.4***		1.3*		n.s.		n.s.		-1.5*	
01/12/2004[5]	n.s.		*-3.1****		n.s.		n.s.		3.2***		5.1***		2.7***		2.4***		n.s.		n.s.		n.s.		-2.3**		-2.6**	
15/12/2004[6]	n.s.		*-5.7****		n.s.		-2.0**		n.s.		5.4***		3.0***		2.1**		n.s.		-1.5*		-2.4***		-2.5***		-3.4***	
29/12/2004[7]	-1.6*		*-4.9****		n.s.		n.s.		3.6***		4.6***		4.8***		2.6***		n.s.		1.6*		n.s.		n.s.		-2.0**	
12/01/2005[8]	-2.6**		*-8.2****		n.s.		*-4.8****		-1.6*		6.4***		5.7***		6.4***		n.s.		n.s.		n.s.		n.s.		n.s.	
26/01/2005[9]	n.s.		*-6.4****		n.s.		*-3.0****		1.3*		6.7***		5.8***		6.5***		n.s.		n.s.		n.s.		n.s.		n.s.	
07/02/2005[10]	-1.8*	-1*	*-6.8****	*-3***	n.s.	n.s.	*-2.5****	n.s.	1.5*	n.s.	5.5***	2*	3.2***	2*	3.7***	n.s.	n.s.	n.s.	-1.7*	n.s.	n.s.	n.s.	-2.5***	n.s.	-1.9**	n.s.
23/02/2005[11]	n.s.	n.s.	*-6.4****	*-3***	1.9*	n.s.	*-1.8***	n.s.	n.s.	n.s.	5.9***	n.s.	3.9***	3*	5.2***	2*	n.s.	n.s.	-1.9**	n.s.	n.s.	n.s.	-1.9**	n.s.	n.s.	n.s.
06/03/2005[12]	n.s.	-2*	*-5.3****	*-12****	n.s.	11*	*-2.6****	n.s.	n.s.	-11***	5.8***	11***	4.6***	n.s.	3.8***	10***	n.s.	n.s.	n.s.	-11***	-1.9**	n.s.	n.s.	-12***	-2.0**	n.s.
24/03/2005[13]	n.s.	n.s.	*-7.5****	*-3***	-2.1**	n.s.	*-4.3**	*-1**	n.s.	n.s.	n.s.	2*	n.s.	n.s.	n.s.	n.s.	1.7*	n.s.	n.s.	n.s.	-3.5***	n.s.	n.s.	n.s.	-5.2**	n.s.
06/04/2005[14]	n.s.	-1*	*-8.2****	*-2***	n.s.	n.s.	*-3.7****	*-7****	n.s.	n.s.	8.6***	2***	6.8***	n.s.	4.4***	2*	1.5*	2***	n.s.	n.s.	-2.9***	1*	-1.8*	-2**	-4.4***	n.s.
22/04/2005	-1.3*	n.s.	*-3.3****	n.s.	n.s.	n.s.	*-2.8****	n.s.	n.s.	n.s.	3.2***	n.s.	n.s.	n.s.	5.4***	n.s.	n.s.	n.s.	n.s.	n.s.	1.9**	n.s.	-2.0**	n.s.	n.s.	n.s.

Superscripts denote approximate chi probability, where * is 0.011 to 0.05, ** is 0.010 to 0.001, *** is <0.001. n.s. denotes an insignificant result.

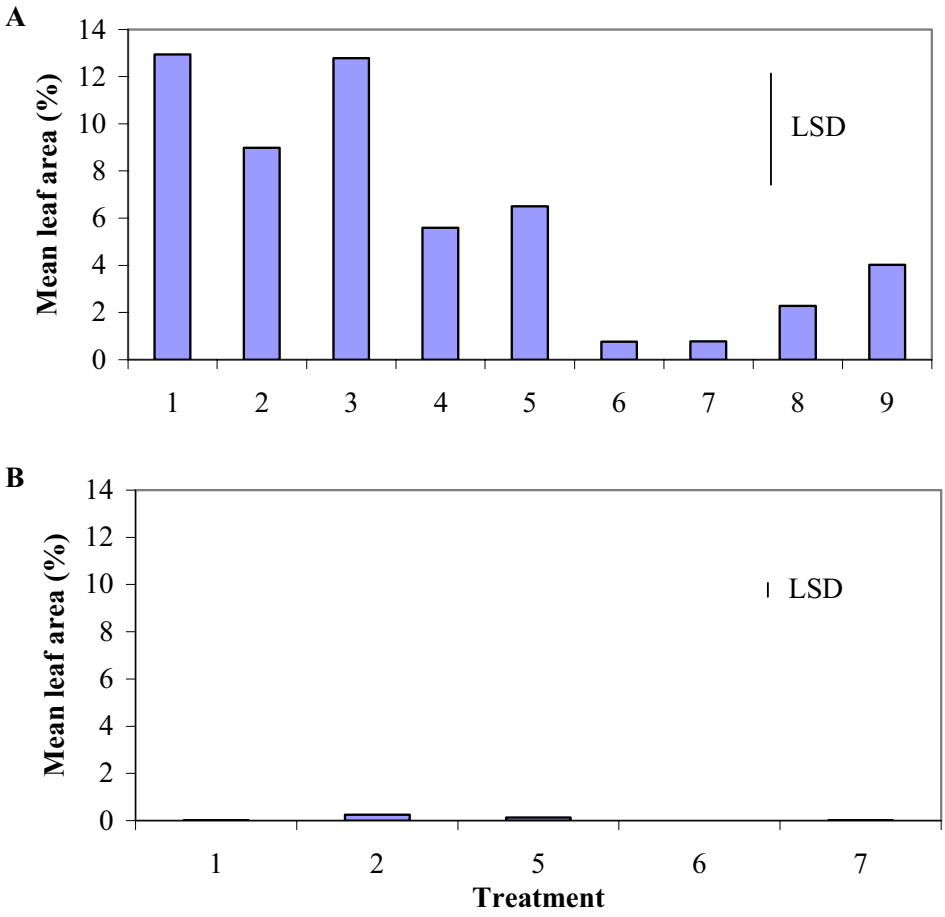
Table 5.6 Summary of ordinal regression analysis of 2005/06 season disease score data of Selva. Disease was scored weekly on a scale of 0 to 6, where 0 represented no symptoms and 6 the plant was deceased. At each observation time the same four plants in each of three replicate plots were examined (n = 12). Superscripted numbers following dates denote the treatment application instance. Estimates consistent over multiple dates are shown in italics.

Treatments	K_2SO_4 (-Si control) vs. water control		Systhane® vs. water control		Systhane® and Kasil 2040® treatment vs. Systhane® control		Kasil 2040® (pH 7.0 + Tween20®) treatment vs. Systhane® control		Kasil 2040® (pH 7.0 + Tween20®) treatment vs. K_2SO_4 (-Si control)	
Date	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit	Foliar	Fruit
04/11/2005[3]	n.d.		n.d.		n.d.		n.d.		n.d.	
18/11/2005[4]	n.s.		n.s.		n.s.		n.s.		n.s.	
05/12/2005[5]	n.s.		n.s.		n.s.		n.s.		n.s.	
19/12/2005[6]	n.s.		n.s.		n.s.		n.s.		n.s.	
02/01/2006[7]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
16/01/2006[8]	n.s.	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.	n.d.
30/01/2006[9]	n.s.	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.	n.d.
13/02/2006[10]	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.	n.d.	n.s.
29/02/2006[11]	n.s.	n.s.	n.s.	n.s.	*-10****	n.s.	*-14.4****	n.s.	n.s.	n.s.
17/03/2006[12]	n.s.	n.d.	n.s.	n.d.	*-2.4**	n.d.	*-6.27***	n.d.	n.s.	n.d.
31/03/2006[13]	n.s.	n.d.	n.s.	n.d.	*-12.1****	n.d.	*-25****	n.d.	n.s.	n.d.
10/04/2006	n.s.	n.d.	n.s.	n.d.	*-2.33**	n.d.	*-13.0****	n.d.	*-9.2****	n.d.

Superscripts denote approximate chi probability, where * is 0.011 to 0.05, ** is 0.010 to 0.001, *** is <0.001. n.s. denotes an insignificant result. n.d. denotes no disease present on the given date and therefore analysis was not possible.

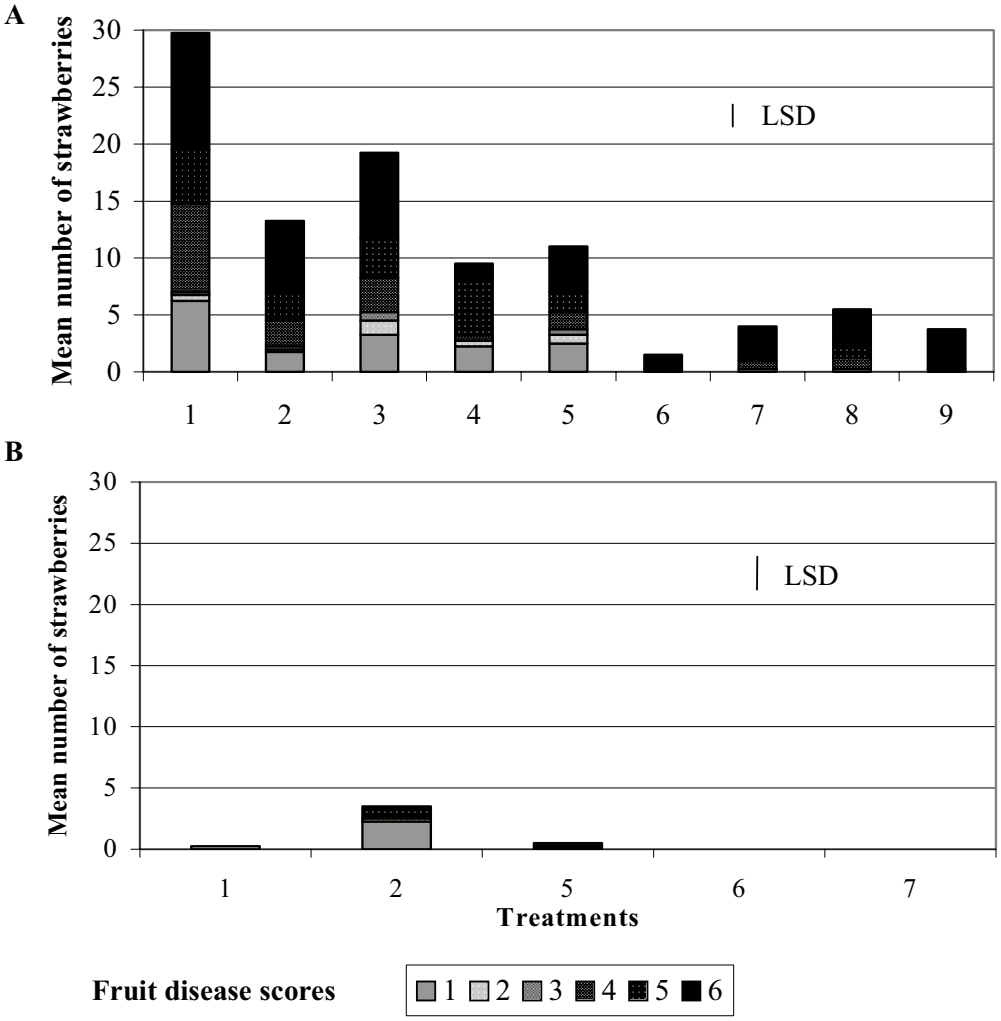
was significantly reduced in the 2005/06 season by Systhane® and Kasil 2040® combined treatment compared with Systhane® alone and there was insufficient levels of powdery mildew on fruit to determine any affect of treatment in this season. ANOVA of affected leaf area also showed similar trends with both the Systhane® alone and combined treatments resulting in significant reduction in affected leaf area compared with the water control in the 2004/05 season (Figure 5.8 A) and no significant effect of treatment in the 2005/06 season (Figure 5.8 B). Of all treatments, Systhane® yielded the fewest number of strawberries affected by powdery mildew in the 2004/05 season, however, all affected fruit scored high for severity of powdery mildew symptom (Figure 5.9 A). The fortnightly combined Systhane® and Kasil 2040® treatment did produce more affected fruit (Figure 5.9 A), however, did not yield a significantly larger number of unmarketable affected fruit (fruit disease scores greater than 2, see also, Table 2.3). There was no significant effect of treatment on the number of fruit affect by powdery mildew or the severity of powdery mildew symptoms on fruit in the 2005/06 season (Figure 5.9 B). Disease in plots treated fortnightly with Systhane® (alone and in combination with Kasil 2040®) was maintained below the economic threshold of 5 % affected leaf area throughout the observation period, between 17/11/2004 and 20/04/2005 (Figure 5.10).

Of the combined Systhane® and Kasil 2040® treatments applied at differing frequencies, ordinal regression analysis and ANOVA of affect leaf area showed the most consistent benefit was achieved with fortnightly applications (Table 5.5 and Figure 5.8 A, respectively). The combined treatment, applied when disease exceeded the economic threshold, appeared to reduce powdery mildew on fruit to a level comparable to the fortnightly application (Figure 5.9 A), however, the ordinal regression analysis of fruit disease reveals this was only the case in one fortnight and not a consistent effect.



1. Water	2. K_2SO_4 (-Si control)
3. Kasil 2040® pH 11.4	4. Kasil 2040® pH 7.0
5. Kasil 2040® pH 7.0 with Tween	6. Systhane®
7. Kasil 2040® + Systhane®, applied fortnightly	
8. Kasil 2040® + Systhane®, applied monthly	
9. Kasil 2040® + Systhane®, applied when severity exceeds economic threshold	

Figure 5.8 Effect of treatment on foliar powdery mildew severity in the 2004/05 (A) and 2005/06 (B) seasons. LSD = 4.828 and 0.2946, respectively. Affected leaf area was measured fortnightly, from 14/11/2004 to 16/04/2005 (n = 16, over 4 replicates). Data presented are means from Selva only.



Treatments

1. Water
2. K_2SO_4 (-Si control)
3. Kasil 2040® pH 11.4
4. Kasil 2040® pH 7.0
5. Kasil 2040® pH 7.0 with Tween
6. Systhane®
7. Kasil 2040® + Systhane®, applied fortnightly
8. Kasil 2040® + Systhane®, applied monthly
9. Kasil 2040® + Systhane®, applied when severity exceeds economic threshold

Figure 5.9 Effect of treatment on the number of strawberries with powdery mildew and symptom severity from cultivar Selva in the 2004/05 (A) and 2005/06 (B) seasons. LSD = 1.049 and 3.527, respectively. Fruit disease severity was scored on a scale of 0 to 6, where 0 represented no symptoms and 6 had superficial mycelium and was misshapen.

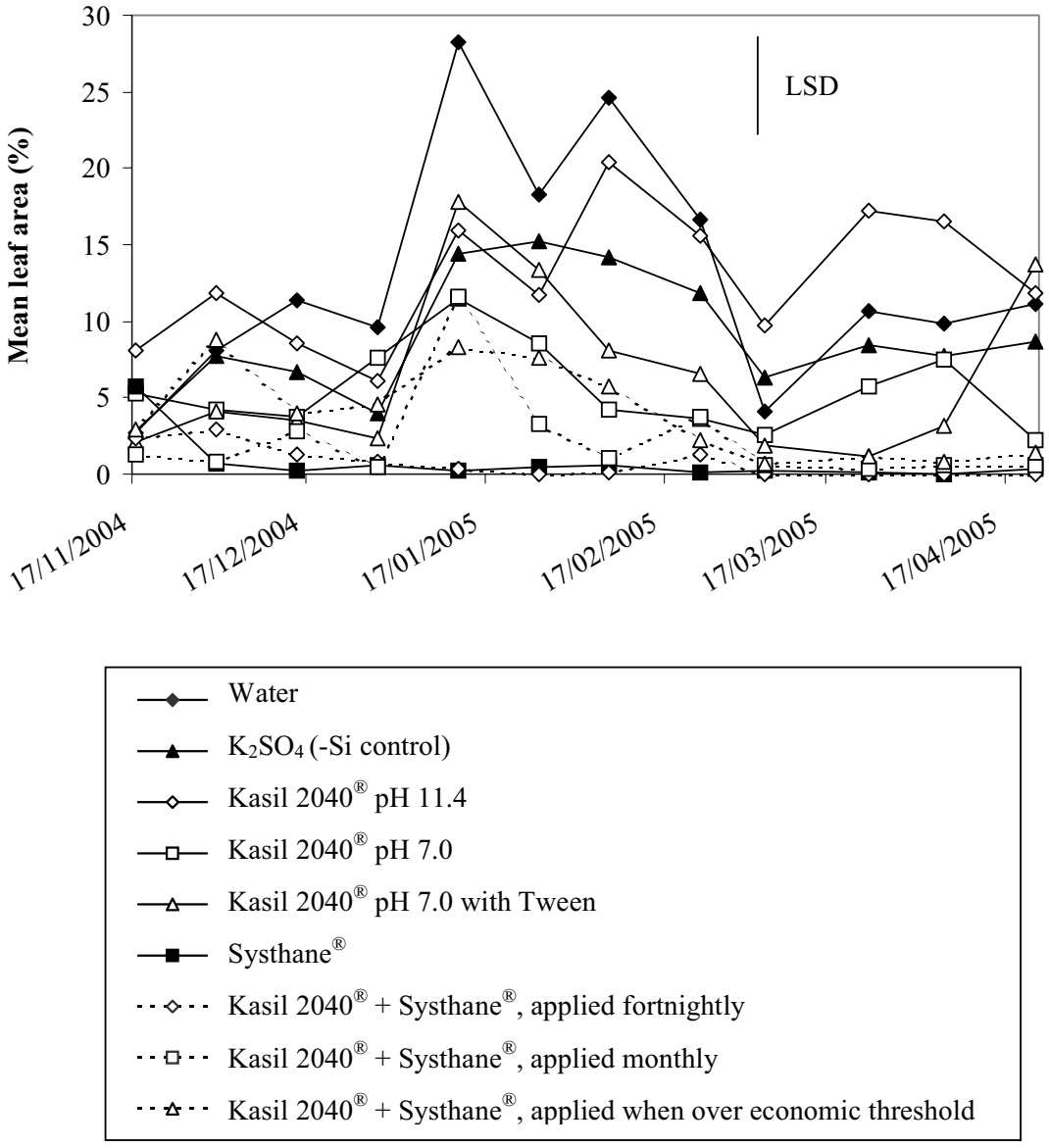
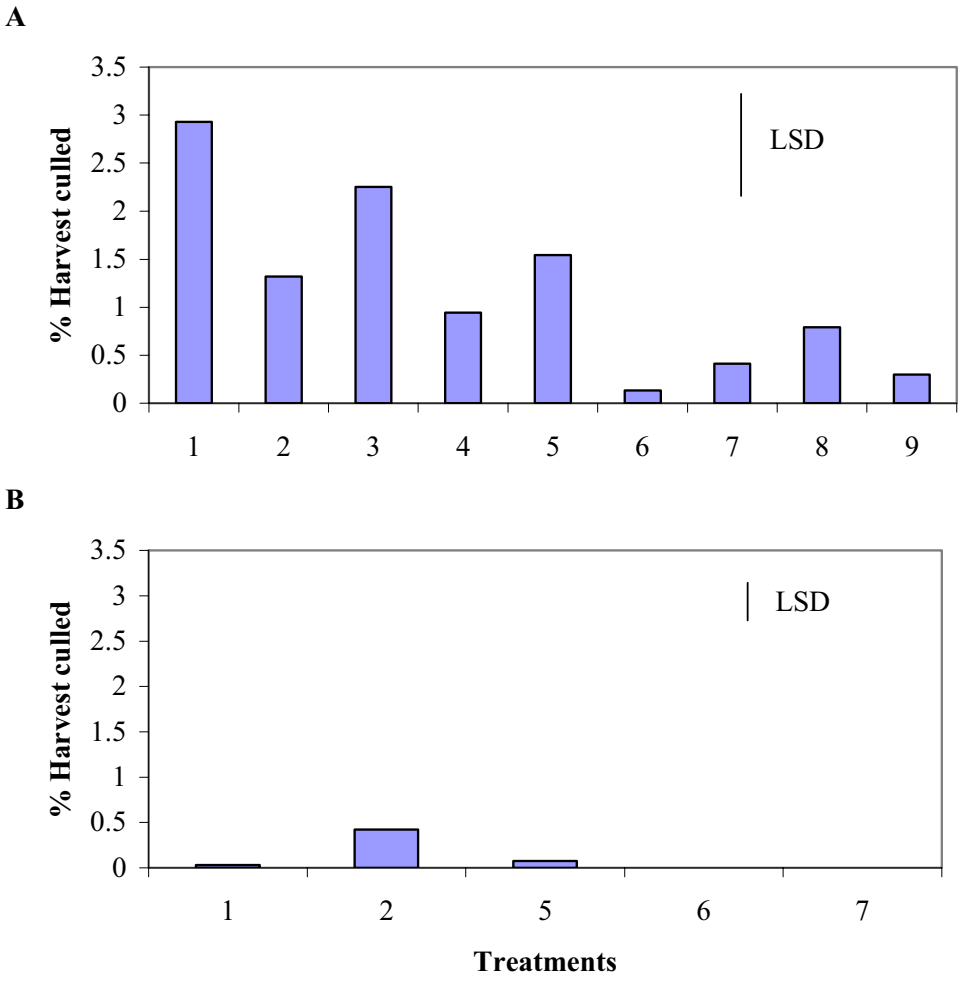


Figure 5.10 The effect of treatment on foliar powdery mildew severity on Selva over the 2004/05 season. Affected leaf area was measured fortnightly, from 14/11/2004 to 16/04/2005 (means from n = 16, over 4 replicates). LSD = 7.279.

Applications of buffered Kasil 2040® (pH 7.0, with and without Tween20®) were shown by ordinal regression analysis to consistently reduce foliar and fruit powdery mildew below the unbuffered Kasil 2040® treatment and generally gave better control of foliar symptoms than both the water and K_2SO_4 controls. However, at no observation did any of the Kasil 2040® treatments reduce foliar or fruit symptoms below that of Systhane®-treated plants (Table 5.5). ANOVA of affected leaf area also showed a significant reduction foliar powdery mildew on plants treated with the buffered Kasil 2040® below that of plants treated with water or unbuffered Kasil 2040® in the 2004/05 season (fprob = <0.001, Figure 5.8 A). The total number of fruit affected by powdery mildew and the numbers unmarketable due to powdery mildew symptoms were also significantly reduced from plants treated with the buffered Kasil 2040® compared with those treated with water, K_2SO_4 or the unbuffered Kasil 2040® (Figure 5.9 A). In the 2005/06 season, there was insufficient levels of disease to show effects of treatments on foliar or fruit powdery mildew symptoms (Figure 5.8 B and 5.9 B, respectively).

The percent of harvest culled due to powdery mildew symptoms in the 2004/05 season was significantly reduced in all plots treated with Systhane® both alone and in combination with Kasil 2040® (F prob = 0.016, Figure 5.11) compared with the water control plots. The harvest culled due to powdery mildew symptoms in plots treated with Systhane® alone were also significantly below that of the nil silicate (K_2SO_4) treated plots. However, the combined treatment did not reduce percent harvest culled due to powdery mildew below that of plots treated with Systhane® alone.

There were obvious differences in yield parameters between the two seasons (Table 5.4). Although data could not be compared statistically, total yield and number of marketable strawberries were higher in the 2004/05 season, however there was also



1. Water
2. K_2SO_4 (-Si control)
3. Kasil 2040® pH 11.4
4. Kasil 2040® pH 7.0
5. Kasil 2040® pH 7.0 with Tween
6. Systhane®
7. Kasil 2040® + Systhane®, applied fortnightly
8. Kasil 2040® + Systhane®, applied monthly
9. Kasil 2040® + Systhane®, applied when severity exceeds economic threshold

Figure 5.11 Effect of treatment on the mean percentage of harvested strawberries culled due to powdery mildew in the 2004/05 (A) and 2005/06 (B) seasons. Percentage calculated weekly. Harvested from 02/01/2006 to 28/04/2006. LSD = 1.076 and 0.424 respectively.

Table 5.7 The effect of treatments on total weight of marketable fruit, the number of marketable fruit, percent unmarketable fruit and the mean fruit weight (of marketable fruit) in the 2004/05 (A) and 2005/06 seasons (B). Means calculated from strawberries harvested between January and April of each year.

A

Treatment	Marketable weight (g)	Number marketable	Percent cull	Mean fruit weight (g)
Water control	9432^{b}	806^{a}	22.24^{ab}	11.51^{b}
K_2SO_4 (-Si control)	7713^{ab}	782^{a}	26.53^{d}	9.74^{a}
Kasil 2040® pH 11.4	7887^{ab}	802^{a}	22.15^{abc}	9.74^{a}
Kasil 2040® pH 7.0	7263^{a}	731^{a}	23.27^{bcd}	9.78^{a}
Kasil 2040® pH 7.0 with Tween	8009^{ab}	770^{a}	18.46^{a}	10.27^{ab}
Systhane®	7292^{a}	741^{a}	26.27^{cd}	9.74^{a}
Kasil 2040® + Systhane®, applied fortnightly	7550^{ab}	745^{a}	21.27^{ab}	10.13^{ab}
Kasil 2040® + Systhane®, applied monthly	7256^{a}	704^{a}	22.48^{abc}	10.25^{ab}
Kasil 2040® + Systhane®, applied when severity exceeds economic threshold	7755^{ab}	755^{a}	24.12^{abc}	10.13^{ab}
Total	**7795**	**760**	**22.98**	**10.14**

B

Treatment	Marketable weight (g)	Number marketable	Percent cull	Mean fruit weight (g)
Water control	5865^{a}	441^{a}	18.45^{a}	13.30^{a}
K_2SO_4 (-Si control)	6151^{a}	468^{a}	19.66^{a}	12.87^{a}
Kasil 2040® pH 7.0 with Tween	6411^{a}	506^{a}	19.34^{a}	12.52^{a}
Systhane®	5481^{a}	396^{a}	17.37^{a}	13.83^{a}
Kasil 2040® + Systhane®, applied fortnightly	5572^{a}	426^{a}	18.14^{a}	12.96^{a}
Total	**5896**	**447**	**18.59**	**13.1**

Superscripts denote statistical significance within seasons at $P<0.05$.

increased percent fruit culled and reduced fruit weight in this season. In the 2004/05 season, there was no significant effect of treatment on the number of marketable strawberries produced. The percent fruit culled was significantly higher in the plots treated with K_2SO_4 or Systhane® alone than the water control. The lowest percent cull was recorded for the buffered Kasil 2040® with Tween 20® treatment, though this was not significantly lower than the water control, unbuffered Kasil 2040®, nor the combined Kasil 2040® and Systhane® treatments. Mean fruit weight from plots treated with K_2SO_4 or Systhane® alone was significantly less than from those treated with water, as was fruit from plots treated with unbuffered and buffered Kasil 2040®. The buffered Kasil 2040® with Tween 20® did not result in significantly smaller fruit. There were no significant effects of treatment on any of the yield attributes in the 2005/06 season.

5.4 Discussion

The above experiments showed that Systhane® controlled powdery mildew in field-grown strawberry. Foliar applications of Kasil 2040® (potassium silicate) reduced powdery mildew and may have potential use in organic production, however, this treatment was not as effective as Systhane®. The combined use of Kasil 2040® and Systhane® in some cases proved to be more effective than either treatment alone. Applications of Stopit® (calcium chloride) did not control powdery mildew.

Regular fortnightly applications of Systhane® reduced the severity of foliar powdery mildew in all the seasons observed, and maintained the severity below the economic threshold on the 2004/05 and 2005/06 seasons. This is in contrast to anecdotal evidence from growers that this product does not give effective control of powdery mildew in their crops, but does concur with field trials conducted by staff of Queensland Department of Primary Industries and Food (Hutton and Gomez, 2005). The significant

reduction of percent fruit culled because of *P. aphanis* mycelium on the receptacle in the 2004/05 season shows that Systhane® also reduced the yield loss from this disease. The beneficial effect of Systhane® in reducing the severity of powdery mildew on fruit and the percent of the harvest culled due to these symptoms was expected to be reflected in the total percent cull for this treatment. However, application of Systhane® significantly increased the percent of total fruit culled compared with the water control, and also reduced mean fruit weight in the 2004/05 season. This suggests Systhane® may have negative effects on yield and supports anecdotal evidence given by farmers that increased number of fruit of unmarketable size are produced with regular application of this fungicide (D. Parker, personal communication, 2006). Similar effects of fungicide on yield have been opbserved in other horticultural crops (Hutcheon *et al.*, 1986; Abbott *et al.*, 1991; Jeffers, 1991; Weiguang *et al.*, 2003).

Systhane® was generally more effective in the later trials when applied with a motorised mister pack rather than when applied with the hand pump pack in the 2003/04 season. Droplet size (Prokop and Veverka, 2006) and spray coverage (Tanigawa *et al*., 1993; Brink *et al.*, 2006) significantly affect the efficacy of fungicides. The mister pack applied smaller droplets, which are less likely to bead off the leaf surface, to both sides of the leaf. This brings into question the efficacy of pesticide applications made by growers and suggests that use of equipment that maximises coverage of leaf area, on both surfaces, may improve the consistency of control with this fungicide.

The lack of significant effect from the calcium chloride treatment, as demonstrated by the results of the preliminary trial, was not unexpected as plants had been fertigated with a relatively rich source of calcium as a matter of course (P. Mason, personal communication, 2003). Calcium is not readily absorbed through the phloem

(Marschner, 1995) and, thus, when applied as a foliar spray, was not expected to provide an additional benefit to foliar nutrition and resistance against fungal infection (Schlegel and Schoenherr, 2004). However, anecdotal evidence suggesting that cultivars that are susceptible to powdery mildew may not efficiently mobilise calcium highlighted the importance of this nutrient in disease control and it is common for calcium products to be added to foliar pesticide treatments applied to strawberry crops to improve post-harvest qualities of the fruit (P. Mason, personal communication, 2003).

The increased severity of powdery mildew when treated with Kasil 2040® in the preliminary trial was attributed to the high pH of this product. When the pH of the diluted Kasil 2040® was measured (after the preliminary trial was concluded) it was found to be 11.4. The unbuffered Kasil 2040® may have affected the physiology of the leaf or fungus, perhaps attributable to the alkaline pH (Kuehny and Morales, 1998; Shi and Sheng, 2005), but the overall effect of this treatment was not clear.

The positive interaction observed between the Systhane® and Kasil 2040® treatments, in terms of reducing powdery mildew severity on Selva, in the preliminary trial may have resulted from Systhane®, a commercial wettable powder systemic fungicide, having a buffering or wetting effect on the Kasil 2040®. Wettable powdered fungicides are relatively well retained on leaves (Cooper, 1993), and some wetting agents alone have been found to reduce powdery mildew symptoms (Cohen *et al.*, 1996). Previous studies have shown that silicate deposits on the leaf surface can inhibit fungal penetration (Bowen *et al.*, 1992), and that silicate is translocated within the leaf tissue to the site of fungal penetration (Chérif *et al.*, 1992b). As Kasil 2040® is a relatively pure potassium silicate and was not supplied in a commercial preparation for application to plants, it was considered that formulation with buffering and wetting

agents might increase efficacy. If the benefit of applying Kasil 2040® in conjunction with Systhane® was the result of commercial additives in the fungicide interacting with the Kasil 2040® rather than a true synergistic effect, then it was believed that the Kasil 2040® would have potential for adaptation for use by conventional and, especially, organic strawberry growers as an alternative fungicide. This was tested in the following seasons.

The buffered Kasil 2040® treatment reduced powdery mildew below the economic threshold of 5 % affected leaf area and significantly reduced the percentage of fruit culled as a result of powdery mildew, in the 2004/05 season, when powdery mildew was severe. There was no additional reduction with the addition of Tween 20® as a wetting agent. Variation between seasons suggested that the effectiveness of this product may be reliant on a relatively heavy load of powdery mildew in a given season. These trials suggest the use of buffered potassium silicate products as an organic treatment for powdery mildew, although further testing and formulation of the compound for commercial use is required.

In the 2004/05 and 2005/06 seasons there was no synergistic effect of combined Systhane® plus Kasil 2040® on foliar severity of powdery mildew as there was in the preliminary trial. However, this treatment did appear to alleviate the adverse effect of Systhane® on the yield of strawberry plants and this may warrant further investigation.

The differences in yield observed between seasons may be attributable to location and weather conditions, though these may also reflect differences in cultural systems and management practises between the two properties. For example, D. Parker uses double row plantings whereas P. Mason uses single. The double row plantings yielded fewer, larger fruit than the single row plantings. Reduced cull (total unmarketable yield) in the latter season was likely to have been a result of planting

density as well as reduced disease pressure as powdery mildew which was well managed in this season's trial plot. There were also differences in pesticide application, reflecting new products that became available for use, such as Flint®. However, contrary to cautions that Flint® applied in combination with oragnosilicate surfactants may cause crop injury (Bayer CropScience, 2007), there was no adverse affect on yield from plants treated with the any of the Kasil 2040® treatments. This product appeared to eradicate powdery mildew outbreaks whereas Systhane® did not. This may also have caused early termination of tertiary and quaternary flowers (D. Parker, personal communication, December 2005).

In summary, fortnightly applications of the fungicide Systhane®, when applied effectively, can control powdery mildew in strawberry crops. Some reduction in yield may be expected from regular use of this fungicide. A buffered potassium silicate product may have potential for increasing yield from strawberry crops and should be investigated further as an organic supplement for managing powdery mildew. Calcium chloride foliar sprays provide little benefit in controlling powdery mildew or improving yield.

Chapter 6 Preliminary identification of candidate genes associated with powdery mildew resistance in Aromas

6.1 Introduction

In the last century there has been significant improvement of strawberry cultivars using classical breeding techniques. Breeding objectives have tended to focus on productivity, fruit size and flavour, although resistance to insect, viral and fungal pests is becoming of greater importance (The University of Calfornia, Strawberry Licencing Program, 2001). While there have been numerous studies of the genetic mechanisms of the inheritance of agronomic traits using diploid strawberry species, study of the cultivated species has been limited due to the complexity of the octoploid nature of the *F.* x *ananassa* genome (Folta *et al.*, 2005). In other polyploids, such as wheat, marker-assisted selection (MAS) has been widely used to assist in breeding programs (Hsam and Zeller, 2002) and the identification and characterisation of molecular markers in *F.* x *ananassa* suitable for MAS would have significant impact on strawberry breeding and increase screening capabilities of new genetic material (see Section 1.5).

Sargent *et al.* (2004a; 2006) have extended on the work of Davis and Yu (1997) to publish a linkage map from an F_2 mapping population of *F. vesca* and *F. nubicola* comprising of 182 SSR markers, spanning 424 cM over the seven linkage groups. Many of these markers have been transferable into cultivars of *F.* x *ananassa* providing a useful reference point for linkage mapping studies of the octoploid species (Sargent *et al.*, 2006). To date, the single linkage map published for *F.* x *ananassa* is based only on 7 RAPD, 11 AFLP and 2 SCAR markers used to map the location of 3 resistance genes to *Phytophthora fragariae* (Haymes *et al.*, 2000). The recent isolation of more than

1500 SSR markers from *F.* x *ananassa* should facilitate saturated mapping of the octoploid species also (Bassil *et al.*, 2006; Keniry *et al.*, 2006).

In the absence of comprehensive genomic information for *Fragaria*, the candidate gene approach has potential to identify genes that are differentially expressed during physiological events, such as fruit ripening (Medina-Escobar *et al.*, 1997; Manning, 1998) and the photoperiodic flowering response (Cekic *et al.*, 2001). However, these studies have been limited to RNA extracted from fruit tissue as strawberry leaf is a very recalcitrant tissue. At the commencement of this investigation the protocol of Manning (1991) was the only RNA extraction method published for strawberry leaf tissue. Recently, Folta *et al.* (2005) have published a large number of ESTs and SSR markers from *F.* x *ananassa* leaf RNA, using an extraction method originally developed for pine cones (Chang *et al.*, 1993). The methods of Kim (2003), developed for RNA extraction of bark were similar to this, and were used in this study.

The aim of this preliminary study was to identify potential candidate genes in Aromas (and absent in Selva) that were differentially expressed in Aromas leaf tissue after inoculation with *P. aphanis*.

6.2 Materials and methods

6.2.1 Plant and fungal material

Selva and Aromas plants were inoculated by placing them in severely powdery mildew affected areas (score 5, see chapter 2) in the field for 2 hours. Un-inoculated and inoculated tissue of the YOLs (midrib removed) was sampled at 3, 4, 5 and 6 dpi. From 3 dpi conidia had germinated, produced haustoria, and resulted in hyphal growth radiating away from the penetration site on susceptible leaf tissue (see chapter 3). Samples were weighed and placed into 1.5 mL microfuge or 15 mL Falcon tubes, snap frozen in liquid nitrogen, then stored at –80 °C until required.

6.2.2 RNA extraction techniques

Four RNA extraction techniques were used before one was found that gave a reasonable yield of high quality total RNA from strawberry. The initial method (Manning, 1991) was also applied to barley youngest emerged blade (YEB) tissue as a positive control. The extraction methods were those of Manning (1991), Porebski *et al.* (1997), RNeasy® plant mini kit (Qiagen) and Kim (2003), in order of investigation.

6.2.2.1 Manning (1991) extraction method

The original Manning procedure (1991) started with 1 g of tissue in a 10 mL Falcon tube. However, these tubes were unsuitable for high-speed centrifugation so the protocol was adapted for a 2 mL microfuge tube (100 mg starting tissue). Tissue was ground to a homogenous powder using a mortar and pestle in the presence of liquid nitrogen. Extraction buffer [200 mM tris-hydroxymethylaminomethane {Tris-HCl} (pH 7.6), 200 mM boric acid, 10 M sodium ethylenediaminetetra acetic acid {Na_2EDTA}, 0.5 % sodium dodecylsulphate {SDS}, 2.0 % β-mercaptoethanol] was then added to cover the tissue (3 – 4 volume of tissue) and left to warm to room temperature. The organic and aqueous phases were separated by mixing with an equal part 1:1 phenol: chloroform and centrifuging at 20000 g for 10 min at 4 °C. The aqueous phase was collected in a clean tube while the organic phase was mixed with a second aliquot of extraction buffer and re-centrifuged. The second aqueous phase was then added to the first. The extracted solution was diluted with 1M sodium acetate buffer (pH 4.5) to a total sodium concentration of 80 mM, then nucleic acids were precipitated by differential concentrations of 2-butoxyethanol (2-BE): 0.4 volume of 2-BE to precipitate out the polysaccharides and the second 0.1 volume to precipitate out the nucleic acids. Precipitation occurred on ice for 30 min and the precipitate was collected by centrifugation at 20000 g for 10 min. The nucleic acid pellet was washed in 2-BE mix

(1:1 2-BE: 200 mM Tris-HCl (pH 7.6), 200 mM boric acid, 10 M Na_2EDTA), followed by 70 % ethanol mix [70 % ethanol with 0.1 M potassium acetate/ acetic acid (pH 6.0)] and 100 % ethanol and then dried under vacuum.

RNA was separated from DNA using 0.3 volume of 12 M LiCl on ice for 1 hour and centrifuged at 11,600 g for 10 min to precipitate the RNA. The pellet was washed twice with 3 M LiCl, then with the 70 % ethanol mix and 100 % ethanol and dried under vacuum before being dissolved in 1 x TE buffer [100 mM Tris-HCl, 10 mM EDTA (pH 8.0)] and stored at –80 °C until required.

6.2.2.2 Porebski *et al.* (1997) extraction method

This method was originally for extraction of DNA from recalcitrant tissues and was adapted for extraction of RNA for these studies. Tissue (500 mg) was ground in liquid nitrogen using a mortar and pestle, then incubated in warmed extraction buffer [100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 2.0 % CTAB, 0.3 % β-mercaptoethanol] and 50 g of polyvinylpolypyrrolidone (PVPP) for 60 min in a 60 °C oven with shaking at 60 rpm. Samples were cooled to room temperature and an equal volume of chloroform: octanol (24:1) was added and mixed on a rotor for 10 min. Phases were separated by centrifugation at 3000 rpm for 20 min. The aqueous phase was collected and mixed with another volume of chloroform: octanol (24:1) and separated. A half volume of 5 M NaCl was mixed with the final aqueous phase, and 2 volumes of ice-cold 95 % ethanol were added, mixed and precipitated at –20 °C for 10 min and then refrigerated overnight. RNA was collected by centrifugation at 3000 rpm for 6 min, and the pellet washed with cold 70 % ethanol, dried in a 37 °C oven and then re-dissolved in 1 x TE buffer overnight at 4 °C. DNA was eliminated by incubating (37 °C) with DNase (10 mg/mL) for an hour and then with proteinase K (1 mg/mL) for a further 30 min. An equal part phenol: chloroform (1:1) was mixed with the DNase-

treated solutions and centrifuged at 14000 rpm for 15 min. The upper phase was collected and 50 µL of TE buffer added to the phenol phase and centrifuged again, with the second upper phase being added to the first. The collected sample was mixed with a 0.1 volume of 2 M sodium acetate and twice the volume of 100 % ethanol, and left to precipitate at –20 °C overnight. The precipitate was collected by centrifugation at 14000 rpm for 15 min and washed with 70 % ethanol, dried, dissolved in 100 µL 1 x TE buffer and stored at –80 °C until required.

6.2.2.3 RNeasy® Plant Mini Kit (Qiagen)

RNA was extracted using the RNeasy® Plant Mini Kit (Qiagen, Australia), as per the manufacturer's protocols. Samples (up to 100 mg) were ground in liquid nitrogen and lysed with the RLC lysis buffer (containing guanidine hydrochloride) included in the kit. The RLC buffer was found to give superior quality RNA compared with the use of the RLT lysis buffer which contains guanidine isothiocyanate. Following this, samples were centrifuged though QIAshredder® units to yield a clean lysate product. This was mixed with ethanol to promote selective binding of the RNA to the RNeasy membrane. The mixture was applied to Qiagen mini spin columns and centrifuged to remove contaminants. The RNA was eluted with RNase-free water, by using the same aliquot twice to maximise the concentration of RNA recovered from the membrane. Product was stored at –80 °C until required.

6.2.2.4 Kim (2003) extraction method

Tissue (100 mg) was ground under liquid nitrogen in a 2 mL Eppendorf tube, using a cooled blunt knitting needle. It was then incubated in extraction buffer (100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 2.0 % CTAB, 2.0 % PVPP, 0.3 % β-mercaptoethanol) at 60 °C for 10 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added and the mixture was homogenised on a rotor for 10 min. Phases were

separated by centrifugation at 13000 rpm for 15 min and the supernatant was added to 0.66 volume of isopropanol, mixed and kept at –20 °C overnight. RNA was collected using an RNeasy® plant mini column (Qiagen, Australia) as per the manufacturer's instructions and stored at –80 °C until required.

6.2.3 Quantification and gel electrophoresis

Samples for quantification were diluted (5 µL into 25 µL) in 10 mM Tris-HCl (pH 8.0) and absorbance (ABS) at 260 and 280 nm was analysed using a Metertech SP8001 UV/VIS spectraphotometer at 260 nm. Total nucleic acid concentration was calculated by multiplying the Abs_{260} by the dilution factor (5) and 40 (1 OD_{260} = 40 ng/µL RNA). Quality of extracted RNA was determined using electrophoresis on a 1.0 % agarose gel stained with 2 µL ethidium bromide. Wells were loaded with 1 µg of each sample with 1 µL loading buffer (0.25 % bromocresol green, 0.25 % xylene cyanol, 30 % glycerol) per 6 µL of sample. Samples were run against a 1 Kb plus DNA ladder (Invitrogen, Australia). Electrophoresis was in 5 x TAE buffer (200 mM Tris-acetate, 10 mM Na_2EDTA in an OWLB1 or OWLB2 tank (Adelab, South Australia) at 100 V using a LKBGPS 200/400 power pack (Pharmacia) for approximately 1 hour or until the bands had migrated to half the length of the gel. Strong bands at 800 and 1200 bp that are characteristic of ribosomal RNA, gave a good indication that the extracted RNA is of suitable quality for further use.

6.2.4 Identification of differentially expressed RNA in Aromas leaf tissue

The mRNA isolated from Aromas leaf tissue inoculated with *P. aphanis*, was compared with uninoculated Aromas leaf tissue using a dot blot method (Davies and Robinson, 2000) to identify genes potentially up-regulated in the presence of *P. aphanis*. The membranes were then probed with cDNA from inoculated and uninoculated Selva leaf tissue. Preparation for the dot blots required isolation of mRNA

and subsequent reverse transcriptase polymerase chain reaction (RT-PCR) to amplify cDNA. To prepare a cDNA library, the cDNAs were integrated into pDNR-LIB vectors (Figure 6.1) and transformed into *E. coli*. The library was then used on dot blots and for subsequent sequencing of candidate genes. A radioactive probe made from the combined extracted cDNA was used to probe the dot blots. This allowed for comparison of the intensity of each cloned mRNA product.

6.2.4.1 Isolation of mRNA

The Creator™ SMART™ cDNA library construction kit (Clontech, Australia) contains a modified oligo (dT) primer (CDSIII/3' PCR primer) which binds specifically with the poly A tail of mRNA (Clontech Laboratories, 2006), removing the need for preparatory isolation of mRNA from the total RNA extracted from leaf tissue.

6.2.4.2 Creation of cDNA libraries

Synthesis of first strand and double stranded cDNA was carried out as per the manufacturer's instructions. The CDSIII/3' PCR primer and SMART™ IV oligonucleotide (1 µL each) were added to 1 µg total RNA in a microcentrifuge tube and the volume made up to 5 µL with RNase-free water. Samples were mixed and contents centrifuged briefly to the bottom of the tube before being incubated at 72 °C in a heating block for 2 min then cooled on ice for 2 min. The following was then added to each tube: 2 µL 5 x first strand buffer [250 mM Tris (pH 8.3), 30 mM $MgCl_2$, 375 mM KCl], 1 µL 20 mM dithiothreitol (DTT), 1 µL 10 mM dNTP mix and 1 µL PowerScript™ reverse transcriptase (Clontech, Australia), making a total volume of 10 µL. Contents were mixed by pipetting and centrifuged to collect sample to the bottom of the tube before being incubated at 42 °C in an air incubator for 1 hour. First strand synthesis was terminated by placing tubes on ice. Surplus product was stored at –20 °C for up to 3 months.

For long-distance PCR (LD-PCR) a 2 µL aliquot of first stand synthesis reaction mixture was transferred to a pre-chilled 0.5 mL tube and combined with 10 µL 10 x Advantage™ 2 PCR buffer, 2 µL 50 x dNTP mix, 2 µL 5' PCR primer, 2 µL CDSIII/3' PCR primer and 2 µL 50 x Advantage™ 2 polymerase mix (Clontech, Australia), and made up to 100 µL with RNase-free water. Contents were mixed by flicking, then collected to the bottom of the tube by centrifuging, and this was placed in a GeneAmp PCR system 9700 PCR thermal cycler (Applied Biosystems, Australia). The thermal cycling program was as follows: 95 °C for 1 min, then 20 cycles of 95 °C for 5 sec and 68 °C for 6 min. A 5 µL aliquot of the PCR product was analysed by electrophoresis (Section 1.2.3). Remaining ds cDNA was stored at –20 °C until use.

In a sterile PCR tube, 50 µL of the amplified ds cDNA product was digested with 2 µL 20 µg/ µL proteinase K to inactivate DNA polymerase. The contents were mixed and collected to the bottom of the tube by centrifugation and then incubated at 45 °C for 20 min. An equal volume of RNase-free water was added to the tube and then 100 µL of phenol: chloroform: isoamyl alcohol was mixed with the contents by inversion for 2 min then centrifuged at 14000 rpm for 5 min. The aqueous phase was pipetted into a clean tube and mixed with a second 100 µL aliquot of phenol: chloroform: isoamyl alcohol and then centrifuged as before. The second aqueous phase was added to 10 µL of 3 M sodium acetate, 1.3 µL of 20 µg/µL glycogen and 260 µL of 95 % ethanol and centrifuged immediately at 14000 rpm for 20 min. The supernatant was pipetted away and discarded, and the pellet washed with 80 % ethanol and air-dried. The pellet was resuspended in 79 µL of RNase free water.

Asymmetrical *Sfi* 1 restriction enzyme sites (A and B, see Figure 6.1) at the 3' and 5' ends of the ds cDNA (incorporated by the Creator™ SMART™ amplification system) meant that a simple digestion, instead of adaptor ligation, was required to

prepare cDNAs for size fractionation and subsequent vector ligation. *Sfi* 1 digestion was achieved by adding 10 µL 10 x *Sfi* buffer, 10 µL *Sfi* restriction enzyme (Clontech, Australia) and 1 µL 100 x BSA to the cDNA product, mixing well and incubating at 50 °C for 2 hours.

Size fractionation was undertaken by excision under UV light. The lane from the 1.0 % agarose electrophoresis gel between 500 and 3000 bp was excised and cut into blocks of a size suitable for extraction using QIAEX™ agarose gel extraction kit (Qiagen, Australia) as per the manufacturer's instructions. This was used as an alternative to the CHROMA SPIN-400 column drip procedure with 1 % xylene cyanol dye as per the Creator™ SMART™ cDNA library construction kit protocols as this procedure yielded no product.

The fractionated products were mixed with 0.1 volume of 3 M sodium acetate (pH 8.0), 1.3 µL of 20 µg/µL glycogen and a 2.5 volume of cold 95 % ethanol (-20 °C) and precipitated at –20 °C overnight, as per the Creator™ SMART™ cDNA library construction kit protocol. The tube was centrifuged at 14000 rpm for 20 min at room temperature and the supernatant removed by pipette and the pellet air-dried. The pellet was resuspended in 7 µL sterile nanopure water and ligated to the Sfi 1 digested dephosphorylated pDNA-LIB vector provided.

The pDNA-LIB vector of the Creator™ SMART™ cDNA library construction kit is a chloramphenicol resistant vector with *Sfi* 1 A and B binding sites (Figure 6.1). The vectors (50 ng) were transformed into 50 µL competent heat-shocked Top 10™ type competent *E. coli* cells (prepared by A. Boettcher in July 2005, as per the Promega® Subcloning notebook) in LB broth (1 % (w/v) Bacto®-tryptone, 0.5 % (w/v) Bacto®-yeast extract, 1 % (w/v) NaCl, pH 7.0). Transformation was achieved by incubating

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

Figure 6.1 Map of the pDNR-LIB vector from the Creator™ SMART™ cDNA library construction kit (Clontech, Australia) showing the multiple cloning sites (MCS) and cut sites, *Sfi* 1 A and B. Reproduced with permission from Clontech, Australia, KB 03/07. Copyright: Clontech Labortories, Inc.

competent cells on ice for 20 min, heat shocking at 42 °C for 45 sec before returning to ice for a further 2 min. LB broth (250 µL) was then added to this reaction and it was incubated at 37 °C with shaking (225 to 250 rpm) for 60 min before 50 µL was plated onto warmed (37 °C) LB agar plates [1 % (w/v) Bacto®-tryptone, 0.5 % (w/v) Bacto®-yeast extract, 1 % (w/v) NaCl, 1.2 % (w/v) Bacto®-agar, pH 7.0] containing 30 µg/mL of chloramphenicol (Cm; Sigma, Australia). Bacterial inoculum was allowed to absorb onto agar and then plates were inverted and incubated at 37 °C overnight. Bacterial colonies (288 per treatment) were transferred from the LB agar plates into 96 well plates of LB/ Cm broth using sterile toothpicks or pipette tips, and incubated overnight at 37 °C with shaking. An equal volume of lysis solution (0.2 M NaOH, 1.0 % SDS) was added to half of the overnight broth culture and incubated at 24 °C for 20 min. An equal volume of 40 % glycerol was added to the remaining overnight broth culture, which was then stored at –80 °C as a reference library.

The pDNA-LIB vector used in the Creator™ SMART™ cDNA library construction kit did not contain a gene (such as the lactate gene) in the multiple cloning site (MCS) to allow for selection against empty plasmids as the common xGal blue/ white screen does. Instead, clones that failed to produce signals with either probe in the dot blot process were assumed to contain empty vectors.

6.2.4.3 Probe preparation

Double stranded cDNA was prepared and amplified from the bulked RNA samples using the Creator™ SMART™ cDNA library construction kit. Samples were purified using the QIAquick™ PCR purification kit (Qiagen, Australia) as per the manufacturer's instructions. Purified products were quantified using spectrophotometry at 260 nm (as per Section 6.2.3) and used to make the radioactive probes for the dot blot screen. Samples were diluted to 45 µL with 1 x TE buffer, and denatured at 95 °C for 3

min. The denatured DNA was then added to Ready-to-go™ DNA labelling beads (Amersham Biosciences, Australia) with 5 μL [α-^{32}P]dCTP (3000 Ci/mM), mixed by pipetting and incubated at 37 °C for 30 min. 0.5 M EDTA (2 μL) was used to terminate the reaction. Unincorporated nucleotides were removed using ProbeQuant™ G-50 micro columns (Amersham Biosciences, Australia) as per the manufacturer's instructions.

6.2.4.4 Dot blot screening of cDNA libraries

Lysed clones were blotted (5 μL) onto duplicated grids (1 cm^2) on N^+ nylon hybridisation membrane and allowed to dry. The membranes were washed with 10x SSC (1.5 M NaCl, 0.15 M sodium citrate), UV cross-linked using the damp dot blot cross-linking program of a GS Genelinker™ UV Chamber (BioRad), and stored between blotting paper sheets in resealable plastic bags at 4 °C until use.

Cross-linked membranes were rolled between sheets of nylon mesh that had been soaked in 2x SSC (0.3 M NaCl, 0.03 M sodium citrate) and carefully placed against the walls of a hybridisation bottle (with 2x SSC), trying to avoid creating air bubbles between the membranes. SSC was replaced with 25 mL hybridisation buffer (0.5 M Na_2HPO_4 (pH 7.2), 7.0 % SDS, 1 mM EDTA) and incubated at 68 °C with rotation for 60 min in a hybridisation oven. After this the hybridisation buffer in the bottle was refreshed. Before being added to the hybridisation bottle, the probe was denatured in a 95 °C waterbath for 10 min (with the lid of the microfuge tube pierced). When the probe was added to the bottle, direct contact with the membrane was avoided. The membranes were incubated at 68 °C with rotation overnight.

Radioactive probes were discarded appropriately, the membranes transferred to a tray and covered with pre-warmed wash solution (1st wash: 2 x SSC/ 0.1 % SDS, 2nd wash: 0.5 x SSC/ 0.1 % SDS, 3rd wash: 0.1 x SSC/ 0.1 % SDS). The membranes were

incubated with each of the wash solutions at 68 °C for 10 min with shaking, and rinsed with running tap water between. The 3rd wash was repeated until the membranes recorded less than 10 counts per second (cps) using a Geiger counter. Membranes were dried on blotting paper and placed inside a clear plastic bag (with only one edge intact) and taped into a cassette. Films were exposed to the membranes in a dark room and kept at –80 °C for 24 to 60 hours. Films were developed using an Agfa CP1000 developer and compared by analysis with Image Master 2D software (version 3.1) to identify grids that differed significantly between films. These results were confirmed by visual observation.

The probes used to probe these membranes first were isolated from Aromas before inoculation and 4 dpi. The membranes were then stripped and re-probed with probes isolated from Selva leaves before inoculation and 4 dpi. This minimised the resources involved in creating replicate membranes and ensured there was no operator error in doing so. Stripping was achieved by applying 70 °C stripping solution [0.1 % SDS, 2 mM EDTA (pH 8.0)] and allowing the membranes to cool to room temperature (approximately 30 min at 22 °C). This procedure was repeated until the membranes yielded no more than 2 cps on a Geiger counter. The dots that showed the greatest differentiation in signal between membranes when probed with the Aromas probes and that were also absent when probed with Selva probes were identified for sequencing and characterisation.

6.2.5 Sequencing of potential genes upregulated in Aromas leaves in the presence of *P. aphanis*

The clones selected for sequencing were re-cultured from the LB/ Cm/ glycerol reference stocks. Reference stock (1 µL) was mixed with 2 mL of fresh LB/ Cm and incubated at 37 °C overnight with shaking. Bacterial cells were collected by

centrifugation at maximum speed for 10 min and the plasmid DNA purified from the *E. coli* cultures using the QIAprep™ spin miniprep kit (Qiagen, Australia) as per the manufacturer's instructions. The products were quantified using spectrophotometry and prepared for sequencing (300 to 600 ng plasmid DNA, 1 μL 3.2 μM primer, diluted to 15.5 μL). Clones were sequenced from both 3' and 5' ends by Ag Genomics, Australia using the BigDye™ terminator v 3.1 (Applied Biosystems, Australia) protocol (Rosenblum *et al.*, 1997).

6.2.6 Database searches for similar sequences

Sequences were examined using Contig Express™ software (Invitrogen). Vector binding sites were found and deleted. Where possible, forward and reverse sequences were compared and mismatches corrected manually using the strongest chromatogram signal. The resulting contigs were compared against the NCBI database using BLASTn and EST nucleotide searches (Altschul *et al.*, 1997). Homology to mRNA or cDNA genes of putative function was considered first, followed by short exact matches to the above. Where the cloned sequences only showed significant homology to ESTs of related species, the complete EST sequence was used for BLASTn analysis to genes of putative function. Sequences that resulted in no significant matches using BLASTn were translated into hypothetical proteins from all open reading frames (ORFs) from 3' to 5' and 5' to 3' using EXPASY software. Resulting amino acid sequences were compared against the NCBI protein database using BLASTp for homology to full proteins and short nearly exact matches (Schäffer *et al.*, 2001). Alignments were generated using Multalin version 5.4.1 (I.N.R.A., France 1996) as per Corpet (1988).

6.2.7 Expression of clones during plant-pathogen interaction

Reverse transcriptase-PCR was used to determine the relative abundance of one of the candidate sequences in leaf tissue extracted from Aromas before inoculation and 12, 24, 48 and 96 hpi with *P. aphanis*.

Primers were designed to amplify the candidate sequence SPIIIG3 and tested to ensure appropriate size (20 to 30 bp), melting temperature (approximately 60 °C) and looping potential using Oligoanalyzer 3.0 and Vector NTI software. Primers were ordered from Geneworks (Australia). The primers were as below:

SP3G6F = CAA GCT AGT AGT CTC ACC AAG AAT TCG AAG

SP3G6R = CAC AAG ATC GAC TCC CCT AGC TTC AC

Reverse transcriptase-PCR was undertaken using the Superscript™ One-step RT-PCR kit with Platinum® Taq (Invitrogen, Australia) following the manufacturer's instructions. Optimal PCR amplification was achieved with 42 cycles. Replicate treatments with control primer for the 18S rRNA were amplified in parallel with the IIIG6 primers. Products were analysed by electrophoresis using 1.0 % agarose gels stained with ethidium bromide (see Section 6.2.3).

6.3 Results

6.3.1 RNA extraction techniques

The differential 2-BE concentration method of Manning (1991) yielded RNA of poor quality (260:280Abs = 1.28) from strawberry leaves. Improved RNA quality was achieved with barley controls (260:280 Abs = 1.32), though yields were still not consistent.

The CTAB DNA extraction method adapted from Porebski *et al.* (1997) produced low yields of RNA (0.7 to 1.1 µg per 100 mg of starting leaf tissue), however the 260:280 Abs ratios (1.4 to 1.56) were still below the ideal of 1.8.

The RNeasy® plant mini kit RNA extraction and the protocol of Kim (2003) produced yields superior to either of the previous procedures, yielding approximately 3 μg of RNA per 100 mg of starting leaf tissue. The 260:280 Abs ratios of these products were greater than the desired 1.8.

When compared using 1.0 % agarose gel electrophoresis stained with ethidium bromide, the quality of RNA extracted using the protocols of Porebski *et al.* (1997) and Kim (2003) was better than that recovered using the RNeasy® plant mini kit (Figure 6.2). The RNA extraction protocol of Kim (2003) was used for all further RNA extraction. Where necessary, the RNeasy® plant mini kit RNA clean-up protocol (with RLC buffer in place of RLT) was used for further purification of extracted samples. RNA extractions of like tissue were bulked for library construction, yielding 1.4 μg and 6.9 μg total RNA for tissue extracted from Aromas leaf tissue, uninoculated and 4 dpi, respectively.

6.3.2 cDNA library construction

Using the Creator™ SMART™ cDNA library construction kit, cDNA and digest products were recovered. However, products were lost during size fractionation using the CHROMA SPIN-400® columns included in this kit. To overcome this problem, sequences between 500 and 3000 bp were extracted from the electrophoresis gel and used for vector ligation and transformation into *E. coli*. Approximately 300 distinct colonies were cultured from each sample (Aromas leaf tissue, uninoculated and 4 dpi), however, of the 288 screened from each sampling time only 75 from the uninoculated sample and 76 from the 4 dpi sample were recombinant.

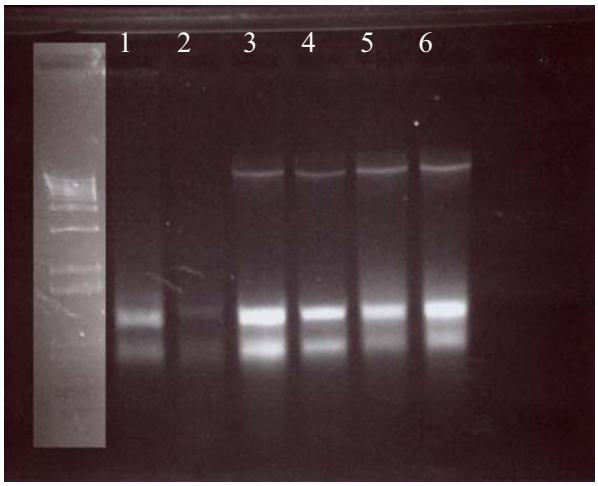


Figure 6.2 Electrophoresis gel of RNA products extracted from strawberry leaf tissue. Samples were extracted from cultivar Aromas, using RNeasy® plant mini kit (lanes 1 and 2) and RNA extraction protocols of Kim (2003) (lanes 3 and 4) and Porebski *et al.* (1997) (lanes 5 and 6. Ladder was a Ready-Load™ 1 Kb plus DNA ladder (Invitrogen, Australia). Intensity of bands indicates relative integrity of extracted rRNA as an indication of sample quality.

6.3.3 Subtractive screening of cDNA libraries

Autoradiographs of the membranes probed with cDNA from Aromas leaf tissue, uninoculated and 4 dpi, showed no signals present in one and not the other. However, there was differentiation in signal between probed films which may indicate increased transcript products (Figures 6.3 and 6.4, compare left plates with right). Few faint signals were produced when the membranes were probed with bulked cDNA from Selva leaf tissue, uninoculated and 4 dpi (Figures 6.5 and 6.6). Four clones produced signals that were more intense and larger in size when probed with 4 dpi Aromas cDNA than with uninoculated Aromas cDNA and that were much fainter from the membranes probed with cDNA extracted from Selva. Clones from plate positions IH6, IIIA8 IIIB6 and IIIG6 were selected and prepared for sequencing. Clones originating from uninoculated Aromas leaf tissue showing difference in signal between probed films were considered a lower priority for sequencing.

6.3.4 Database searches for similar genes

Of the four clones, only SPIH6 showed significant homology to publically available sequences using BLASTn (Table 6.1). The full sequence recovered from the SPIH6 clone was 183 bp. The sequence generated from clone SPIH6 aligned to a portion of a chloroplastic psbM-trnD intergenic spacer gene from *Prunus* species, with the highest significance to *P. hortulana* (Figure 6.7). This sequence also aligned closely to ESTs from other Rosaceae species (Table 6.1, Figure 6.7).

The sequence recovered from clone SPIIIA8 was partial and only 41 bp long. In a BLASTn of genes of known function, SPIIIA8 showed short exact sequence alignments to a chloroplast inverted repeat region and an mRNA associated with endobetaglucanase synthesis, both of which were from *F.* x *ananassa* (Table 6.2).

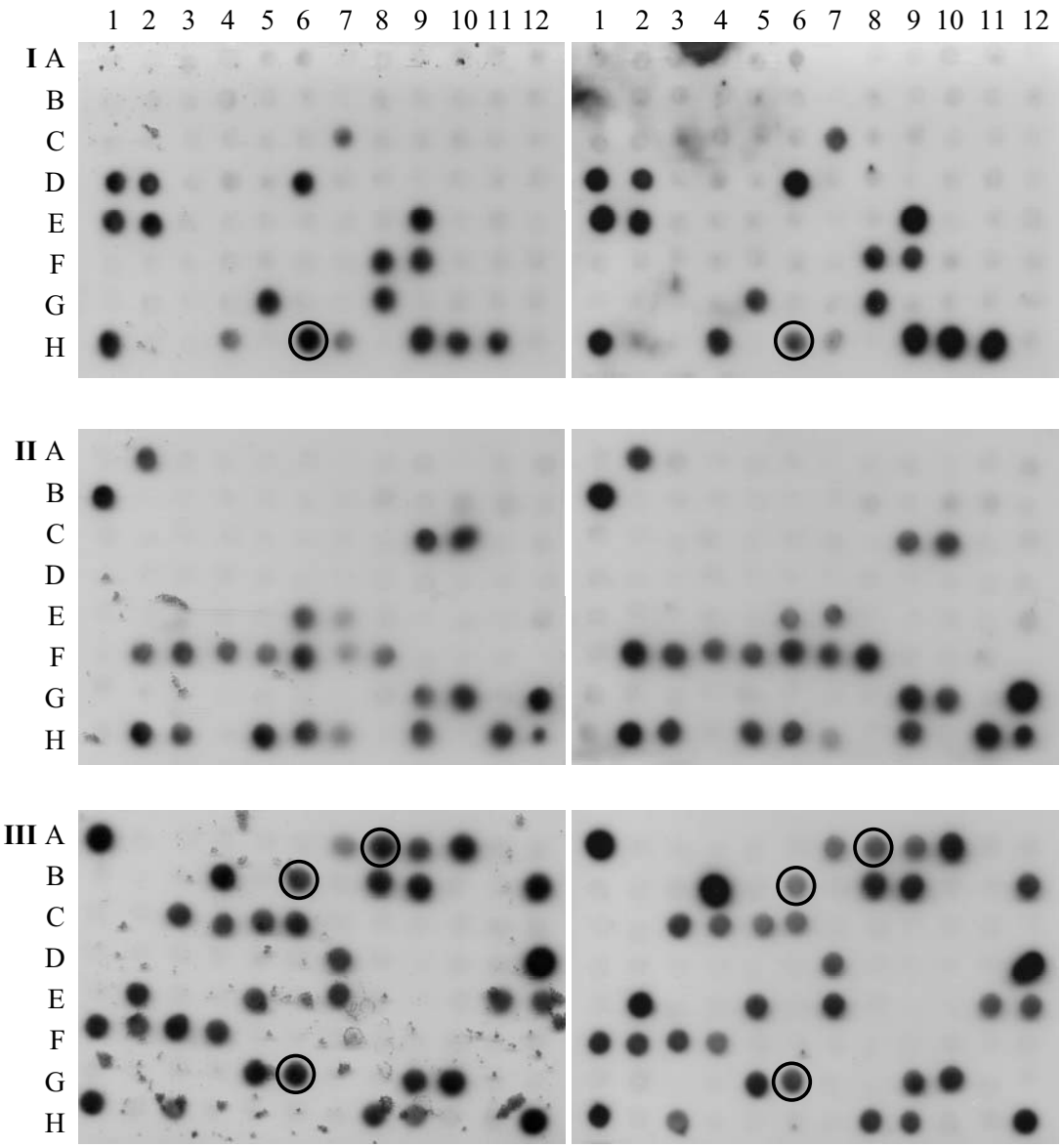


Figure 6.3 Dotblot film of clones from Aromas leaf tissue 4 dpi with *P. aphanis* probed with cDNA from 4dpi (left) and uninoculated (right) Aromas leaf tissue. Clones H6 from plate I and A8, B6 and G6 from plate III (circled) were identified as having stronger signals when probed with the 4dpi probe than with uninoculated probe.

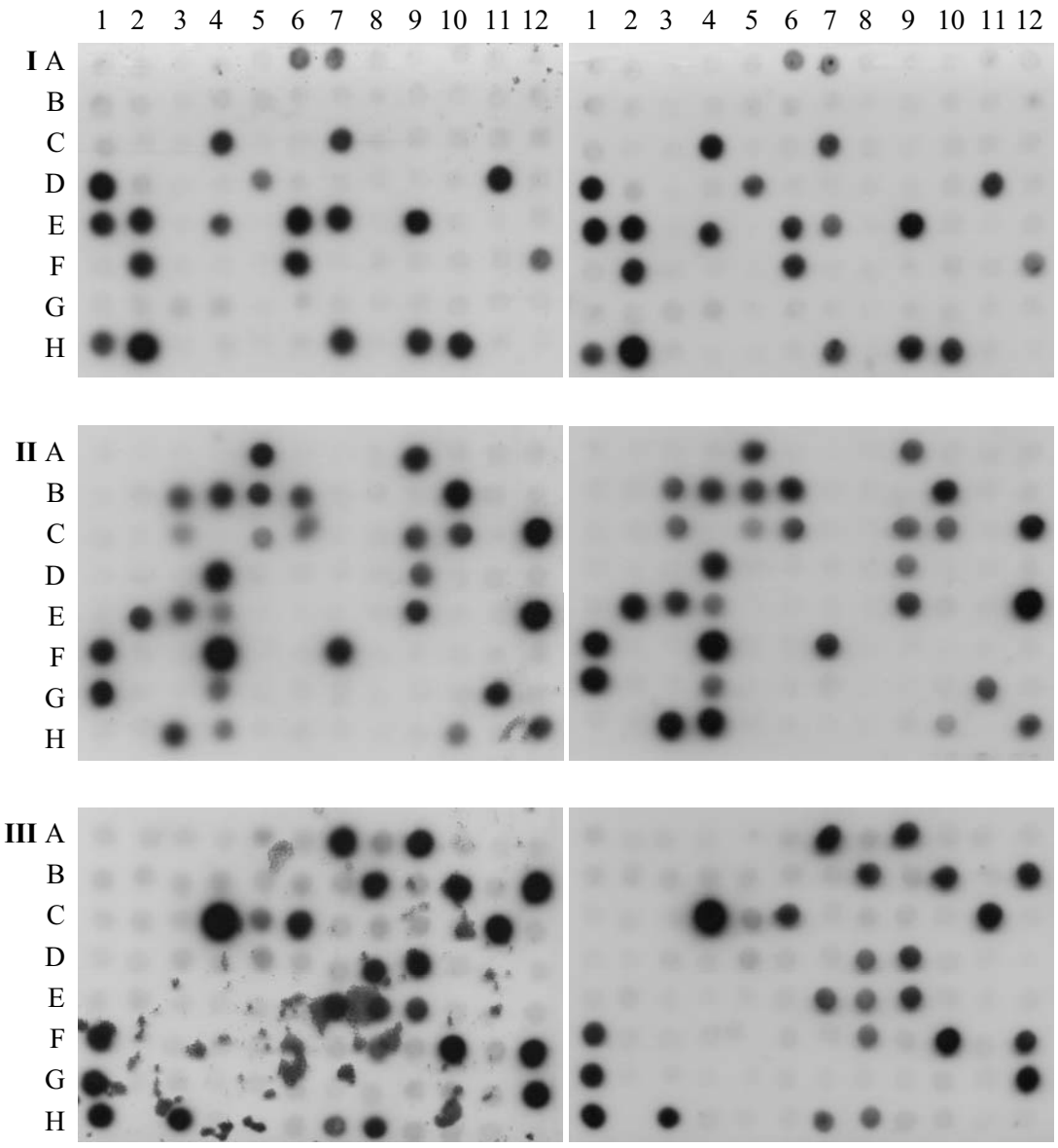


Figure 6.4 Dotblot film of clones from uninoculated Aromas leaf tissue probed with cDNA from inoculated (left) and uninoculated (right) Aromas leaf tissue.

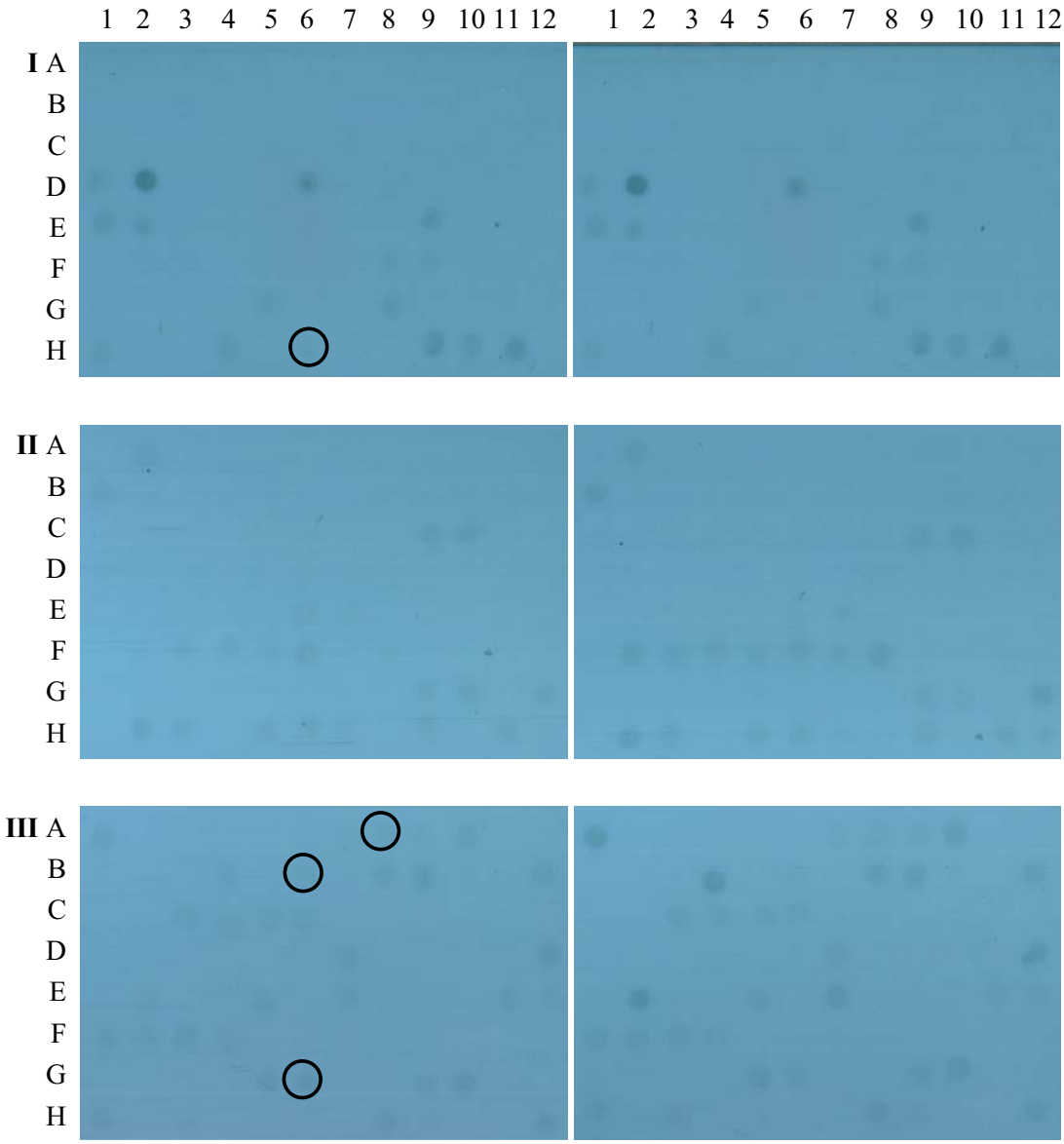


Figure 6.5 Dotblot film of clones from Aromas leaf tissue 4 dpi with *P. aphanis* probed with cDNA from inoculated (left) and uninoculated (right) Selva leaf tissue. Circles show clones chosen for sequencing and characterisation.

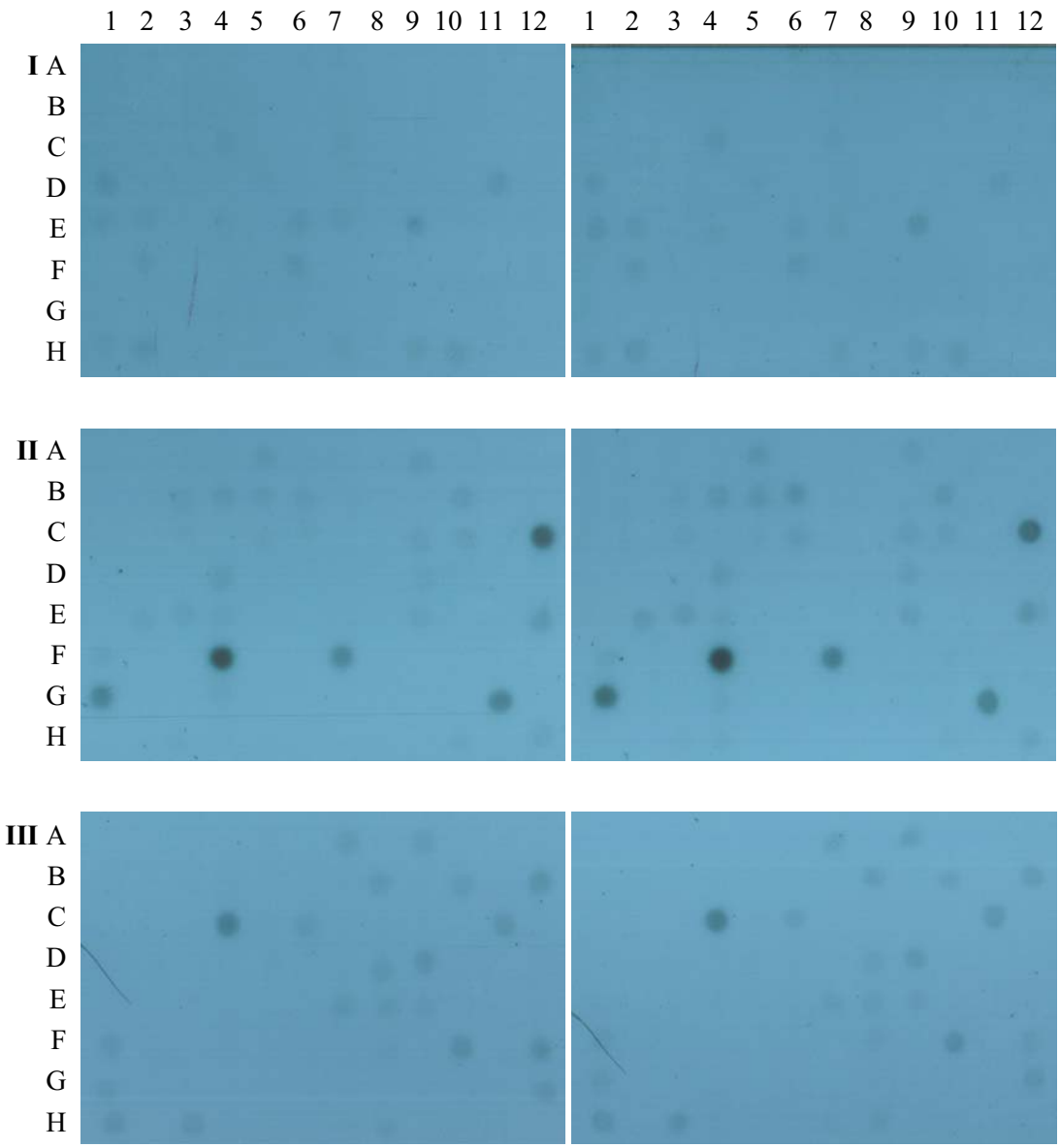


Figure 6.6 Dotblot film of clones from uninoculated Aromas leaf tissue probed with cDNA from inoculated (left) and uninoculated (right) Selva leaf tissue.

Table 6.1 Homology of clone SPIH6 (183 bp, complete sequence for clone), isolated from Aromas leaf tissue 4 dpi with *Podosphaera aphanis*, to the NCBI nucleotide and EST databases.

Species	Putative function	Accession number	Homology	Overlap	E-value	Database
Prunus hortulana	Chloroplast psbM-trnD intergenic spacer gene (partial)	AY727109.1[a]	93 %	130 bp	$2.00E^{-44}$	Nucleotide
Prunus virginiana	Chloroplast psbM-trnD intergenic spacer gene (partial)	AY727108.1[a]	93 %	130 bp	$2.00E^{-44}$	Nucleotide
Prunus nigra	Chloroplast psbM-trnD intergenic spacer gene (partial)	AY727111.1[a]	92 %	130 bp	$4.00E^{-42}$	Nucleotide
Rosa sp.	No known function	EC587995.1[b]	94 %	138 bp	$8.00E^{-52}$	EST
Malus x *domestica*	No known function	CO729284.1[b]	91 %	138 bp	$3.00E^{-42}$	EST

[a] Shaw *et al.* (2005), [b] Unpublished

Figure 6.7 Alignment of sequence SPIH6 to sequences from the NCBI nucleotide and EST databases. SPIIIG6 was isolated from Aromas leaf tissue 4 dpi with *Podosphaera aphanis*. Alignment to sequences AY727109.1, AY727111.1, AY727108.1, (Chloroplast psbM-trnD intergenic spacer gene from *P. hortulana, P. virginiana* and *P. nigra*, respectively. N.B. these sequences extend to 985 bp, 770 bp shown), CO729284.1 (EST of no known function from *Rosa* sp.) and EC587995.1 (EST of no known function from *M.* x *domestica*) are shown (see Table 6.1).

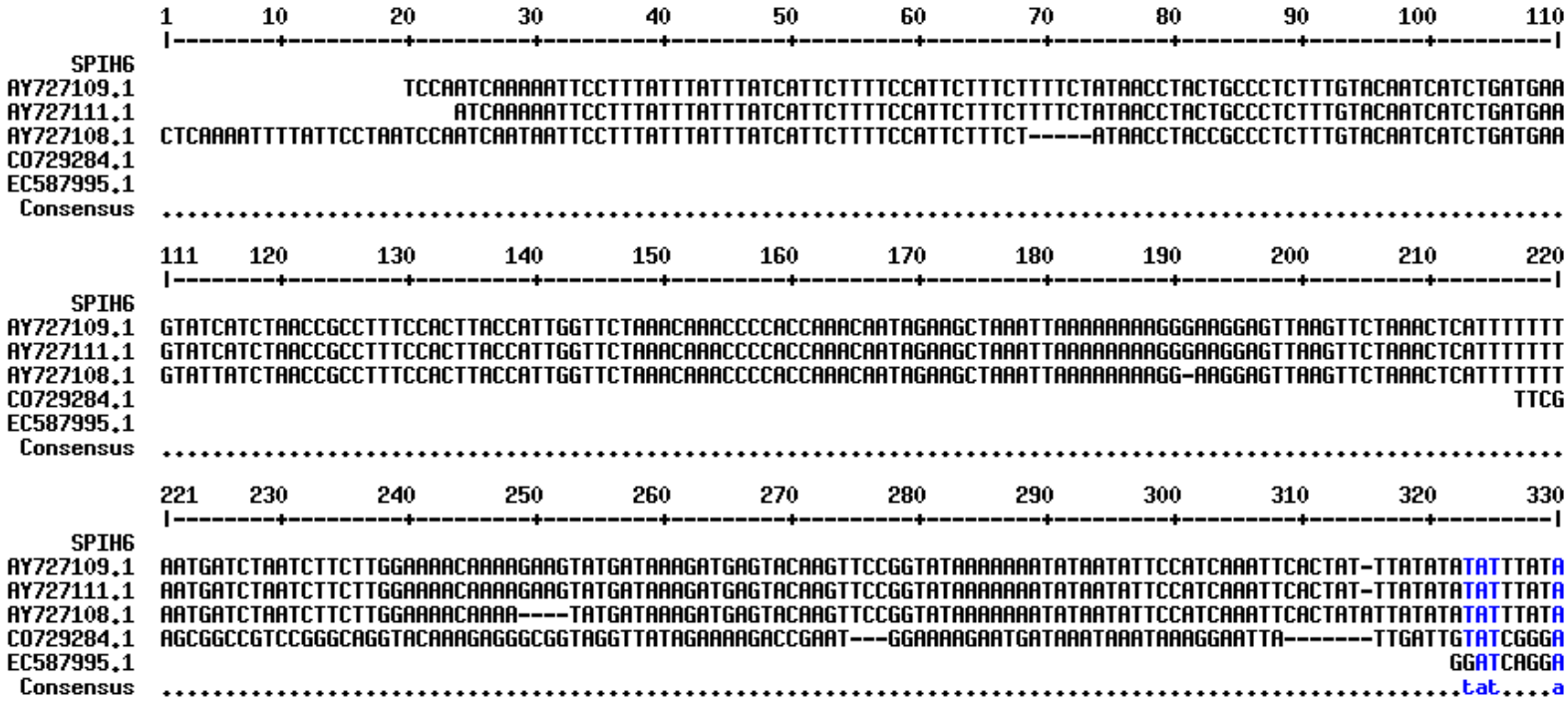


Figure 6.7 Continued

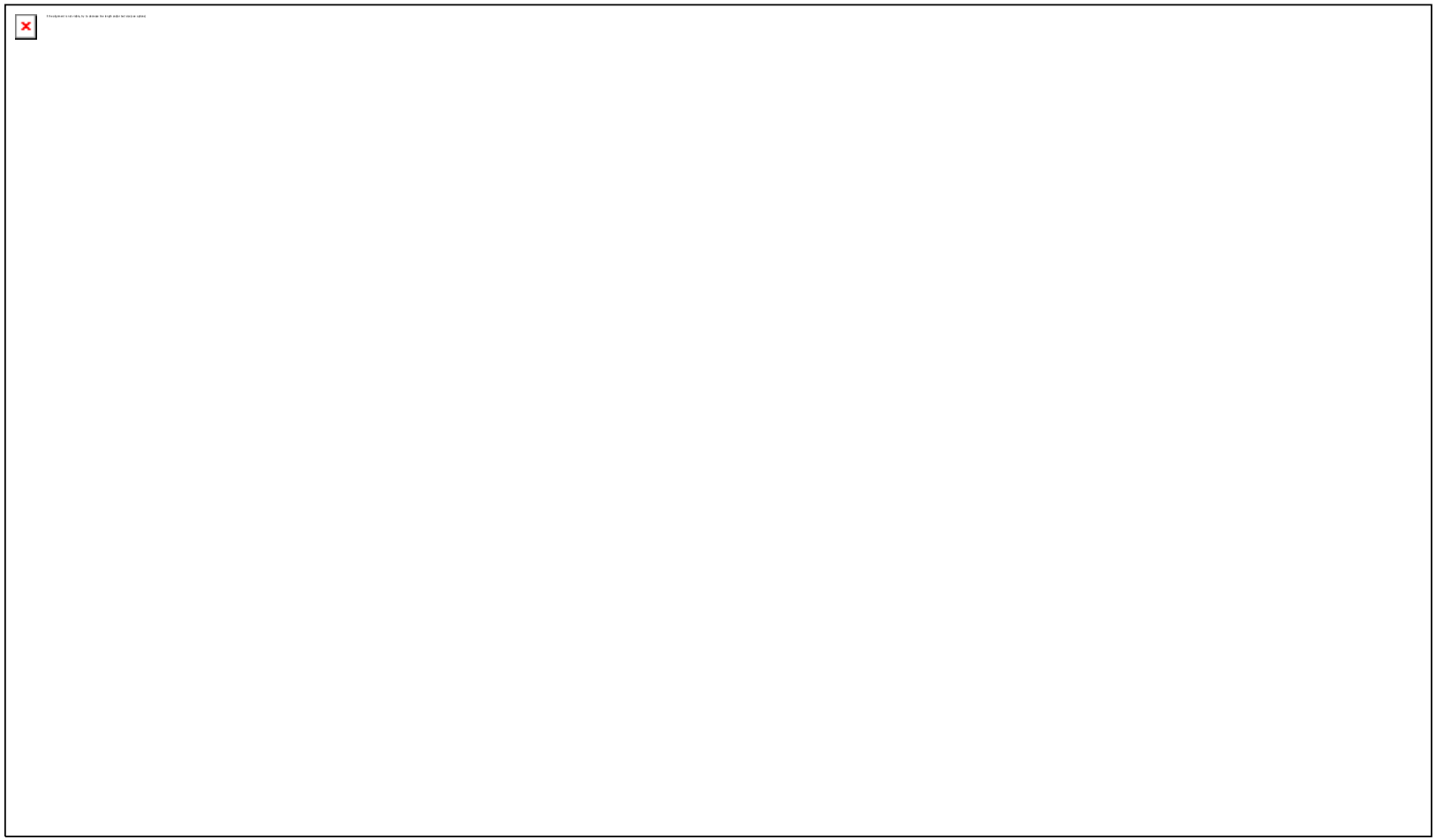


Table 6.2 Homology of clone SPIIIA8 (41 bp, partial sequence for clone), isolated from Aromas leaf tissue 4 dpi with *Podosphaera aphanis*, to the NCBI nucleotide database. * indicates short exact matches only

Species	Putative function	Accession number	Homology	Overlap	E-value	Database
F. x *ananassa*	Chloroplast inverted repeat region	DQ768221.1[a]	100 %	13 bp*	0.98	Nucleotide (*Fragaria*)
F. x *ananassa*	Endobetaglucanse synthesis	AJ414709.1[b]	100 %	12 bp*	3.9	Nucleotide (*Fragaria*)
Zea mays	mRNA, stress induced	DQ244476[c]	100 %	17 bp*	2.9	Nucleotide
Arabidopsis thaliana	Phytosulfokine precursor (complete)	AB074573.2[d]	100 %	17 bp*	2.9	Nucleotide
Clarkia concinna	Linalool synthase (complete)	AF067602[e]	100 %	17 bp*	2.9	Nucleotide
F. x *ananassa*	No known function	CO381894.1[b]	94 %	18 bp*	0.25	EST

[a] Dhingra and Folta (2005), [b] Unpublished, [c] Jia *et al.* (2006), [d] Yang *et al.* (2001), [e] Cseke *et al.* (1998).

Homology to stress induced mRNAs and genes associated with protein synthesis was also found in other species (Table 6.2). However, these matches were all less than 20 bp. An EST search using this sequence showed short exact sequence alignments to an EST from *F.* x *ananassa* (24 hours after treatment with salicylic acid) [Accession number (AN): CO381894.1; Table 6.2]. When the longer *F.* x *ananassa* EST was used for BLASTn analysis, homology to 18s ribosomal proteins from wheat (alignment was 86 % over 105 bp, AN: AY123418.1), capsicum (alignment was 87 % over 100 bp, AN: AY496116.1) and *Arabidopsis* (alignment was 93 % over 49 bp, AN: AY064680.1) was found.

The sequence from clone SPIIIB6 resulted in no significant matches to the NCBI nucleotide database, though when compared against the EST database showed significant alignment with a number of ESTs from *Fragaria* and *Rosa* species (Table 6.3 and Figure 6.8), including *F.* x *ananassa* cv. Strawberry Festival, 24 hours after being treated with salicylic acid (AN: CO816658.1). This sequence aligned with the central portion of a PsbW gene from the photosystem II reaction centre of *Retama raetam.*

The sequence for the SPIIIG6 clone was 402 bp and showed no significant homology to any sequences available on the NCBI nucleotide database. When small exact matches were considered this sequence showed exact alignment with a 21 bp sequence for a putative glycine-rich protein from *Arabidopsis thaliana* (Table 6.4). When compared against the NCBI EST database, SPIIIG6 showed synteny to an EST of *Fragaria vesca* and *Rosa* sp. With no known function (Table 6.4, Figure 6.9). When the *F. vesca* EST was used for BLASTn analysis there were small exact matches to genomic DNA from rice (*O. sativa*) (Table 6.4), but no significant homology to any

Table 6.3 Homology of clone SPIIIB6 (110 bp, partial sequence for clone), isolated from Aromas leaf tissue 4 dpi with *Podosphaera aphanis*, to the NCBI EST database. No significant homology to the nucleotide database was observed.

Species	Putative function	Accession number	Homology	Overlap	E-value	Database
Fragaria x *ananassa*	No known function	CO816658.1[a]	95 %	70 bp	$2.00E^{-23}$	EST
Fragaria vesca	No known function	DY672144.1[a]	94 %	71 bp	$1.00E^{-21}$	EST
Rosa luciae	No known function	EC589291.1[a]	92 %	71 bp	$1.00E^{-15}$	EST

[a] Unpublished

Figure 6.8 Alignment of sequence SPIIIB6 to sequences from the NCBI EST database. SPIIIB6 was isolated from Aromas leaf tissue 4 dpi with *Podosphaera aphanis*. Alignments to sequences CO816658.1 (*F.* x *ananassa*, no known function), DY672144.1 (*F. vesca*, no known function) and EC589291.1 (*Rosa luciae*, no known function. Sequence extends to 4570 bp, only shown to 1040 bp) shown (see Table 6.3).

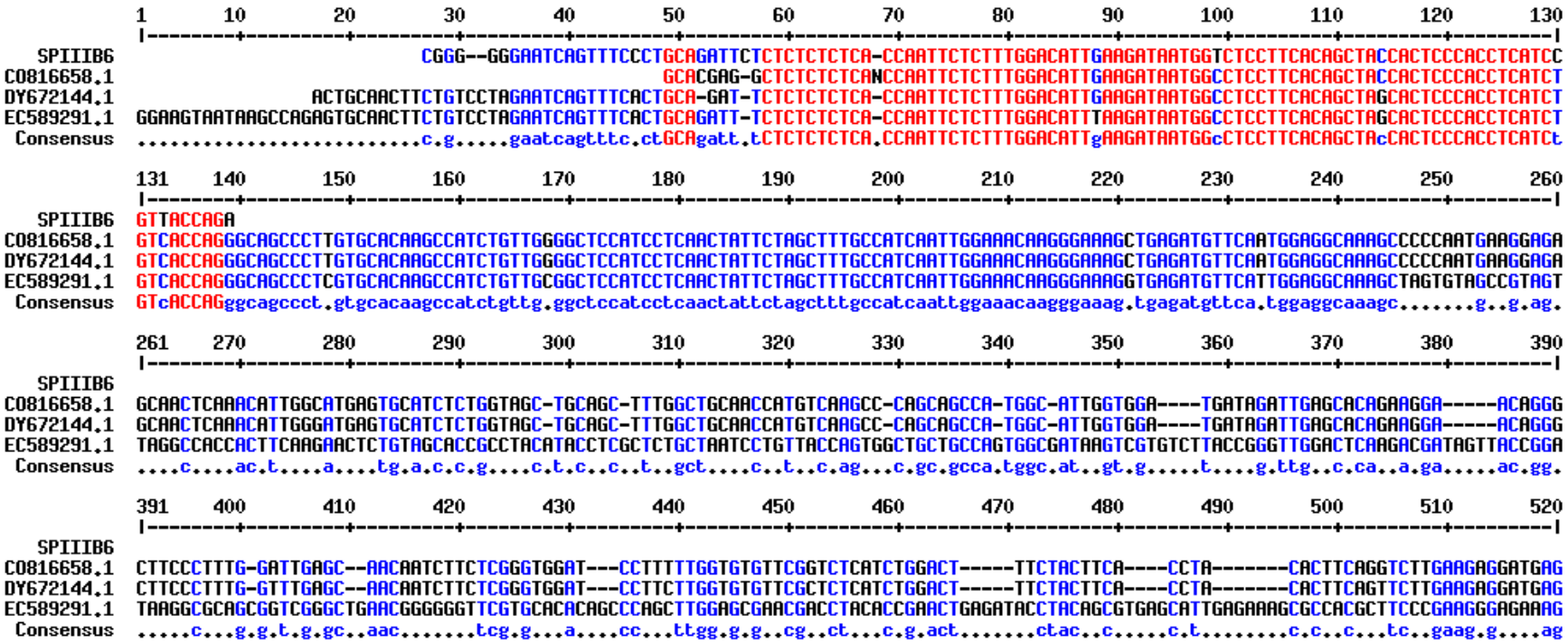


Figure 6.8 Continued

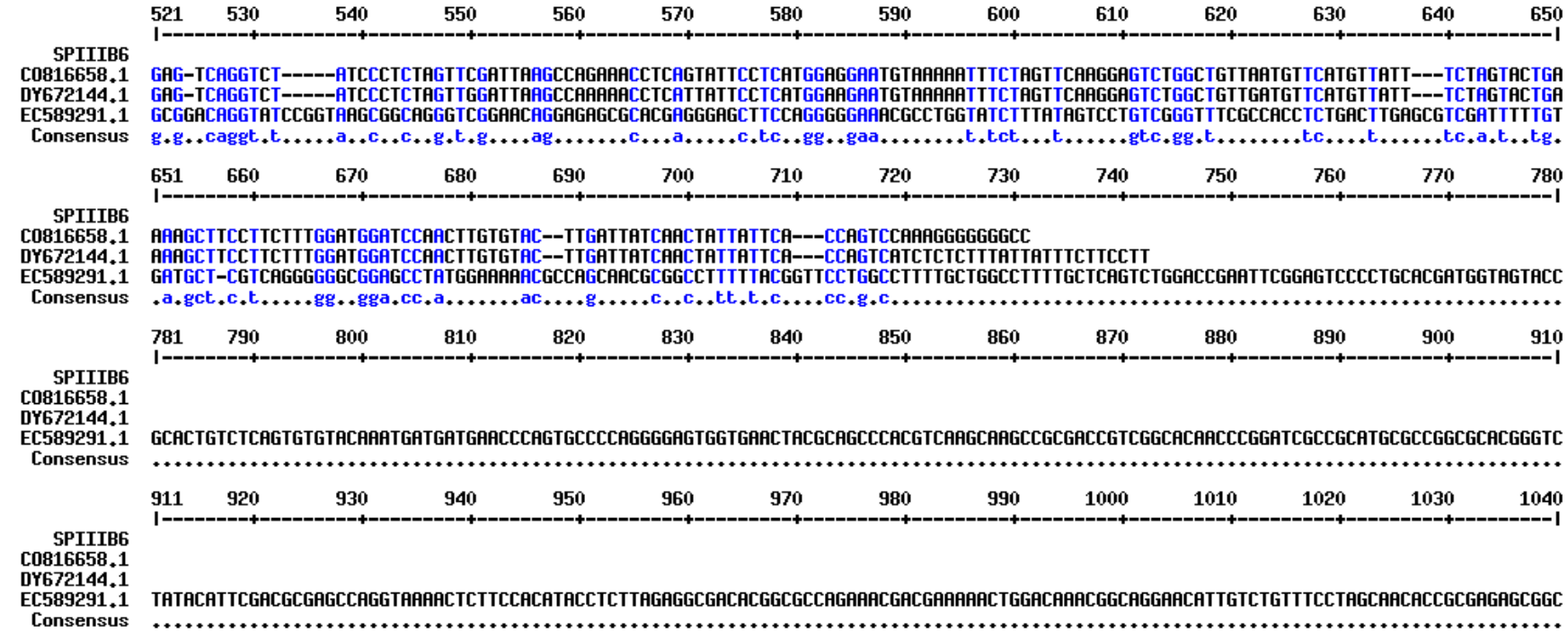
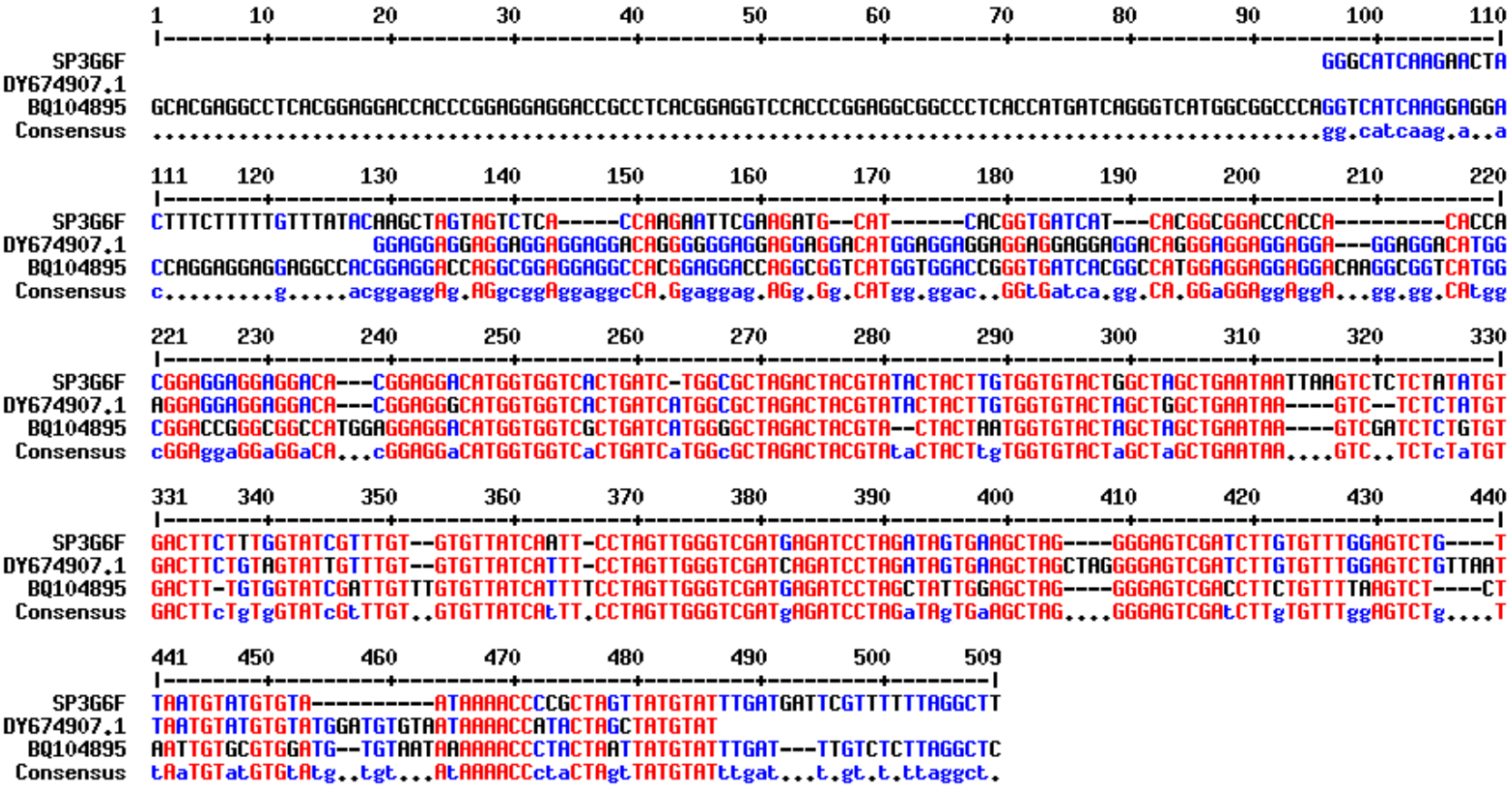


Table 6.4 Homology of clone SPIIIG6 (406 bp, complete sequence for clone), isolated from Aromas leaf tissue 4 dpi with *Podosphaera aphanis*, to the NCBI nucleotide and EST databases.

Species	Putative function	Accession number	Homology	Overlap	E-value	Database
Oryza sativa	No known function	NM 001054069.1[a]	96 %	29 bp*	0.006	Nucleotide
Arabidopsis thaliana	Glycine-rich protein	AK228184.1[b]	100 %	21 bp*	1.3	Nucleotide
Fragaria vesca	No known function	DY672144.1[b]	91 %	213 bp	$2.00E^{-64}$	EST
Rosa sp.	No known function	BQ104895[b]	88 %	241 bp	$9.00E^{-20}$	EST

[a] Ohyangi *et al* (2006), [b] Unpublished

Figure 6.9 Alignment of sequence SPIIIG6 to sequences from the NCBI EST database. SPIIIG6 was isolated from Aromas leaf tissue 4 dpi with *Podosphaera aphanis*. Alignments to sequences DY674907.1 (*F. vesca*, no known function) and BQ104895 (*Rosa* sp., no known function) are shown (see Table 6.4).



genes of known or putative function. Translations of this sequence from all possible reading frames were made. Only two reading frames contained both Met and Stop codons with more than 2 amino acids between. The 3rd reading frame from 5' to 3' resulted in a protein sequence with a 24 amino acid sequence rich in glycine (54.2 %) and histidine (37.5 %). This protein chain showed short nearly exact amino acid sequence alignments to both a cation efflux protein from *Kineococcus radiotolerans*, a glycine and histidine rich antimicrobial peptide called shep-GRP from *Capsella bursa-pastoris* and the glycine-rich protein from *A. thaliana* that corresponded to the nucleotide sequence match from the nucleotide BLAST (Table 6.5). The glycine rich proteins (GRPs) from *C. bursa-pastoris* and *A. thaliana* shared a conserved domain, unique to GRPs, that preceded alignment with the candidate sequence (Figure 6.10).

6.3.5 RT-PCR glycine-histidine rich peptide

RT-PCR was carried out to confirm the relative abundance of the SPIIIG6 sequence in the samples used to create the dot blot clones and to establish an expression profile relative to time after inoculation with powdery mildew. Expression of SPIIIG6 was much higher at 4 dpi than in the uninoculated sample in an initial RT-PCR (Figure 6.11 A). Replicated RT-PCR analysis showed upregulation of this sequence from 48 to 96 hpi (Figure 6.11B)

6.4 Discussion

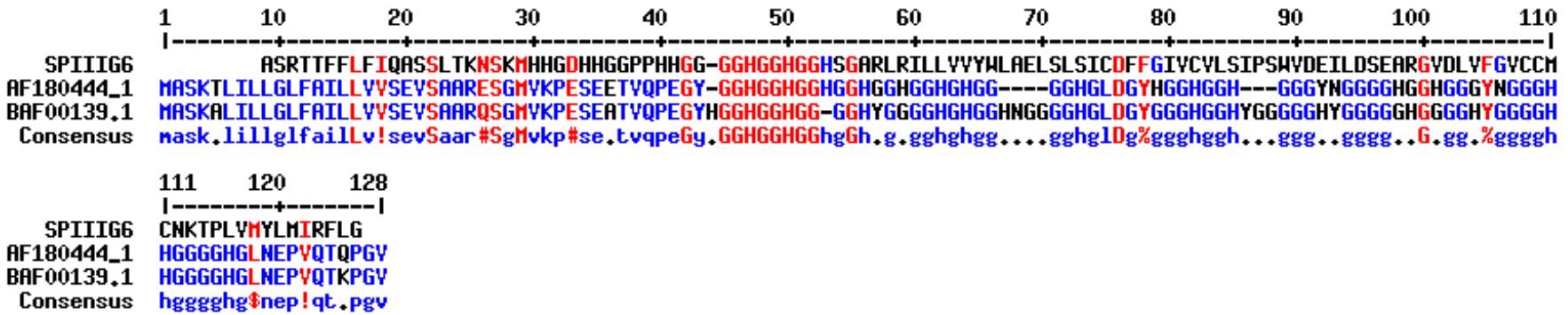
The purpose of this preliminary study was to identify differentially expressed genes during challenge of the strawberry cultivar Aromas by the powdery mildew pathogen *P. aphanis*. Four potential candidate sequences were investigated and may be useful in future studies that characterise resistance of Aromas to *P. aphanis*.

Table 6.5 BLASTp analysis of amino acid sequences translated from candidate sequence SPIIIG6 to proteins of known function on the NCBI protein database. SPIIIG6 was isolated from Aromas leaf tissue that were up-regulated 4 days after being inoculated with *Podosphaera aphanis*.

Species	Putative function	Accession number	Homology	Overlap	E-value
Kineococcus radiotolerans	Cation efflux protein	EAM76243.1[a]	76 %	25 aa	$3.00E^{-02}$
Capsella bursa-pastoris	Antimicrobial peptide (Shep-GRP)	AF180444.1[b]	61 %	31 aa	0.016
Arabidopsis thaliana	Glycine-rich protein	BAF00139.1[a]			

[a] Unpublished, [b] Park *et al.*, (2000).

Figure 6.10 Alignment of clone sequence SPIIIG6 to the NCBI protein database. SPIIIG6 was isolated from Aromas leaf tissue 4 dpi with *Podosphaera aphanis*. Alignment to protein sequences AF180444.1 (Antimicrobial GRP from *C. bursa-pastoris*) and BAF00139.1 (GRP from *A. thaliana*) shown (see Table 6.5).



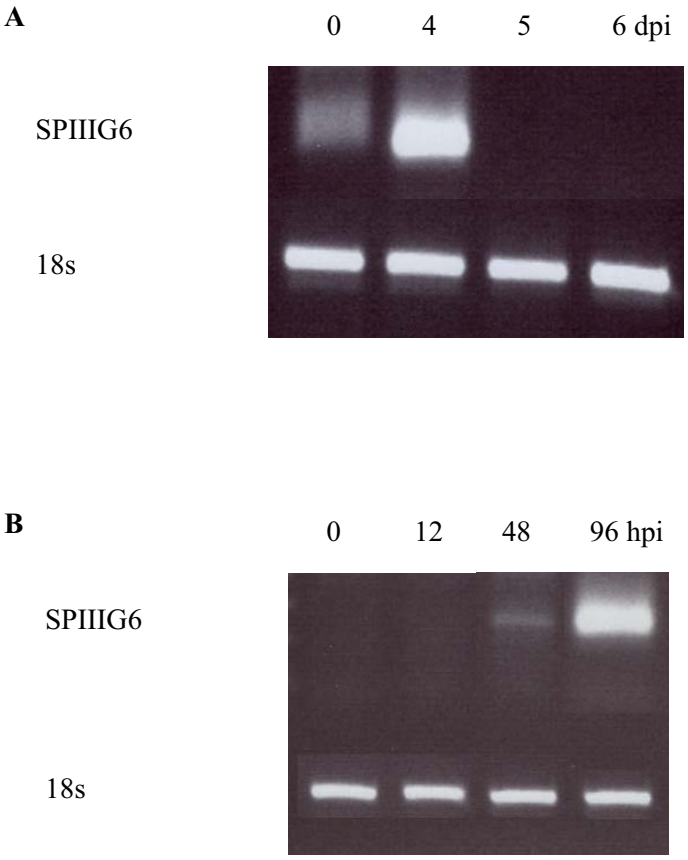


Figure 6.11 Expression of candidate sequence SPIIIG6 in Aromas leaf tissue inoculated with *Podosphaera aphanis*. **A.** uninoculated control and 4, 5 and 6 dpi with *P. aphanis* (lanes 1 to 4, respectively) and **B.** unioculated and 12, 48 and 96 hpi with *P. aphanis* with SPIIIG6 and 18s primers.

Most dot blot studies identify clones that are amplified by one probe and not another (Davies and Robinson, 2000). In this study there were no signals present in one film and not the other. There were, however, a number of signals that differed in the intensity of signal between films. The absence of complete differentiation in signals between the probed blots of this study may be explained by the nature of powdery mildew infection on Aromas. Firstly, primary inoculum appears to be carried latent on plant material, so it is possible that the uninoculated treatment, while it was not purposefully inoculated, was carrying a small amount of powdery mildew inoculum. Also, while Aromas shows field resistance to powdery mildew infection, powdery mildew colonies have been observed to develop on individual plants, though only during favourable environmental conditions and times of high disease pressure, to a lesser extent than most strawberry cultivars. This suggests that the resistance of Aromas to powdery mildew is likely to be polygenic and relatively constitutive, as is the case with powdery mildew resistance in many other plant species (Nelson *et al.*, 1995; Dirlewanger *et al.*, 1996). Thus, the reasoning behind selection of signals that were relatively stronger in the infected probed blot, in the absence of true presence/ absence signals.

Of the four that were investigated three (SPIH6, SPIIIA8 and SPIIIB6) showed homology to genes involved in regulating physiological processes such as photosynthesis. During times of stress, such as pathogen attack, photosynthesis is up-regulated to provide energy for defence mechanisms (Pennypacker *et al.*, 1994; Gibly *et al.*, 2004; Bechtold *et al.*, 2005; Li *et al.*, 2006). However, genes such as these are likely to be constitutive and common between genomes of different cultivars.

The SPIIIG6 sequence produced significant alignment with a *F. vesca* EST. The molecular map of this diploid species is close to being saturated (Sargent *et al.*, 2006).

There have been in excess of 1500 ESTs from a number of *F.* x *ananassa* cultivars recently added to the NCBI database (Folta *et al.*, 2004; Folta *et al.*, 2005; Keniry *et al.*, 2006). That the SPIIIG6 sequence did not match any of these ESTs from *F.* x *ananassa* may suggest that this sequence is either from an area of the genome where the available ESTs are sparse or that Aromas has this conserved material from *F. vesca*, one of the wild relatives of *F.* x *ananassa*, whereas other cultivars may not. Further research would allow for use of this sequence in mapping studies may enrich genetic maps of this species.

When the SPIIIG3 sequence was translated, the corresponding protein showed a high proportion of glycine and histidine amino acids. Many stress-related proteins are rich in glycine and histidine, including antimicrobial proteins (Park *et al.*, 2000). However, most GRPs have a conserved domain in the first 40 aa which was not found in the sequence recovered from this cloning technique. The cloned sequence is not complete and therefore gene walking, RACE-PCR or a similar molecular technique may allow for discovery of a translated GRP conserved domain, however, time constraints prevented studies of this nature and the initial translation does not show this domain. Future studies could also include the characterisation of this gene in other cultivars, to determine if it is unique to cultivars that are resistant to powdery mildew and how widely distributed over the *F.* x *ananassa* germplasm it is. Studies of the inheritance of this potential marker could also assist to characterise the mode of action it has in the powdery mildew resistance response of Aromas. The discovery of SPIIIG6 up-regulation in Aromas between 2 and 4 dpi with *P. aphanis* suggests this sequence may code for a protein that is involved in the disease response of Aromas to this pathogen. Futher research is required to establish the function this protein may have in preventing infection.

In conclusion, this work has showed potential for identification of candidate genes associated with powdery mildew resistance of Aromas. Studies conducted in this manner are not complicated by the octoploidy of the *F.* x *ananassa* genome and the uncertain polysomony, as inheritance studies can be.

Chapter 7 General discussion

South Australian strawberry growers suffer losses in most seasons due to powdery mildew resulting from the use of susceptible cultivars (namely Selva) in production and the limited availability of effective fungicides for treatment of this disease. While production of resistant cultivars may provide a useful long-term option, at present there are no cultivars that are suitable. In the short-term, South Australian strawberry growers would benefit from new means for managing powdery mildew on Selva. This study was designed to provide growers with tools for management of this disease by improving knowledge of epidemiology and treatments. In particular, the environmental conditions conducive to epidemic in South Australian crops were determined, the effect of conventional and novel fungicide treatments assessed, the benefit of crop nutrition with potassium, calcium and silicon in minimising disease determined and preliminary investigation of the resistance of cultivar, Aromas, made.

7.1 Predicting and understanding vulnerability to powdery mildew

While powdery mildew affects most of the southern strawberry production regions in Australia, local environmental conditions in South Australia are particularly conducive to powdery mildew. This and the long period and continuous fruiting cycle of day-neutral cultivars produced in South Australia make this disease difficult manage. Disease is severe in most seasons. Furthermore, Selva, the predominant cultivar produced in this region is very susceptible to powdery mildew. In these studies, specific environmental conditions have been identified as conducive to powdery mildew epidemics (Chapter 3). These hypotheses originated from the knowledge accumulated from detailed studies of germination (Peries, 1962b; Jhooty and McKeen, 1964; Mukerji, 1968; Sivapalan, 1993a), colony development (Peries, 1962a; Miller *et al.*, 2003) and conidial dispersal (Blanco *et al.*, 2004) of *P. aphanis*. The correlation of

these conditions with powdery mildew epidemics in commercial crops needs further validation, preferably using a network of onsite weather stations and disease observations throughout the year. If validated, new and existing data could be used to generate a predictive model that South Australian strawberry growers could use to manage powdery mildew with strategic fungicide application. Such a model may also have application to production regions with similar environmental conditions around the world, such as Spain.

Having identified conditions that promote disease, there is also potential for manipulation of conditions within the crop canopy to reduce conidial dispersal and germination. For instance, the use of overhead irrigation could act to wash the conidia from the air (Peries, 1962a; Strand, 1994; Blanco *et al.*, 2004), increase RH in the crop canopy thus reducing conidiation (Adams *et al.*, 1986) and also leave free water on the leaf surface thus preventing germination (Peries, 1962a; Schnathorst, 1965; Perera and Wheeler, 1975; Sivapalan, 1993a). In future studies, the use of sensors within the canopy of the crop would allow for accurate measurement of the effect of such treatments and spatial spread of epidemics. This would also allow for the assessment of the effect of altered fertilisation on crop density and comparison of the microclimate between resistant and susceptible cultivars.

Indeed, differences in architecture between the two cultivars were observed. Aromas had longer and more erect petioles and peduncles than Selva and it is expected that this would influence both the number of conidia trapped by each cultivar (Russell, 1975) and subsequent development (Schnathorst, 1965). Increased movement of air within the canopy and exposure of leaves and developing fruit to sunlight would decrease RH and increase temperature both within the canopy and at the surface of leaves and fruit, factors that are known to inhibit development of fungal pathogens.

However, detached leaf inoculation studies showed limited attachment of conidia to Aromas leaves and no colony development, suggesting Aromas leaves have some mechanism of preventing establishment of colonies on leaves. Colonies were found sporadically on leaves of Aromas plants in the field, but only when disease on Selva was severe, and even then colonies remained small and did not persist (Chapter 2). Furthermore, preliminary molecular studies of Aromas leaf tissue suggest the involvement of an antimicrobial protein in the resistance response, however, this requires validation with further study.

It is also important to have the ability to predict the first occurrence of disease (Hardwick, 2006; Hau and de Vallavieille-Pope, 2006), however the source of primary inoculum remains one of the uncertainties in epidemiology of strawberry powdery mildew (Amsalem *et al.*, 2006; Peries, 1962b). Planting material (stolons), which is not sanitised prior to planting, has generally been assumed to carry conidia, cleistothecia or mycelium (Blanco *et al.*, 2004), however, *P. aphanis* has also been found growing on eucalypts (Cunnington, 2003). Eucalypt trees are commonly found on or near strawberry farms in South Australia. Indeed, stands of eucalypts, up to 15 m in height, bordered most of the trial sites used in these studies (within 20 m) with an occasional mature tree located amongst the crop plantings. If the powdery mildew pathogen found on eucalypts has been correctly identified as the same fungus that causes powdery mildew on strawberry plants then this has serious implications for management of this disease, especially given the potential for wind dissemination of conidia from such a height (Hau and de Vallavieille-Pope, 2006). If this is indeed the case, then a priority for future research will be to establish if this fungus grows and sporulates on *Eucalyptus* species growing in the local strawberry production area and that the same strains of fungus in turn infect strawberry plants. Such research would involve morphological,

cross infection and molecular studies. Novel management strategies incorporating this knowledge could involve fungicide treatment of the eucalypt trees, introduction of biological control agents, or replacement of border strips with non-host trees. The eucalypts adjacent to strawberry plots could even provide a refuge for introduced biological control agents that would otherwise be adversely affected by chemical applications to strawberry crops.

It is important for consumer image that disease is minimised in food crops. These studies have shown that when powdery mildew is poorly managed in strawberry crops, the resulting fruit can have mycelial growth on the receptacle and often do not form in the desired conical shape (Chapter 3). The biotrophic nature of *P. aphanis* means that infection on harvested fruit will not cause decay as would be expected of necrotrophic fungi, however, the effect on shelf life is uncertain. While mildly affected strawberries are still marketable the skin can have a furry texture, a quality not favoured by consumers of fresh fruit. The characterisation of powdery mildew on harvested fruit, which closely associated with that on leaves, may also be more suitable than the time-consuming observations on plants for use in larger scale trials of powdery mildew severity.

The knowledge gained from these studies provides new tools for further research of strawberry powdery mildew and combined with strategic treatment applications, should facilitate management of powdery mildew in South Australian strawberry crops. The main reason for using forecasting in disease management is that treatment can be applied in times when disease is predicted to threaten crops. Reduced fungicide use has health and safety as well as economic benefits, reducing exposure to non-target organisms, operators and consumers. The economic benefits should be two-fold, with

reduced treatment costs and increased market prices as a result of meeting customer demand for produce grown with reduced chemical input (Hardwick, 2006).

7.2 Fungicide reduces disease but affects berry development

At present, the only fungicides available to strawberry growers in South Australia for powdery mildew treatment are Systhane® and Flint®, the latter only being registered for use immediately prior to the 2005/06 season. This limited range gives cause for concern as there was evidence of severe disease in 2006 even with both fungicides available (M. Naradisorn, personal communication, 2007).

Although growers believed that Systhane® could not be used to effectively control powdery mildew (D. Parker, R. Lewis and P. Mason, personal communication, 2003), the results of this study showed that when applied fortnightly, Systhane® effectively controlled disease when inoculum potential was low (Chapter 5). Improved management of this disease may be achieved by reserving this fungicide for use following periods when conditions are conducive to powdery mildew (Chapter 3), however, optimisation of the timing and number of Systhane® treatments needed at designated periods to sustain economic levels of disease is required.

As seen with regular use of other fungicides on strawberry crops (Kovach *et al.*, 2000), this study showed adverse effects on yield from regular use of Systhane®, supporting anecdotal evidence from growers (D. Parker, personal communication, 2005). This highlights the importance of reducing the frequency of application especially in seasons when there is little disease, ultimately achieving the outcome of reducing both the costs of fungicide treatment and losses incurred from unmarketable fruit.

The use of Kasil 2040®, a potassium silicate product, in conjunction with Systhane® appeared to alleviate the adverse effect of Systhane® on yield to some extent

without affecting disease control (Chapter 5). Alone, Kasil 2040® treatment gave some control of powdery mildew, but this was not as good as that achieved with Systhane®. However, even this limited control would be useful to the increasing number of organic strawberry growers in South Australia and around the world if a similar product, produced using means acceptable to organisations that approve inputs for organic agriculture (usually via physical extraction from crude potassium salts), was available.

In seasons where disease is severe, improved management may be achieved if a number of fungicides that control powdery mildew using different modes of action were available to growers, to be used in rotation for treatment. This strategy should also minimise the selection for mutants resistant to a particular fungicide. Any novel alternative fungicide and/or biological control agent proven to be effective in field conditions could be adopted as a component of an integrated disease management strategy for this disease. *P. persimilus* is already used by many strawberry growers as part of an integrated pest management approach for control of two spotted mite (P. Mason, personal communication, 2003), suggesting that alternatives to spraying with fungicide would be readily adopted.

7.3 Improved nutrition with calcium helps prevent powdery mildew in strawberry

Optimised fertilisation with nutrients may improve resistance to powdery mildew as well as yield and flavour of strawberries produced. The observation that calcium nutrition status of Selva and Aromas leaves affected the development of *P. aphanis* described in these studies (Chapter 4) supports anecdotal evidence of the involvement of calcium in resistance of strawberry to powdery mildew (B. Morrison, personal communication, 2003; P. Mason, personal communication, 2003). Furthermore, Aromas achieved a higher foliar concentration than did Selva when

fertilised with the same rate of calcium. This has also been observed in the short-day cultivars, Kiewa (powdery mildew resistant) and Adena (susceptible) (B. Morrison personal communication, 2003). This response warrants further characterisation in the cultivars used in these studies and in other cultivars to assess the extent to which calcium efficiency is associated with powdery mildew resistance in *F*. x *ananassa*.

Studies of fertilisation using potassium silicate were inconclusive. Indeed, the concentrations of this salt used in these studies to fertilise strawberry plants may have induced foliar toxicity symptoms, which have not previously been documented in strawberry (Chapter 4). However, this does imply that silicate was metabolised by the treated plants and that studies involving more comprehensive rates of fertilisation with silicate may show improved yield and disease resistance in strawberry plants as reported by Miyake and Takahashi (1986) and Wang and Tzeng (1998).

Nutrient utilisation is known to affect post-harvest qualities such as flavour in strawberries (Eaves and Leefe, 1962; Wang and Galletta, 1998; Dunn, 2003; Wójcik and Lewandowski, 2003; Dunn and Able, 2004) as well as disease resistance in many plant species (Bayles and Aist, 1987; Yamazaki and Hoshina, 1995; Yamazaki *et al*., 1996; 2000). Differences in nutrient utilisation between cultivars are common (May *et al*., 1993). Growers have observed that new cultivars can show improved yield when fertilised differently from Selva (D. Parker, personal communication, 2005). However, new cultivars are not distributed with specific fertilisation guidelines. The recommendations of 4.5 mg calcium and 12.5 mg potassium per g dry weight for Aromas and 6.0 mg calcium and 10.0 mg potassium per g dry weight of leaf material for Selva will provide a starting point for developing fertilisation guidelines for these cultivars (Chapter 4). Fertigation studies incorporating these new recommendations for foliar concentrations of calcium and potassium are required to assess the efficiency of

fertilisation rates used at present. Research could be extended to consider seasonal fluctuation in foliar nutrient concentration (May *et al.*, 1994) and how this relates to strawberry flavour (Dunn, 2003). Knowledge of the dynamics of nutrient uptake would allow for synchronisation of nutrient supply with seasonal requirement (Tagliavini, 2005) and ultimately optimisation of the quality of strawberries on supermarket shelves.

7.4 Breeding a powdery mildew resistant cultivar for South Australia

South Australian growers benefit from market dominance during the months of March to May when supply from other regions of Australia is low. This is the main reason that South Australian growers have been reluctant to replace Selva, as this cultivar yields well during these months. In the foreseeable future, labelling of fresh strawberries should include the cultivar name (N. Greer, personal communication, 2004). Cultivars that consistently have better flavour than Selva are likely to obtain premium prices. To remain competitive in the Australia-wide market, it is becoming increasingly urgent for South Australian growers to produce strawberries that meet customer expectations of high quality, flavoursome fruit. There is no more important time than the present for renewed effort to develop superior yielding, powdery mildew resistant cultivars specifically for South Australian production.

At this time there is no breeding for this purpose, nor controlled evaluation of new cultivars bred in other regions of the world (such as Spain and the U.S.A.) for production in South Australia. While the cultivar Aromas is resistant to powdery mildew (Chapter 2) it has not been readily adopted by South Australian strawberry growers as it yields few fruit late in the season, and the strawberries produced are often poor in flavour. It is anticipated that improved nutrition would improve local production, however, breeding using Selva and a powdery mildew resistant cultivar, such as Aromas, for progeny that are resistant to powdery mildew and produce late

season yields is, perhaps, the best long-term solution for South Australian strawberry production. Characterisation of genes conferring resistance to powdery mildew and increased uptake and utilisation of calcium would also contribute to breeding of improved cultivars for South Australian production. Determination of the phenotypic and genetic characteristics involved in the resistance of this cultivar would aid in the generation and evaluation of progeny toward breeding for powdery mildew resistant strawberry cultivars. If growers could be convinced of potential profitability of new cultivars developed in Australia and abroad for production in South Australia, then increased adoption of new improved cultivars would result. This, combined with specific fertilisation guidelines, would improve production, marketability of fruit and lead to expansion of South Australian strawberry production, which has remained stagnant for a number of years (ABS, 2006).

Successful breeding relies on knowledge of the inheritance of traits of interest. A saturated map of the cultivated species will aid studies of inheritance, which to date have been difficult to interpret (Lerceteau-Köhler *et al.*, 2003). In order to understand the octoploid nature of *F.* x *ananassa* there have been increasing efforts to map the genome of this species and those related to it (Davis and Yu, 1997; Haymes *et al.*, 2000; Cekic *et al.*, 2001; Viruel *et al.*, 2002; James *et al.*, 2003; Lerceteau-Köhler *et al.*, 2003; Albani *et al.*, 2004, Cipriani and Testolin, 2004; Hadonou *et al.*, 2004; Sargent *et al.*, 2004a; Folta *et al.*, 2005; Keniry *et al.*, 2006; Sargent *et al.*, 2006). While a candidate gene approach has been used to identify genes in fruit (Deng and Davis, 2001) and has shown potential for use with RNA from leaves (Chapter 6), QTL studies with a saturated map would allow association of markers to traits of interest and facilitate MAS breeding of new strawberry cultivars. In the case of breeding for powdery mildew resistance, screening of progeny can be heavily time and resource consuming. MAS

using associated markers would allow a higher throughput of material in the early evaluation stages, thus minimising resources required in subsequent disease screening of potential cultivars.

7.5 Managing mildew could be the key to success

The results of this study have provided tools for South Australian strawberry growers by which they may manage powdery mildew in their crops effectively, efficiently and with minimal chemical input. If validated, the environmental conditions conducive to powdery mildew, identified in these studies, should be used as the basis for a prediction system for application of fungicides and alternatives for improved control of powdery mildew in strawberry crops. This treatment strategy, used in conjunction with nutrition regimes to maximise yields and disease resistance, should facilitate effective management of powdery mildew in South Australian strawberry crops and aid productivity and marketability of strawberries in the Australian fresh berry market. Exploration of alternative sources of disease resistance and development of cultivars suitable for South Australian production will enhance the success of this industry in years to come.

References

Abbott, J. D., B. D. Bruton and C. L. Patterson (1991) Fungicidal inhibition of pollen germination and germ-tube elongation in muskmelon. *HortScience* 26: 529 – 530.

Adams, G. C., Jr., T. R. Gottwald and C. M. Leach (1986) Environmental factors initiating liberation of conidia of powdery mildews. *Phytopathology* 76: 1239 - 1245.

Agrios, G. N. (1997) *Plant Pathology*. San Diego, Academic Press.

Aist, J. R. and W. R. Bushnell (1991) Invasion of plants by powdery mildew fungi, and cellular mechanisms of resistance. In, *The fungal spore and disease initiation in plants and animals* (eds G. T. Cole and H. C. Hoch). Plenum Press, New York, 321 - 345.

Aist, J. R., R. E. Gold, C. J. Bayles, G. H. Morrison, S. Chandra and H. W. Israel (1988) Evidence that molecular components of papillae may be involved in ml-o resistance to barley powdery mildew. *Physiological and Molecular Plant Pathology* 33: 17 - 32.

Aist, J. R. and H. W. Israel (1986) Elemental composition of barley coleoptile papillae in relation to their ability to prevent penetration by *Erysiphe graminis*. *Physiological and Molecular Plant Pathology* 29: 69 - 78.

Albani, M. C., N. H. Battey and M. J. Wilkinson (2004) The development of ISSR-derived SCAR markers around the Seasonal Flowering Locus (SFL) in *Fragaria vesca*. *Theoretical and Applied Genetics* 109: 571 - 579.

Albregts, E. E. and C. M. Howard (1978) Elemental composition of fresh strawberry fruit. *Journal of American Society of Horticultural Science* 103: 293 - 296.

Albregts, E. E., C. M. Howard and C. K. Chandler (1991) Strawberry responses to potassium rate on a fine sand soil. *HortScience* 26: 135 - 138.

Allardice, P. (1991) *Strawberries*. Hill of Content Publishing Company Pty Ltd, Melbourne, Australia.

Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller and D. J. Lipman (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389 - 3402.

American Society of Testing Materials (1995) Standard practise for maintaining constant relative humidity by means of aqueous solutions. *Annual Book of ASTM Standards* 11.03: 572 - 574.

Amsalem, L., S. Freeman, D. Rav-David, Y. Nitzani, A. Sztejnberg, I. Pertot and Y. Elad (2006) Effect of climatic factors on powdery mildew caused by *Sphaerotheca macularis* f. sp. *fragariae* on strawberry. *European Journal of Plant Pathology* 114: 283 - 292.

Arulsekar, S. and R. Bringhurst (1981) Genetic model for the enzyme marker PGI in diploid California *Fragaria vesca*. *Journal of Heredity* 73: 117 - 120.

Ashley, M. V., J. A. Wilk, S. M. N. Styan, K. J. Craft, K. L. Jones, K. A. Feldheim, K. S. Lewers and T. L. Ashman (2003) High variability and disomic segregation of microsatellites in the octoploid *Fragaria virginiana* Mill. (Rosaceae). *Theoretical and Applied Genetics* 107: 1201 - 1207.

Australian Bureau of Statistics (2005) Value of agricultural commodities produced, Australia 2003 - 04. Publication number: 7503.0, http://www.ausstats.abs.gov.au/ausstats/, accessed: 08/092006.

Australian Bureau of Statistics (2006) Agricultural commodities, Australia, 2004 - 05. Publication number: 7121.0. http://www.ausstats.abs.gov.au/ausstats/, accessed: 08/09/2006.

Australian Natural Resources Atlas (2006) Soils - An overview - Australia. http://audit.ea.gov.au/ANRA/land/, accessed: 15/09/2006.

Australian Pesticides and Veternary Medicines Authority (2006) http://www.apvma.gov.au, accessed: 07/09/2006.

Australian Soil Resource Information System (2006) CSIRO Land and Water. http://www.asris.csiro.au/mapping/viewer.htm, accessed: 15/09/2006.

Baker, K. F. (1957) *The UC system for producing healthy container-grown plants*. University of California, Division of Agricultural Sciences, Agricultural Experiment Station, Extension Service.

Bassil, N. V., M. Gunn, K. M. Folta and K. Lewers (2006) Microsatellite markers for *Fragaria* from 'Strawberry Festival' expressed sequence tags. *Molecular Ecology Notes* 6: 473 - 476.

Bayer CropScience (2007) Flint® application tips. http://www2.bayercropscience.ca/Products/Fungicides/Flint.aspx, accessed: 26/10/2007.

Bayles, C. J. and J. R. Aist (1987) Apparent calcium mediation of resistance of an ml-o barley mutant to powdery mildew. *Physiological and Molecular Plant Pathology* 30: 337 - 345.

Bechtold, U., S. Karpinski and P. M. Mullineaux (2005) The influence of the light environment and photosynthesis on oxidative signalling responses in plant-biotrophic pathogen interactions. *Plant Cell and Environment* 28: 1046 - 1055.

Bélanger, R. R., P. A. Bowen, D. L. Ehret and J. G. Menzies (1995) Soluble silicon: Its role in crop and disease management of greenhouse crops. *Plant Disease* 79: 329 - 336.

Bélanger, R. R. and C. Labbé (2002) Control of powdery mildews without chemistry: prophylactic and biological alternatives for horticultural crops. *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 256 – 267.

Bell, J. A. and D. W. Simpson (1994) The use of isoenzyme polymorphisms as an aid for cultivar identification in strawberry. *Euphytica* 77: 113 - 117.

Bennett, M. D., P. Bhandol and I. J. Leitch (2000) Nuclear DNA amounts in angiosperms and their modern uses - 807 new estimates. *Annals of Botany* 86: 859 - 909.

Berglund, R., B. Svensson and U. Gertsson (2006) Impact of plastic mulch and poultry manure on plant establishment in organic strawberry production. *Journal of Plant Nutrition* 29: 103 - 112.

Blaich, R. and H. Grundhöfer (1998) Silicate incrusts induced by powdery mildew in cell walls of different plant species. *Journal of Plant Diseases and Protection* 105: 114 - 120.

Blanco, C., B. de los Santos, C. Barrau, F. T. Arroyo, M. Porras and F. Romero (2004) Relationship among concentration of *Sphaerotheca macularis* conidia in the air, environmental conditions and the incidence of powdery mildew in strawberry. *Plant Disease* 88: 878 - 881.

Blanco-Portales, R., N. Medino-Escobar, J. A. López-Ráez, J. A. González-Reyes, J. M. Villaba, E. Moyano, J. l. Caballero and J. Muñoz-Blanco (2002) Cloning,

expression and immunololocalization pattern of a cinnamyl alcohol dehydrogenase gene from strawberry (*Fragaria* x *annanasa* cv. Chandler). *Journal of Experimental Botany* 53: 1723 - 1734.

Bonoli, M., C. Rosati, G. Perrotta, F. Mourgues, G. Cipriani, L. Manzecchi and W. Faedi (2004) Strawberry fruit quality: Biochemical and molecular aspects. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 359 - 362.

Boughey, A. S. (1949) The ecology of fungi which cause economic plant diseases. *Transactions of the British Mycological Society* 32: 179 -189.

Bould, C. (1964) Leaf analysis as a guide to the nutrition of fruit crops V. Sand culture N, P, K, Mg experiments with strawberry (*Fragaria* spp.). *Journal of the Science of Food and Agriculture* 15: 474 - 487.

Bowen, P., J. Menzies, D. Ehret, L. Samuels and A. Glass (1992) Soluble silicon sprays inhibit powdery mildew development on grape leaves. *Journal of American Society of Horticultural Science* 117: 906 - 912.

Boyce, B. R. and D. L. Matlock (1966) Strawberry nutrition. *Nutrition of fruit crops: tropical, sub-tropical, temperate, tree and small fruits* (ed N. F. Childers). Horticultural Publications, The State University of Rutgers, New Brunswick, 518 - 548.

Brahm, R. U., B. Urno and R. P. d. Oliveira (2005) Reaction of strawberry cultivars to powdery mildew under greenhouse conditions. *Revista Brasileira de Fruticultura* 27: 219 - 221.

Braun, U., R. T. A. Cook, A. J. Inman and H.-D. Shin (2002) The taxonomy of the powdery mildew fungi. *The powdery mildews: A comprehensive treatise* (eds R.

R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). APS Press, St. Paul, Minnesota, U.S.A. 13 - 55.

Brink, J. C., G. Holz and P. H. Fourie (2006) Effect of fungicide spray cover on *Botrytis cinerea* infection in grape bunches. *South African Journal of Enology and Viticulture* 27: 51 - 56.

Broadley, R. H. (1992) *Protect your strawberries*. Queensland Government, Brisbane, Australia.

Brown, J. K. M. (2002) Comparative genetics of avirulence and fungicide resistance in the powdery mildew fungi. *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 56 - 65.

Bureau of Meteorology (2006) Australian Weather. Department of Environment and Water Resources, Australian Government. http://www.bom.gov.au/, accessed: 15/09/2006.

Bush, D. (1995) Calcium regulation in plant cells and its role in signalling. *Annual Review of Plant Physiology and Plant Molecular Biology* 46: 95 - 122.

Byrne, J. M., M. K. Hausbeck and B. D. Shaw (2000) Factors affecting concentrations of airborne conidia of *Oidium* spp. among poinsettias in a greenhouse. *Plant Disease* 84: 1089 - 1095.

Carver, T. L., R. J. Zeyen and G. G. Ahlstrand (1987) The relationship between insoluble silicon and success or failure of attempted primary penetration by powdery mildew (*Erysiphe graminis*) germlings on barley. *Physiological and Molecular Plant Pathology* 31: 133 - 148.

Cekic, C., N. H. Battey and M. J. Wilkinson (2001) The potential of ISSR PCR primer pair combinations for genetic linkage analysis using the seasonal flowering locus in *Fragaria* as a model. *Theoretical and Applied Genetics* 103: 540 - 546.

Celio, G. J. and M. K. Hausbeck (1998) Conidial germination, infection structure formation and early colony development of powdery mildew on poinsettia. *Phytopathology* 88: 105 - 113.

Cengiz, K., B. E. Ak and D. Higgs (2003). Response of salt-stressed strawberry plants to supplementary calcium nitrate and/or potassium nitrate. Journal of Plant Nutrition 26: 543 - 560.

Chang, S., J. Puryear and J. Cairney (1993) A simple and efficient method for isolating RNA from pine trees. *Plant Molecular Biology Reporter* 11: 113 - 116.

Chérif, M., N. Benhamou, J. G. Menzies and R. R. Bélanger (1992a) Silicon induced resistance in cucumber plants against *Pythium ultimum*. *Physiological and Molecular Plant Pathology* 41: 411 - 425.

Chérif, M., J. G. Menzies, N. Benhamou and R. R. Bélanger (1992b). Studies of silicon distribution in wounded and *Pythium ultimum* infected cucumber plants. *Physiological and Molecular Plant Pathology* 41: 371 - 385.

Chérif, M., J. G. Menzies, D. L. Ehret, C. Bogdanoff and R. R. Bélanger (1994). Yield of cucumber infected with *Pythium aphanidermatum* when grown with soluble silicon. *HortScience* 29: 896 - 897.

Chiu, T. F. and C. Bould (1976) Effects of calcium and potassium on ^{45}Ca mobility, growth and nutritional disorders of strawberry plants (*Fragaria* spp.) *Journal of Horticultural Science* 51: 525 – 531.

Chow, K. K., T. V. Price and B. C. Hanger (1992) Nutritional requirements for growth and yield of strawberry in deep flow hydroponic systems. *Scientia horticulturae* 52: 95 - 104.

Cipriani, G. and R. Testolin (2004) Isolation and characterisation of microsatellite loci in *Fragaria*. *Molecular Ecology Notes* 4: 366 - 368.

Clarke, D. D. and A. Akhkha (2002) Population genetics of powdery mildew - natural plant pathosystems. *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 200 - 218.

Clavero, I., J. Gálvez, E. González-Benito, J. F. Sánchez-Sevilla, C. Soria and J. M. López-Aranda (2004) Strawberry germplasm conservation: The collection at IFAPA-CIFA (Málaga, Spain). *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 527 - 530.

Clontech Laboratories Inc. (2006) The Creator™ SMART™ cDNA library construction kit user manual. Protocol PT3577-1.

Cohen, R., D. Shtienberg and M. Edelstien (1996) Suppression of powdery mildew (*Sphaerotheca fuliginea*) in cucumber by the detergent Zohar LQ-215. *European Journal of Plant Pathology* 102: 69 - 75.

Cohen, Y., H. Eyal and J. Hanania (1990) Ultrastructure, autoflourescence, callose deposition and lignification in susceptible and resistant muskmelon leaves infected with the powdery mildew fungus *Sphaerotheca fuliginea*. *Physiological and Molecular Plant Pathology* 36: 191 - 204.

Collinge, D. B., P. L. Gregersen and H. Thorndal-Christensen (2002) The nature and role of defense response genes in cereals. *The powdery mildews: A*

comprehensive treatise (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 146 - 160.

Collins, N. C., A. Sadanandom and P. Schulze-Lefert (2002). Genes and molecular mechanisms controlling powdery mildew resistance in barley. *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 134 - 145.

Congiu, L., M. Chicca, R. Gella, R. Rosi and G. Bernacchia (2000) The use of random amplified polymorphic DNA (RAPD) markers to identify strawberry varieties: a forensic application. *Molecular Ecology* 9: 229 - 232.

Cook, R. T. A., A. J. Inman and C. Billings (1997) Identification and classification of powdery mildew anamorphs using light and scanning electron microscopy and host range data. *Mycological Research* 101: 975 - 1002.

Cooper, J. A. H., F. R. (1993) Effect of surface tension on the retention of various pesticides by apple leaves. *Journal of Environmental Science and Health Part B Pesticides Food Contaminants and Agricultural Wastes* 28: 487 - 503.

Corke, A. T. K. and V. W. L. Jordan (1978) Powdery mildews of bush and soft fruits. *The powdery mildews* (ed D. M. Spencer) Academic Press Inc. Ltd., London, England, 347 - 358.

Cseke, L., N. Dudareva and E. Pichersky (1998) Structure and evolution of linalool synthase. *Molecular Biology and Evolution* 15: 1491 – 1498.

Cunnington, J. H., S. Takamatsu, A. C. Lawrie and I. G. Pascoe (2003) Molecular identification of anamorphic powdery mildews (*Erysiphales*). *Australasian Plant Pathology* 32: 421 - 428.

Dai, N., S. Slotzky, M. Petreikov, Y. Yeslson, Z. Tanami and A. A. Schaffer (2004) Suppression of AGPase in transgenic strawberry plants abolishes the leaf starch diurnal cycle, but has no effect on normal vegetative and reproductive growth. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 79 - 82.

Dale, A. and T. M. Sjulin (1990) Few cytoplasms contribute to North American strawberry cultivars. *HortScience* 25: 1341 - 1342.

Darnell, R. L., D. J. Cantliffe, D. S. Kirschbaum and C. K. Chandler (2003) The physiology of flowering in strawberry. *Horticultural Reviews* 28: 325 - 349.

Darrow, G. M., D. H. Scott and A. C. Goheen (1954) Relative resistance of strawberry varieties to powdery mildew at Beltsville, Maryland, 1954. *Plant Disease Reporter* 38: 864.

Datnoff, L. E., G. H. Snyder and G. H. Korndörfer (2001) *Silicon in Agriculture*. Elsevier Science BV, Amsterdam.

Daubeny, H. A. (1959) Relative resistance of some strawberry varieties, species, selections and seedling populations to powdery mldew at Agassiz and Abbotsford, British Columbia. *Canadian Journal of Botany* 43: 1253 - 1255.

Daubeny, H. A. (1961) Powdery mildew resistance in strawberry progenies. *Canadian Journal of Plant Science* 41: 239 - 243.

Daugaard, H. (2001) Nutritional status of strawberry cultivars in organic production. *Journal of Plant Nutrition* 24: 1337 - 1346.

Davies, C. and S. P. Robinson (2000) Differential screening indicates a dramatic change in mRNA profiles during grape berry ripening: Cloning and characterisation of cDNAs encoding putative cell wall and stress response proteins. *Plant Physiology* 122: 803 - 812.

Davik, J. and B. I. Honne (2005) Genetic variance and breeding values for resistance to a wind-borne disease [*Sphaerotheca macularis* (Wallr. ex Fr.)] in strawberry (*Fragaria* x *ananassa* Duch.) estimated by exploring mixed and spatial models and pedigree information. *Theoretical and Applied Genetics* 111: 256 - 264.

Davis, T. M. and H. Yu (1997) A linkage map of the diploid strawberry *Fragaria vesca*. *Journal of Heredity* 88: 215 - 221.

Davis, T. M., H. Yu, K. M. Haigis and P. J. McGowan (1995) Template mixing: a method of enhancing detection and interpretation of codominant RAPD marker. *Theoretical and Applied Genetics* 91: 582 - 588.

Degani, C., L. J. Rowland, A. Levi, J. A. Hortynski and G. J. Galletta (1998) DNA fingerprinting of strawberry (*Fragaria* x *ananassa*) cultivars using randomly amplified polymorphic DNA (RAPD) markers. *Euphytica* 102: 247 - 253.

Degani, C., L. J. Rowland, J. A. Saunders, S. C. Hokanson, E. L. Ogden, A. Golan-Goldhirsh and G. J. Galletta (2001) A comparison of genetic relationship measures in strawberry (*Fragaria* x *ananassa* Duch.) based on AFLPs, RAPDs and pedigree data. *Euphytica* 117: 1 - 12.

Deng, C. and T. M. Davis (2001) Molecular identification of the yellow fruit color (c) locus in diploid strawberry: a candidate gene approach. *Theoretical and Applied Genetics* 103: 316 - 322.

Dhingra, A. and K. M. Folta (2005) ASAP: Amplification, sequencing and annotation of plastomes. *BMC Genomics* (Online) 6: 175.

Dik, A. J., M. A. Verhaar and R. R. Bélanger (1998) Comparison of three biological control agents against cucumber powdery mildew (*Sphaerotheca fuliginea*) in semi-commercial-scale glasshouse trials. *European Journal of Plant Pathology* 104: 413 - 423.

Dirlewanger, E., T. Pascal, C. Zuger and J. Kervella (1996) Analysis of molecular markers associated with powdery mildew resistance genes in peach (*Prunus persica* (L.) Batsch) x *Prunus davidiana* hybrids. *Theoretical and Applied Genetics* 93: 909 - 919.

Drawert, F., F. Giorg and G. Staudt (1974) On the electrophoretical differentiation and classification of proteins. IV. Disc electrophoresis and isoelectrical focusing in polyacrylamide gels of proteins and enzymes from various species, varieties and hybrid species of strawberries (*In german*). *Zeitschrift fur lebenmittel-untersuchung und -forschung* 156: 129 - 138.

Dunn, J. (2003) The effect of calcium nutrition on post-harvest strawberry quality and botrytis fruit rot development. Honours Thesis, The University of Adelaide, Australia.

Dunn, J. and A. J. Able (2004) Pre-harvest calcium effects on sensory quality and calcium mobility in strawberry. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 307 - 312.

Eaves, C. A. and J. S. Leefe (1962) Note on the influence of foliar sprays of Ca on the firmness of strawberries. *Canadian Journal of Plant Science* 42: 746 - 747.

Ellingboe, A. H. (2001) Plant-pathogen interactions: Genetic and comparative analysis. *European Journal of Plant Pathology* 107: 79 - 84.

Engelhard, B. (2004) The impact of weather conditions on the behavior of powdery mildew in infecting hop (*Humulus*). *Proceedings of the First International Humulus Symposium*, Corvallis, Oregon, U.S.A. 111 - 116.

Epstein, E. (1999) Silicon. *Annual Review of Plant Physiology and Plant Molecular Biology* 50: 641 - 664.

Evans, K. J., D. L. Whisson and E. S. Scott (1996) An experimental system for characterizing isolates of *Uncinula nector*. *Mycological Research* 100: 675 - 680.

Ficke, A., D. M. Gadoury, R. C. Seem, D. Godfrey and I. B. Dry (2004) Host barriers and responses to *Uncinula necator* in developing grape berries. *Phytopathology* 94: 438 - 445.

Folta, K. M., M. Staton, P. Stewart, J. Sook, D. H. Bies, C. Jesdurai and D. Main (2005) Expressed sequence tags (ESTs) and simple sequence repeat (SSR) markers from octoploid strawberry (*Fragaria* x *ananassa*). *BMC Plant Biology* 5: 12.

Folta, K. M., P. Stewart and D. H. Bies (2004) Development of an EST database for octoploid strawberry (*Fragaria* x *ananassa*, cv. Strawberry Festival). Unpublished.

Food and Agriculture Organization of the United Nations (2006) FAOSTAT Agricultural statistics database. http://faostat.fao.org, accessed: 12/09/2006.

Foulongne, M., T. Pascal, F. Pfeiffer and J. Kervella (2003) QTLs for powdery mildew resistance in peach x *Prunus davidiana* crosses: consistency across generations and environments. *Molecular Breeding* 12: 33 - 50.

Galletta, G. J. and J. L. Maas (1990) Strawberry genetics. *HortScience* 25: 871 - 879.

Gay, J. L. and J. M. Manners (1987) Permeability of the haustorium-host interface in powdery mildews. *Physiological and Molecular Plant Pathology* 30: 389 - 399.

Genc, Y., G. K. McDonald, et al. (2004) Differential expression of zinc efficiency during the growing season of barley. *Plant and Soil* 263: 273 - 282.

Gibly, A., A. Bonshtien, V. Balaji, P. Debbie, G. B. Martin and G. Sessa (2004) Identification and expression profiling of tomato genes differentially regulated

during a resistance response to *Xanthomonas campestris* pv. *vesicatoria*. *Molecular Plant Microbe Interactions* 17: 1212 - 1222.

Gidoni, D., M. Rom, T. Kunik, M. Zur, E. Izak, S. Izhar and N. Firon (1994) Strawberry-cultivar identification using randomly amplified polymorphic DNA (RAPD) markers. *Plant Breeding* 113: 339 - 342.

Gooding, H. J., R. J. McNicol and D. MacIntyre (1981) Methods of screening strawberries for resistance to *Sphaerotheca macularis* (Wall ex Frier) and *Phytophthora cactorum* (Leb. and Cohn). *Journal of Horticultural Science* 56: 239 - 245.

Graham, J., R. J. McNicol and J. W. McNicol (1996) A comparison of methods for the estimation of genetic diversity in strawberry cultivars. *Theoretical and Applied Genetics* 93: 402 - 406.

Graham, R. D. (1983) Effects of nutrient stress on susceptibility of plants to disease with particular reference to the trace elements. *Advanced Botanical Research* 10: 221 - 276.

Graham, R. D. and M. J. Webb (1991) Micronutrients and disease resistance and tolerance in plants. In, *Micronutrients in agriculture*. Soil Science Society of America, Madison, USA: 329 - 370.

Green, J. R., T. L. W. Carver and S. J. Gurr (2002) The formation and function of infection and feeding structures. *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 66 - 82.

Grove, G. G., R. J. Boal and L. H. Bennett (2000) Managing powdery mildew of cherry in Washington orchards and nurseries with spray oils. *Plant Health Progress* (Online).

Gubler, W. D., M. Rademacher, S. J. Vasquez and C. S. Thomas (1999) Control of powdery mildew using the UC Davis powdery mildew risk index. *APS Net* (Online).

Gupta, P. K., H. S. Balyan, P. C. Sharma and B. Ramesh (1996) Microsatellites in plants: A new class of molecular markers. *Current Science* 70: 45 - 54.

Hadonou, A. M., D. J. Sargent, F. Wilson, C. M. James and D. W. Simpson (2004) Development of microsatellite markers in *Fragaria*, their use in genetic diversity analysis, and their potential for genetic linkage mapping. *Genome* 47: 429 - 438.

Hancock, J. F. (1999) *Strawberries*. CABI Publishing, New York, U.S.A.

Hancock, J. F. and J. J. Luby (1993) Genetic resources at our doorstep: The wild strawberries. *Bioscience* 43: 141 - 148.

Hardwick, N. V. (2006) Disease forecasting. In, *The Epidemiology of Plant Diseases* (Eds B. M Cooke, D. Gareth Jones and B. Kaye) Springer, Netherlands. 239 – 267.

Harrison, R. E., J. L. Luby, G. R. Furnier and J. F. Hancock (2000) Differences in the apportionment of molecular and morphological variation in North American strawberry and the consequences for genetic resources management. *Genetic Resources and Crop Evolution* 47: 647 - 657.

Hau, B. and C. de Vallavieille-Pope (2006) Wind-dispersed diseases. In, *The Epidemiology of Plant Diseases* (Eds B. M Cooke, D. Gareth Jones and B. Kaye) Springer, Netherlands. 387 – 416.

Haymes, K. M., W. E. V. d. Weg, P. Arens, J. L. Maas, B. Vosman and A. P. M. D. Nijs (2000) Development of SCAR markers linked to a *Phytopthora fragariae* resistance gene and their assessment in European and North American

strawberry genotypes. *Journal of the American Society for Horticultural Science* 125: 330 - 339.

Haynes, R. J. and K. M. Goh (1987) Effects of nitrogen and potassium applications on strawberry growth, yield and quality. *Communications in Soil Science and Plant Analysis* 18: 457 - 472.

Hearne, C. M., S. Ghosh and J. A. Todd (1992) Microsatellites for linkage analysis of genetic traits. *Trends in Genetics* 8: 288 - 294.

Hecht-Buchholz, C. (1979) Calcium deficiency and plant ultrastructure. *Communications in Soil Science and Plant Analysis* 10: 67 - 81.

Herrington, M. E. and C. K. Chandler (2004) Strawberry breeding in a subtropical environment. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 441 - 447.

Hoagland and Arnon (1938) The water-culture method for growing plants without soil. Berkley.

Hokanson, S. C. and J. L. Maas (2001) Strawberry Biotechnology. *Plant Breeding Reviews* 21: 139 - 180.

Hollomon, D. W. and J. E. Wheeler (2002) Controlling powdery mildews with chemistry. *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 249 - 255

Horn, N. L., K. R. Burnside and R. B. Carver (1972) Powdery mildew of strawberry. *Plant Disease Reporter* 56: 368.

Hospital, K. (2003) Marker-assisted breeding. In, *Plant Molecular Breeding* (eds H. J. Newbury). Blackwell Publishing, Birmingham, U.K. 30 - 59.

Howard, C. M. and E. E. Albregts (1982) Cleistothecia of *Sphaerotheca macularis* on strawberry plants in Florida. *Plant Disease* 66: 261 - 262.

Hsam, S. L. K. and F. J. Zeller (2002) Breeding for powdery mildew resistance in common wheat (*Triticum aestivum* L.). *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 219 – 238.

Hsu, C. S., R. Watkins, A. T. Bolton and L. P. S. Spangelo (1969) Inheritance of resistance to powdery mildew in the cultivated strawberry. *Canadian Journal of Genetic Cytology* 11: 426 - 438.

Hutcheon, J. A., J. Coyle, M. E. Holgate and R. J. W. Byrde (1986) Effects of fungicide on long-term cropping and fruit quality of apple. *Plant Pathology* 35: 249 – 253.

Hutton, D. and A. Gomez (2005) Fungicides for powdery mildew control. Queensland Department of Primary Industries. http://www2.dpi.qid.gov.au/horticulture/17940.html, accessed: 17 August 2006.

Jahn, M., H. M. Munger and J. D. McCreight (2002) Breeding curcubit crops for powdery mildew resistance. *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 239 – 248.

James, C. M., F. Wilson, A. M. Hadonou and K. R. Tobutt (2003) Isolation and characterisation of polymorphic microsatellites in diploid strawberry (*Fragaria vesca* L.) for mapping, diversity studies and clone identification. *Molecular Ecology Notes* 3: 171 - 173.

Jarvis, W. R., W. G. Gubler and G. G. Grove (2002) Epidemiology of powdery mildews in agricultural pathosystems. *The powdery mildews: A comprehensive treatise*

(eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 169 – 199.

Jeffers, S. N. (1991) Effects of fungicides applied during bloom on yield, yield components and storage rots of cranberry. *Plant Disease* 75: 244 – 250.

Jhooty, J. S. and W. E. McKeen (1964) Studies on powdery mildew of strawberry caused by *Sphaerotheca macularis*. *Phytopathology* 55: 281 - 285.

Jia, J., J. Fu, J. Zheng, X. Zhou, J. Huai, J. Wang, M. Wang, Y. Zhang, X. Chen, J. Zhang, J. Zhao, Z. Su, Y. Lv and G. Wang (2006) Annotation and expression profile analysis of 2073 full-length cDNAs from stress-induced maize (*Zea mays* L.) seedlings. *Plant Journal* 48: 710 - 727.

Johanson, F. (1980) *"Hunger" in strawberries*. K & H Printers, Everett, Washington.

Johanson, F. D. and R. B. Walker (1963) Nutrient deficiencies and foliar composition of strawberries. *American Society for Horticultural Science* 83: 431 - 439.

John, M. K., H. A. Daubeny and F. D. McElroy (1975) Influence of sampling time on elemental composition of strawberry leaves and petioles. *Journal of American Society of Horticultural Science* 100: 513 - 517.

Kanto, T., A. Miyoshi and T. Ogawa (2004) Suppressive effect of potassium silicate on powdery mildew of strawberry in hydroponics. *Journal of General Plant Pathology* 70: 207 - 211.

Karasuda, S., S. Tanaka, H. Kajihara, Y. Yamamoto and D. Koga (2003) Plant chitinase as a possible biocontrol agent for use instead of chemical fungicide. *Bioscience Biotechnology and Biochemistry* 67: 221 - 224.

Kaya, C., H. Kirnak, D. Higgs and K. Saltali (2002) Supplementary calcium enhances plant growth and fruit yield strawberry cultivars grown at high (NaCl) salinity. *Scientia horticulturae* 93: 65 - 74.

Keniry, A., C. J. Hopkins, E. Jewell, B. Morrison, G. C. Spangenberg, D. Edwards and J. Batley (2006) Identification and characterization of simple sequence repeat (SSR) marker from *Fragaria* x *ananassa* expressed sequences. *Molecular Ecology Notes* 6: 319 - 322.

Kersey, M. J. and Z. W. Luo (2003) Mapping, characterisation and deployment of quantitative trait loci. In, *Plant Molecular Breeding* (ed H. J. Newbury). Blackwell Publishing, Birmingham, U.K. 1 - 29.

Kew, G., K. Wetherby, T. Zimmermann and T. Meissner (2004) Estimation of readily available waterholding capacity using filed hand texture. *Supersoil 2004: Proceedings of the 3rd Australian New Zealand Soils Conference*, University of Sydney, Australia. The Regional Institute Ltd.

Kim, S. (2003) RNA extraction protocol for basal angiosperms. http://www.flmnh.ufl.edu/soltislab/RNA_extraction.pdf, accessed: 27 January.

Kirsch, R. K. (1958) The importance of interaction effects in fertilizer and lime studies with strawberries. *American Society for Horticultural Science* 73: 181 - 188.

Kirschbaum, D. S., A. M. Borquez, S. L. Quipildor, M. Correa, H. Magen and P. Imas (2004) Nitrogen requirements of drip irrigated strawberries grown in subtropical environments. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 93 - 96.

Kiss, L. (2003) A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. *Pest Management Science* 59: 475 - 483.

Koh, S., A. André, H. Edwards, D. Ehrhardt and S. Somerville (2005) *Arabidopsis thaliana* sucellular responses to compatible *Erysiphe cicoracearum* infections. *The Plant Journal* 44: 516 - 529.

Kovach, J., R. Petzoldt and G. E. Harman (2000) Use of honey bees and bumble bees to disseminate *Trichoderma harzianum* 1295-22 to strawberries for *Botrytis* control. *Biological Control* 18: 235 – 242.

Kuehny, J. S. and B. Morales (1998) Effects of salinity and alkalinity on pansy and impatiens in three different growing media. *Journal of Plant Nutrition* 21: 1011 - 1023.

Kunihisa, M., N. Fukino and S. Matsumoto (2004) Development of PCR-RFLP marker on strawberry and the identification of cultivars and their progeny. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 517 - 521.

Kunoh, H. (2002) Localized induction of accessibility and inaccessibility by powdery mildew. *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 126 - 133.

Kunoh, H., K. Kung and H. Ishzaki (1985) Cytological studies of the early stages of powdery mildew on barley and wheat. XI. Autofluorescence and haloes at penetration sites of appressoria of *Erysiphe graminis hordei* and *Erysiphe pisi* on barley coleoptiles. *Canadian Journal of Botany* 63: 1535 - 1539.

Kunoh, H., R. L. Nicholson, H. Yosioka, N. Yamaoka and I. Kobayashi (1990) Preparation of the infection court by *Erysiphe graminis*: Degradation of the host cuticle. *Physiological and Molecular Plant Pathology* 36: 397 - 407.

Kunoh, H., K. Yamamori and J. Ishizaki (1983) Cytological studies of early stages of powdery mildew in barley and wheat. IX. Effect of various inorganic salts on the occurance of autoflourescence at penetration sites of appressoria of *Erysiphe graminis hordei*. *Canadian Journal of Botany* 61: 2181 - 2185.

Landry, B. S., L. Rongqi and S. Khanizadeh (1997) Classification of 75 strawberry cultivars and breeding lines using RAPD markers. *Proceedings of the Third International Strawberry Symposium*, Beltsville, Maryland, U.S.A. *Acta Horticulturae* 439: 101 - 104.

Laxalt, A. M. and T. Munnik (2002) Phospholipid signalling in plant defence. *Current Opinion in Plant Biology* 5: 1 - 6.

Lerceteau-Köhler, E., G. Guérin, F. Laigret and B. Denoyes-Rothan (2003) Characterisation of mixed and polysomic inheritance in the octoploid strawberry (*Fragaria* x *ananassa*) using AFLP mapping. *Theoretical and Applied Genetics* 107: 619 - 628.

Lerceteau-Kohler, E., A. Moing, G. Guérin, C. Renaud, M. Maucourt, D. Rolin, P. Roudeillac and B. Denoyes-Rothan (2004) QTL analysis for sugars and organic acids in strawberry fruits. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 573 - 577.

Lewers, K. S., S. M. N. Styan, S. C. Hokanson and N. V. Bassil (2005) Strawberry GenBank-derived and genomic simple sequence repeat (SSR) markers and their utility with strawberry, blackberry, and red and black raspberry. *Journal of American Society of Horticultural Science* 130: 102 - 115.

Li, C., Y. Bai, E. Jacobsen, R. Visser, P. Lindhout and G. Bonnema (2006) Tomato defense to the powdery mildew fungus: differences in expression of genes in susceptible, monogenic- and polygenic resistance responses are mainly in timing. *Plant Molecular Biology* 62: 127 - 140.

Lieten, P. (2004) Effect of K:Ca:Mg ratio on performance of 'Elsanta' strawberries grown on peat. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 397 - 400.

Lineberry, R. A. and L. Berkhart (1950) Degree of calcium saturation of soil in relation to growth and calcium content of strawberry plants. 455 - 466.

Lines, R., G. Kelly, M. Milinkovic and B. Rodoni (2004) Runner certification and virus elimination in commercial strawberry cultivars in Australia. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 253 - 254.

López-Medina, J., A. Palacio-Villegas and E. Vázquez-Ortiz (2004) Misshaped fruit in strawberry, an agronomic evaluation. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 77 - 78.

Lyngkjær, M. F., T. L. W. Carver and R. J. Zeyen (1997) Suppression of resistance to *Erysiphe graminis* f.sp. *hordei* conferred by the mlo5 barley powdery mildew resistance gene. *Physiological and Molecular Plant Pathology* 50: 17 - 36.

Maas, J. (1984) *Compendium of strawberry diseases*. The American Phytopathological Society Press, St. Paul, Minnesota, U.S.A.

MacLaughlan, J. B. (1978) Data on the inheritance of resistance to powdery mildew in the cultivated strawberry. *Scientia Horticulturae* 8: 43 - 49.

Magarey, P. A., J. R. Dixon, J. Sippo, J. Hook and R. McGeachy (2005) Cropwatch on-line: a website for grapegrowers to manage disease and pests more effectively. *Proceedings of the 15th Australasian Plant Pathology Society Conference*, Geelong, Victoria, Australia. 190.

Magarey, P. A., M. F. Wachtel, P. C. Weir and R. C Seem (1991) A computer-based simulator for rational management of grapevine downy mildew (*Plasmopara viticola*). *Plant Protection Quarterly* 6: 29 – 33.

Mahaffee, W. F., C. S. Thomas, W. W. Turechek, C. M. Ocamb, M. E. Nelson, A. Fox and W. D. Gubler (2003) Responding to an introduced pathogen: *Podosphaera macularis* (hop powdery mildew) in the Pacific Northwest. *Plant Health Progress* (Online) doi:10.1094/PHP-2003-1113-07-RV.

Makus, D. J. and J. R. Morris (1998) Preharvest calcium applications have little effect on mineral distribution in ripe strawberry fruit. *HortScience* 33: 64 – 66.

Manning, K. (1991) Isolation of nucleic acids from plants by differential solvent precipitation. *Analytical Biochemistry* 195: 45 - 50.

Manning, K. (1998) Isolation of a set of ripening-related genes from strawberry: their identification and possible relationship to fruit quality traits. *Planta* 205: 622 - 631.

Marschner, H. (1995) *Mineral nutrition of higher plants*. Academic Press, London.

Marshall, M. R., M. G. Smart, J. R. Aist and H. W. Israel (1985) Chlortetracycline and barley papilla formation: localization of calcium and alteration on the response induced by *Erysiphe graminis*. *Canadian Journal of Botany* 63: 876 - 880.

Martinsson, M., A. Kwast, G. Cieslinski and W. Treder (2004) Impact of production systems and fertilizer application on yield and quality of strawberries. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 59 - 64.

Mattner, S. W., R. K. Grounder, R. C. Mann, I. J. Porter, J. N. Matthiessen, Y. L. Ren and M. Sarwar (2004) Ethanedinitrile (C_2N_2) - A novel soil fumigant for strawberry production. *Proceedings of the Fifth International Strawberry*

Symposium, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 197 - 203.

May, G. and M. Pritts (1990) Strawberry Nutrition. *Advances in Strawberry Production* 9: 10 - 23.

May, G. M., M. P. Pritts, M. J. Kelly (1994) Seasonal patterns of growth and tissue nutrient content in strawberries. *Journal of Plant Nutrition* 17: 1149 - 1162.

May, G. M., M. P. Pritts and M. J. Kelly (1993) Phosphorus, zinc, and boron influence yield components in 'Earliglow' strawberry. *Journal of American Society of Horticultural Science* 118: 43 - 49.

McNicol, R. J. and H. J. Gooding (1979) Assessment of strawberry clones and seedlings for resistance to *Sphaerotheca macularis* (Wall ex. Frier). *Horticultural Research* 19: 35 - 41.

Medina, J. J., L. Miranda, F. Romero, B. D. L. Santos, F. Montes, J. M. Vega, J. I. Páez, J. Bascón, C. Soria and J. M. López-Aranda (2004) Seven years work on alternatives to methyl bromide (MB) for strawberry production in Huelva (Spain). *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 205 - 209.

Medina-Escobar, N., J. Cárdenas, V. Valpuesta, J. Muñoz-Blanco and J. L. Caballero (1997) Cloning and characterisation of cDNAs from genes differentially expressed during the strawberry fruit ripening process by a MAST-PCR-SBDS method. *Analytical Biochemistry* 248: 288 - 296.

Mengel, K. (2007) Potassium. In, *Handbook of plant nutrition* (eds A. V. Barker, D. J. Pilbeam). CRC Press, Taylor and Francis Group, Boca Raton, Florida, U.S.A. 91 – 120.

Menzel, C. M. and G. K. Waite (2004) The performance of strawberry plugs in Queensland. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 217 - 224.

Menzies, J., P. Bowen, D. Ehret and A. Glass (1992) Foliar applications of potassium silicate reduce severity of powdery mildew on cucumber, muskmelon and zucchini squash. *Journal of American Society of Horticultural Science* 117: 902 - 905.

Menzies, J. G., D. L. Ehret, A. D. M. Glass and A. L. Samuels (1991) The influence of silicon on cytological interactions between *Sphaerotheca fuliginea* and *Cucumis sativus*. *Physiological and Molecular Plant Pathology* 39: 403 - 414.

Miller, P. W. and G. F. Waldo (1957) Relative resistance of some strawberry varieties and selections to powdery mildew at Cornvallis, Oregon. *Plant Disease Reporter* 41: 23 - 24.

Miller, T. C., W. D. Gubler, S. Geng and D. M. Rizzo (2003) Effects of temperature and water vapor pressure on conidial germination and lesion expansion of *Sphaerotheca macularis* f. sp. *fragariae*. *Plant Disease* 87: 484 - 492.

Miller, T. C., W. D. Gubler, F. F. Laemmlen, S. Geng and D. M. Rizzo (2004) Potential for using *Lecanicillium lecanii* for the suppression of strawberry powdery mildew. *Biocontrol Science and Technology* 14: 215 - 220.

Mims, C. W., K. A. Liljebjelke and E. A. Richardson (1995) Surface morphology, wall structure, and initial adhesion of conidia of the powdery mildew fungus *Uncinuliella australiana*. *Phytopathology* 85: 352 - 358.

Miner, G. S., E. B. Poling, D. E. Carroll and L. A. Nelson (1997) Influence of fall nitrogen and spring nitrogen - Potassium applications on yield and fruit quality

of 'Chandler' strawberry. *Journal of the American Society for Horticultural Science* 122: 290 - 295.

Miyake, Y. and E. Takahashi (1986) Effect of silicon on the growth and fruit production of strawberry plants in a solution culture. *Soil Science and Plant Nutrition* 32: 321 - 326.

Mohan, M., S. Nair, A. Bhagwat, T. G. Krishna, M. Yano, C. R. Bhatia and T. Sasaki (1997) Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding* 3: 87 - 103.

Moisander, J., M. Herrington, D. Hutton and N. Greer (2004) Effect of micro-propagation on the health status of strawberry planting material for commercial production of strawberry runners for Queensland. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 271 - 273.

Mukerji, K. G. (1968) *Sphaerotheca macularis*. *C.M.I. Descriptions of Pathogenic Fungi and Bacteria* 188.

Nelson, M. D., W. D. Gubler and D. V. Shaw (1995) Inheritance of powdery mildew resistance in greenhouse-grown versus field-grown California strawberry progenies. *Phytopathology* 85: 421 - 424.

Nelson, M. D., W. D. Gubler and D. V. Shaw (1996) Relative resistance of 47 strawberry cultivars to powdery mildew in California greenhouse and field environments. *Plant Disease* 80: 326 - 328.

Nicholson, R. L., H. Kunoh, T. Shiraishi and T. Yamada (1993) Initiation of the infection process by *Erysiphe graminis*: Conversion of the conidial surface from hydrophobicity to hydrophilicity and influence of the conidial exudate on the

hydrophobicity of the barley leaf surface. *Physiological and Molecular Plant Pathology* 43: 307 - 318.

Nicot, P. C., M. Bardin and A. J. Dik (2002) Basic methods for epidemiological studies of powdery mildews: Culture and preservation of isolates, production and delivery of inoculum and disease assessment. In, *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 83 - 99.

Nielsen, K. A., R. L. Nicholson, T. L. W. Carver, H. Kunoh and R. P. Oliver (2000) First touch: An immediate response to surface recognition in conidia of *Blumeria graminis*. *Physiological and Molecular Plant Pathology* 56: 63 - 70.

Northcote, K. H. (1983) Australian soil landscape regions: Central southern region. In, *Soils: an Australian viewpoint*. C.S.I.R.O. Division of Soils. Academic Press, London, U.K. 211 - 217.

Ohyanagi, H., T. Tanaka, H. Sakai, Y. Shigemoto, K. Yamaguchi, T. Habara, Y. Fujii, B. A. Antonio, Y. Nagamura, T. Imanishi, K. Ikeo, T. Itoh, T. Gojobori and T. Sasaki (2006) The rice annotation project database (RAP-DB): Hub for *Oryza sativa* spp. *japonica* genome information. *Nucleic Acids Research* (Online) 34: D741 – D744.

Orchard, W. R. and M. C. J. Adrichem (1957) Relative resistance of some strawberry species, varieties and selections to powdery mildew at Saanichton, British Columbia. *Plant Disease Reporter* 41: 945 -947.

Osbourn, A. E. (1996) Preformed antimicrobial compounds and plant defense against fungal attack. *The Plant Cell* 8: 1821 - 1831.

Ostatek-Boczynski, Z. A. and M. B. Haysom (2003) The determination of Si in sugarcane tissue using a microwave-assisted acid and alkali digestion procedure. *Tools for nutrient and pollutant management: Applications to agriculture and environmental quality* (eds L. D. Currie and J. A. Hanly). Occasional Report No. 17. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand.

Park, C. J., C. B. Park, S. Hong, H. Lee, S. Y. Lee and S. C. Kim (2000) Characterisation and cDNA cloning of two glycine- and histine-rich antimicrobial peptides from the roots of shepherd's purse, *Capsella bursa-pastoris*. *Plant Molecular Biology* 44: 187 - 197.

Pascholati, S. F., H. Yoshioka, H. Kunoh and R. L. Nicholson (1992) Preparation on the infection court by *Erysiphe graminis* f.sp. *hordei*: cutinase is a component of the conidial exudate. *Physiological and Molecular Plant Pathology* 41: 53 - 59.

Peñalosa, J. M., C. Cadahia, M. J. Sarro and A. Masaguer (1994) Improvement of strawberry nutrition in sandy soils by addition of manure, calcium and magnesium. *Journal of Plant Nutrition* 17: 147 - 153.

Pennypacker, B. W., D. P. Knievel, M. L. Risius and K. T. Leath (1994) Photosynthetic photon flux density x. pathogen interaction in growth of alfalfa infected with *Verticillium albo-atrum*. *Phytopathology* 84: 1350 - 1358.

Perera, R. G. and B. E. J. Wheeler (1975) Effect of water droplets on the development of *Sphaerotheca pannosa* on rose leaves. *Transactions of the British Mycological Society* 64: 313 - 319.

Peries, O. S. (1962a) Studies on strawberry mildew, caused by *Sphaerotheca macularis* (Wallr. Ex Fries) Jaczewski. I. Biology of the fungus. *Annals of Applied Biology* 50: 211 - 224.

Peries, O. S. (1962b) Studies on strawberry mildew, caused by *Sphaerotheca macularis* (Wallr. Ex Fries) Jaczewski. II. Host-parasite relationships on the foliage of strawberry varieties. *Annals of Applied Biology* 50: 225 - 233.

Perkins-Veazie, P. (1995) Growth and ripening of strawberry fruit. *Horticultural Reviews* 17: 267 - 297.

Pilbeam, D. J. and P. S. Morley (2007) Calcium. In, *Handbook of plant nutrition* (eds A. V. Barker and D. J. Pilbeam). CRC Press, Taylor and Francis Group, Boca Raton, Florida, U.S.A. 121 – 144.

Porebski, S., G. Bailey and B. Baum (1997) Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter* 15: 8 - 15.

Porter, I. J., S. W. Mattner, J. Banks and P. Fraser (2004) Impact of global methyl-bromide phase-out on the sustainability of strawberry industries. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 179 - 185.

Potter, D., J. J. Luby and R. E. Harrison (2000) Phylogenetic relationships among species of *Fragaria* (Rosaceae) inferred from non-coding nuclear and chloroplast DNA sequences. *Systematic Botany* 25: 337 - 348.

Prats, E., T. L. W. Carver, M. F. Lyngkjær, P. C. Roberts and R. J. Zeyen (2006) Induced inaccessibility and accessibility in the oat powdery mildew system: insights gained from used of metabolic inhibitors and silicon nutrition. *Molecular Plant Pathology* 7: 47 - 59.

Prokop, M. and K. Veverka (2006) Influence of droplet spectra on the efficiency of contact fungicides and mixtures of contact and systemic fungicides. *Plant Protection Science* 42: 26 - 33.

Pryce-Jones, E., T. Carver and S. J. Gurr (1999) The roles of cellulase enzymes and mechanical force in host penetration by *Erysiphe granimis* f.sp. *hordei*. *Physiological and Molecular Plant Pathology* 55: 175 - 182.

Reuter, D. J. and J. B. Robinson (1997) *Plant Analysis: An Interpretation Manual*, CSIRO Publishing, Australia.

Reynolds, A. G., L. J. Veto, P. L. Scholberg, D. A. Wardle and P. Haag (1996) Use of potassium silicate for the control of powdery mildew [*Uncinula necator* (Scwein) Burrill] in *Vitis vinifera* L. cultivar Bacchus. *American Journal on Enology and Viticulture* 47: 421 - 428.

Ringli, C., B. Keller and U. Ryser (2001) Glycine-rich proteins as structural components of plant cell walls. *Cellular and Molecular Life Sciences* 58: 1430 - 1441.

Robinson, A. J., C. G. Love, J. Batley, G. Barker and D. Edwards (2004) Simple sequence repeat marker loci discovery using SSR primer. *Bioinformatics Applications Note* 20: 1475 - 1476.

Röpenack, E. v., A. Parr and P. Schulze-Lefert (1998) Structural analyses and dynamics of soluble and cell wall-bound phenolics in a broad spectrum resistance to the powdery mildew fungus in barley. *Journal of Biological Chemistry* 273: 9013 - 9022.

Rosenblum, B. B., L. G. Lee, S. L. Spurgeon, S. H. Khan, S. M. Menchen, C. R. Heiner and S. M. Chen (1997) New due-labelled terminators for improved DNA sequencing patterns. *Nucleic Acids Research* 25: 4500 - 4504.

Russell, G. E. (1975) Deposition of *Erysiphe graminis* f. sp. *hordei* conidia on barley varieties of differing growth habit. *Phytopathologische Zeitschrift* 84: 316 – 321.

Salmeron, J., B. Vernooij, K. Lawton, C. Kramer, C. Frye, M. Oostendorp, G. Knauf-Beiter and T. Staub (2002) Powdery mildew control through transgenic expression of antifungal proteins, resistance genes and systemic acquired resistance. *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 268 – 288.

Samuels, A. L., A. D. M. Glass, D. L. Ehret and J. A. Menzies (1991) Distribution of silicon in cucumber leaves during infection by powdery mildew fungus (*Sphaerotheca fuliginea*). *Canadian Journal of Botany* 69: 140 - 146.

Sargent, D., T. Davis, K. Tobutt, M. Wilkinson, N. Battey and D. Simpson (2004a) A genetic linkage map of microsatellite, gene-specific and morphological markers in diploid *Fragaria*. *Theoretical Applied Genetics* 109: 1385 – 1391.

Sargent, D. J., N. H. Battey, M. J. Wilkinson and D. W. Simpson (2004b) The detection of QTL associated with vegetative and reproductive traits in diploid *Fragaria*: A preliminary study. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 471 - 474.

Sargent, D. J., J. Clarke, D. W. Simpson, K. R. Tobutt, P. Arús, A. Monfort, S. Vilanova, B. Denoyes-Rathan, M. Rousseau, K. M. Folta, N. V. Bassil and N. H. Battey (2006) An enhanced microsatellite map of diploid *Fragaria*. *Theoretical and Applied Genetics* 112: 1349 - 1359.

Sarooshi, R. A. and G. C. Cresswell (1994) Effects of hydroponic solution composition, electrical conductivity and plant spacing on yield and quality of strawberries. *Australian Journal of Experimental Agriculture* 34: 529 - 535.

Saure, M. C. (2005) Calcium translocation to fleshy fruit: its mechanism and endogenous control. *Scientia Horticulturae* 105: 65 - 89.

Saxena, G. K. and S. J. Locascio (1968) Fruit quality of fresh strawberries as influenced by nitrogen and potassium nutrition. *Proceedings of the American Society of Horticultural Science* 92: 354 - 362.

Schäffer, A. A., L. Aravind, T. L. Madden, S. Shavirin, J. L. Spouge, Y. I. Wolf, E. V. Koonin and S. F. Altschul (2001) Improving the accuracy of PSI-BLAST protein database searches with composition-based statistics and other refinements. *Nucleic Acids Research* 29: 2994 - 3005.

Schestibratov, K. A. and S. V. Dolgov (2004) Genetic engineering of strawberry cv. Firework and cv. Selekta for taste improvement and enhanced disease resistance by introduction of thauII gene. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 475 - 481.

Schlegel, T. K. and J. Schoenherr (2004) Mixing calcium chloride with commercial fungicide formulations results in very slow penetration of calcium into apple fruits. *Journal of Plant Nutrition and Soil Science* 167: 357 - 362.

Schnathorst, W. C. (1965) Environmental relationships in the powdery mildews. *Annual Review of Phytopathology* 3: 343 - 366.

Seigner, E., A. Lutz, H. Radic-Miehle, S. Seefelder and F. G. Felsenstein (2004) Breeding for powdery mildew resistance in hop (*Humulus* L.): Strategies at the hop research center, Huell, Germany. *Proceedings of the First International Humulus Symposium*, Corvallis, Oregon, U.S.A. *Acta Horticulturae* 668: 19 - 30.

Serçe, S. and J. F. Hancock (2005) Inheritance of day neutrality in octoploid species of *Fragaria*. *Journal of American Society of Horticultural Science* 130: 580 - 584.

Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling and R. L. Small (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142 – 166.

Shi, D. and Y. Sheng (2005) Effect of various salt-alkaline mixed stress conditions on sunflower seedlings and analysis of their stress factors. *Environmental and Experimental Botany* 54: 8 - 21.

Simpson, D. W. (1987) The inheritance of mildew resistance in everbearing and day-neutral strawberry seedlings. *Journal of Horticultural Science* 62: 329 - 334.

Sivapalan, A. (1993a) Effect of water on germination of powdery mildew conidia. *Mycological Research* 97: 71 - 76.

Sivapalan, A. (1993b) Effects of impacting rain drops on the growth and development of powdery mildew fungi. *Plant Pathology* 42: 256 - 263.

Sjulin, T. M. and J. Robbins (1986) Selection for virus tolerance in strawberry. *Journal of American Society of Horticultural Science* 111: 458 - 464.

Strand, L. L. (1994) *Integrated pest management for strawberries*. The Regents of the University of California, Oakland, California, U.S.A.

Tagliavini, M., E. Baldi, P. Licchi, M. Antonelli, G. Sorrenti, G. Baruzzi and W. Faedi (2005) Dynamics of nutrients uptake by strawberry plants (*Fragaria* x *ananassa* Dutch.) grown in soil and soilless culture. *European Journal of Agronomy* 23: 15 - 25.

Takahashi, K., J. R. Aist and H. W. Israel (1985) Distribution of hydrolytic enzymes at barley powdery mildew encounter sites: implications for resistance associated

with papilla formation in a compatible system. *Physiological Plant Pathology* 27: 167 - 184.

Tanigawa, M., T. Nakano, T. Hagihara, K. Okayama and S. Sezaki (1993) Relationship between the effect of fungicides on powdery mildew (*Sphaerotheca humuli*) and their deposits on strawberry leaves. *Journal of Pesticide Science* 18: 135 – 140.

Treder, W. and G. Cieslinski (2005) Effect of silicon application on cadmium uptake and distribution in strawberry plants grown on contaminated soils. *Journal of Plant Nutrition* 28: 917 - 929.

Ulrich, A., M. Mostafa and W. Allen (1980) *Strawberry deficiency symptoms: A visual and plant analysis guide to fertilization*. The Regents of the University of California, Berkeley, California, U.S.A.

University of California, Strawberry Licensing Program (2001) Aromas strawberry cultivar. http://www.ucop.edu/ott/strawberry/Aromascultivar.htm, accessed: 10/04/2003.

Verhaar, M. A., T. Hijwegen and J. C. Zadoks (1996) Glasshouse experiments on biocontrol of cucumber powdery mildew (*Sphaerotheca fulignea*) by the mycoparasites *Verticillium lecanii* and *Sporothrix rugulosa*. *Biological Control* 6: 353 - 360.

Veronese, P., M. T. Ruiz, M. A. Coca, A. Hernandez-Lopez, H. Lee, J. I. Ibeas, B. Damsz, J. M. Pardo, P. M. Hasegawa, R. A. Bressan and M. L. Narasimhan (2003) In defense against pathogens both plant sentinels and foot soldiers need to know the enemy. *Plant Physiology* 131: 1580 - 1590.

Viruel, A., D. Sánchez and P. Arús (2002) An SSR and RFLP linkage map for the octoploid strawberry (*Fragaria* x *ananassa*). *10th International Plant and*

Animal Genome Conference, San Diego, California, U.S.A. http://www.intl-pag.org/pag/10/abstracts/PAGX_P660.html, accessed: 10/04/2003

Vogel, J. and S. Somerville (2002) Powdery Mildew of *Arabidopsis*: A model system for host-parasite interactions. In, *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 161 – 168.

Vogel, J. P., T. K. Raab, C. Schiff and S. C. Somerville (2002). PMR6, a pectate lyase-like gene required for powdery mildew susceptibility in *Arabidopsis*. *The Plant Cell* 14: 2095 - 2106.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. v. d. Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407 - 4414.

Voth, V., K. Urin and R. S. Bringhurst (1967) Effect of high nitrogen applications on yield, earliness, fruit quality and leaf composition on California strawberries. *Proceedings of the American Society of Horticultural Science* 91: 249 - 256.

Wang, S. Y. (2003) Dr. Shiow Y. Wang - Plant Physiologist. Recent accomplishments. http://www.barc.usda.gov/psi/fl/wang.html, Accessed: 14/07/2003.

Wang, S. Y. and G. J. Galletta (1998) Foliar application of potassium silicate induces metabolic changes in strawberry plants. *Journal of Plant Nutrition* 21: 157 - 167.

Wang, S. Y. and S. Lin (2002) Compost as soil supplement enhanced plant growth and fruit quality of strawberry. *Journal of Plant Nutrition* 25: 2243 - 2259.

Wang, S. Y. and D. D. S. Tzeng (1998) Methionine-riboflavin mixtures with surfactants and metal ions reduce powdery mildew infection in strawberry plants. *Journal of American Society of Horticultural Science* 123: 987 - 991.

Weiguang, Y. S., E. Law and H. Y Wetzstein (2003) Fungicide sprays can injure the stigmatic surface during receptivity in almond flowers. *Annals of Botany* 91: 335 – 341.

Weir, R. G. and G. C. Cresswell (1993). *Temperate and Subtropical Fruit and Nut Crops*. Reed International Books Australia Pty Ltd, Melbourne, Australia.

Werner, D. and R. Roth (1983). Silica metabolism. In, *Encyclopaedia of Plant Physiology* (eds A. Läuchli and R. L. Bieleski). Springer-Verlag, Berlin and New York. 15B: 682 - 694.

Wilhelm, S. (1961). *Diseases of strawberry; A guide for the commercial grower*. University of California, Division of Agricultural Sciences, Berkeley, California, U.S.A.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531 - 6535.

Williams, M. N. V., N. Pande, S. Nair, M. M and J. Bennett (1991) Restriction fragment length polymorphism analysis of polymerase chain reaction products amplified from mapped loci of rice (*Oryza sativa* L.) genomic DNA. *Theoretical and Applied Genetics* 82: 489 - 498.

Wójcik, P. and M. Lewandowski (2003) Effect of calcium and boron sprays on yield and quality of "Elsanta" strawberry. *Journal of Plant Nutrition* 26: 671 - 682.

Yamazaki, H. and T. Hoshina (1995) Calcium nutrition affects resistance of tomato seedlings to bacterial wilt. *HortScience* 30: 91 - 93.

Yamazaki, H., O. Ishizuka and T. Hoshina (1996) Relationship between resistance to bacterial wilt and nutrient uptake in tomato seedlings. *Soil Science and Plant Nutrition* 42: 203 - 208.

Yamazaki, H., S. Kikuchi, T. Hoshina and T. Kimura (2000) Calcium uptake and resistance to bacterial wilt of mutually grafted tomato seedlings. *Soil Science and Plant Nutrition* 46: 529 - 534.

Yang, H., Y. Matsubayashi, K. Nakamura and Y. Sakagami (2001) Diversity of *Arabidopsis* genes encoding precursors for phytosulfokine, a peptide growth factor. *Plant Physiology* 127: 842 – 851.

Zeyen, R. J., T. L. W. Carver and M. F. Lyngkjaer (2002) Epidermal cell papillae. In, *The powdery mildews: A comprehensive treatise* (eds R. R. Bélanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver). American Phytopathological Society Press, St. Paul, Minnesota, U.S.A. 107 - 125.

Zhang, Z., C. Henderson and S. J. Gurr (2004) *Blumeria graminis* secretes an extracellular catalase during infection of barley: potential role in suppression of host defence. *Molecular Plant Pathology* 5: 537 - 547.

Appendix 1

Palmer, S., A. J. Able, E. S. Scott and J. Stangoulis (2004) The effect of foliar-applied Ca and Si on the severity of powdery mildew in two strawberry cultivars. *Proceedings of the Fifth International Strawberry Symposium*, Coolum Beach, Queensland, Australia. *Acta Horticulturae* 708: 135 - 139.

Palmer, S., A. J. Able, E. S. Scott and J. Stangoulis (2005) Powdery mildew on strawberries. *Proceedings of the 15th Australasian Plant Pathology Society Conference*, Geelong, Victoria, Australia. 346.

Appendix 2

Analyses of RO and tap water from glasshouse.

Element	Concentration	RO water	Tap water
Fe	µg/l	<0.1	22.10
Mn	µg/l	0.79	1.33
B	µg/l	9.29	41.36
Cu	µg/l	6.01	69.18
Mo	µg/l	< 0.1	< 0.1
Co	µg/l	< 1.0	< 1.0
Ni	µg/l	<1.0	1.59
Zn	µg/l	2.19	30.40
Ca	µg/l	2.28	13076.19
Mg	µg/l	<1.0	9500.80
Na	µg/l	119.63	51556.78
K	µg/l	<1.0	3969.50
P	µg/l	<1.0	7.61
S	µg/l	0.94	106559.63
Al	µg/l	0.57	29.98
Cd	µg/l	< 0.1	< 0.1

Appendix 3

7.6 Introduction

At the commencement of the nutrition studies of strawberry cultivars, Selva and Aromas, an experimental system recommended by local plant nutrition researchers was employed. The literature of strawberry nutrition did not suggest that such a system would be unsuitable for strawberry culture, however, systems had to be adapted to provide suitable experimental conditions for calcium and potassium deficiency in these cultivars to develop. Outlined below are the unsuccessful systems trailed as much knowledge was acquired in their failure.

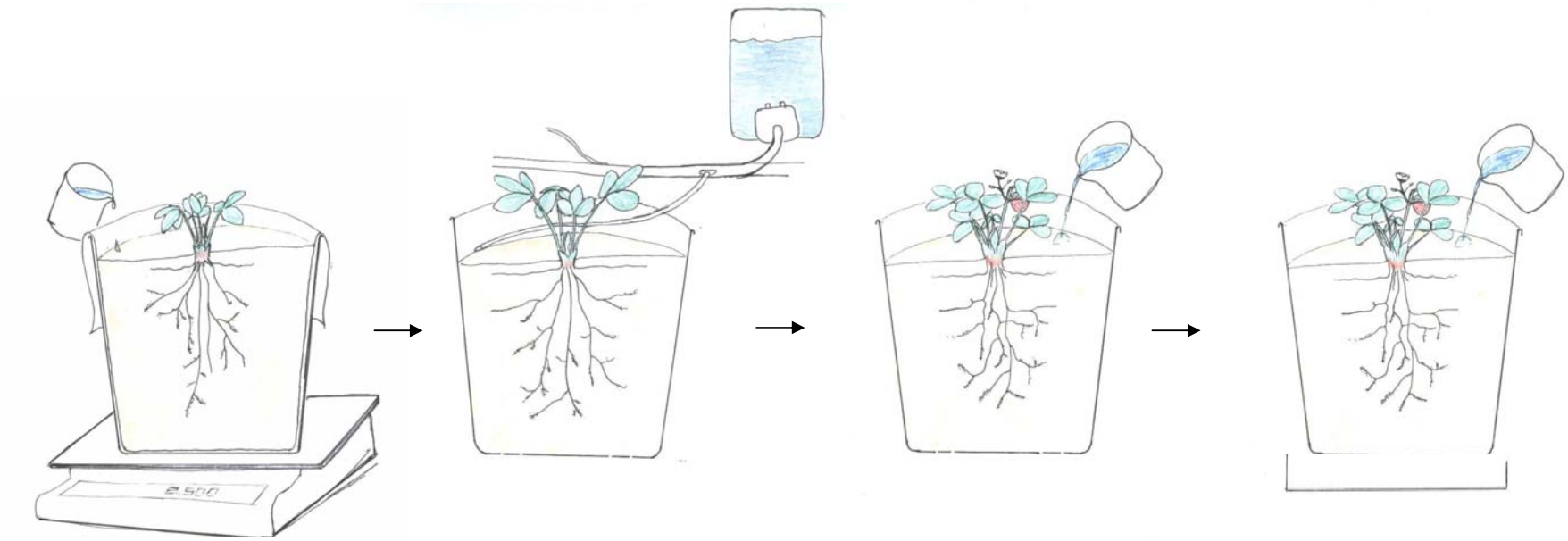
7.7 Materials and Methods

In order to determine a suitable experimental system for critical nutrient studies of strawberry plants a series of experimental systems were trialed (Figure A3.1). These included a non-draining system (planted in May 2003), automatic fertigation system (December 2003), and controlled volume fertigation system (first planted in March 2005, then in May and November 2005, for methodology and results, see Chapter 4).

7.7.1 Use of a non-draining system for critical nutrient studies

Initial experiments were set up using a non-draining system. This design assumed that negligible amounts of all nutrients were available in the potting medium and that pots were contained and thus all of the nutrients, which were mixed through the sand prior to planting, were available to the plant for growth. This experiment was kept in a growth chamber with controlled day length and temperature.

For the non-draining experiment, Mount Compass sand (pH 5.1) was prepared by drying and sifting out the organic matter before being weighed into 5 kg lots in



Non-draining system, manually watered by weight

Free draining system, automatically fertigated

Free-draining system, manually fertigated by volume

Free-draining with catchment, manually fertigated by volume

Figure A3.1 Flow diagram of the nutrition experimental systems (in consecutive order) used in studies of potassium and calcium nutrition of the strawberry cultivars, Selva and Aromas.

plastic bags. Nutrient solutions were added to the sand at the rates required (Table A3.1) and allowed to dry at 30 °C overnight. Before planting, the weight of each bag was measured and 20 mL of RO water was added to reduce transplanting stress. Runners were prepared by removing the roots up to 10 cm from the crown and soaking the remaining roots in water for a few minutes. Once planted, water was added up to 10 % of field capacity (the starting weight + 42.5 g of water). Pots were watered to this weight twice per week.

The treatments for potassium were 26, 40, 60, 80 and 160 mg/L potassium applied as 0.14 M K_2SO_4 solution. Other nutrients were applied as outlined in Table 4.1 A. The pH was raised to 6.25 using 1000 mg of $CaCO_3$ (applied dry) per kg of sand. A completely randomised block design with four replicate pots of each cultivar in each treatment was used in this experiment.

The treatments for calcium were 100, 200, 300, 400 and 800 mg/L calcium, applied as $CaSO_4$ (dry). Other nutrients were applied as outlined in Table 4.1 B. The pH was raised to 6.25 using 80 mg of KOH (in solution) per kg of sand. A completely randomised block design with four replicate pots of each cultivar in each treatment was used in this experiment.

7.7.2 Use of an automatic fertigation system for critical nutrient studies

The second experimental system used was designed to be free-draining to avoid the problems encountered with the non-draining system and to imitate field conditions, where plants are watered and fed soluble fertiliser via irrigation lines (commonly known as fertigation). The experiment using this system was planted on the 05/12/2003 and terminated on the 30/04/2004.

Table A3.1 The nutrient solutions added to sand for non-draining experiments to examine the effect of **A.** potassium and **B.** calcium on yields of strawberry cultivars, Selva and Aromas. *Added one month after planting (see Results).

A. Potassium experiment treatments

Compound	Stock	Solution applied	Rate of application
	g/L	mL/kg soil	final content, mg/kg soil
$CaCO_3$			1000
$Ca(NO_3)_2$	143	5	715
KH_2PO_4	75	1.2	90
$MgSO_4$	18	5	90
H_3BO_3	0.05	2	0.1
$CoSO_4$	0.5	2	1
$CuSO_4$	2.5	2	5
$FeSO_4$	0.7	2	1.4
MoO_3	0.005	1	0.005
$MnSO_4$	1.5	2	3
$NiSO_4$	0.075	2	0.15
$ZnSO_4$*			1

B. Calcium experiment treatments

Compound	Stock	Solution applied	Rate of application
	g/L	mL/kg soil	final content, mg/kg soil
$Ca(NO_3)_2$	143	2.9	409.4
KH_2PO_4	75	1.2	90
KOH	40	2	80
KNO_3	75	5	375
$MgSO_4$	18	5	90
H_3BO_3	0.05	2	0.1
$CoSO_4$	0.5	2	1
$CuSO_4$	2.5	2	5
$FeSO_4$	0.7	2	1.4
MoO_3	0.005	1	0.005
$MnSO_4$	1.5	2	3
$NiSO_4$	0.075	2	0.15
$ZnSO_4$*			1

Strawberry runners were planted in Golden Grove sand in free-draining pots (see Figure A3.1). Golden Grove sand was used instead of Mount Compass sand because it only contained limited organic matter and thus, did not require sieving prior to use. There were eight pots per treatment (four of each cultivar). These pots were watered via irrigation lines from a 20 L tank raised above the level of the pots. The gravity-fed controllers were set to water once per day for 2 min, delivering approximately 2.375 L to each pot over a fortnight. Treatments were based on quarter strength (0.25) Hoagland's solution. All treatment solutions were made with the standard volumes of $MgSO_4$, $FeSO_4$, and micronutrient solution (Mn, Cl, Zn, Cu, Mo, Co, and Ni) found in 0.25 Hoagland's solution (Hoagland and Arnon, 1938).

The potassium treatments were 40, 60, 80, 100 and 150 % of the concentration of potassium (Table A3.2 A) in 0.25 Hoagland's solution (93.8, 140.8, 187.7, 234.6 and 351.9 mg/L potassium, respectively).

The calcium treatments were 40, 60, 80, 100 and 150 % of the concentration of calcium (Table A3.2 B) in 0.25 Hoagland's solution (96.2, 144.3, 192.4, 240.5 and 360.7 mg/L calcium, respectively).

7.8 Results

7.8.1 Use of non-draining system for critical nutrient studies

Plants grew relatively fast in the controlled environmental conditions, though after approximately a month started to present abnormal foliar symptoms. These included marginal leaf necrosis, interveinal chlorosis in the young leaves, and reddening of the petioles as shown in Figure 4.2. These symptoms were consistent over both potassium and calcium experiments and all treatment concentrations. Roots of the surviving plants were examined and compared with those of healthy plants and the former were smaller and brown (Figure A3.2 D).

Table A3.2 The composition of solutions used in the automatically fertigated experiments. The effect of **A**. potassium and **B**. calcium treatment on strawberry cultivars, Selva and Aromas was examinted. Solutions used were 1 M (unless specified) and volumes were in mL added to make up 20 L of 0.25 Hoagland's solution.

A

	40 %	60 %	80 %	100 %	150 %
KH_2PO_4	5	5	5	5	5
$Ca(NO_3)_2$	25	25	25	25	25
KNO_3	9.25	9.25	9.25	9.25	9.25
NH_4NO_3	7.9	7.9	7.9	7.9	7.9
K_2SO_4 (0.6M)	0	5	10	15	27.5

B

	40 %	60 %	80 %	100 %	150 %
KH_2PO_4	5	5	5	5	5
$Ca(NO_3)_2$	12	12	12	12	12
KNO_3	25	25	25	25	25
NH_4NO_3	13	13	13	13	13
$CaSO_4$ (0.015M)	0	389	778	1167	2139

Figure A3.2 Symptoms observed in the non-draining system. **A.** Marginal leaf necrosis, **B.** Chlorosis in young leaves, **C.** Reddening of the petioles and **D.** Discolouration of root system (normal on left, abnormal on right). Symptoms shown were representative of those observed over all treatments.

Samples of symptomatic tissue, non-symptomatic tissue from affected plants and from healthy plants grown in the glasshouse were analysed in order to determine the cause of the symptoms. The nutrient analysis (Table A3.3) indicated that affected plants appeared to be lower in zinc than healthy tissue. This was consistent with the symptoms of interveinal chlorosis in the younger leaves. Closer examination of the base nutrient recipe revealed that zinc was inadvertently omitted from the base fertiliser mix. Five mg of zinc was added to the sand in the hope that these plants would recover. However, most of these plants died in the weeks following. Even so, nutrient analysis demonstrated that calcium treatment did result in increased concentration of foliar calcium compared with that of plants the same cultivar and age grown in UC soil in glasshouse conditions (Table A3.3).

7.8.2 Use of an automatic fertigation system for assessing the nutrition requirements of cultivars Selva and Aromas

7.8.2.1 Use of the automatically fertigated system to assess potassium nutrition

After 5 months of treatment, no plants presented with symptoms of deficiency for potassium. The concentration of potassium in the YOLs decreased when treated with the limited potassium treatments compared with the complete 0.25 Hoagland's solution (Figure 4.3, f prob = 0.085). However, the mean concentrations of potassium recorded ranged from 19.8 to 23.1 mg/g, in excess of the critical concentration of 10.0 mg/g potassium and the concentrations expected to induce potassium deficiency (1.0 to 5.0 mg/g potassium), as determined by Ulrich *et al.* (1980). The results suggested that the foliar potassium of Aromas was lower than Selva when the same treatment was applied (f prob = 0.072) (Figure A3.3). The concentrations of calcium, boron and phosphorus also differed significantly between cultivars (f probs = 0.003, <0.001 and

Table A3.3 ICP-OES analysis of symptomatic and non-symptomatic leaf tissue from plants grown in non-draining experiments (maintained in a growth room), compared with that of plants grown in free-draining pots of UC soil (maintained in the glasshouse). Leaves were sampled randomly over treatments and pooled for analysis. Ranges defined as sufficient by Ulrich *et al.* (1980) are given in italics, * indicates discrepancy between sources of literature.

Plant health	Tissue health	Cultivar	Fe mg/kg	Mn mg/kg	B mg/kg	Cu mg/kg	Zn mg/kg	Ca mg/g	Mg mg/g	Na mg/kg	K mg/g	P mg/g	S mg/kg
			50 – 3000	*30 – 700*	*35 – 200*	*3 – 30*	*20 – 50*	*4.00 – 27.00**	*3.00 – 7.00*	*100 - 4000*	*10.00 – 60.00*	*1.50 – 13.00**	*>1000*
Healthy	Healthy	Aromas	82	270	42	6.4	22	10.00	4.30	290	30.00	10.90	5500
Healthy	Healthy	Selva	84	340	38	5.5	22	82.00	3.30	440	26.00	7.30	4200
Affected	Non-symptomatic	Aromas	67	191	23	3.9	15	23.00	3.20	35	22.00	4.10	2800
Affected	Non-symptomatic	Selva	84	185	21	5.3	17	14.10	2.60	52	23.00	4.00	2700
Affected	Symptomatic	Aromas	71	260	31	5.0	14	32.00	4.20	95	31.00	4.20	4200
Affected	Symptomatic	Selva	114	430	29	4.4	13	26.00	4.00	191	31.00	4.30	4000

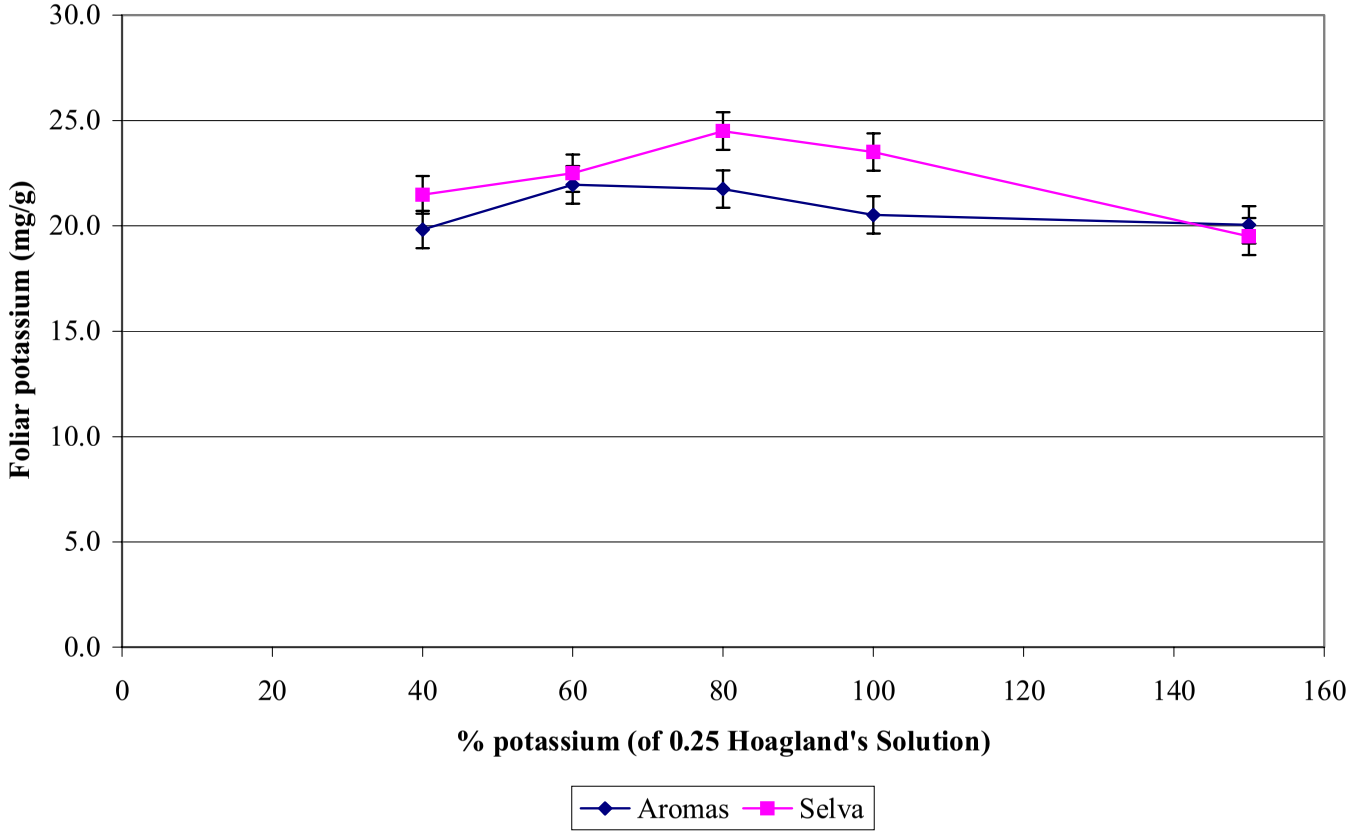


Figure A3.3 The effect of potassium treatment on the foliar concentration of potassium in cultivars, Aromas and Selva. Data taken after 21 weeks supplied with 40 to 150 % of the potassium in 0.25 complete Hoagland's solution via the automatically fertigated system.

0.015, respectively) (Table A3.4). The mean leaf concentration of Aromas was less than Selva for calcium (5.4 and 6.7 mg/g, respectively) and for boron (39.8 and 50.2 mg/kg), though more for phosphorus (6.2 and 5.6 mg/g). A significant increase in mean magnesium concentration was observed with decreasing potassium treatment in both cultivars (Table A3.4 A, f prob = 0.031), but the concentrations of potassium and magnesium were not significantly correlated (R^2 = 0.154). However, there were insufficient replicates (n = 4) to determine statistical significance from the data collected.

7.8.2.2 Use of the automatically fertigated system to assess calcium nutrition

After 5 months of treatment, no plants presented with symptoms of deficiency for calcium. The concentration of calcium in the YOLs decreased with the limited calcium treatments compared with the complete 0.25 Hoagland's solution (Figure A3.4, f prob = 0.095). However, the mean concentration of calcium recorded ranged from 5.0 to 6.2 mg/g, which was above the critical concentration of 3.0 mg/g and the range expected to induce calcium deficiency symptoms (0.8 to 0.2 mg/g potassium) in strawberry plants as defined by Ulrich *et al*. (1980). No significant effect of cultivar on foliar calcium concentration was observed (Table A3.5, f prob = 0.962). However, potassium concentrations were significantly lower in Aromas than Selva (20.2 and 22.8 mg/g, respectively, f prob = 0.004), as was the mean boron concentrations (42.3 and 52.2 mg/kg, respectively, f prob = 0.002) (Table A3.5). The mean phosphorus concentration was significantly higher in Aromas than Selva (5.3 and 5.0 mg/g, respectively, f prob = 0.017). The mean magnesium concentrations in plants of both cultivars that received 40 to 60 % of the standard calcium in 0.25 Hoagland's solution were significantly reduced, though there was no significant correlation between foliar concentrations of calcium and magnesium (Table A3.5, R^2 = 0. 244).

Table A3.4 ICP-OES analysis of leaves and fruit from the automatically fertigated experiments to examine the effect of potassium on cultivars Selva and Aromas. Samples were taken on the 30/04/2005, after 21 weeks of treatment. Concentrations expected to cause deficiency in strawberry are given in italics for leaves (Ulrich *et al.*, 1980) and receptacle (Chiu and Bould, 1976) tissues. * discrepancy in literature, *?* unknown.

		Foliar					**Fruit**	
Cultivar	Potassium treatment (% 0.25 Hoagland's solution)	B mg/kg	Ca mg/g	Mg mg/g	K mg/g	P mg/g	Ca mg/kg	K mg/g
		<35	*<4.00*	*<3.00*	*<10.00*	*<3.00**	*<600*	*?*
Aromas	150	34	5.28	3.33	20.05	6.28	194	3.31
	100	42	5.30	3.03	20.53	5.63	83	0.99
	80	41	5.45	3.48	21.75	6.33	69	0.94
	60	39	5.23	3.38	21.95	6.60	116	2.33
	40	43	5.80	3.48	19.83	6.33	254	2.53
Selva	150	41	5.55	2.90	19.50	5.10	90	1.50
	100	49	6.15	3.28	23.50	6.13	78	1.20
	80	55	8.05	3.55	24.50	5.43	89	1.42
	60	55	6.18	3.05	22.50	5.88	97	1.50
	40	51	7.60	3.88	21.48	5.53	98	1.39

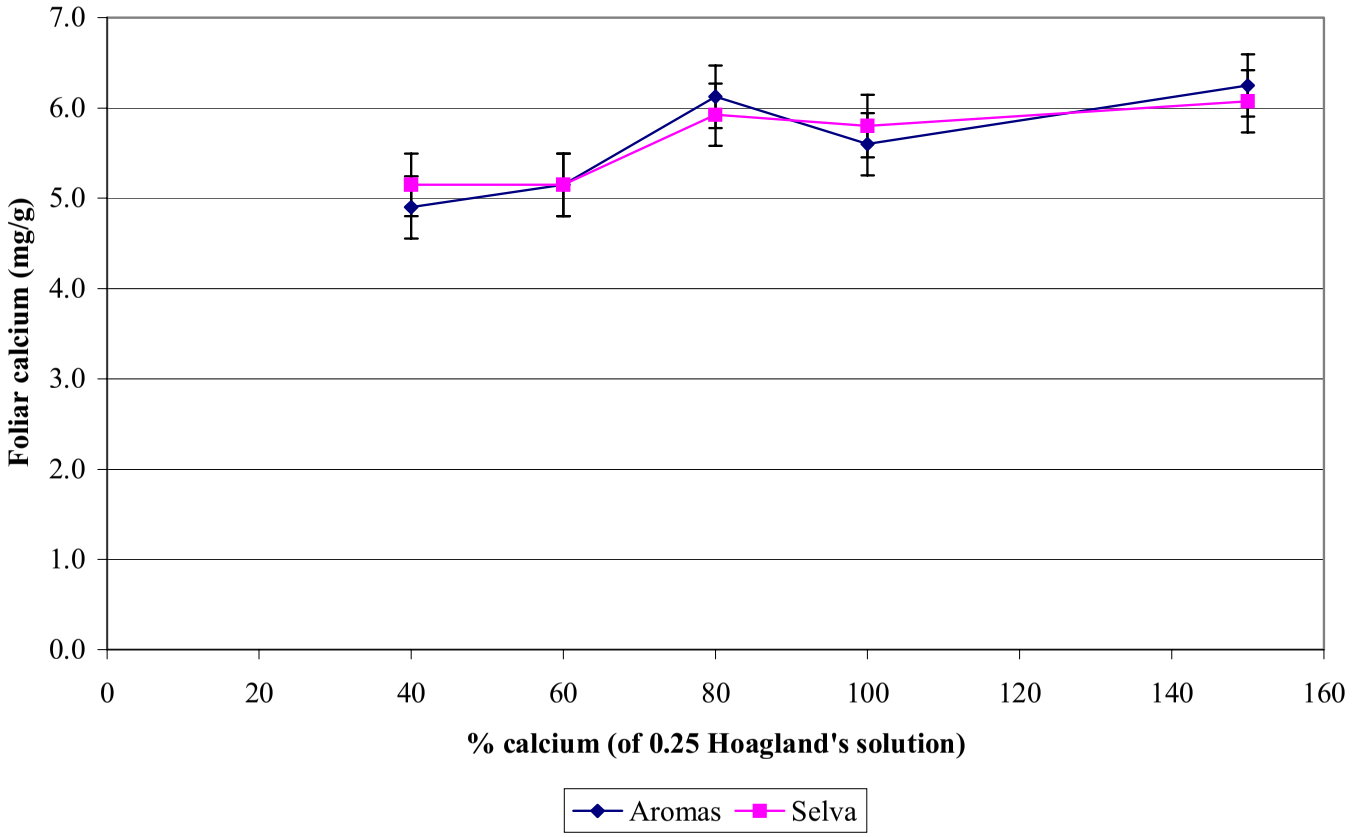


Figure A3.4 The effect of treatment on the foliar concentration of calcium in cultivars, Aromas and Selva. Data taken after 21 weeks supplied with 40 to 150 % of the potassium in 0.25 complete Hoagland's solution via the automatically fertigated system.

Table A3.5 ICP-OES analysis of leaves and fruit from the automatically fertigated experiments to examine the effect of calcium on cultivars Selva and Aromas. Samples were taken on the 30/04/2005, after 21 weeks of treatment. Concentrations expected to cause deficiency in strawberry are given in italics for leaves (Ulrich *et al.*, 1980) and receptacle (Chiu and Bould, 1976) tissues. * discrepancy in literature, *?* unknown.

		Foliar					**Fruit**	
Cultivar	Calcium treatment (% 0.25 Hoagland's solution)	B mg/kg	Ca mg/g	Mg mg/g	K mg/g	P mg/g	Ca mg/kg	K mg/g
		<35	*<4.00*	*<3.00*	*<10.00*	*<3.00**	*<600*	*?*
Aromas	150	44	6.25	3.18	20.15	4.43	76	0.98
	100	40	5.60	3.43	20.78	5.38	93	1.23
	80	43	6.13	3.75	20.00	5.60	97	1.10
	60	42	5.15	3.73	21.13	5.83	82	1.01
	40	42	4.90	4.25	19.13	5.43	99	0.93
Selva	150	58	6.08	2.78	24.25	5.23	97	1.28
	100	49	5.80	2.95	22.75	5.55	78	1.06
	80	50	5.93	3.18	22.75	4.93	111	1.49
	60	52	5.15	2.98	21.68	4.55	171	3.12
	40	52	5.15	3.75	22.75	4.73	123	1.33

7.9 Discussion

Difficulty with the experimental systems employed for these studies resulted in the adaptation of a system that accommodates for the long fruiting season and fertilisation sensitivity of strawberry plants. The experiment using the non-draining system, commonly used for nutrition studies of other plant species (as by Genc and McDonald, 2004), caused most of the strawberry plants to die. However, a lot was learnt about the nutritional requirements of strawberry plants by looking into what caused these deaths. As well as zinc deficiency, caused by an accidental omission from the base nutrient recipe, persistent symptoms of marginal leaf necrosis and petiole colouring were observed using this system. Initially, waterlogging, pH and salt toxicity were considered the likely causes, so alternative systems that would not cause the same problems with strawberry plants were designed. In the non-draining system sand on the surface of the pot regularly appeared very dry (regardless of frequency of watering) and wet at the base of the profile. In the Ca experiments pH where KOH was used to adjust the pH instead of $CaCO_3$, leaching occurred, resulting in a profile that ranged from 7.5 at the top to 4.5 at the bottom of the pot (data not shown). The symptoms of marginal leaf necrosis observed resembled those of salt toxicity (Ulrich *et al.*, 1980), and occasional reoccurrence of these symptoms after application of fertiliser pellets to glasshouse-maintained plants led to the hypothesis that over-fertilisation may have caused these symptoms. Further research revealed descriptions and photos of fertiliser burn in strawberries (Weir and Cresswell, 1993) that matched the observed symptoms exactly. This led to the conclusion that the non-draining system, which contains all the nutrients at the time of planting, was excessive for normal strawberry growth, and that the correct course of action was to find an alternative experimental system for strawberry plants. The free-draining pot with catchment tray would be recommended

for future glass-house based nutrition experiments of strawberry. Further research should include adaptation of the fertiliser recommendation for use in fertigated field production of strawberry crops.

Appropriate base levels for potassium and calcium were not reached in the automatically fertigated system. The lowest treatments used with this system were 40 % of the potassium or calcium in 0.25 Hoagland's solution. This was the level of potassium supplied by the K_2PO_4 in Hoagland's recipe. Analysis of the YOL samples that were taken showed that the 40 % potassium treatment reduced foliar potassium concentrations, but did not affect growth or induce deficiency symptoms over the 6 months that this experiment was run. It was suggested that this experiment should have included a 0 % potassium control. In future experiments, CaPOH was used as a source of phosphate as per Hoagland and Arnon (1938). The 40 % calcium treatment also had no effect on growth or foliar symptoms.