Epidemiology and control of powdery mildew (*Podosphaera aphanis*) on strawberry

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Abstract

Strawberry powdery mildew, caused by *Podosphaera aphanis,* has the potential to cause over 20% yield loss, particularly where strawberries are grown under cover. A holistic approach to the control of strawberry powdery mildew (*P. aphanis*) is important, since the disease is never absent from the crop. The new disease assessment key was developed to assess strawberry powdery mildew (the old one is for assessing red blotches on leaves, See appendix 8). The results (Chapter 3) showed that the disease is present in the crop when new plants are bought in from a propagator， with 14% of strawberry crowns were infected by *P. aphanis* in July 2013 and 4% of the strawberry plants had symptoms of powdery mildew in pre-assessment of plants for the 2013 Si nutrient fertigation field experiment. Control measures used in one growing season reduced the disease carry-over, thus reducing the initial inoculum in the following season. The use of a late autumn fungicide spray and a fungicide spray before the plants were covered by fleece in spring reduced the number and maturity of overwintering chasmothecia, thus contributing to a reduction in initial inoculum.

The use of silicon (Si) nutrient (foliar spray and root treatment) also suppressed strawberry powdery mildew development (Chapter 4). The results of Area Under Disease Progress Curve (AUDPC) and the rate of epidemic growth curve (*r)* in 2012 indicated that the high concentration of Si nutrient foliar spray inhibited (r = 0.002, AUDPC = 44) the epidemic build-up of *P. aphanis* better than the low concentration of Si nutrient (r = 0.012, AUDPC = 51) and untreated (r = 0.018,

AUDPC = 70). The Si nutrient root treatment (AUDPC = 12.8) was better in inhibiting strawberry powdery mildew development than the Si nutrient foliar spray treatment. Moreover, the high concentration of Si nutrient foliar spray resulted in fewer chasmothecia compared to the untreated. Si nutrient foliar spray and root treatments increased the concentration of Si in the plants and produced physiological changes in the plants, including wax formation on the adaxial leaf surface, greater leaf thickness and cuticle layer and increased $Brix^0$ value in plants, which all were associated with reduced disease incidence. The integrated use of all these control strategies suppresses disease development so that control is achieved with less use of conventional fungicides.

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Jin, X. L., Hall, A. M., Fitt, B. D. L. and Huang, Y. J. 2013. The role of chasmothecia in the initiation of epidemics of powdery mildew (*Podosphaera aphanis*) and the role of silicon in controlling the epidemics on strawberry. *Aspects of Applied Biology* 119, *Fruit and Roots: A Celebration and Forward Look*, 151-155. Association of Applied Biologists. 271

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Chapter 1– General introduction

1.1 Introduction

The strawberry plant is perennial and a member of the rose family (Rosaceae) (Maas, 1998), with asexual stolon reproduction (Fig. 1.1). The genus *Fragaria* was first included in pre-Linnaean literature by C. Bauhin (1623) (Hummer et al., 2011). Antoine Nicolas Duchesne (1747-1827) considered that in evolutionary terms *Fragaria vesca*, the wood strawberry, was the species from which all other species evolved (Kingsbury, 2009). Furthermore, Duchesne (1766) indicated that *Fragaria* × *ananassa*, the "pineapple strawberry" is a hybrid between *F. virginiana* and *F. chiloensis* and has been the most commonly cultivated strawberry in Europe since 1850 (Hummer et al., 2011; Maas, 1998).

The crown of the strawberry plant is a short thickened stem (Fig. 1.1) with leaves and axillary buds growing from the stem (Maas, 1998). New leaves and flowers arise from the crown in the early spring (Fig. 1.1a) (Poling, 2001). Runners (stolons) emerge from the crown and develop leaves and roots (daughter plants). The roots develop quickly if they lie on moist soil or water (Maas, 1998).

The leaves that develop from the crown have long petioles (Fig. 1.1). The leaf is an important organ to produce food for the plant through photosynthesis. Metabolic products (sugars) are translocated from leaves to maturing fruit and

other plant parts to support the growth of the whole plant. *Fragaria* × *ananassa* leaves are mostly trifoliate; however, some cultivars have four or five leaflets, which demonstrates a close relationship to *F. chiloensis* (Maas, 1998). Moreover, the leaf thickness differs between different varieties (personal observation).

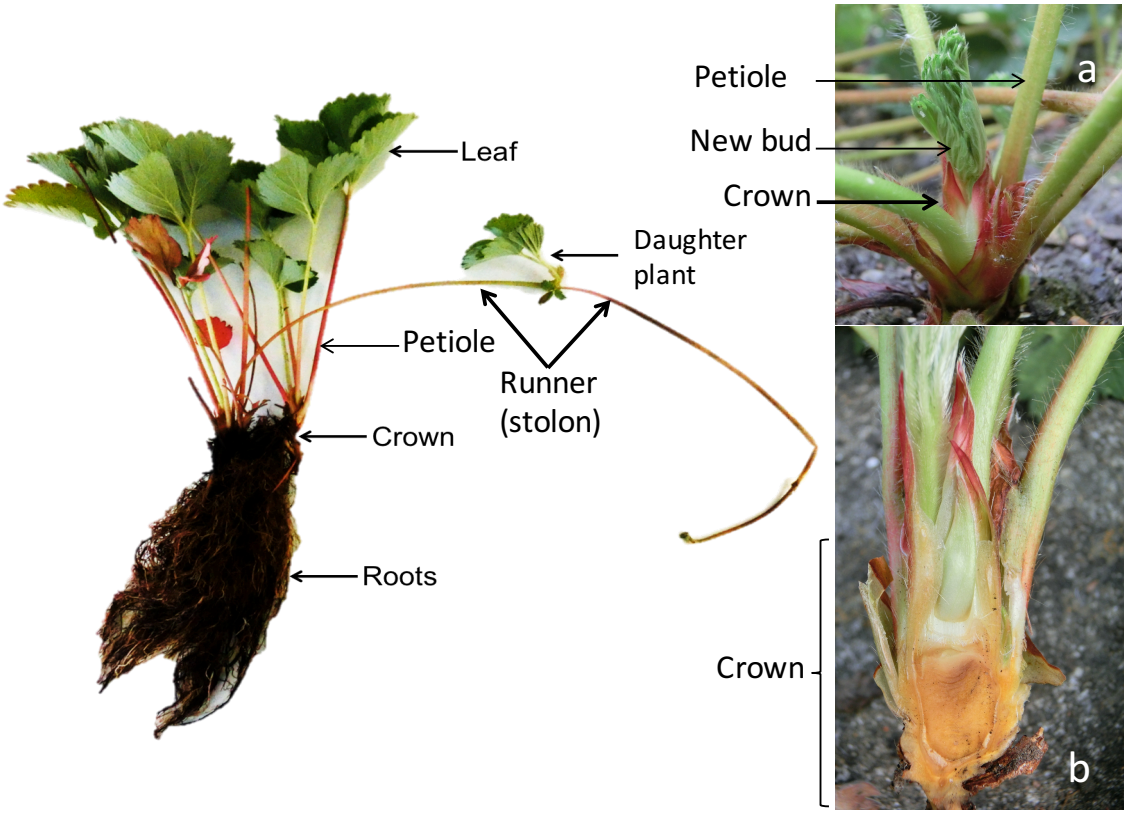


Figure 1.1: The anatomy of a strawberry plant. (a) The growing point of the strawberry (crown), the new stems and branches start to grow from the crown. (b) Lengthwise cross section of crown.

Furthermore, strawberry leaves have a great number of stomata (300-400/mm^2) (Maas, 1998). Stomata are commonly a route for infection by some pathogens that cause leaf diseases such as leaf spot, leaf rust and leaf scorch but not strawberry powdery mildew. The number of leaves is directly linked to strawberry fruit production in the following year. Thus, a reduction in leaf area caused by

diseases or pests has a direct effect on the subsequent yield (Maas, 1998). Strawberry plants flower in spring and autumn; the flower normally has five petals (Fig. 1.2c). The stamens discharge pollen to fertilize the stigma (Fig. 1.3). The strawberry seeds on the fruit surface are actually the achenes of strawberry plants (Fig. 1.4). However, the receptacle becomes swollen and forms the centre of the fruit (Fig. 1.2g & 1.4) (Bowes, 1996). Strawberry plants that form flower buds in autumn are called June bearers (early, mid and late summer), whilst ever-bearers (July-October) form flower buds in March and fruit two or more times per season (Maas, 1998). Moreover, there are many other strawberry plant species grown in home gardens around the world (Strawberryplants, 2010).

Strawberries are now grown worldwide and in many countries they are grown as a perennial crop. Strawberry fruit yield and quality is affected by many factors; important environmental factors include temperature, humidity, sunlight and rainfall; other features are the winter cold hardiness and disease resistance, the soil conditions and humidity. Since 1993, the use of polyethylene tunnels for strawberry production has increased in the UK. The use of polyethylene tunnels reduces some of the losses associated with strawberry production, which extends the strawberry production season and improves fruit quality in the harvest season (Dodgson et al., 2008). However, as a result of polyethylene tunnel use, environmental conditions are now favourable for strawberry powdery mildew (*Podosphaera aphanis*), which is now common all over the world (Dodgson et al., 2008). It has the potential to cause over 20% yield loss because it reduces photosynthesis and increases transpiration, particularly when strawberries are

grown under cover. Normally strawberry growers use fungicides to control its spread; however, this adds an extra cost onto production (Dodgson et al., 2008).

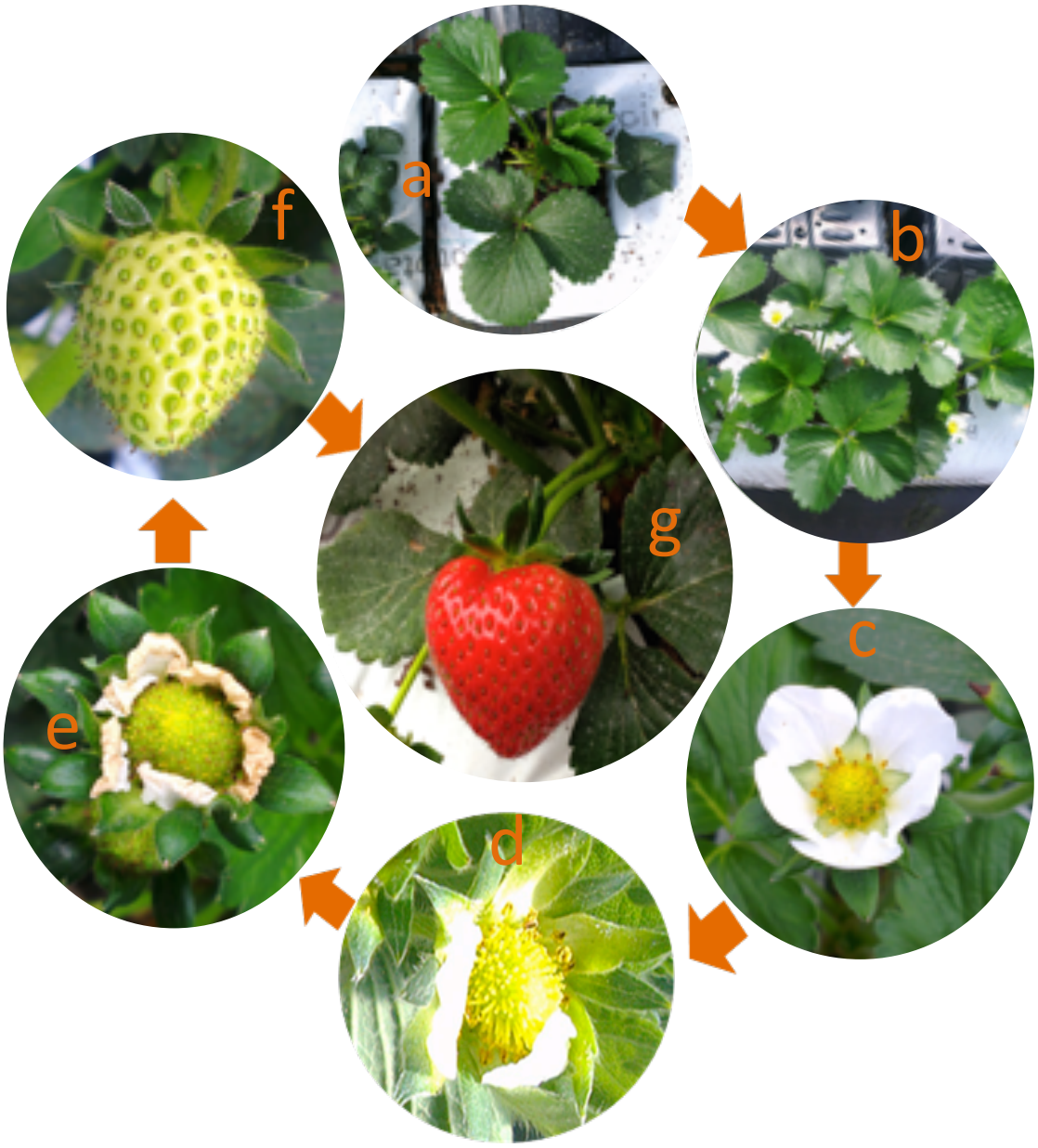


Figure 1.2: The growth of a strawberry plant: (a) young strawberry plant; (b) flowering; (c) (d) strawberry flower; (e) (f) immature fruit; (g) mature fruit.

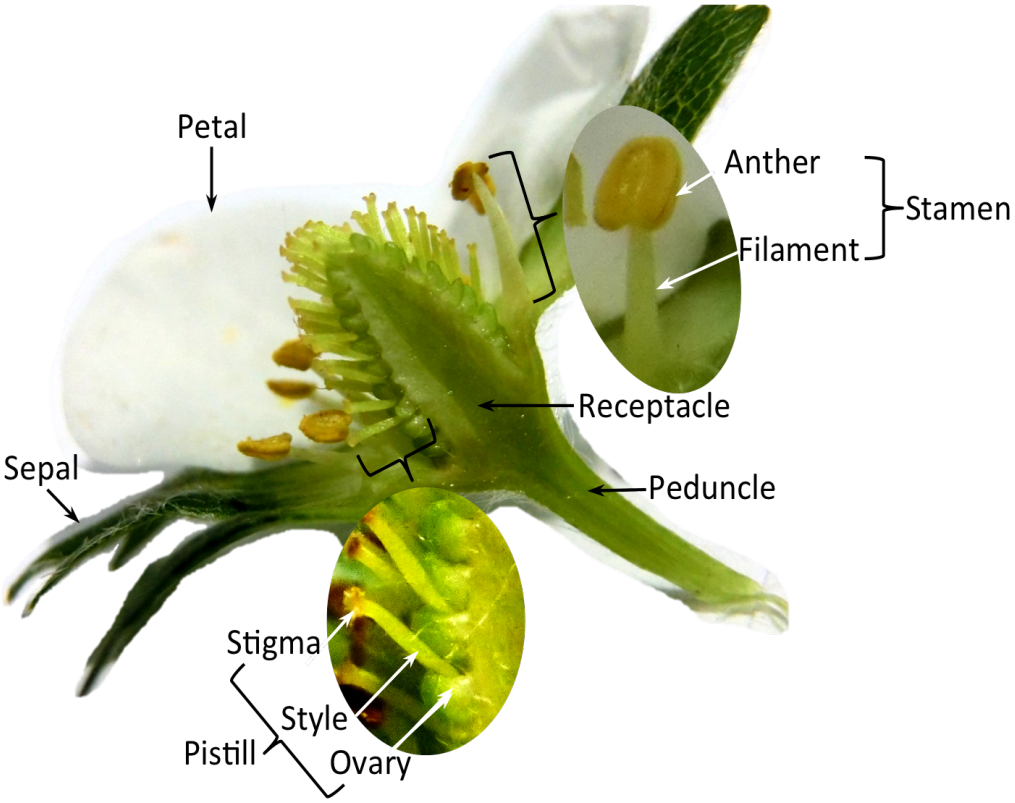


Figure 1.3: A cross section of a strawberry flower.

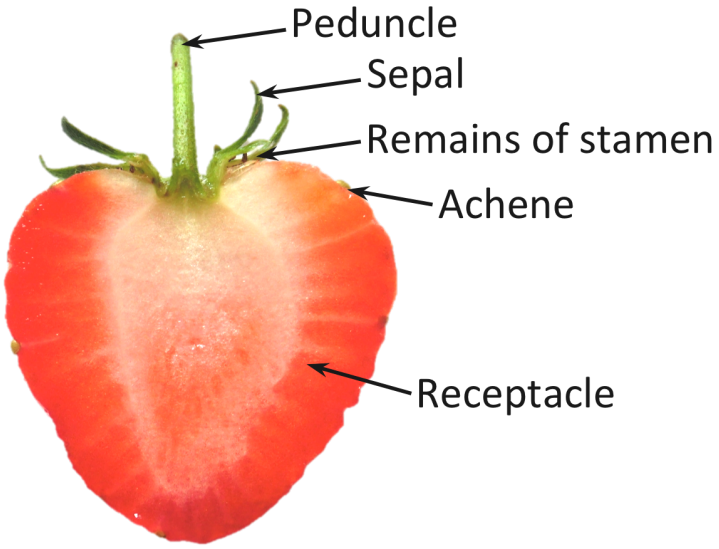


Figure 1.4: A cross-section of a strawberry fruit.

1.2 World strawberry production

Currently, the genus *Fragaria* embraces 20 named wild species, including three natural hybrid species *F. moschata, F. viridis* and *F. chiloensis* and two commercial species cultivated worldwide *F. xvescana* R. Bauer & A. Bauer and *F. ananassa* Duch. ex Lamarck (Hummer et al., 2011). The wild species are distributed across northern Europe and North America, with 24 *Fragaria* species recognized worldwide and 13 species found in China (Table 1.1) (Liang et al., 2011). Chinese and mid-Asian species are still under-studied and require further investigation, as China is the Vavilov centre of diversity for strawberry. The history and evolution of these species has been estimated according to the distribution of particular ploidy levels within central Europe (Hummer et al., 2011).

According to the Food and Agriculture Organization (FAO), currently more than 75 countries produce large numbers of strawberry fruits (Table 1.2). The Americas (North, South and Central) have always been the largest producers (Fig. 1.5). Major strawberry producing countries are USA, Turkey, Spain, Egypt and Mexico in central America (Fig. 1.6). Spain still has a high production but the increase has been slow. The annual production of Spain was 275K tons in 2010, which was 12% less than that 10 years ago (Wu et al., 2012). In contrast, in Turkey, Egypt, and Mexico production grew substantially in the same period. The production of strawberries in Turkey was stable from 2009 to 2013; thus, it became the second largest strawberry producer in the world (Fig. 1.6). However, Spain is still the largest world trader (Binard, 2013).

Table 1.1: *Fragaria* species and their global distribution area.

Species	Ploidy	Distribution
F. bucharica Losinsk	2x	Western Himalayas
F. chinensis Lozinsk.	2x	China
F. daltoninana Gay	2x	China (Tibet)
F. iinumae Makino	-	Japan
F. mandshurica Staud*t*	2x	China
F. nilgerrensis Schlect	2x	China
F. nipponica Makino	-	Japan
F. nubicola Lindl	2x	China (Tibet)
F. pemtaphylla Lozinsk.	2x	China
F. vesca L.	2x	China
F. virdis Duch	2x	China (Xinjiang)
F. bifera Duch	-	France, Germany
F. corymbosa Lozinsk.	4x	China
F. gracilis Lozinsk.	4x	China
*F. moupinensis (*French) Card.	4x	China
F. orientalis Lozinsk.	4x	China
F. tibetica Staudt et Dickor *é*	4x	China
F. xbringhurstii Staudt	5 (9x)	California
F. natural pentaploid	5x	China
F. moschata Duch	6x	Euro-Siberia
F. chiloensis (L.) Miller	8x	Western N. America. Hawaii. Chile
F. virginiana Mille*r*	-	North America
F. ananassa Duch. ex Lamarck	-	Cultivated worldwide
F. xananassa subsp. Cuneifolia	-	North-western N. America
F. iturupensis Staudt	10x	Iturup Island, Kurile Island
F. virginiana subsp. Platypetala Miller	-	Oregon, United States
F. xvescana R. Bauer & A. Bauer	-	Cultivated worldwide

- Not known. Adapted from: Hummer et al. (2011) & Liang et al. (2011)

Table 1.2: World strawberry production 2009-2012.

Areas	Production (tonnes)			
	2009	2010	2011	2012
World	4,596,586.2	4,352,869.1	4,328,129.4	4,516,810.4
Africa	612,731.0	393,616.0	366,754.0	398,967.0
Americas	1,678,995.4	1,699,243.4	1,724,353.4	1,934,361.4
Asia	767,328.5	798,374.65	746,434.0	829,223.0
Europe	1,503,385.3	1,426,174.0	1,453,238.0	1,316,950.0

Source: Food and Agriculture Organization (FAO) (2013)

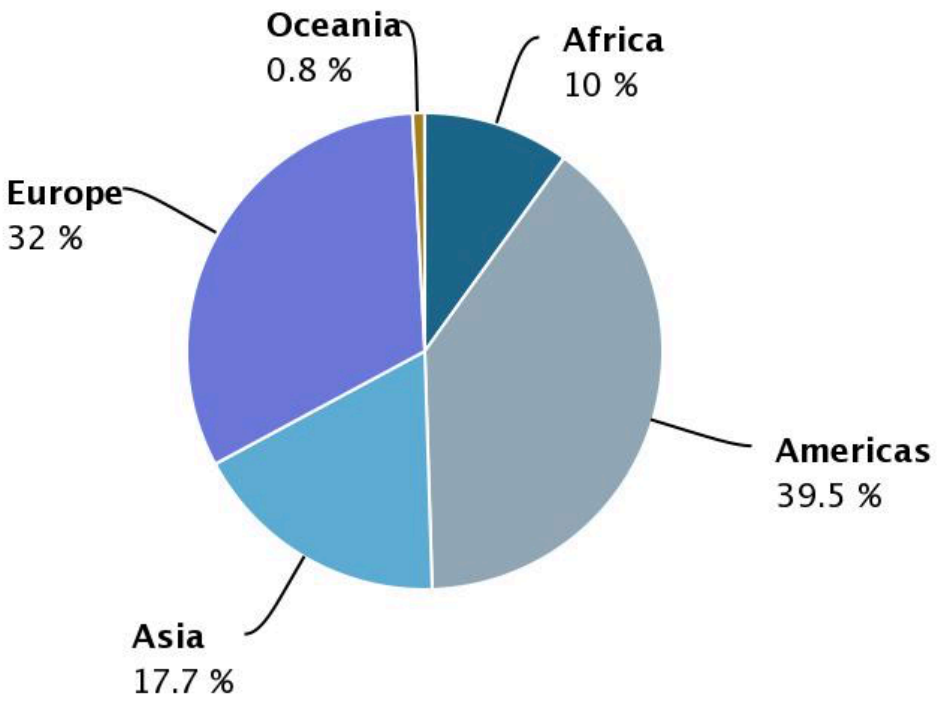


Figure 1.5: Share of worldwide strawberry production by region.

Source: FAO (2013)

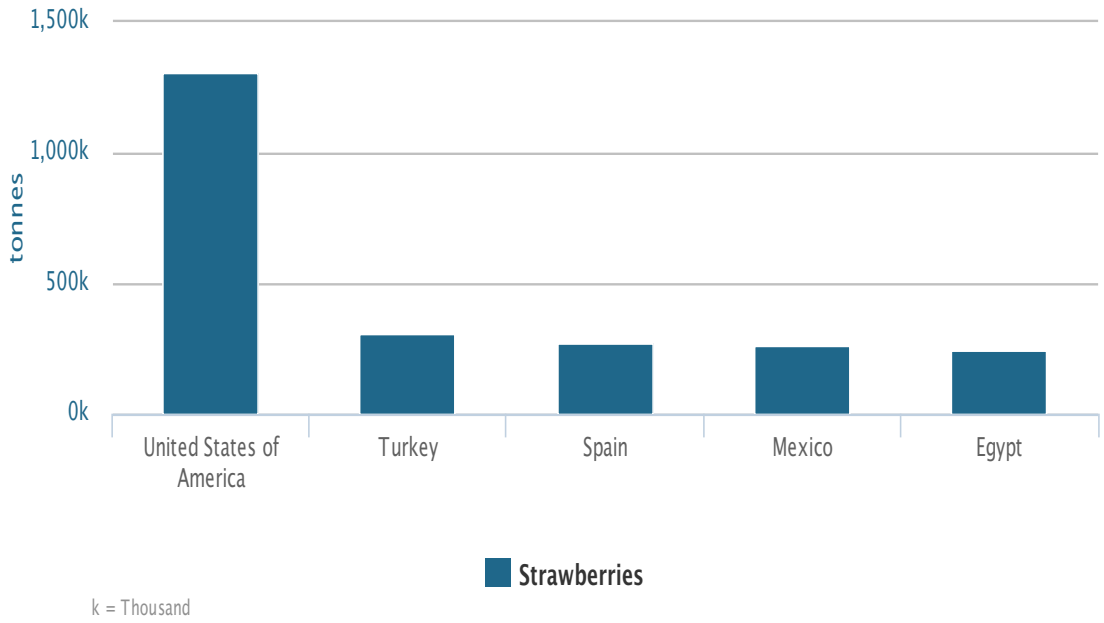


Figure 1.6: Strawberry production by the top five producing countries (2009-2013). Source: FAO (2013)

Production of strawberries in the UK

The UK strawberry industry has expanded rapidly over the last two decades. The output has increased through the introduction of new varieties and new growing systems, which have improved productivity and fruit quality. UK growers supply 85% of the UK market from May to October (Dogson, 2007), having increased consumer demand in recent years. This has been achieved by adopting new cultivars and new technologies, such as portable tunnels and harvesting rigs (Anon, 2008). Almost 85% of UK strawberries are grown under polyethylene tunnels, either in soil in raised beds covered with a polyethylene mulch or in bags and troughs that have a coir-based substrate (Anon, 2012).

The total area of soft fruit grown in the UK is 1% less than that in 2012 at 9.5 thousand hectares (Anon, 2014). The area of strawberries grown has remained constant, showing a 1% decrease in 2013 compared to 2012. In 2013, strawberries accounted for a market worth £70 million (75 % of the overall soft fruit market). Over the past decade, the value of UK strawberries has increased by £46 million. This was mostly due to an increase in intensity of production (Fig. 1.7) (Anon, 2014).



Figure 1.7: UK strawberry production, imports and exports (2000-2013)

Source: Department for the Environment Food and Rural Affairs (DEFRA) (2014)

East Malling Research (EMR) has released 17 new varieties since 1984; hence sales of EMR cultivars are over 20 million plants per annum. The cultivar development programme is funded by the industry, which contributes £275,000

per annum for breeding at EMR. EMR does not carry out many trials on commercial farms (Anon, 2008). Moreover, Driscoll's of Europe and Berry Gardens Ltd. work exclusively together to market Driscoll's varieties in the UK market all year round (Anon, 2010). A few of Driscoll's unique varieties are: Driscoll® Pasadena™, Driscoll® Amesti™, Driscoll®Magdalena™, Camarillo, Driscoll® Lusa™ and Driscoll El Dorado (Anon, 2013). Driscoll's varieties became the most popular variety in Europe (Anon, 2013).

The major diseases of strawberry (Table 1.3) in the UK are grey mould (*Botrytis cinerea*), black spot (*Colletotrichum acutatum*) and powdery mildew (*Podosphaera aphanis*), which can all be controlled by fungicide sprays. Red core (*Phytophthora fragariae*), crown rot (*Phytophthora cactorum*) and wilt (*Verticillium dahliae*) are controlled by soil fumigation (Anon, 2002). The dominant cultivar Elsanta has been grown throughout Northern Europe and is susceptible to majority of these pathogens. Hence, it is important that this cultivar is replaced by others, which need fewer chemical inputs for successful production (Anon, 2002).

Table 1.3: Major strawberry diseases in the UK

Disease and Causal agent	Symptoms
Botrytis *Botrytis cinerea*	Commonly known as grey mould, botrytis is found on old leaf debris, young leaves, leaf petioles, flower buds, petals and flower stalks as well as ripening fruits and ripe fruits
Powdery mildew *Podosphaera aphanis*	This fungus is found on leaves, flower stalks, flowers and fruit
Blackspot *Colletotrichum acutatum*	This is mainly a disease of ripe fruit, but the pathogen can also attack leaves, petioles, stolons and the crown, where it can remain almost symptomless until conditions favour it.
Leaf spot *Mycospharella fragariae*	Lesions develop on leaves, petioles, stolons and fruit. Leaf lesions are most common and are found on the upper and lower sides of the leaf
Leaf blotch *Zythia fragariae*	Mainly a disease of the leaf, although lesions can spread to fruiting stalks, resulting in shrivelling of stalk tissues and subsequent loss of fruit
Red core *Phytophthora fragariae*	A soil-borne oomycetesl disease that affects the roots, causing root death, plant collapse and plant death
Crown rot *Phytophthora cactorum*	A soil-borne oomycetes disease that affects the plant crown
Verticillium wilt *Verticillum dahliae*	A soil-borne disease that affects plant roots and reduces the ability of plants to take up water from the soil
Angular leaf spot *Xanthomonas fragariae*	A bacterial disease that is not currently a major problem in the UK
Xanthomonas arboricola pv. fragariae	Dry brown necrotic leaf spots and large brown V-shaped lesions around the leaf margins, mid rib and major leaf veins. These spots differ from those of angular leaf spot (caused by *Xanthomonas fragariae*) in that they are dry rather than being water-soaked in appearance.
Strawberry green petal	A phytoplasma spead by leafhoppers
Strawberry mottle virus	Spread by the strawberry aphid and melon-cotton aphid; this in the most common virus of strawberries
Strawberry crinkle virus	A virus spread by the strawberry aphid.
Strawberry mild yellow edge virus	Transmitted by the strawberry aphid; this virus often occurs together with one or more of the other strawberry viruses.
Strawberry vein-banding virus	Transmitted by the strawberry aphid, this is the least common of the aphid-borne viruses
Arabis mosaic virus	Transmitted by the free living nematode *Xiphinema diversicaudatum*
Nematodes Eelworms	Minute worm-like invertebrates, which can only be identified by nematologists using a microscope.
Nematodes Various genera and species	Minute parasitic worm-like creatures applied as drench or spray. Nematodes do carry diseases.

Adapted from: Anon, (2010)

Propagation and growth of strawberry plants

Strawberries are normally propagated from runner plants or by tissue culture techniques. However, the breeders use seed for propagation when they are trying to develop new varieties (Maas, 1998). Most of the propagators sell runner plants, which are from the mother plants, to strawberry growers. Propagation of strawberry plants is highly regulated by the Plant Health Propagation Scheme (PHPS), which is operated by the Food and Environment Research Agency (FERA) in the UK (Anon, 2012). The strawberry certification scheme (from PHPS) has five levels of approval: foundation, Super Elite, Elite, A, Approved health, with the objective of producing healthy strawberry plants (See Appendix 1 for detail). Strawberry plants are guaranteed to be virus free from the propagator but not free from other diseases.

Planting

Strawberry plants can be grown in many ways, typically in raised beds with an irrigation system laid below the plastic film that prevents weed growth, or in coir bags (Fig. 1.8) (Ellis et al., 2006). In early spring, strawberry growers plant new strawberry plants that are bought from the propagator or some bare root plants which have been kept over-wintered and cold-stored at -1.5 °C. Strawberry plants are planted in coir bags or soil under polyethylene tunnels and in open fields. The plants are planted approximately 45cm apart down the row with 100cm between rows in fields. In addition, the stolons produced by the mother plant are removed

to encourage further crown development and increase the yield in the next season. The open field strawberry plants are in the ground for three winters and four harvests (Ellis et al., 2006).

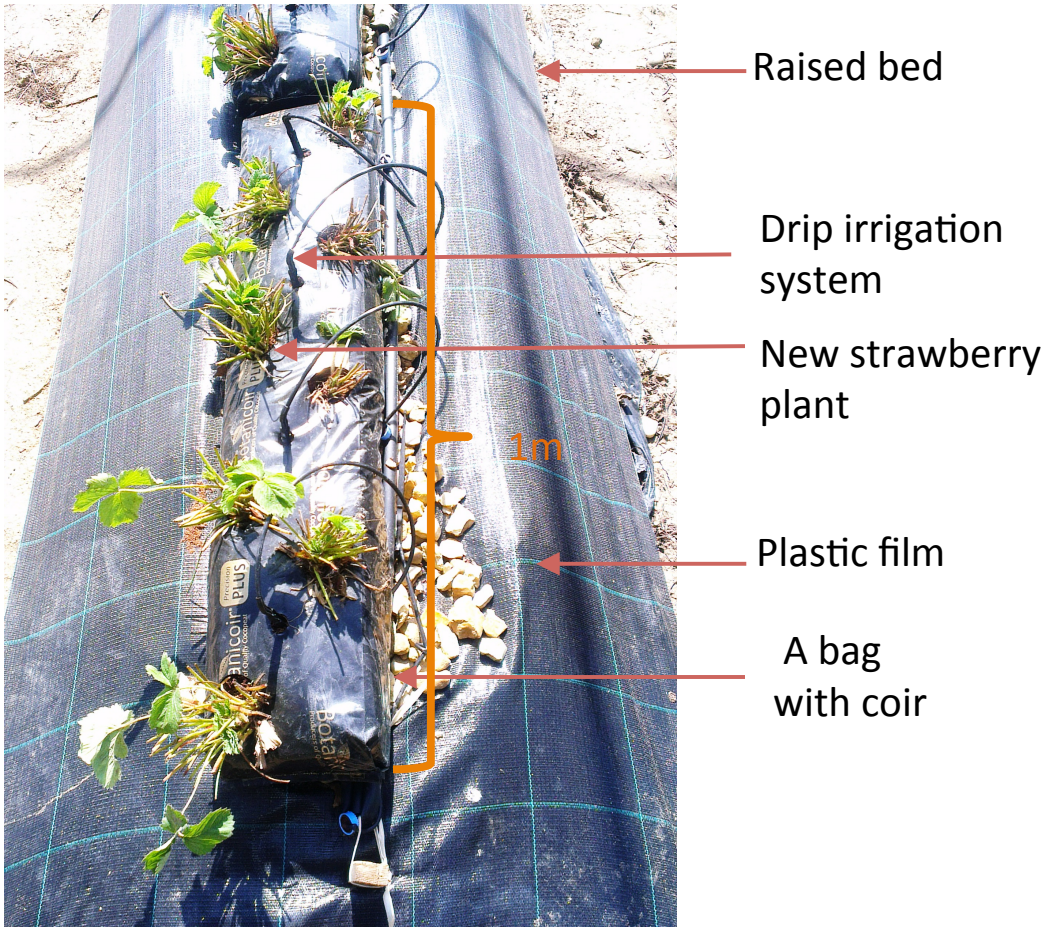


Figure 1.8: Strawberry plants planted in a coir bag on a raised bed with a drip irrigation system in June 2013.

Watering/irrigation

Trickle irrigation and drip irrigation are the most common types of irrigation used on growing strawberries (Ellis et al., 2006). In the UK, strawberry growers use

trickle irrigation and drip irrigation to deliver water and nutrients to strawberry plants via fertigation tubes (Fig. 1.8). The amount of irrigation applied should be based on the crop requirement for water, which is important to ensure that the crop is not stressed. If the watering is overhead, it may encourage the development of grey mould (*Botrytis cinerea*), which can lead to fruit rotting. Using a drip system to irrigate means the leaves and fruit can remain dry and greatly reduces the amount of grey mould (CALU, 2007). Furthermore, growers use the irrigation system to apply fertilizers; hence it reduces costs and the need to enter the field with equipment (Anon., 2008).

Mulch, fleece and net door

Mulch and fleece are used to warm the soil, to protect the plants from frost and to help them to retain moisture for a period of up to 6 weeks from mid-February before tunnels are covered with polyethylene (Fig. 1.9). Mulch is made of plastic with holes (1x1 cm) in it. Fleece is more like material or fabric. There are net doors at the ends of tunnels to retain the heat inside the tunnels. Thus, when the temperature is cold (< 10 ºC) outside the net door is shut and when the temperature is >20 ºC the net door is rolled up (Fig. 1.10). The strawberry plants are covered by fleece and mulch to increase the soil temperature and encourage flowering between February and April (Fig. 1.9). Generally, the fleece and mulch are removed from the plant row in early spring in March/April, depending on the temperature. Mulch is removed completely and fleece used to cover plants only when the overnight temperature is likely to be < 6 ºC.



Figure 1.9: Strawberry beds covered with mulch and fleece under polyethylene tunnel.



Figure 1.10: Strawberry beds covered with mulch and fleece under a polyethylene tunnel with the net door shut.

Use of polyethylene tunnels

Polyethylene (Spanish) tunnels have been used in commercial strawberry production in the UK since 1993 (Dodgson et al., 2008). They were adopted to advance the first harvest date to a date earlier in the year and therefore to extend the harvest season (Fig. 1.11), which enabled UK growers to compete with overseas suppliers. Moreover, the proportion of strawberry fruits that are Grade 1 have increased from 50% to more than 90% since the use of polyethylene tunnels started (Brook, 2009). Currently 85% of strawberries sold in supermarkets are produced under polyethylene tunnels (Fatema, 2009). The tunnels can prevent rain damage to the crop and stop mud splash to decrease the spread of grey mould caused by *Botrytis cinerea* (Anon, 2009). They also facilitate the use of biological control methods to control pests and reduce the need for pesticides, which is more environmental friendly (CALU, 2007).

Moreover, the strawberry plants remain in the field for up to four cropping seasons (June-bearing) or one cropping season (Ever bearer). The polyethylene sheets are rolled up and kept by the side of tunnel each winter and are put back on in early spring. The strawberry plants are covered with mulch and fleece in the early spring each year under polyethylene tunnels to force early cropping and harvest (Dodgson et al., 2008). However, the environmental conditions in tunnels are conducive to strawberry powdery mildew, which requires warm temperature (15 - 25 ºC) and high humidity (> 97%) (Dodgson et al., 2008).

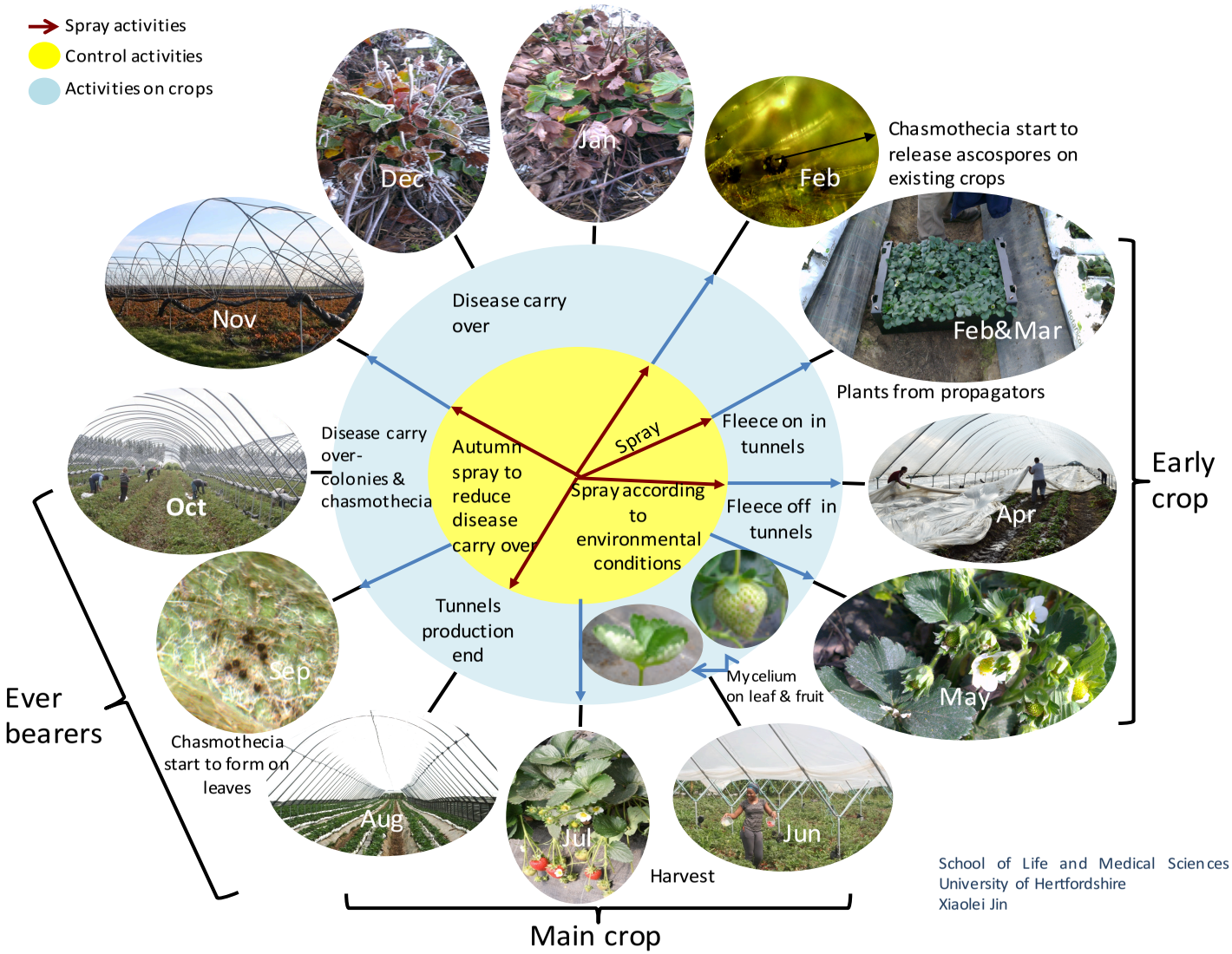


Figure 1.11: The UK strawberry farming system during the year, showing the times for harvest of early crop, main crop and ever bearer strawberries, farming activities and fungicide schedule (yellow cycle) to prevent strawberry powdery mildew and the stages in development of strawberry powdery mildew during the year.

Weed control

Weed control is one of the critical tasks for strawberry growers, as weeds compete with the crop for light, water and nutrients, which reduces crop establishment, crop density, flower bud initiation, fruit size, results in unequal fruit ripening and reduces the harvest yield (Anon, 2008). Weed control is best achieved through a combination of cultivation practices and the use of herbicides. Herbicides are used before soil beds are prepared (CALU, 2007). Thus, the selection of herbicides, proper timing and rates of application are factors critical to a weed control programme (Ellis et al., 2006). A single herbicide is often not effective in controlling all weed species present and the use of a single herbicide for long periods will result in herbicide-resistant weed species. Thus, using more than one type of herbicide in the course of a growing season will help reduce the occurrence of herbicide-resistant weed species (Anon, 2008). During harvesting period, hand weeding is done. In addition, polyethylene film can be laid up to 4 weeks before planting; this encourages weed seedlings to emerge and die. Although, using woven polypropylene sheets can be more expensive they last for a long time, saving physical work and reducing costs (CALU, 2007).

Harvesting and the pack-house

When strawberry pickers are picking in the field they pay particular attention to size and shape of strawberries and to whether strawberries are clean and free of disease and damage (Fig. 1.12a) (Armstrong, 2004). Plastic trays are taken to

the field and each tray holds 6 or 8 punnets. The punnets hold 250 or 450g of strawberries (Bearer & Bearer, 2014). From the fields, strawberries are taken as quickly as possible to a cold store (Fig. 1.12b). The pack-house (20 ºC) has the cold store (4 ºC) to keep the fruit in perfect condition. The pack-house is the place for staff to control fruit quality, measure fruit weight (Fig. 1.12c), seal plastic film on top of strawberry punnets by machine (Fig. 1.12e) and make a final check (Fig. 1.12f) before all of the sealed strawberry punnets are returned into the cold store.

Pests and diseases of strawberry

Strawberries are affected by many kinds of insect pests and diseases (Table 1.3). For example, the roots can be infested by vine weevil, which may lead to plant death, especially in spring when rapid growth occurs (CALU, 2007). Leaves and crowns can be infested with aphids, which result in leaf curling and deformation. Slugs can damage the fruit by making holes in them and rendering the fruit unsaleable. Strawberry seed beetles can eat the seeds from the outside of the berry, which spoils the appearance of the fruit (Maas, 1998). Different varieties of strawberry have different levels of resistance to fungal and oomycete attack (Maas, 1998). Root and crown rot caused by *Phytophthora* species generally occur in heavier soils. Grey mould (*Botrytis cinerea*) is the most common fungal disease that harms the developing fruit. After introduction of the polyethylene tunnel in the UK a reduction of up to 50% in the use of fungicides to control grey mould and black spot has been recorded. Where polyethylene tunnels are used,

Botrytis is controlled but powdery mildew becomes a major problem. Powdery mildew (*Podosphaera aphanis*) can damage the leaves and spread to fruits.

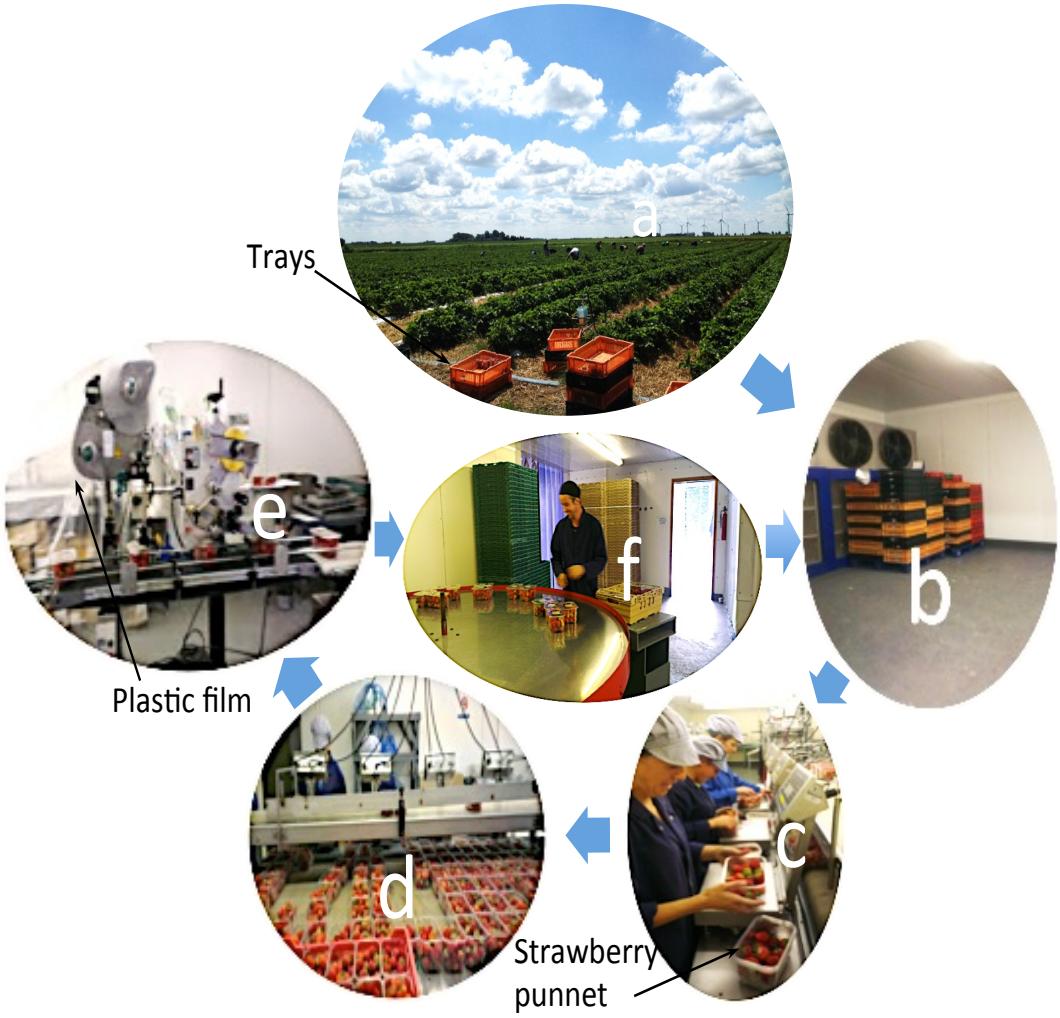


Figure 1.12: The cycle of storing strawberries in the pack-house and cold store; a) pickers picking strawberries in the open field; b) cold room to store harvested strawberries; c) checking strawberry weight and quality; d) e) sealing punnets with heat-sealed plastic film by machine ; f) final check.

1.3 *Erysiphales* (powdery mildews)

Erysiphales (powdery mildews) was first published by Salmon (1900). The second taxonomic treatment of this group of plant pathogenic fungi that cause powdery mildews appeared as the " Monograph of the *Erysiphales"* in 1987 (Braun, 1987 cited in Braun & Cook, 2012). The group is composed of 16 genera and about 650 pathogen species; almost 10,000 host species of powdery mildews worldwide were listed by Amano (1986). The *Erysiphales* are a group of obligately biotrophic fungi; thus they cannot be cultured on artificial media (Braun & Cook, 2012). Moreover, "the taxonomy of the *Erysiphales* has generally been based on the morphological characteristics of fresh and herbarium specimens" (Takamatsu et al*.,* 2009).

Figure 1.13 shows the tree of the phylogeny of tribes and genera of *Erysiphales;* it has been adapted from an analysis by Braun et al. (2006) based on 28S rDNA data (Braun & Cook, 2012). Furthermore, Braun and Takamatsu (Braun & Cook, 2012) stated that about 86% of species are hosts of *sect*. *Podosphaera* and 50% of its hosts belong in the Rosaceae family *(*Bélanger et al., 2007).

Colonisation and symptom development

Generally, the *Erysiphales* can be simply identified as agents of diseases that form white patches or coverings of mycelium on leaves, stems and sometimes

on flowers and fruits. The mycelium goes onto the upper and lower surface of the leaf. As the disease progresses, the colony get larger and denser as large numbers of conidium (asexual stage) are formed, and the mildew may spread up and down the length of the plant (Braun & Cook, 2012). Furthermore, powdery mildew can also cause deformations of stems and leaves, disfiguration and distortion (Braun & Cook, 2012). Every conidiophore forms a conidium in 24 h and those conidia are mature by mid-day but this is not the case for in *Erysiphale. betae* (Braun & Cook, 2012).

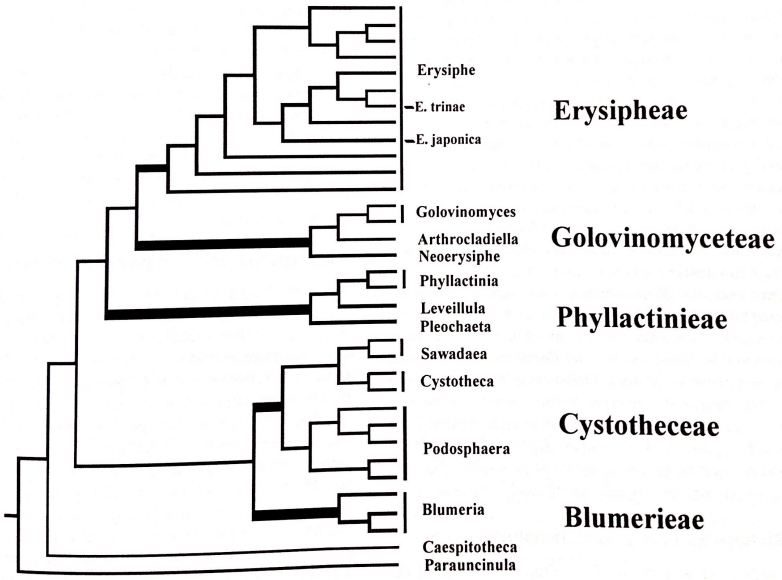


Figure 1.13: The tree of the phylogeny of tribes and genera of *Erysiphales;* it is adapted from an analysis by Braun et al. (2006) based on 28S rDNA data set for 40 taxa of the *Erysiphales* covering all known tribes and out-group taxons.

Source: Braun & Cook (2012)

General life cycle of *Erysiphales*

Generally, *Erysiphales* have an asexual cycle and a sexual cycle (Fig. 1.14). Conidia, as asexual organs, can be distinguished between species according to their shape and germination type (Braun & Cook, 2012). Conidiophores of *Erysiphales* typically arise from superficial hyphae and conidia are one-celled, colourless, maturing singly or in chains. Ascoma development is normally uniform in all *Erysiphales*. Mycelium in or on infected plant tissue could remain dormant during the winter, such as *Podosphaera leucotricha* on apple which can overwinter on the bud scales. Mycelium then start to grow and sporulate with regrowth of the host plant in the spring and then infecting the new growth.

Braun introduced the special term chasmothecium (chasma + thecium = slit + fruiting body) that in order to denominate a special type of fruiting body within the *Erysiphaceae,* which opens by a natural opening curve. The sexual organs are one-celled structures formed on lateral branches of the mycelium, which can are gamocytes, the female ascogonium and male androgamocyte (Braun & Cook, 2012). The female ascogonium and male androgamocyte generally enclose or line up beside each other (Fig. 1.14). Moreover, species in *Podosphaera* sect. *Sphaerotheca* contain only one single ascus in the chasmothecium (Braun & Cook, 2012).

Reviews of resistance

a) R genes – effect plant physiology, race specific resistance give rise to

pathotypes in the pathogen (unlikely to be durable) (Lucas, 1998). *P. aphanis* has no pathotypes or physiological races (Braun & Cook, 2012).

b) Polygenic resistance – governed by many genes, may be physiological or morphological (probably durable) (Lucas, 1998).

c) Third type which morphological but can give resistance to several pathogen (durable) (Lucas, 1998). *P. aphanis* has no recorded.

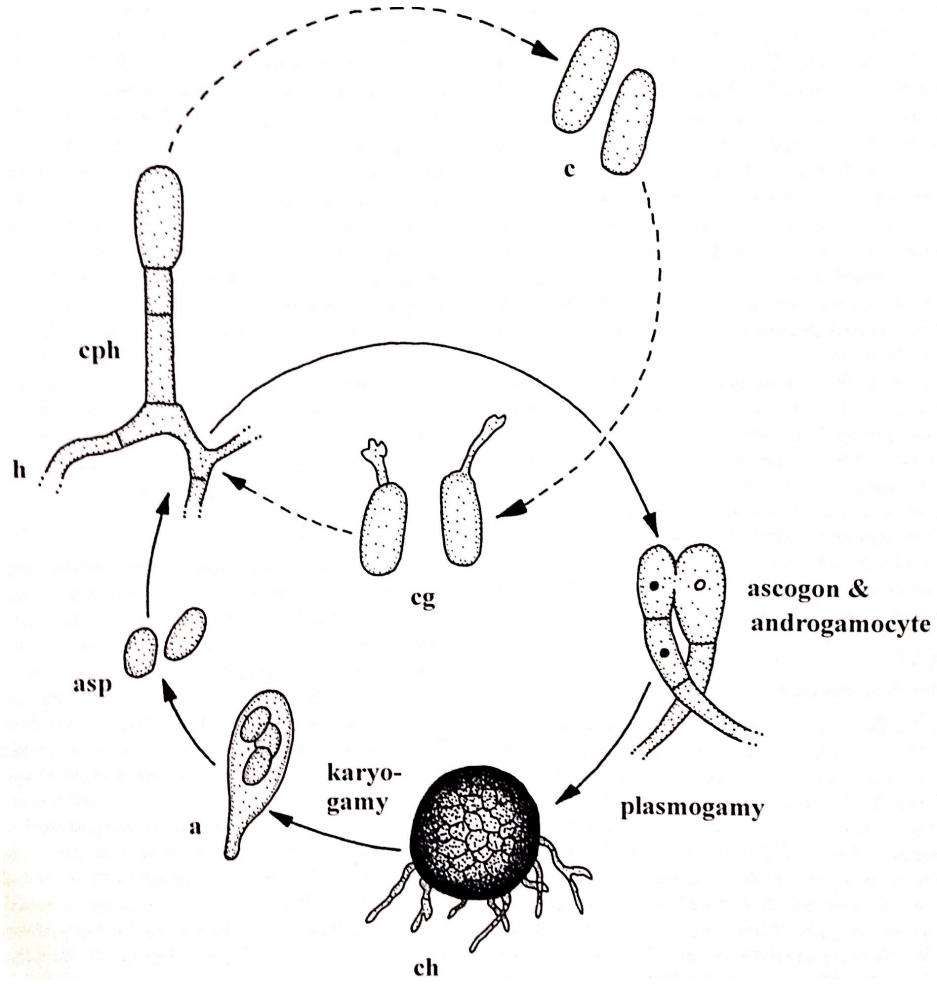


Figure 1.14: The general life cycle of *Erysiphales* (powdery mildews), schematic (asp = ascospores, a = ascus, ch = chasmothecium, c = conidia, cg = conidia with germ tubes, cph = conidiophores, h = hypha). Source: Braun & Cook (2012)

1.4 Strawberry powdery mildew (*Podosphaera aphanis*)

Strawberry powdery mildew is a widespread disease all over the world (Appendix 6). The fungus causing strawberry powdery mildew has had several name changes and been reclassified from *Sphaerotheca humuli* (Cooke, 1906 cited in Braun & Cook, 2012) to *Sphaerotheca macularis* (Braun, 1982e) and finally to *Podosphaera aphanis* (Braun & Cook, 2012). It was first recorded in 1898 and 1900 (Moore, 1959). The disease affects leaves, flowers and fruits, thus reducing strawberry yield, both in quantity and quality. *Podosphaera aphanis* is a biotroph, which has two stages, the asexual stage and the sexual stage (Schumann & D'arcy, 2012). A large number of conidia are produced from the conidial chain (Fig. 1.15a, b); these conidia result from the asexual reproduction and form a visible white powder (Glawe, 2008).

The chasmothecia of *Podosphaera aphanis* as sexual stage appear in late summer and autumn in response to changes in environmental conditions. The mycelia begin to produce chasmothecia on the lower leaf surface to survive inclement conditions over winter and begin the life cycle again in the following spring when conditions conducive for disease occur. The chasmothecia may drop onto debris or in soil which there is not much evidences about it. Salmon (1900) reported that strawberry powdery mildew was common in England in 1900 but was not able to find chasmothecia (cited in Gadoury et al., 2010). However, the survival structures of strawberry powdery mildew are the chasmothecia, in which sexual reproduction occurs over winter (Glawe, 2008). The mature

chasmothecium contains one ascus and eight ascospores (Fig. 1.15c, d), which form a visible round black case on the lower leaf surface (Putnicki, 2009). There is not much evidences about how ascospores are released into the air for dispersal.

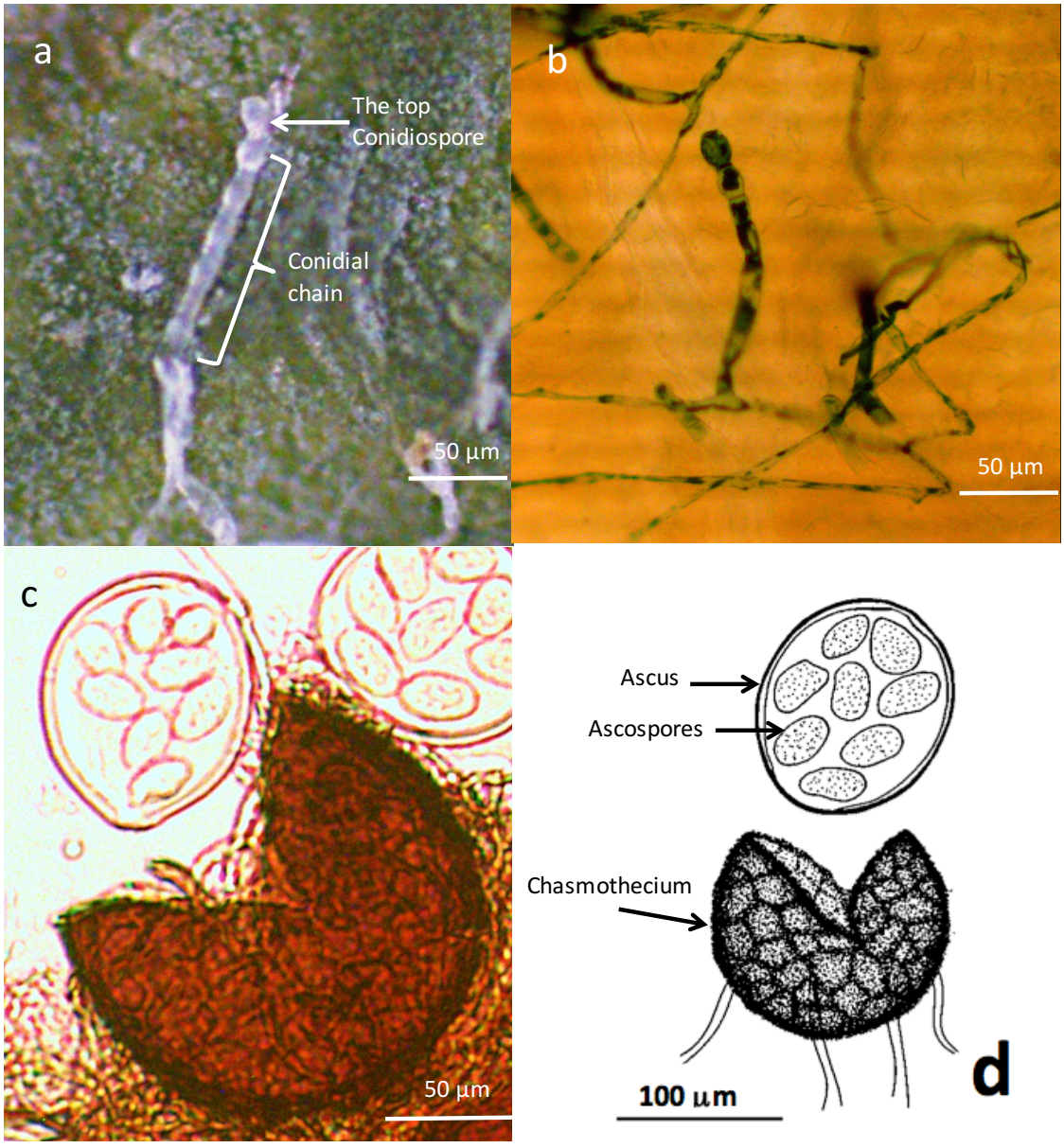


Figure 1.15: *Podosphaera aphanis* (a) conidial chain; (b) conidial chain stained with trypan blue; (c)(d) a chasmothecium of *Podosphaera aphanis* with one ascus and eight ascospores inside.

Colonisation and symptom development

The disease affects leave, buds, flowers, fruits, runners, peduncles and petioles (Fig. 1.16) (Agrios, 2005). In addition, the infected green fruit does not ripen and if it ripens it remains soft, with a shorter shelf life and small seeds (Amsalem et al., 2006). The healthy leaves should be flat. If they start to curl, this may be an indication of the presence of the pathogen (Dodgson et al., 2008).

Initially, the leaves curl up like spoons (cupping) and may appear to have red blotches, depending on the variety (Fig. 1.16a, b). The leaf surface is covered by white fungal mycelium that spreads from the lower surface to the upper surface (Fig. 1.16c). Eventually, susceptible flower buds develop pink petals and the flower buds cannot open. The diseased flowers can be deformed, resulting in low concentrations of pollen and the flowers may wilt and fall (Fig. 1.16e, f) and this leads to fruit deformation. Hence, the strawberry colour and ripening can be affected and fruit surfaces may be covered by white ‘powder’, making the fruit unsaleable (Fig. 1.16g) (Dodgson et al., 2008).

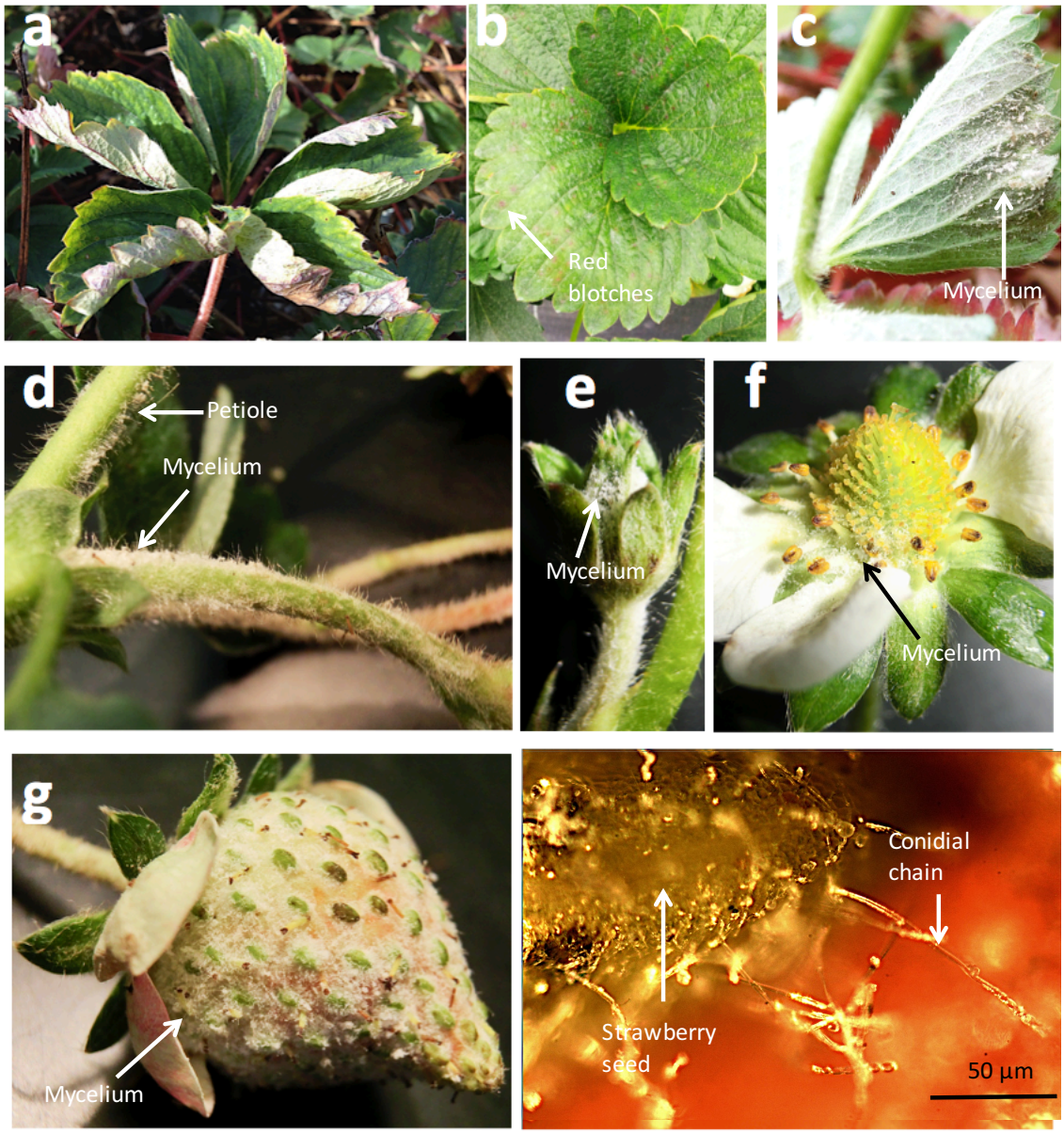


Figure 1.16: Symptoms of strawberry powdery mildew; a) cupping; b) characteristic red blotches; c) fungal mycelium on a leaf; d) infected peduncles and petioles with mycelium; e) infected flower bud with mycelium; f) infected flower with mycelium; g) infected immature fruit with mycelium; h) enlarged strawberry seed with conidial chain and hyphae around it.

Conditions suitable for growth of *Podosphaera aphanis*

For strawberry powdery mildew to occur, specific environmental conditions are required (Drandarevski et al., 1978). Strawberry powdery mildew produces conidia 7-14 days after infection under favourable temperature and humidity conditions (Dodgson et al., 2008). The optimum temperature is 15-25 °C (Table 1.4). At temperatures < 5 °C or > 30 °C no conidia form (Drandarevski et al., 1978). When the relative optimum relative humidity is > 97%, powdery mildew epidemics can be extremely serious (Dodgson et al., 2008). Under the appropriate environmental conditions, large numbers of conidia are produced and spread by wind onto new leaves, flowers and fruits (Glawe, 2008). Conidium germination occurs on healthy, dry leaf surfaces. Chasmothecia develop slowly when temperature is 20 - 25 °C; however, they develop more rapidly when temperature is 13 - 15 °C (Asalf et al., 2013).

Table 1.4: Summary of conditions that affect stages in the life cycle of *Podosphaera aphanis*

Variable		Germination	Infection	Sporulation
Temperature (°C)	minimum optimum maximum	2 15–25 30	5 15–30 30	13 20 35
Relative humidity (%)	minimum optimum maximum	8 97–100 100	No effect No effect No effect	No effect No effect No effect
Presence of free water (immersion time hours)	minimum optimum maximum	NA 0 24	No effect No effect No effect	No effect No effect No effect
Time of day (hours)	minimum optimum	No effect No effect	No effect No effect	20:00–8:00 12:00–16:00

Source: Dodgson et al., (2008)

Life cycle of Podosphaera aphanis

The asexual conidiospores of *P. aphanis* (Fig. 1.17) are produced from the upper surface of the mother cell and are released from the top of the conidial chain by wind or insects; conidial dimensions are ≤ 20-45 x 10-26 μm; germ tubes are unbranched (Braun & Cook, 2012). These conidia germinate and initiate infections. Production of conidia is critical to starting the secondary cycle of disease and ensuring the progress of the epidemic from May to September. In the autumn, the pathogen mycelia start to produce chasmothecia for survival through inclement conditions over winter. The cycle begins again the following spring when conditions favourable for disease occur.

Chasmothecia are the sexual structures of *P. aphanis* that over-winter. They contain one ascus and eight ascospores. Chasmothecia are usually about 60-120 μm in diameter (Braun & Cook, 2012). Appendages are normally produced from the lower half of the chasmothecium. Germinating ascospores produce germ tubes, which penetrate into the epidermis of leaves. After penetration, haustoria are formed inside the cells and mycelium starts to grow on the leaf surface. Furthermore, fully developed ascospores are almost colourless and generally ≤ 16-30 · 11-21 μm in length and width (Braun & Cook, 2012).

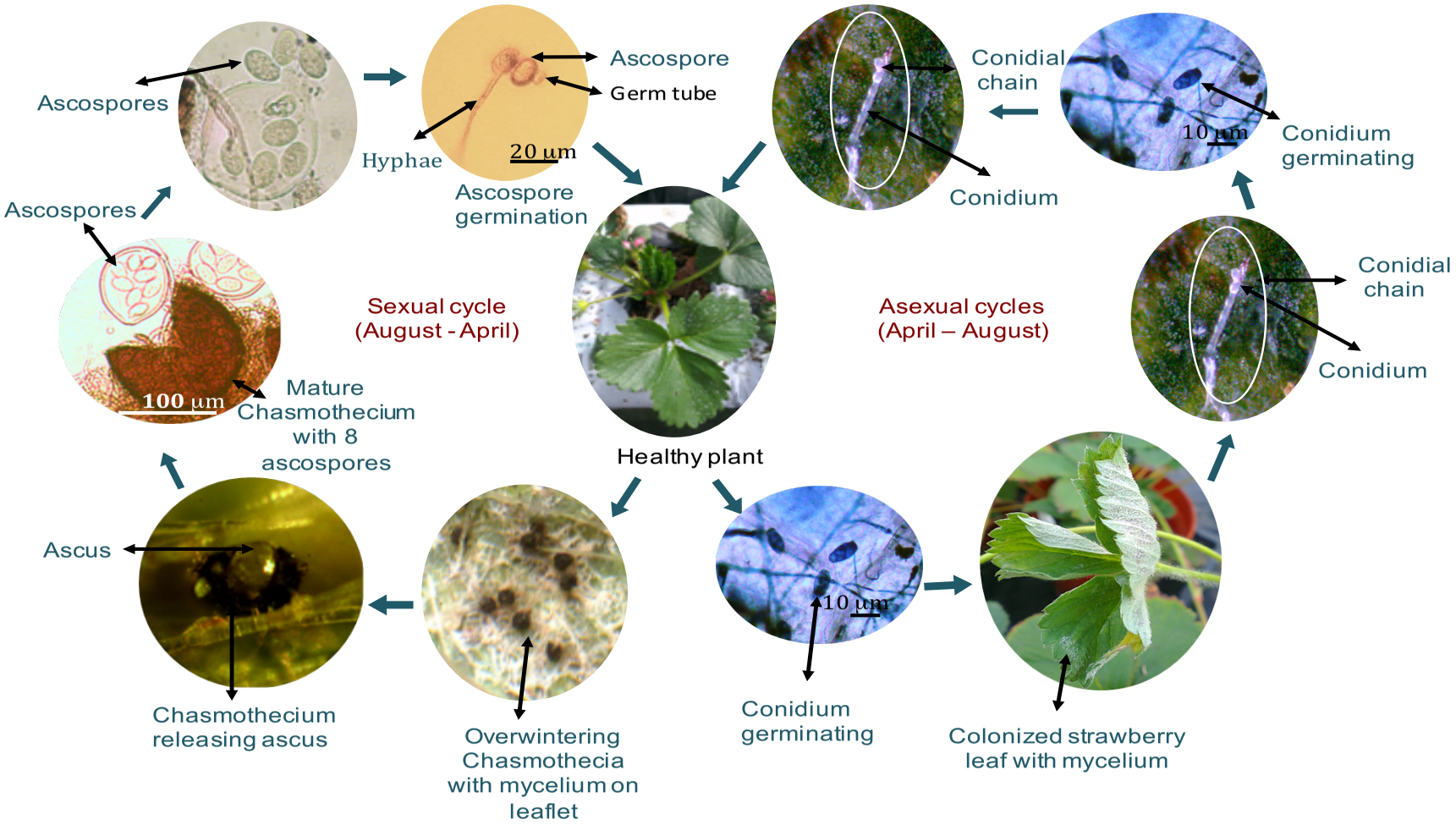


Figure 1.17: Life cycle of *Podosphaera aphanis*. The asexual conidia are released from the top of the conidial chain. These germinate and initiate infections. Production of conidia is key to creating the secondary cycle of infection and the development of the epidemic. In the autumn the mycelia begin to produce chasmothecia to survive inclement conditions and begin the cycle again the following spring when disease conducive conditions occur. Chasmothecia, produce one ascus and eight ascospores. Germinating ascospores produce germ tubes which penetrate the epidermis of the leaves where haustoria are formed inside the cells and mycelium starts to grow. Over-wintering chasmothecia contain one ascus an eight ascospores for primary infection. (The drawing life cycle is in Appendix 7).

1.5 Integrated control of strawberry powdery mildew

A strategy for integrated control of strawberry powdery mildew does not eliminate all fungicides but it aims to reduce the frequency and amount of fungicides used. This can be achieved in part by using alternative products including chemical control and non-chemical control (see Box 1). Strawberry powdery mildew caused by *P. aphanis* poses a constant threat to strawberry production worldwide, including the UK. Whether the crop is produced in glasshouses, under polyethylene tunnels or in open fields, this disease is a potential problem depending on the cultivar grown and the prevailing environmental conditions. Infection by *P. aphanis* causes a loss in both yield quantity and quality. Strawberry production in the UK is very efficient; in the last 20 years the total yield has doubled and the area cropped has halved. The harvest period has been extended from 4 weeks in June and July to 20 weeks from early May to October (Dodgson et al., 2008). This has been achieved in part by the use of a range of production systems, careful choice of cultivars, as well as use of fungicides and precision use of fertilisers.

However, strawberry powdery mildew poses a persistent problem and there are only a small number of resistant or tolerant varieties available. Furthermore, Table 1.5 shows that the number of fungicides available to growers is relatively small and most of them cannot be used immediately before harvest because of the harvest interval, which may be up to fourteen days (Dodgson et al., 2008). Thus, strawberry growers use potassium bicarbonate combined with silicon

nutrient to control powdery mildew. Potassium bicarbonate is an ideal product to use at the time fruit is being picked when fungicides are not allowed to be used on crops (Dodgson et al., 2008).

Box1: Chemical control and non-chemical control of strawberry powdery mildew

Chemical control:

- Use of fungicides (Table 1.5)
- Rule-based prediction system to optimise fungicide applications (Dodgson, 2007)
- Use of potassium bicarbonate (Dodgson, 2007; Fatema, 2014)
- Use of potassium silicate (Kanto et al, 2004)
- Use of silicon nutrient (Fatema, 2014)
- Propagation phase: “A ‘clean-up’ spray pre-lifting may help to control disease before storage and subsequent planting by growers.” (Dodgson et al., 2008)

Non-chemical control:

- Bio-control (Appendix 2)
- Cultural control: reduce the density of the strawberry plants in the tunnel; growers should not feed or irrigate excessively; avoid high nitrogen use (Dodgson et al., 2008).
- Crop resistance: growing resistant varieties.
- Tunnel management: keep optimal environmental conditions for fruit production whilst also avoiding conditions that are conducive to powdery mildew development (Dodgson et al., 2008).

Table 1.5: Summary of control agents currently approved for use in UK strawberry crops

Causal agent and Diseases	Fungicide
Black spot *Colletotrichum acutatum*	azoxystrobin (off-label), boscalid + pyraclostrobin, cyprodinil + fludioxonil (qualified, minor use)
Botrytis *Botrytis cinerea*	boscalid + pyraclostrobin, captan, chlorothalonil (off-label), cyprodinil + fludioxonil, fenhexamid, *Gliocladium catenulatum* (bio-control), iprodione, mepanipyrim, pyrimethanil, thiram
Collar rot	fenamidone + fosetyl-aluminium (moderate control from foliar spray)
Crown rot *Phytophthora cactorum*	dimethomorph (moderate control), fenamidone + fosetyl- aluminium (moderate control from foliar spray)
Didymella stem rot	*Gliocladium catenulatum* (bio-control)
Fusarium	*Gliocladium catenulatum* (bio-control)
Leaf spot *Mycospharella fragariae*	chlorathalonil (off-label)
Phytophthora	fenamidone + fosetyl-aluminium, *Gliocladium catenulatum* (bio - control)
Powdery mildew *Podosphaera aphanis*	boscalid + pyraclostrobin, bupirimate, fenpropimorph (off-label), kresoxim-methyl, myclobutanil, penconazole (off-label), quinoxyfen (off-label) sulphur
Pythium	fenamidone + fosetyl-aluminium, *Gliocladium catenulatum* (bio - control)
Red core *Phytophthora fragariae*	fenamidone + fosetyl-aluminium
Rhizoctonia	*Glocladium catenulatum* (bio-control)

Adapted from: Lainsbury, 2015 *off-label=no longer registered for use

Rule-based prediction system to optimise fungicide applications

A rule-based prediction system has been developed that has the potential to reduce the number of fungicide applications applied by the growers and ensure that fungicide applications are not too frequent. Work from 2004 to 2008 funded by HDC, and further work (Dodgson, 2007; Dodgson et al., 2008) led to the production of a rule-based prediction system based on environmental conditions favourable to conidial germination and disease development.

The prediction system uses farm weather stations with temperature and humidity probes in the vicinity of the crop under polyethylene tunnels. When a strawberry field is at greatest risk of infection by *P. aphanis*, it operates with a warning period of 48 hours to allow time for a fungicide spray to control *P. aphanis* (Dodgson, 2007). Germination of conidia occurs only when temperature is > 15 ºC and germination also requires an optimum humidity of > 60% (Table 1.4). The prediction system (Fig. 1.18) requires an elapsed period of 144 cumulative hours that has both of the required parameters. The system is set to zero hours at the start of the season, and then reset to zero after each time that the crop is treated with a fungicide (Dodgson et al., 2008).

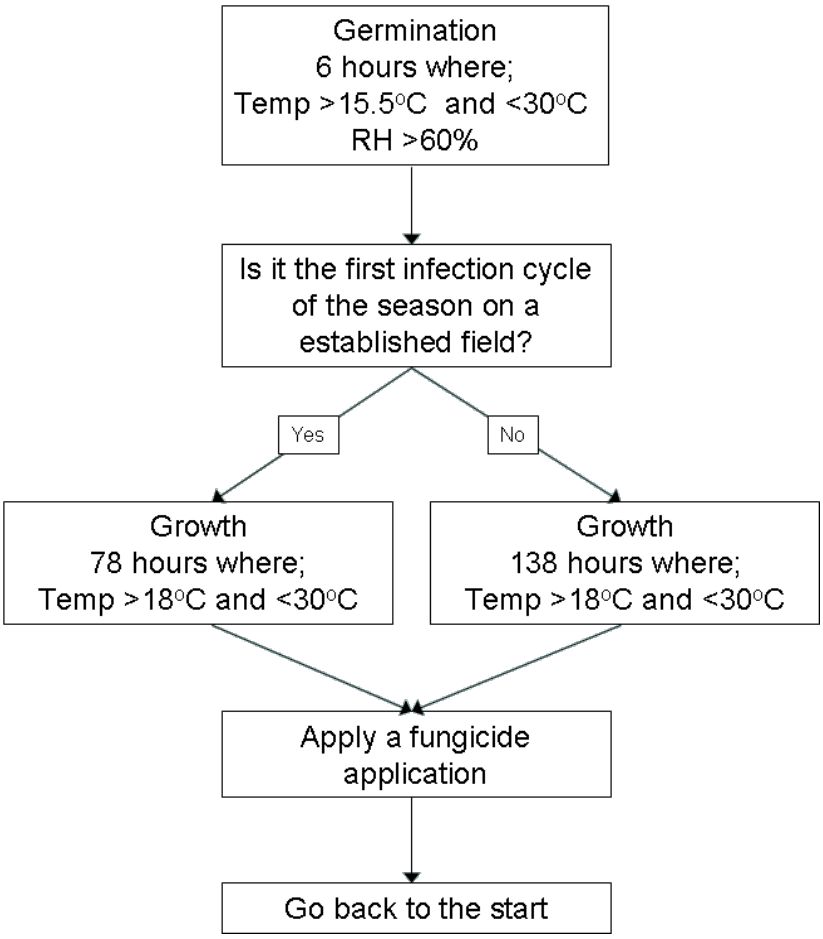


Figure 1.18: The rule-based prediction system is based on conidial data and constant monitoring of the temperature and humidity in a tunnel but only 'counting' the number of hours when the temperature and humidity are suitable for disease development. Source: Dodgson et al. (2007)

Potassium bicarbonate

Potassium (K) is an important basic nutrient for all plants. It increases root growth, enhances uptake of water and nutrients, decreases respiration to prevent energy losses, increases protein and inhibits crop diseases (Datnoff et al., 2007). Huber

and Arny (1985) reported that K-deficient plants are more susceptible to many pathogens than K-sufficient plants, which suggests that K affects host resistance more than it directly affects the pathogen (cited in Datnoff et al., 2007). Potassium bicarbonate is a slightly basic, white crystalline powder. Potassium bicarbonate is soluble in water; however, it is not soluble in alcohol. It is used as a contact fungicide in several crops, such as grapevine, pome and stone fruit, berries and soft fruit, vegetables and cereals (Homma et al., 1981 cited in Reuveni et al., 1996). Potassium bicarbonate was found to be effective in controlling established infection of powdery mildews. Potassium bicarbonate given good control of powdery mildew on soft fruit such as strawberry (Cross and Berrie, 2006 cited in Dodgson, 2007). Dodgson (2007) indicated that potassium bicarbonate mixed with silicon nutrient could enhance the inhibition of strawberry powdery mildew development. Prodorutti et al. (2014) also stated that the spray application with potassium bicarbonate significantly reduced the incidence and severity of powdery mildew compared with the untreated. However, Dodgson (2007) indicated that it was only effective when there was direct contact with the fungal pathogen (Dodgson, 2007).

1.6 Silicon

Silicon (Si) is one of the most abundant elements in soil, both in terms of weight and number of atoms (Ma & Takahashi, 2002). Si dioxide constitutes about 60% of the earth's crust and more than 50% of the soils have Si dioxide in them; it is the second most abundant element in the soil (Epstein, 1994; Datnoff et al.,

2007). Si always exists as silica (Si dioxide) or silicates in nature that are combined with different metals. Moreover, the concentration of Si in soil is between 3.5 to 40 Si L^{-1} in the form of silicic acid (Ma & Takahashi, 2002).

Many plants are able to take up Si, depending on the plant species. The Si concentration accumulated in the biomass can range from 1% to > 10%, depending on the plant species; when the concentration of Si is >1% of the dry weight then that plant is considered as a Si accumulator (Datnoff et al, 2007). The strawberry plant is classified as a non-accumulator. However, Fatema (2014) suggested that the more Si that was applied the greater the concentration of Si absorbed by the strawberry plants in both foliar and root treatment. Although Si has not been considered as vital to plants (for lack of data), species such as *Equisetum* spp. and some of the Diatomaceae cannot survive without Si uptake (Datnoff et al., 2007).

In Japan, the Ministry of Agriculture, Forestry and Fisheries started field trials on slag in 1952. Slag contains a sufficient amount of Si, but free of toxic components (Ma & Takahashi, 2002). The results of those trials suggested the slag has beneficial effects. Therefore, silicate fertilizer was first produced in Japan and Japan is the first country to use slag as a Si source for crops. Slag is also used as a lime material in European and American countries (Ma & Takahashi, 2002). Moreover, Si compounds are ingredients in fertilizers; they promote growth in vegetables, which improves production and enhances the quality of arable soils. Si can mitigate and reduce the toxicity of heavy metals such as Mn, Al, Cr, Cd,

Hg and other poisons, and can also protect plants against pathogen infection, enhancing stress resistance (Lewin & Reimann, 1969).

Si and plant disease

Rice is a very important crop and the significance of Si in rice production was demonstrated in Japan about 80 years ago (Ma & Takahashi, 2002). Application of Si has proved to be effective for control of plant diseases. Table 1.6 shows some of the plant diseases suppressed by Si. High concentrations of Si can prevent fungal spore germination and inhibit mycelial growth (Barker & Pilbeam, 2006). Silicate has been known to suppress plant disease in Japan since the 1920s (Kawashima, 1927). Soluble Si causes physical and physiological changes in plants such as thicken the epidermal cell layer, which can result in an increase in plant resistance against pathogens (Kanto et al., 2007). Si has been observed to control a number of diseases and to protect plants from fungal attack because the pathogen spores deposited on the plant epidermis cannot then penetrate the epidermal cell layer; Si forms a strong physical barrier against penetration by fungal hyphae (Lewin & Reimann, 1969). Si has been shown to increase the yield of strawberry fruit and to enhance their market value (Miyake & Takahashi, 1986). It was also found to suppress other powdery mildews on grapes and roses (Bowen et al., 1992 & Lanning, 1960). Recent research shows that potassium silicate also suppresses powdery mildew on melon when it is supplied to the roots (Dallagnol et al., 2012). It has also been found that there is Si-induced resistance against powdery mildew in rose (Shetty et al., 2012). However, there is lack of

evidence about the mechanism of action of both silicon and potassium bicarbonate and the direct effects on fungi.

Table 1.6: Effects of Si on plant diseases

Crop species	Disease	Pathogen	Effect of Si on disease
Barley	Powdery mildew	*Erysiphe graminis* f. sp. hordei	Decrease
Barley	Black point	*Alternaria* spp.	Decrease
Corn	Stalk rot	*Pythium aphanidermatum, Fusarium moniliforme*	Decrease
Melon	Powdery mildew	*Podosphaera (*sect*. sphaerotheca) xanthii*	Decrease
Rice	Blast	*Magnaporthe grisea*	Decrease
Rice	Brown spot	*Cochliobolus miyabeanus*	Decrease
Rice	Sheath blight	*Thanatephorus cucumeris*	Decrease
Rice	Leaf scald	*Monographella albescens*	Decrease
Rice	Stem rot	*Magnaporthe salvinii*	Decrease
Rice	Grain discoloration	Many fungal species	Decrease
Rose	Powdery mildew	*Podosphaera pannosa*	Decrease
Sorghum	Anthracnose	*Colletotrichum graminicola*	Decrease
Sugarcane	Rust	*Puccinia melanocephala*	No effect
Strawberry	Powdery mildew	*Podosphaera aphanis*	Decrease
Wheat	Powdery mildew	*Blumeria graminis*	Decrease
Wheat	Brown rust	*Puccinia recondita*	No effect
Wheat	Foot rot	*Fusarium* spp.	Decrease
Wheat	Leaf spot	*Phaeosphaeria nodorum*	Decrease
Pea	Leaf spot	*Mycosphaerella pinodes*	Decrease
Soybean	Stem canker	*Diaporthe phaseolorum*	Decrease

Adapted from: Barker & Pilbeam (2006)

1.7 Rationale, aims and objectives

Rationale

Strawberry powdery mildew is a potentially serious disease of strawberries, causing loss in yield and quality. The development and role of *P. aphanis* chasmothecia in overwintering and initial infection in the spring is unclear from the literature. Furthermore, fungicides are not generally used to control chasmothecial production during the autumn, winter or spring. It is hypothesised that spraying fungicides to decrease numbers of chasmothecia in the crop in the non-harvest season will decrease the amount of *P. aphanis* inoculum in the spring. However, there are a limited number of fungicides available. Growers are under pressure to use fewer fungicides in order to reduce residues in fruit. Growers are routinely using potassium bicarbonate in a Si nutrient, and there is anecdotal evidence that Si nutrient reduces disease susceptibility. Thus, it is hypothesised that using Si nutrient will change morphology and physiology to give reduces disease susceptibility in experiments.

Aims

The aims of the project were to: i) investigate the importance of chasmothecia and the chasmothecial development, the sexual, overwintering stage of *P. aphanis* in powdery mildew epidemics on strawberries, investigate the hypothesis that spraying fungicides to decrease numbers of chasmothecia in the crop in the

non-harvest season will decrease the amount of *P. aphanis* inoculum in the spring and to investigate the source of disease in plants that came from a propagator, ii) investigate the hypothesis that the application of Si nutrient reduces disease susceptibility and changes on morphology and physiology of strawberry plants.

Objectives

i) Aim (chapter 3);

1. Develop a scoring method for mycelium coverage of *P. aphanis* (method in chapter 2)
2. Follow the development and progression of *P. aphanis* in the field in order to investigate the correlation between the size and colour of the chasmothecia and the maturation of chasmothecia, relationship to colony coverage
3. Assess the development and maturation of the chasmothecia in the field in order to Identify the favourable temperature and humidity for acsospore release and germination in different year's experiment
4. Investigate the effect of fungicides on development of chasmothecia in the field
5. Identify the favourable temperature and humidity for the development of chasmothecia in the field and laboratory in comparison to the published literature
6. Assess initial inoculum from propagators

ii) Aim (chapter 4);

1. Follow the build-up and progression of *P*. *aphanis* in the field to Investigate the efficacy of Si nutrient (foliar spray application and root treatment through fertigation tube) in reducing disease susceptibility to strawberry powdery mildew
2. Identify the effects of Si nutrient in changing morphology and physiology of strawberry plants
3. Quantify the Si concentration in Si nutrient treated and untreated strawberry plant tissue and investigate the correlation between the si concertation in plant and disease level
4. Location of Si in strawberry plant tissue

Chapter 2 – General materials and methods

2.1 Experiment site

Data for this project were gathered from a commercial strawberry grower in Wisbech and from experiments in two glasshouses belonging to the University of Hertfordshire. Experimental field trials in commercial production were established at Maltmas Farm located near Wisbech in Cambridgeshire (PE14 0HS) (Fig. 2.1 & Appendix 3), a company owned by Harriet and Henry Duncalfe on a Cambridgeshire County Council farm. The total area of the farm is 86 ha. In summer 2013, 27 ha were dedicated to soft fruit production, of which 10 ha were for strawberry production and 17 ha for raspberry production. The remaining area is in an arable rotation, of which 28 ha were winter wheat, 10 ha winter oil seed rape, 15 ha spring wheat and 6 ha sugar beet in 2013.

New strawberry varieties are developed and released almost every year. Therefore, strawberry growers can choose different varieties for their farm. Criteria that guide variety choice include market requirement, yield, length of fruiting season, early fruit (June bearers) or sustained fruit production (Ever bearers) (Table 2.1). Furthermore, fruit size, colour, shape, appearance, brix (sugar content) and flavour are important. At Maltmas Farm, strawberry crops are grown both in open fields (soil grown) (Chapter 1, Fig. 1.12a, 2.2) and under

polyethylene tunnels (Chapter 1, Fig. 1.9 & 1.10, 2.3) (in substrate, generally coir). The harvest period is from April to October. The grower normally uses fleece and mulch to cover the strawberry beds in February in order to encourage early flowering under polyethylene tunnels and in open fields. A fertigation system is used in all the strawberry tunnels and fields at Maltmas Farm; this enables delivery of nutrients and water to the strawberry plants.

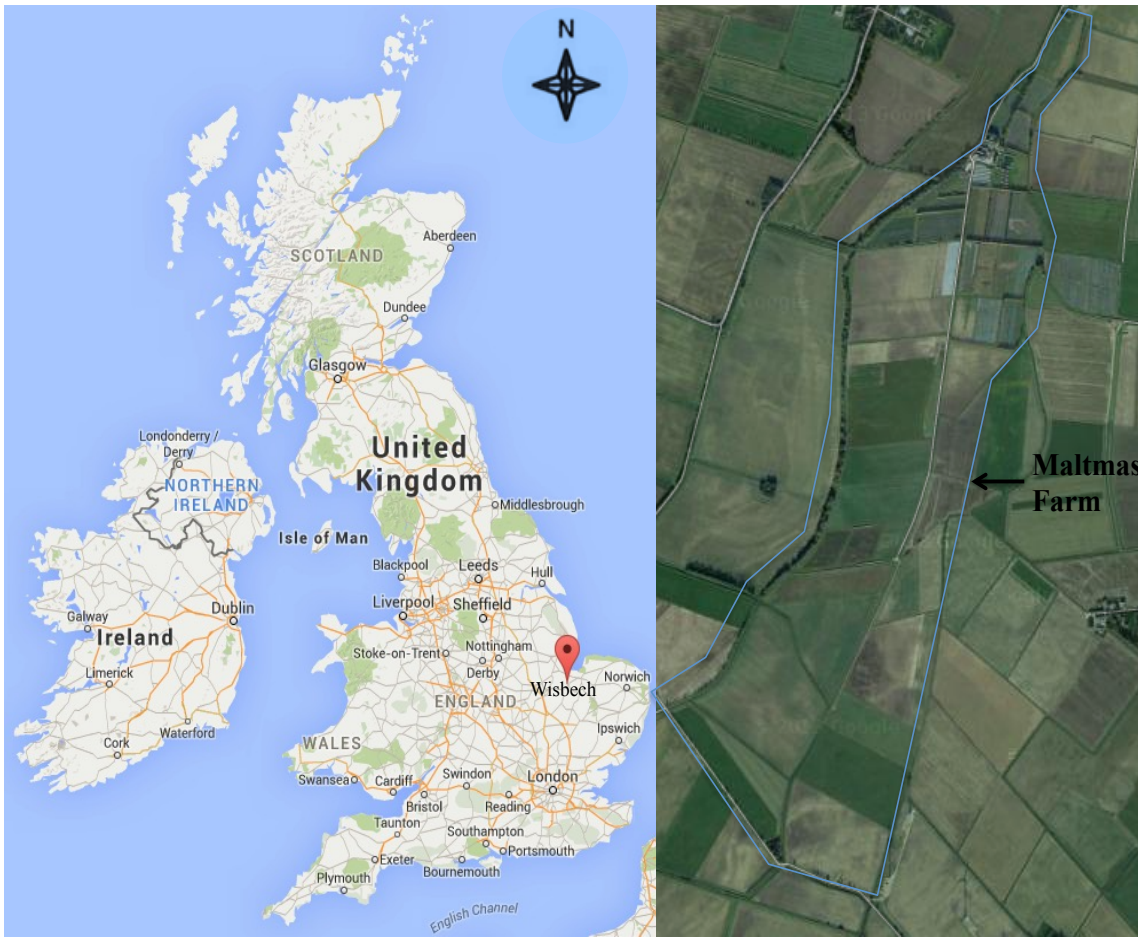


Figure 2.1: Map showing the study site (marked). The map (left) showing Wisbech at north east Cambridgeshire. The map (right) showing the area of Maltmas farm. Source: Google map (2014)

Table 2.1: The comparison of June bearers and Ever bearers

Strawberry Type	Example of Varieties used	Features
June bearers	Virbrant, Rumba, Elegance, Alexandra, Sonata	Always the first fruits to ripen. Easy to grow in many climates. Plants produce many runners.
Ever bearers	Shelley, Amesti	Bloom and set fruit all season, especially between June-November. Plants grow in early spring; high yield variety; produce few runners.



Figure 2.2: Strawberry beds in Diamante field，Maltmas farm during winter in 2012.

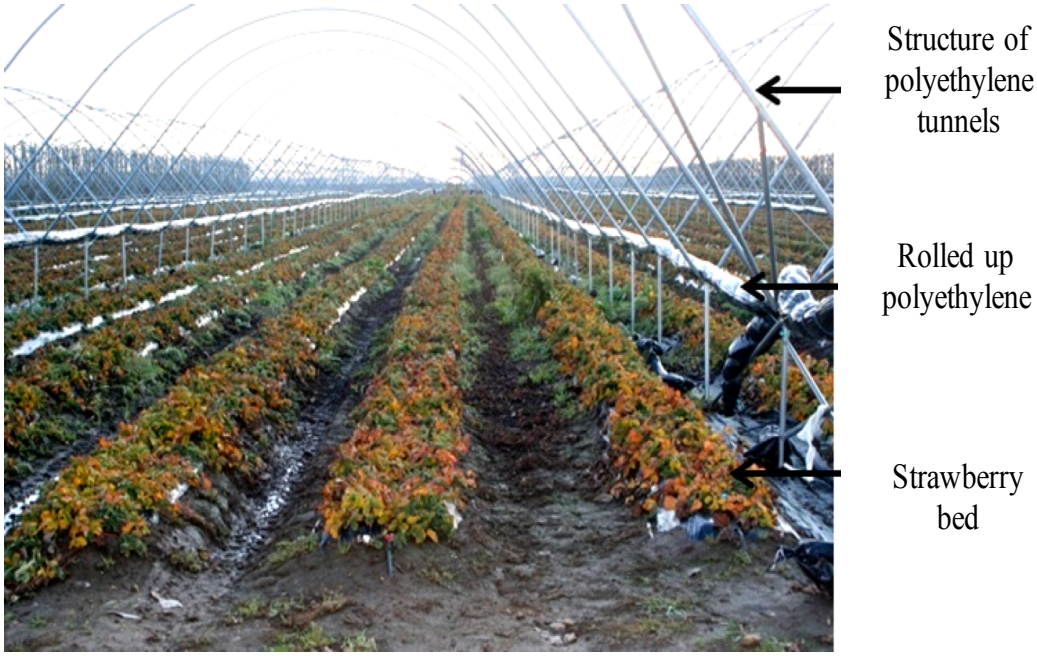


Figure 2.3: Strawberry beds in tunnels with no polyethylene covering at Maltmas farm during winter in 2013.

The strawberry growers select different strawberry varieties to supply the market over an extended period. June bearer varieties were already planted in Diamante and Amelia fields (open fields) (Appendix 3, Map B) and the strawberry plants stay in the ground for 3 winters and 4 harvests. The first harvested crops are between May and June. June bearers were also grown in Ladybird field (under polyethylene tunnels (Appendix 3, Map B). In Roadside and Blackberry fields (both under polyethylene tunnels), annual strawberry varieties (Ever bearers) were grown only for one season and they had a long harvest season from June to November. Field names and positions are shown on maps in Appendix 3, Map B. In 2013 and 2014, 6 ha of the variety Sonata were grown in Diamante field and Amelia field and 4 ha of other varieties (Virbrant, Rumba, Amesti and Shelley) were grown under polyethylene tunnels. The farm supplies Grade 1 strawberries to major UK supermarkets.

The field experiments were carried out from 2011 to 2014 on the fields at Maltmas Farm (Table 2.2). The experimental field experiments were designed to review the effects of different fungicide treatments on development of *P. aphanis* chasmothecia and the development of chasmothecia under different environment conditions each year (see chapter 3) in Diamante, Amelia, Ladybird and Barn fields. The Si nutrient foliar spray experiments were conducted in Diamante and Amelia fields in 2012 and 2013 and the Si nutrient fertigation experiment was conducted in Roadside field in 2013.

2.2 Field experiment methods

Leaf sample, collection and storage

Samples of leaves, petioles and roots were collected from experimental field experiments. The timing of collection varied; weekly, once every two weeks or monthly (see Chapters 3 & 4). Strawberry leaves were sampled by using scissors to cut the stalks and then placed in marked, labelled zip-locked sample bags. The samples were brought back to the College Lane campus, University of Hertfordshire (UH) (AL10 9AB), Hatfield, and stored in a cold room (+ 4 °C) in sealed boxes in the CP Snow building. The leaves sampled were assessed in the laboratory for disease as soon as possible. For further details, see individual chapters (Chapters 3 & 4).

Identification of *Podosphaera aphanis* symptoms in crops

The first sign of infection by *Podosphaera aphanis* is leaf cupping (Chapter 1, Fig. 1.16a). Cupping of the leaf could be seen in the crops. The over-wintering chasmothecial development field experiment involved random sampling (the random sample was taken across a zigzag of the strawberry bed) of cupped leaves in every experimental strawberry bed in the field. Sampling and assessing of cupped leaves for disease severity was also done in the Si nutrient spray experiment (see Chapter 4).

Table 2.2: Field experiments carried out from 2011 to 2014 at Maltmas Farm.

Year	Field name	Cultivation type	Variety	Experiments
2011-2012	Diamante	Open field	Elegance Alexandra Sonata (June bearers)	▪ Chasmothecial development with different fungicide treatments (Chapter 3) ▪ Temperature and humidity monitoring (x 2 Tinytag loggers)
	Ladybird	Tunnel	Elegance (June bearer)	▪ Chasmothecial development with different fungicide treatments (Chapter 3) ▪ Temperature and humidity monitoring (x 2 Tinytag loggers)
2012-2013	Diamante	Open field	Elegance Alexandra Sonata (June bearers)	▪ Predication system experiment and observation of chasmothecial development (Chapter 3) ▪ Temperature and humidity monitoring (x 2 Tinytag loggers)
	Diamante	Open field	Elegance Alexandra Sonata (June bearers)	▪ Si nutrient foliar spray experiment (Chapter 4) ▪ Temperature and humidity monitoring (x 2 Tinytag loggers)
2013-2014	Amelia	Open field	Sonata (June bearer)	▪ Si nutrient foliar spray experiment (Chapter 4) Chasmothecial development (Chapter 3) ▪ Temperature and humidity monitoring (x 2 Tinytag loggers)
	Roadside	Tunnel	Shelley (Ever bearer)	▪ Si nutrient fertigation experiment (Chapter 4) ▪ Temperature and humidity monitoring (x 2 Tinytag loggers)
	Barn	Tunnel	Rumba (June bearer)	▪ Observation of chasmothecial development (Chapter 3)

Temperature and humidity monitoring

Tinytag plus 2 – TGP-4500 loggers with built-in sensors from Gemini Data Loggers (Chichester, West Sussex, PO19 8UJ, weather-proofed from manufacture) were used to monitor temperatures (over a range from -25°C to +85°C) and relative humidity (RH from 0 to 100%) (Anon, 2014). Tinytag loggers were used all year in all experimental field experiments (Fig. 2.4). Tinytag loggers were placed in the strawberry beds to monitor the temperature and humidity in field experiments (Table 2.2). Two Tinytags loggers were set up to record temperature and humidity, one was set up for monitoring temperature and humidity every 10 minutes and another was set up for monitoring every 30 minutes. The temperature and humidity data were downloaded monthly in the over-wintering chasmothecial development experiments and every week in the Si nutrient foliar spray field experiments and Si nutrient fertigation field experiment.

Glasshouse experiments

Three glasshouse experiments were carried out in two glasshouses in 2011-2014 (Table 2.3). Strawberry plants were potted at the UH Hatfield College Lane campus glasshouse near the CP Snow building (AL10 9AB) and at the UH Bayfordbury Research Station, Hertford (SG13 8LD) (Fig. 2.5). The John Innes No. 3 and New Horizon multi-purpose compost were used for planting strawberry

plants in the glasshouse. Strawberry plants were watered automatically when the sensor detected that the mat was dry at the Hatfield campus glasshouse. At the Bayfordbury glasshouse, the automatic watering was set for 30 minutes twice a day for watering the strawberry plants. The lights (ValuTek™ 400W Metal Halide Low Bay Light) and heating were off during summer time and the vents in the roof opened automatically when the temperature was > 25°C. In winter, the lights were on for 12 hours per day and the heating was on for 24 hours a day to maintain a temperature above freezing.



Figure 2.4: Example of two Tinytag data loggers sited on a strawberry bed in Roadside field (2013).

For the 2011 (Table 2.3) glasshouse experiment, 20 strawberry plants were planted at the Bayfordbury glasshouse; the plants were divided into four treatments of five plants each. All plants were placed randomly on the bench after treatment. Three different timings were used for spraying the Si nutrient; were weekly, once every 2 weeks and once every 4 weeks. Two strawberry leaves were sampled from each strawberry plant (for method, refer to 2.2.1) every week.

The 2012 and 2013 glasshouse experiments were done in the Hatfield glasshouse. The fifty strawberry crowns (cv. Elegance) were provided by Maltmas Farm from a propagator and were planted in the Hatfield glasshouse in February 2013. These plants were monitored weekly from April to July for powdery mildew development. In 2014, the strawberry plant runners were planted in new pots and disconnected from the parent plants for the Si nutrient foliar spray and root treatment experiments in the Bayfordbury glasshouse. All plants were assessed weekly for powdery mildew. For further details, see individual chapters (Chapters 3 & 4).

Table 2.3: Glasshouse experiments carried out from 2011 to 2014

Year	Location	Variety	No of plants	Treatments	Assessment
2011	Bayfordbury	Elsanta (June bearer)	20	1. 0.25 % (v/v) Si nutrient (foliar spray) 2. 1.25 % (v/v) Si nutrient (foliar spray) 3. 2.5 % (v/v) Si nutrient (foliar spray) 4. *DI Water (control) (foliar spray)	• Leaf hair assessments (See Chapter 4) • Si concentration (See Chapter 4)
2012	Hatfield	Elegance (June bearer)	20	None	• Assess powdery mildew development on strawberry plant
2013	Hatfield	Elegance (June bearer)	50	None	• Assess powdery mildew development on strawberry plant from propagator (See Chapter 3)
2014	Bayfordbury	Runner of Elegance from 2013 (June bearer)	60	1. 0.017 % (v/v) Si nutrient (root) 2. 0.1 % (v/v) Si nutrient (foliar spray) 3. 0.1% (v/v) Si nutrient (root) 4. 0.25 % (v/v) Si nutrient (foliar spray) 5. DI Water (foliar spray) 6. Untreated(control)	• Assess powdery mildew development on strawberry plant (See Chapter 4) • Si concentration (See Chapter 4) • Biomass (See Chapter 4)

*DI water = deionized water



Figure 2.5: The glasshouse experiment at the UH Bayfordbury Research Station in 2014

2.3 Laboratory methods

Development of a new disease assessment key

The MAFF Strawberry Powdery Mildew Key 8.1.1 (Appendix 8) considered only cupping and red blotches as key symptoms in identifying a *P. aphanis* infection. It is not for identifying mycelium growth on strawberry leaves. Therefore, a new disease assessment key was needed for identifying mycelium growth in the strawberry field. The strawberry leaves were viewed using a dissection microscope (Nikon Labophot microscope, circa 1980s) at both 10(eyepiece)×1(objective) and 10(eyepiece)×3(objective) magnification to

assess the percentage area covered by powdery mildew according to the new assessment key (Fig. 2.6) and the number of colonies was counted on each leaf. The assessment key was developed by assessing the mycelial growth of *P. aphanis* on the strawberry leaves in a damp chamber. The mycelial development on the strawberry leaves was observed every day and photos were taken every two days. Therefore, the photos for the assessment key were drawn according to the photos of strawberry leaves with different percentage areas covered by powdery mildew. ImageJ was used to calculate the percentage areas covered by powdery mildew for each photo. The photographs of strawberry leaves with different percentage areas covered by powdery mildew were selected to give examples with 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60% or 80% of the area covered by powdery mildew.

ImageJ is a public domain Java image-processing program inspired by NIH Image for the Macintosh computer (Anon, 2014). It can calculate area and pixel value statistics of user-defined selections and can measure distances, area and angles. Therefore, all of the leaf sample areas were measured with the ImageJ software (Fig. 2.7). The photo of a leaf sample has to be taken with the leaf alongside a ruler in order to get the known distance then the ImageJ can measure the leaf area in cm^2. Moreover, the colony area (per cm^2) on leaf could then be calculated with an equation as leaf area divided by the colony number; colony area per cm^2 = colony number (count every colony on the leaf) / leaf area (cm^2) (e.g. 0.23 per cm^2 = 15/65 cm^2).

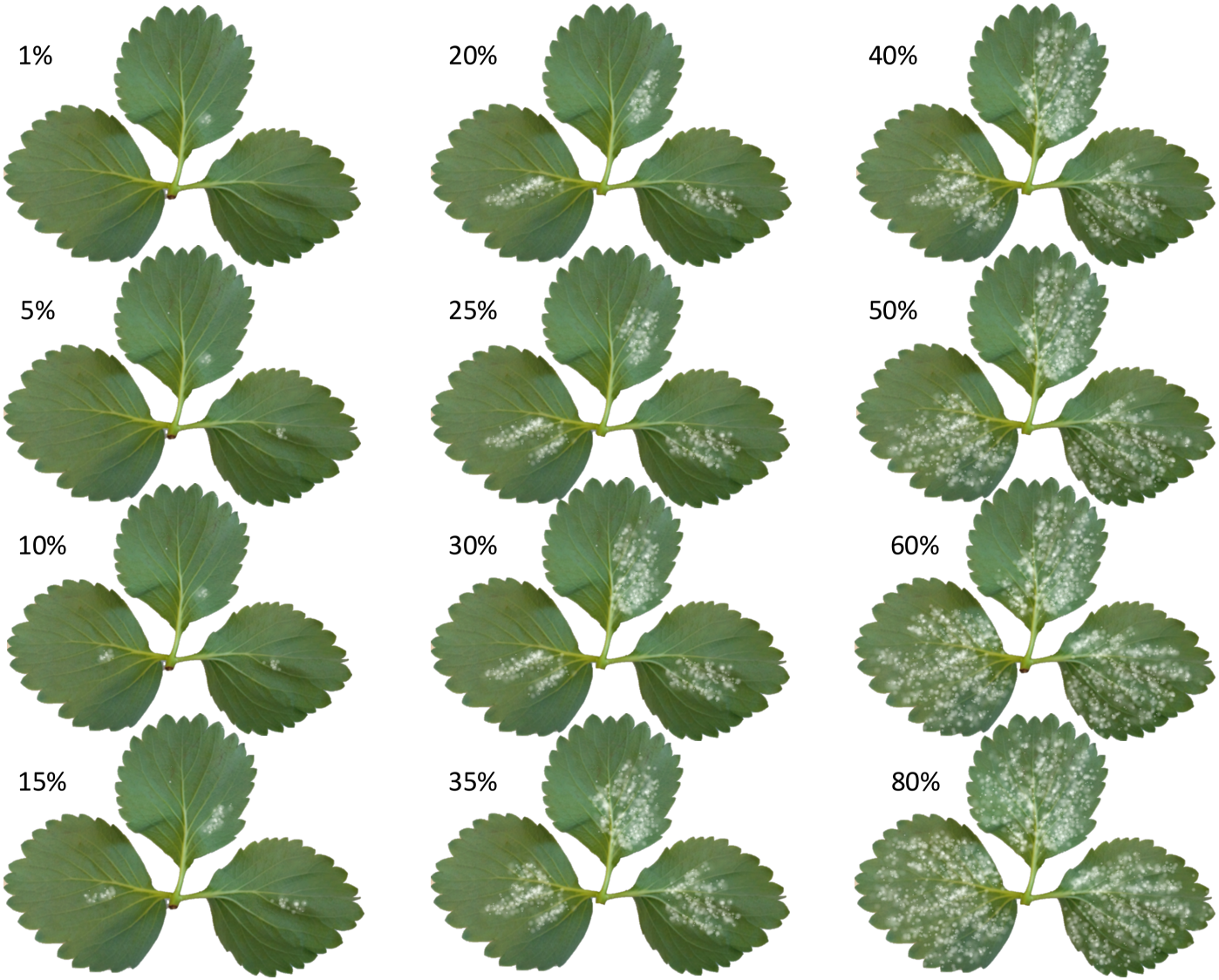


Figure 2.6: New Strawberry powdery mildew assessment key.

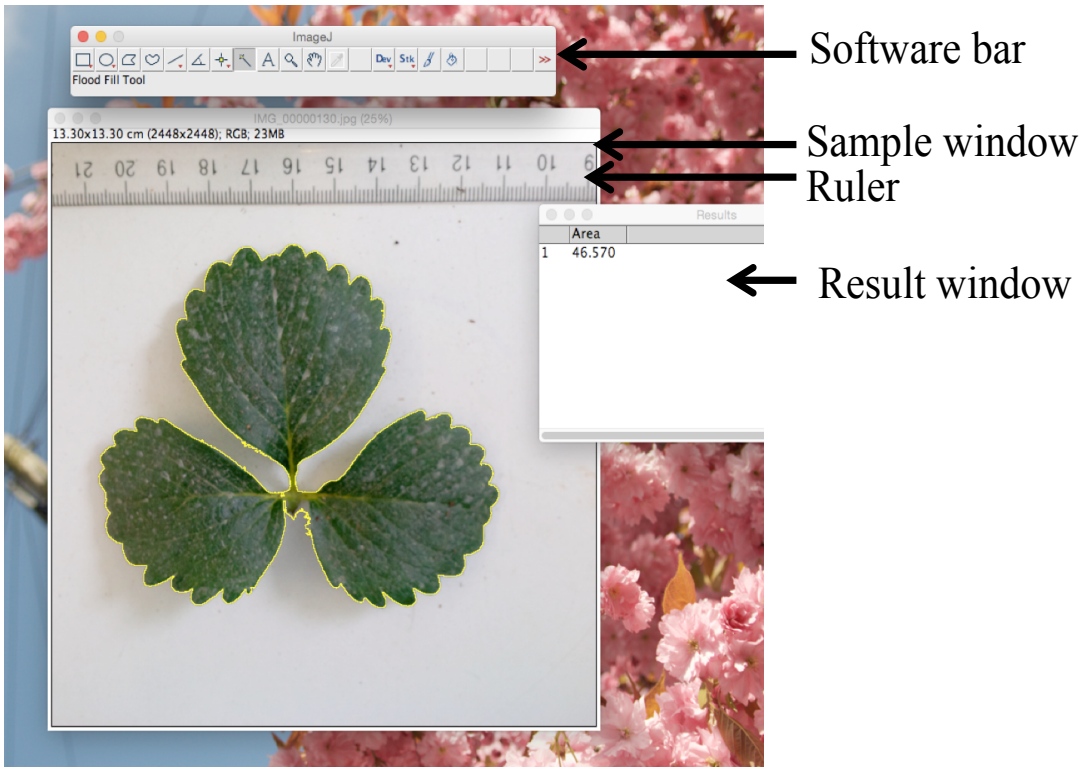


Figure 2.7: The area of a strawberry leaf was measured with the ImageJ software.

The use of the damp chambers

The damp chamber was a plastic container; 35 cm long, 15cm high and 20cm wide (Fig. 2.8), which was used to provide favourable environmental conditions for strawberry powdery mildew development. A tissue paper wetted with DI water was placed at the bottom of the damp chamber in order to create 100% humidity in the damp chamber. Then infected strawberry leaves were placed on the damp tissue (approximately 10 to 20 strawberry leaflets). The damp chamber was placed at 20°C in the laboratory at normal day light cycle. The mycelial and

chasmothecial development on the strawberry leaves was observed daily or every two days for between one months and two, according to the experiments.

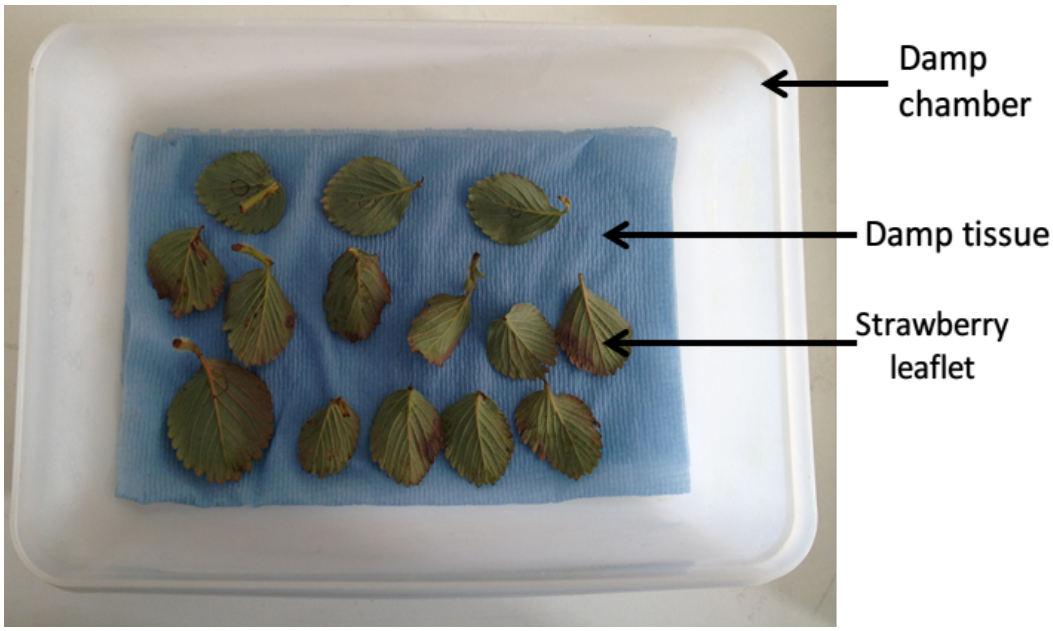


Figure 2.8: Infected strawberry leaflets were placed in a damp chamber to observe mycelial and chasmothecial development.

Preparation of glass microscope slides

Materials required for preparation of slides were industrial methylated spirits (IMS), slides, dissecting needles, cover glasses, forceps and dropper bottle with fungal mounting fluid (85% lactic acid and 15% glycerol). IMS was sprayed onto the bench to sterilize the bench surface. Sterilized dissecting needles or forceps were used to place the samples (e.g. chasmothecia, leaf strips or sections, see Chapters 3 & 4) on the centre of the slide in one or two drops of fungal mounting fluid. A cover glass was added by lowering it at an angle onto the top of the fungal

mounting fluid containing the sample. If necessary, more fungal mounting fluid was added to one edge of the cover glass until the underside of the cover glass was all wetted. Excess fungal mounting fluid was drawn off with a filter paper or absorbent paper if the slide was too wet. Nail varnish was used as a sealer for the cover glass when the sample needed to be kept permanently for storage, reference or imaging.

Preparation of media

Tap Water Agar (TWA)

TWA was used as the basal support medium in the slide culture method and for stimulation of sporulation (Harris, 1986). The preparation requires 15g of agar made up to 1L with tap water. A laboratory balance was used to measure 15g of agar powder, then a weighing boat was used to put 7.5g into each of two-1L glass bottles. A measuring cylinder was used to measure 500ml tap water twice in order to pour 500ml tap water into each bottle containing agar powder. The solution was mixed well; the bottle was labelled and the cap was twisted on loosely, then it was autoclaved (138Pka at 120°C/15 minutes). After autoclaving, the agar was allowed to cool to 40°/45° and poured into Petri dishes.

Photographs with microscopes

Obtaining images of specimens viewed by a microscope required the following equipment; a microscope with an eyepiece, a laboratory digital camera (GXCAM-5 ID1122) for the eyepiece (minimum ×3 optical zoom), a USB cable and a laptop computer (Fig. 2.9). The technique was as follows:

1. Use the microscope, examine the specimen by naked eye and select the area of interest and magnification required.
2. Adjust the light source to maximum intensity.
3. Remove the microscope eyepiece and then place the camera into the eyepiece tube. Link the camera with the laptop using a USB cable; open the camera software on the laptop computer; the image is seen on the screen.
4. Adjust the magnification of the microscope to obtain get a clear image. If the image is still dark, blurry or grainy, go to the setting and then click on 'effect' or 'adjust' to set the exposure, contrast and saturation; at the same time adjust the microscope light, then the image will be much clearer.
5. When the image is really clear, a photograph can be taken, and the image can be previewed; if it is not satisfactory, readjust the setting and retake it.

SEM images

The SEM images of chasmothecia, leaf wax and leaf thickness were taken by Jean Devonshire in the Rothamsted Centre (for method, refer to Appendix 4).

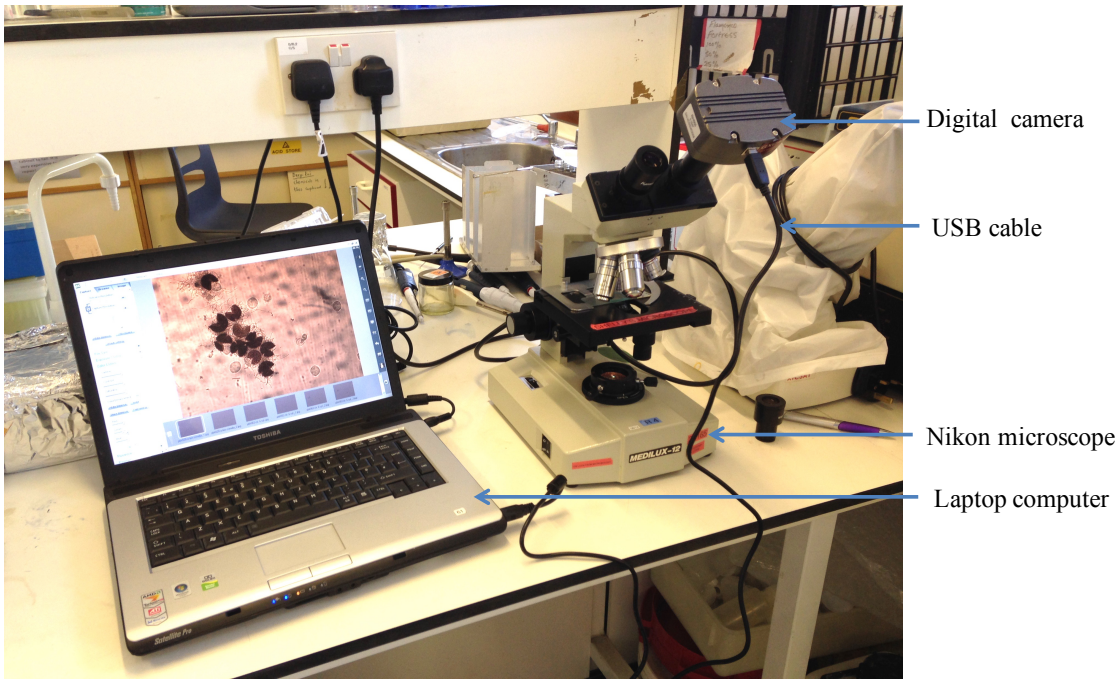


Figure 2.9: Photographs with microscopes in the laboratory. A microscope with eyepiece, a digital camera (GXCAM-5 ID1122) for the eyepiece, USB cable and a laptop computer on the bench.

2.4 Data analyses

Data were saved as Microsoft Excel files. Preliminary analysis, calculation of means etc., was done by Microsoft Excel (2015). Graphs were produced using Microsoft Excel (2015). Analysis of variance (ANOVA) and regression was done using Microsoft Excel data analysis (2015). All of the raw data and data analysis were stored into a CD (as attached).

Chapter 3 – Initiation of strawberry powdery mildew epidemics

3.1 Introduction

The aims of this chapter were to assess the development and maturation of the chasmothecia throughout the year under normal farm conditions and to investigate the use of routine fungicide sprays during the winter to control powdery mildew. Moreover, investigation was carried out to assess the disease level of *P. aphanis* on plants as they came to the farm from the propagator.

In the UK strawberries are normally grown as a perennial crop; the strawberry plants are in the ground for three winters and four harvests. Control of the disease requires a holistic approach throughout the year. The survival structures of strawberry powdery mildew are chasmothecia, which overwinter. When conditions are unsuitable for mycelial growth, the chasmothecia, as sexual overwintering bodies, can provide a route for inoculum to survive between strawberry production seasons (Gourley, 1979). Chasmothecia were first reported in the UK in 1960 (Asalf et al., 2013). Mature chasmothecia form visible round black cases on the leaf (Putnicki & Glawe, 2009). The mature chasmothecia contain eight ascospores within one ascus (Chapter 1, Fig. 1.15c, d). The initiation of chasmothecium formation begins when the androgamocyte and ascogonium are formed from the mycelium of compatible isolates of opposite mating types (Fig. 3.1 A, B). Once the chasmothecia have reached half of their

mature diameter, appendages grow out from the ascocarp wall. The appendages are largely directed downwards towards the mildew colony and become enmeshed with pannose mycelium of the mildew colony on the leaf surface (Fig. 3.1 C, D) (Asalf et al., 2013).

The chasmothecia of *P. aphanis* appear in late summer and autumn in response to changes in environmental conditions (Dodgson et al*.,* 2008). Jin and Hall (2012) and Farooq et al. (2007) demonstrated that the sexual stages of the fungus, the chasmothecia, are produced from August in one year until March/April in the following year. Jin and Hall (2012) also showed that use of fungicides in autumn and spring reduced the numbers of chasmothecia, which led to a reduction in the number of mature chasmothecia. In addition, a fungicide used in the spring reduces the number of germinating ascospores, thus reducing the initial inoculum for epidemic development in April (Jin & Hall, 2012).

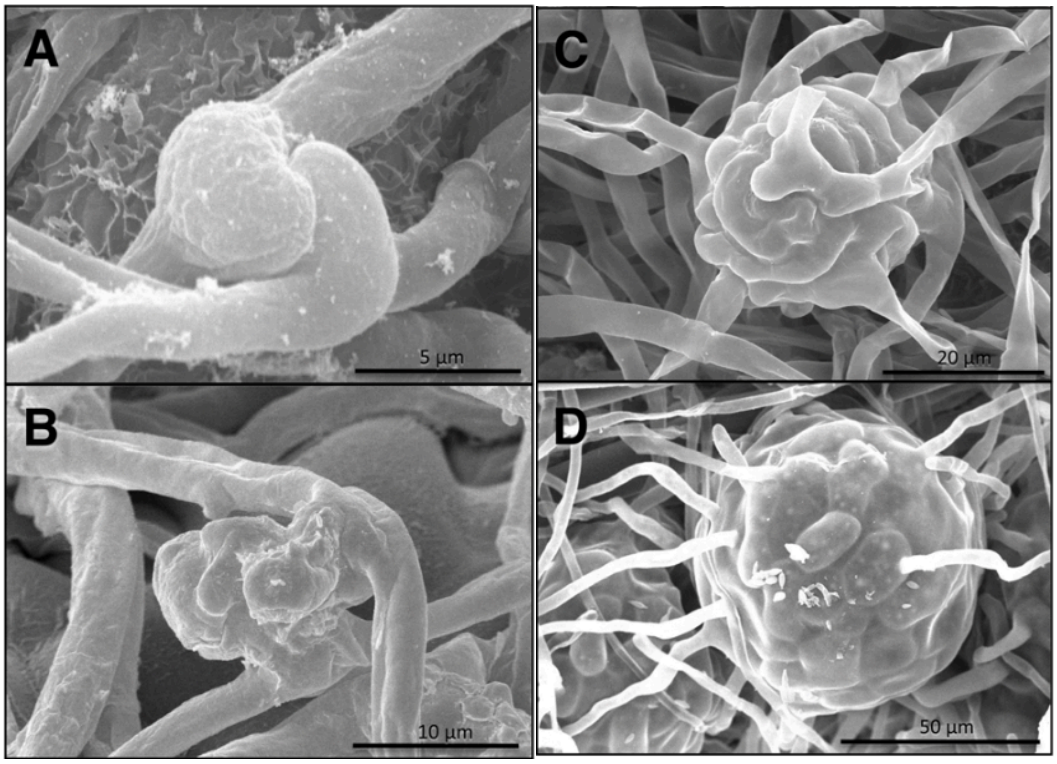


Figure 3.1: Developmental stages of chasmothecia of *Podosphaera aphanis*. A, Initial coiling of androgamocyte and ascogonium following contact by hyphae of compatible mating types; B, fusion of antheridium and ascogonium to form ascocarp initial; C, early formation of hyphal outgrowths from the ascocarp wall; and D, enmeshment of the hyphal outgrowths in the pannose mycelium of the mildew colony as the chasmothecium reached its mature diameter. Source: Asalf et al. (2013)

3.2 Aims and objectives

Aims

The aims of the project were to: i) investigate the importance of chasmothecia and the chasmothecial development, the sexual, overwintering stage of *P. aphanis* in powdery mildew epidemics on strawberries, ii) investigate the hypothesis that spraying fungicides to decrease numbers of chasmothecia in the crop in the non-harvest season will decrease the amount of *P. aphanis* inoculum in the spring and iii) investigate the source of disease in plants that came from a propagator.

Objectives

1. Develop a scoring method for mycelium coverage of *P. aphanis* (method in chapter 2)
2. Follow the development and progression of *P*. *aphanis* in the field in order to investigate the correlation between the size and colour of the chasmothecia and the maturation of chasmothecia, relationship to colony coverage
3. Assess the development and maturation of the chasmothecia in the field in order to Identify the favourable temperature and humidity for acsospore release and germination in different year's experiment

4. Investigate the effect of fungicides on development of chasmothecia in the field
5. Identify the favourable temperature and humidity for the development of chasmothecia in the field and laboratory in comparison to the published literature
6. Assess initial inoculum from propagators

3.3 Materials and Methods

The development of chasmothecia from August 2011 to August 2012, September 2012 to April 2013, August 2013 to April 2014 was assessed and quantified for the numbers of chasmothecia (mature and immature) and the maturity of the chasmothecia in different fields (Ladybird field, Diamante field, Amelia field and Barn field).

3.3.1 The development of chasmothecia and maturation in field experiments

Three field experiments were used to monitor chasmothecial development from 2011 to 2014 (Table 3.1). Strawberry leaves were collected from two fields in August 2011-July 2012; Ladybird field (13 sprays with commercial fungicides, refer to

Table 3.2 and Appendix 15) under polyethylene tunnel and the other Diamante field (8 sprays with commercial fungicides, refer to

Table 3.2 and Appendix 15), an open field at Maltmas Farm near Wisbech. Ladybird field was covered with fleece in October and November 2011 and with fleece and mulch in March and April 2012. The tunnels were covered with

polyethylene from March 2012 until after harvest (June - August) to an enable early harvest. Diamante field was not covered with polyethylene, fleece or mulch. Ten cupped leaves were randomly sampled from each 30m length of a 150m strawberry bed (five replicates) and taken to the laboratory for assessment of percentage area covered with disease and chasmothecial development (refer to Chapter 2，2.2.1). Two Tinytag monitors were used to monitor field temperature and humidity for all field experiments (refer to Chapter 2，2.2.3).

From September 2012 to April 2013, the chasmothecial development and percentage area of leaves covered with disease were assessed in Diamante field. The experiment was laid out in four strawberry beds; two beds were sprayed according to the farm commercial fungicide schedule (refer to

Table 3.2 and Appendix 15) and two beds were untreated. Twenty-five cupped leaves (two replicates) were randomly sampled every month from four beds for assessment from a 30 m length of each of two untreated strawberry beds and two treated beds (Fig. 3.2). Two Tinytag monitors were used to monitor field temperature and humidity for all field experiments (refer to Chapter 2，2.2.3).

Table 3.1：Chasmothecial development field experiments carried out from 2011 to 2014 at Maltmas Farm

Year	Location	Variety	Treatments	Experiments
2011-2012	Diamante and Ladybird fields, Maltmas farm, Wisbech	Elegance Alexandra Sonata (June bearer)	Diamante field (8 sprays with commercial fungicides) Ladybird field (13 sprays with commercial fungicides)	• Chasmothecial development with different fungicide treatments • Temperature and humidity monitoring (x 2 Tinytag loggers)
2012-2013	Diamante field, Maltmas farm, Wisbech	Elegance Alexandra Sonata (June bearer)	• Untreated • Commercial fungicides	• Chasmothecial development with different fungicide treatments • Temperature and humidity monitoring (x 2 Tinytag loggers)
2013-2014	Amelia field Barn field Maltmas farm, Wisbech	Sonata (June bearer) Shelley (Ever bearer	• Untreated • Commercial fungicides	• Chasmothecial development with different fungicide treatments • Temperature and humidity monitoring (x 2 Tinytag loggers)

Table 3.2: Commercial fungicides schedule at Maltmas farm in the Ladybird field and Diamante field during August 2011-July 2012

Fungicides spray timetable (08/2011-07/2012)			
Ladybird field		Diamante field	
08/11/11	THIANOSAN DG	08/11/11	THIANOSAN DG
18/02/12	THIANOSAN DG		
	ROVRAL FLO		
10/04/12	SYSTHANE 20EW	10/04/12	THIANOSAN DG
	FORTRESS		
30/04/12	SIGNUM	03/05/12	SIGNUM
08/05/12	SIGNUM	05/05/12	SWITCH
11/05/12	SWITCH	11/05/12	SWITCH
	SYSTHANE 20EW		SYSTHANE 20EW
28/05/12	TOPAS		
05/06/12	AMISTAR		
10/07/12	SYSTHANE 20EW	10/07/12	SYSTHANE 20EW
	FORTRESS		FORTRESS

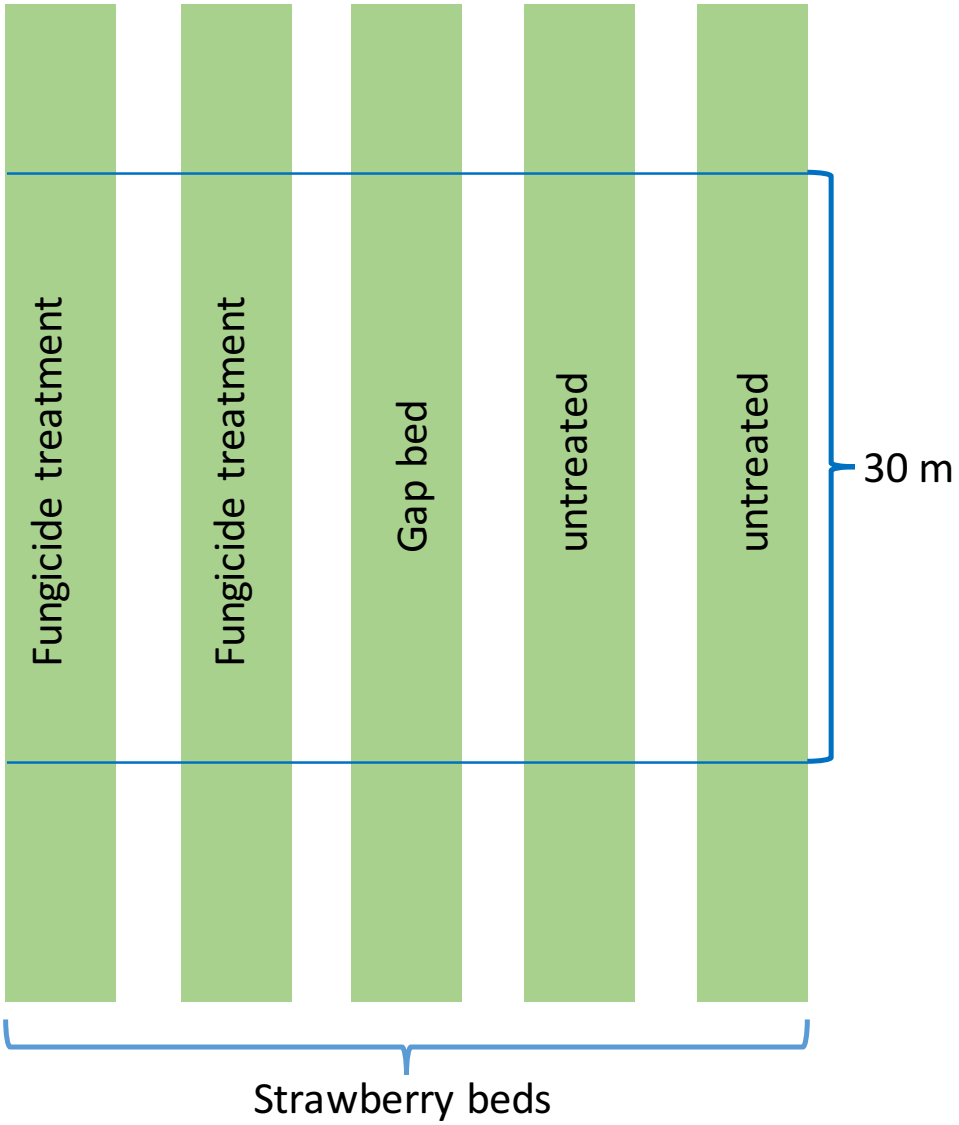


Figure 3.2: The layout of the chasmothecial development experiment in Diamante field in Aug 2012 – Apr 2013.

3.3.2 **Assessment of chasmothecia development and maturation**

Use assessment key to assess the percentage area of leaves covered with disease (see Chapter 2, 2.3.1) and the number of chasmothecia (mature and

immature) per leaf, the leaves were viewed using a dissection microscope (Nikon Labophot microscope, circa 1980s) at 10(eyepiece)×1(objective) or 10(eyepiece)×3(objective) magnification to identify leaves with chasmothecia or without chasmothecia and to assess the colour of the chasmothecial shell. The colours of the chasmothecia are related to their maturity; the mature chasmothecia are generally dark brown at 10×1or 10×3 magnification (Putnicki & Glawe, 2009). However, the colour of the mature chasmothecial shell is different when assessed under 10x10 magnification , compared to 10×1 or 10×3 magnification . Therefore, the colour of the mature chasmothecial shell was recorded as three colours, dark brick, rust and sienna (Anon, 1969), in order to investigate the interaction between the colour of the mature chasmothecial shell and their maturity (Fig. 3.3). A tally chart was then used to record the chasmothecial development for each experiment (Table 3.3).

Five dark brown chasmothecia were selected from one leaflet with chasmothecia; thus 250 chasmothecia (i.e. 50 leaflets) were assessed each field experiment each month (2011-2014). Chasmothecia were then placed on a slide with one drop of fungal mounting fluid and covered by a cover slip (see Chapter 2, 2.3.3). Five chasmothecia per leaflet were observed under 10×10 magnification (Nikon, YS100) for maturity, i.e. when eight ascospores were present in the ascus. The chasmothecia and ascospores were measured for length and width using an eyepiece graticule at 10×10 magnification . Thus, the cross-sectional areas of the chasmothecia were calculated by using equation $s = \pi r^2$, where r is the average length and width of chasmothecia divided by two.

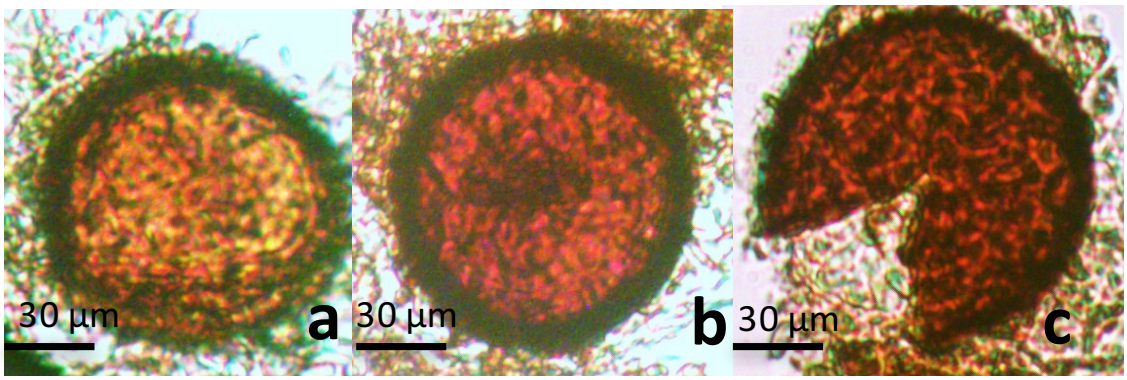


Figure 3.3: The different colours of the chasmothecial shell at 10x10 magnification . a) sienna shell b) rust shell c) dark brick shell.

Table 3.3: A tally chart was used to record the assessment of chasmothecial development in field experiments.

% of disease cover	±Chasmothecia	No. of chasmothecia (per leaf)	Length (µm)	Width (µm)	Shell colour (dark brick/rust /sienna)	No. mature	Size of ascospore
			(Assessments were recorded from 5 chasmothecia per leaflet)				
%	Yes/No		X µm	Y µm			µm

3.3.2.1 Assessment of germinating ascospores on strawberry leaflets

To assess the number of ascospores and germinating ascospores on leaflets, twenty leaflets (i.e. four replicates) with chasmothecia were selected form the leaf samples were sampled for each treatment. Leaflets were cleared (to remove colouration) in 80% IMS for 48 h to get a clear view of ascospore germinating on leaflets (Fig. 3.4a). Then leaflets were washed with running tap water, placed in Petri dishes and submerged in 0.1% trypan blue (Fig. 3.4c). Leaflets were left to stain for 24 h in the laboratory (20°C), then washed with running tap water and cut into two or four strips. The strips (Fig. 3.4d) were made into slides, abaxial surface up. The assessments were viewed using the microscope at 10×10 or 10×40 magnification to count ascospore on each strip (Fig. 3.4e & f) .

3.3.3 Effects of temperature and relative humidity on chasmothecial development

3.3.3.1 Chasmothecia development in damp chamber

Leaflets with white immature chasmothecia were placed in a damp chamber (see Chapter 2, 2.3.2) at 20°C and relative humidity (>97%) to assess the progress of their maturation. The leaflets were observed every 2 days to assess the colour change and maturity of chasmothecia. The assessments were done using the microscope at 10×1 or 10×3 magnification.

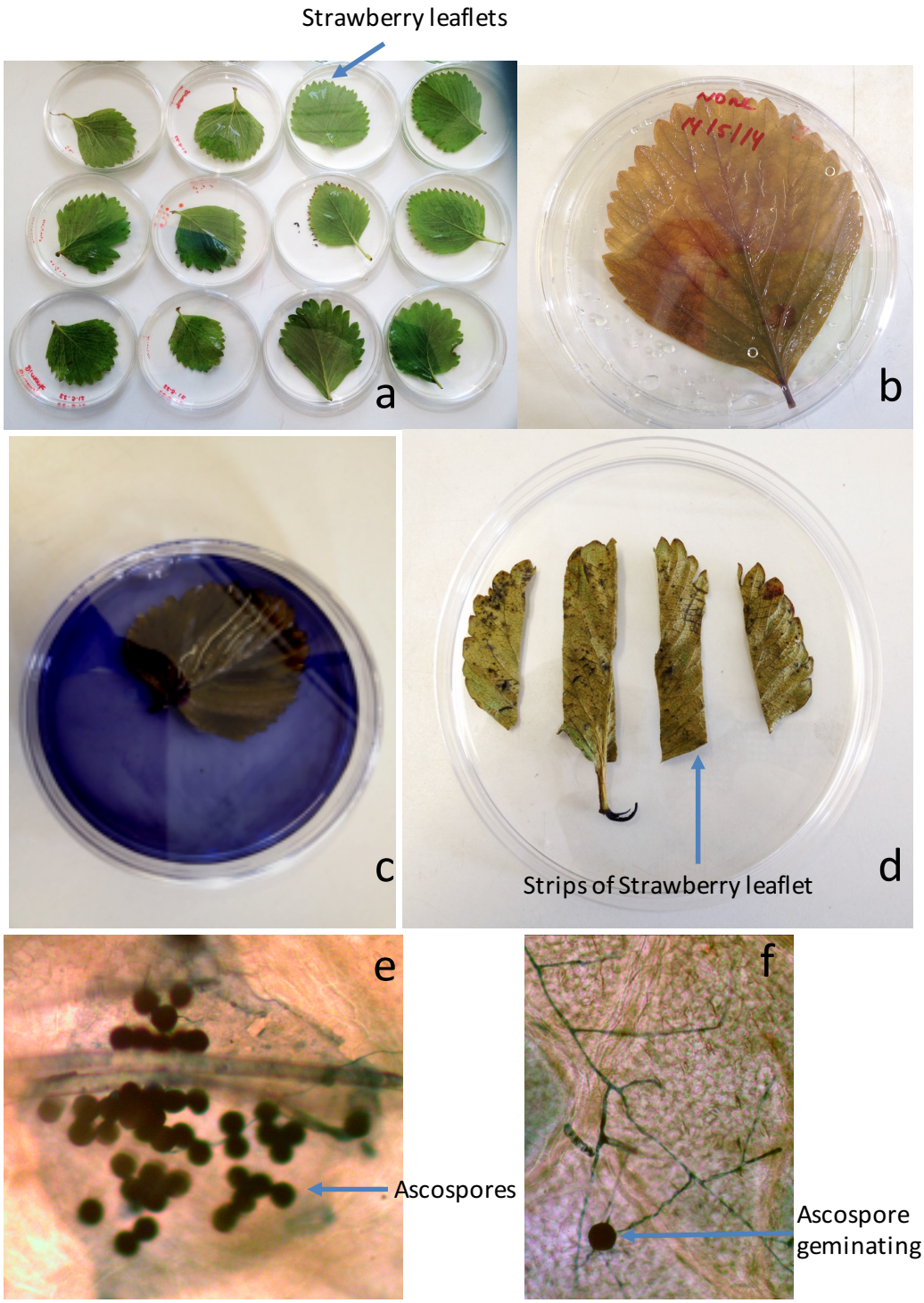


Figure 3.4: (a) Leaflets were cleared in 80% Industrial Methylated Spirit (IMS) for 48 h; (b) leaflet was taken out from 80% IMS and rinsed with running tap water; (c) leaflets stained in 0.1% trypan blue for 24 h; (d) leaflet cut into 4 strips; (e) stained ascospores on leaflet; (f) stained ascospore geminating on leaflet.

3.3.3.2 The development of mature chasmothecia at different temperatures

Leaflets with mature chasmothecia were cut into small strips, with approximately 15 to 20 chasmothecia on each strip. The strips with chasmothecia were sterilized with IMS. Vaseline was used to stick the strips on the lid of tap water agar (TWA) Petri dishes (Fig. 3.5). The use of TWA was to create relative humidity (>97%) for chasmothecial development. Three different storage methods were compared for chasmothecia, which had been kept either in the deep freezer at -20°C for eighteen months (sample A), or in the cold room for one month at 4°C (sample B) or in the cold room for one week at 4°C (sample C) (three replicates for each sample). The samples were removed from storage and placed at different temperatures of 15ºC, 20ºC, 25ºC and 30ºC to find the optimal temperature for chasmothecial natural opening curve to occur, for ascus release and the release of ascospores on agar. The assessments were done using the microscope at 10×10 or 10×40 magnification .

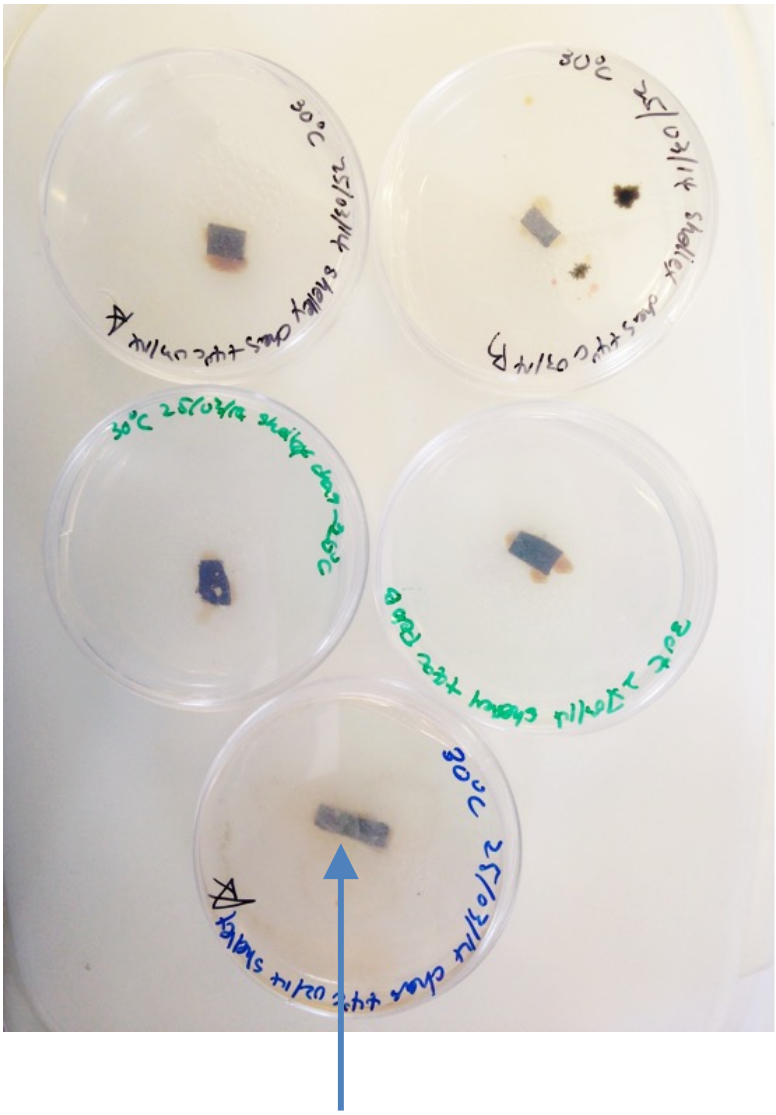


Figure 3.5: The strips with chasmothecia were placed on TWA in Petri dishes.

3.3.3.3 Assessment of diseases on new strawberry plants from propagator

The 2013 glasshouse experiment was done in the College Lane Campus (UH) glasshouse. The strawberry crowns (cv. Elegance) from a propagator were

planted in the glasshouse in February 2013. These plants were assessed weekly for 4 months for powdery mildew development. The runners were disconnected from the parent plants and repotted. Runner plants were also assessed for powdery mildew development weekly. Moreover, the plants for the Si nutrient fertigation experiment were assessed for strawberry powdery mildew symptoms on arrival at the growers. *[In addition, the strawberry leaf spot (*Mycosphaerella fragariae)* was also assessed on the the plants for the Si nutrient fertigation experiment (Appendix 10)].

3.3.4 **Statistical analysis**

The significant difference of the numbers of chasmothecia (mature and immature) and the numbers of mature chasmothecia between fields in each experiment (2011-2014) were analysed using two-sample binomial test. Two-sample binomial test was done using R version 3.1.3.

3.4 Results

3.4.1 The development of chasmothecia in the 2011-2012 field experiment

In 2011-2012, the results between figure 3.6 and 3.7 showed that there were significantly ($P < 0.01$) greater numbers of chasmothecia (mature and immature) and greater numbers of mature chasmothecia in Diamante field, which had received 8 fungicides sprays (Table 3.2 & Appendix 15), compared with Ladybird field, which had received 12 fungicide sprays. The binomial test also showed there was a significant difference in September 2011 ($P = 0.001$) and March 2012 ($P < 0.05$) between Diamante field and Ladybird field (Appendix 9). This result indicated that fewer chasmothecia developed when there was more frequent fungicide spraying.

Moreover, there was a significant correlation ($P < 0.001$) between the percentage area covered with disease on leaves and the number of chasmothecia (Table 3.4), which showed that fewer chasmothecia were formed where there was a smaller percentage area covered with disease than where there was greater area covered with disease.

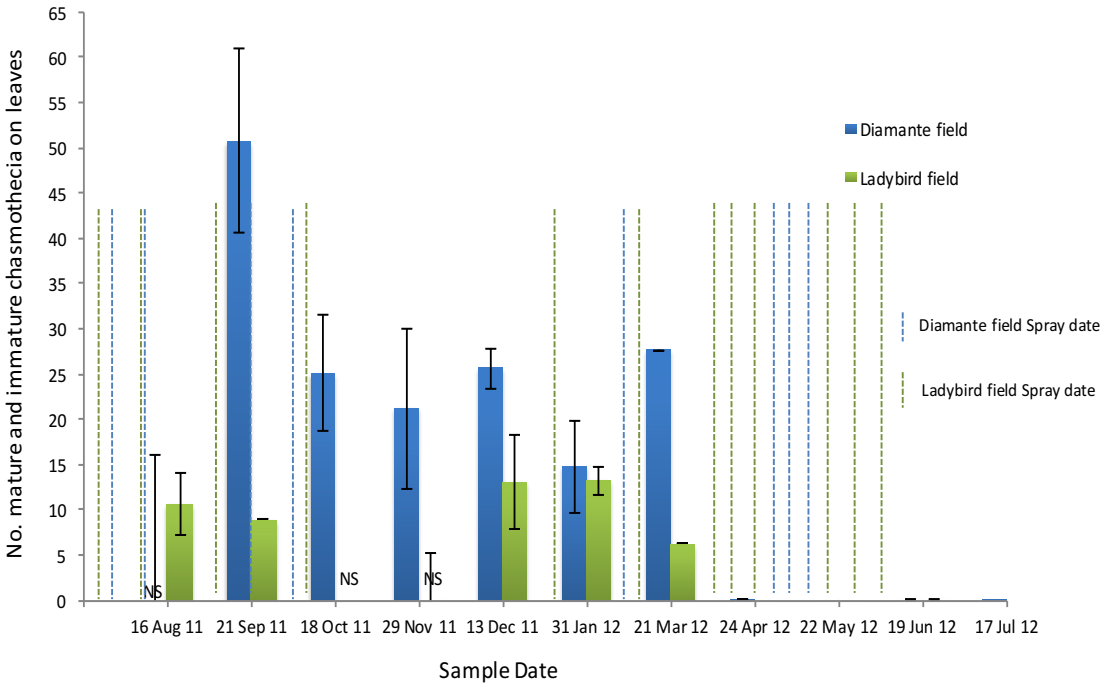


Figure 3.6: The average number (mature and immature) of *Podosphaera aphanis* chasmothecia per leaflet on 50 strawberry leaves (cv. Elsanta, Alexandra & Sonata) sampled each month from Diamante field (open field) (8 sprays with fungicide) and Ladybird field (polyethylene tunnel) (13 sprays with fungicide) ($P < 0.01$) from August 2011 to July 2012 at Maltmas Farm near Wisbech in Cambridgeshire (see Appendix 15 for spray dates in the two fields). NS= did not sample. Bars represent standard errors of mean; 4 degrees of freedom.

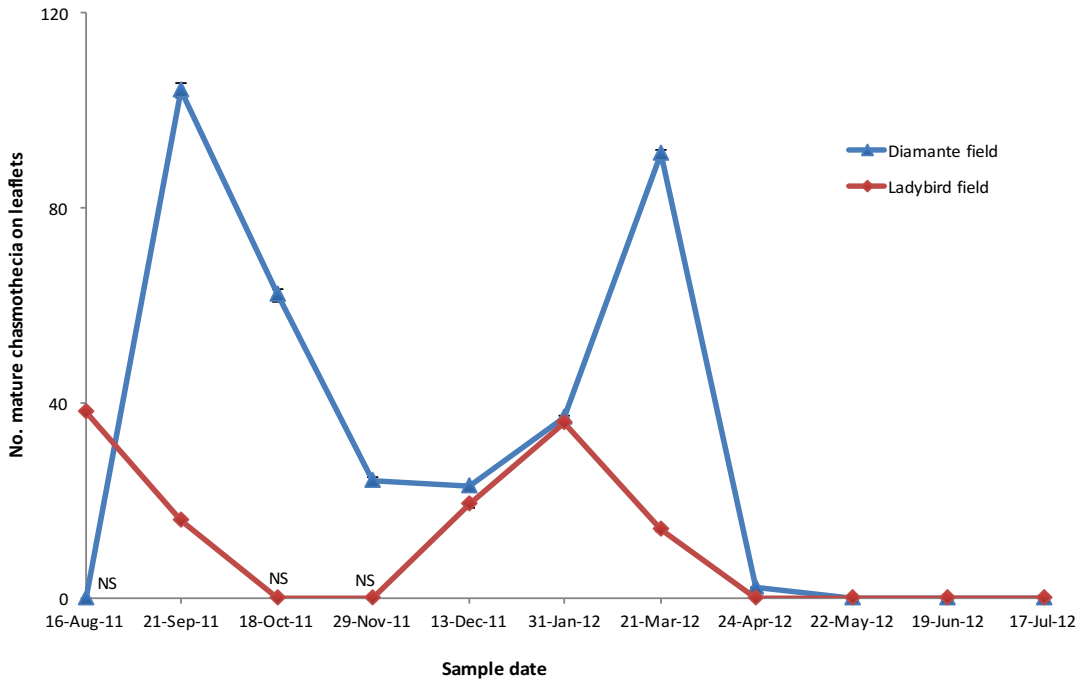


Figure 3.7: The average number of mature *P. aphanis* chasmothecia per leaflet from 50 strawberry leaflets sampled (cv. Elsanta, Alexandra & Sonata) each month from Diamante field (open field) (8 sprays with fungicide) and Ladybird field (polyethylene tunnel) (13 sprays with fungicide) ($P < 0.01$) from August 2011 to July 2012 at Maltmas Farm near Wisbech in Cambridgeshire (see Appendix 15 for spray dates in the two fields). NS= did not sample. Bars represent standard errors of mean; 4 degrees of freedom.

Table 3.4: The results of regression analysis between number of chasmothecia (immature and mature) on the percentage of disease cover on leaves in Ladybird field (polyethylene tunnel) (13 sprays with fungicide) or Diamante field (open field) (8 sprays with fungicide) in 2011-2012.

	No. chasmothecia (immature and mature)		
	R	R^2	*P*
Ladybird field			
% disease cover	0.78	0.61	< 0.001
Diamante field			
% disease cover	0.90	0.80	< 0.001

Figures 3.8 and 3.9 showed that when the relative humidity in Diamante field was greater than that in Ladybird field, the temperature in Diamante field was lower than that in Ladybird field, especially in December, January and March (from Aug 2011 to July 2012). The chasmothecia developed rapidly when the temperature was < 20 ºC and humidity > 70% in Diamante field, which indicated that the sexual stage started in autumn when temperature decreased to < 20 ºC (Fig. 3.8). The amount of disease in Diamante field was greater than that in Ladybird field, which again indicated that higher percentage of disease cover where more chasmothecia were formed (Fig. 3.8 & 3.9). Thus, this demonstrated that the Ladybird field had less chasmothecia because the amount of disease had been

less than in the Diamante field (Fig. 3.9). Furthermore, the different environmental conditions in the two fields brought in an additional variable. The temperature in the Ladybird field was higher than in the Diamante field and the humidity was lower.

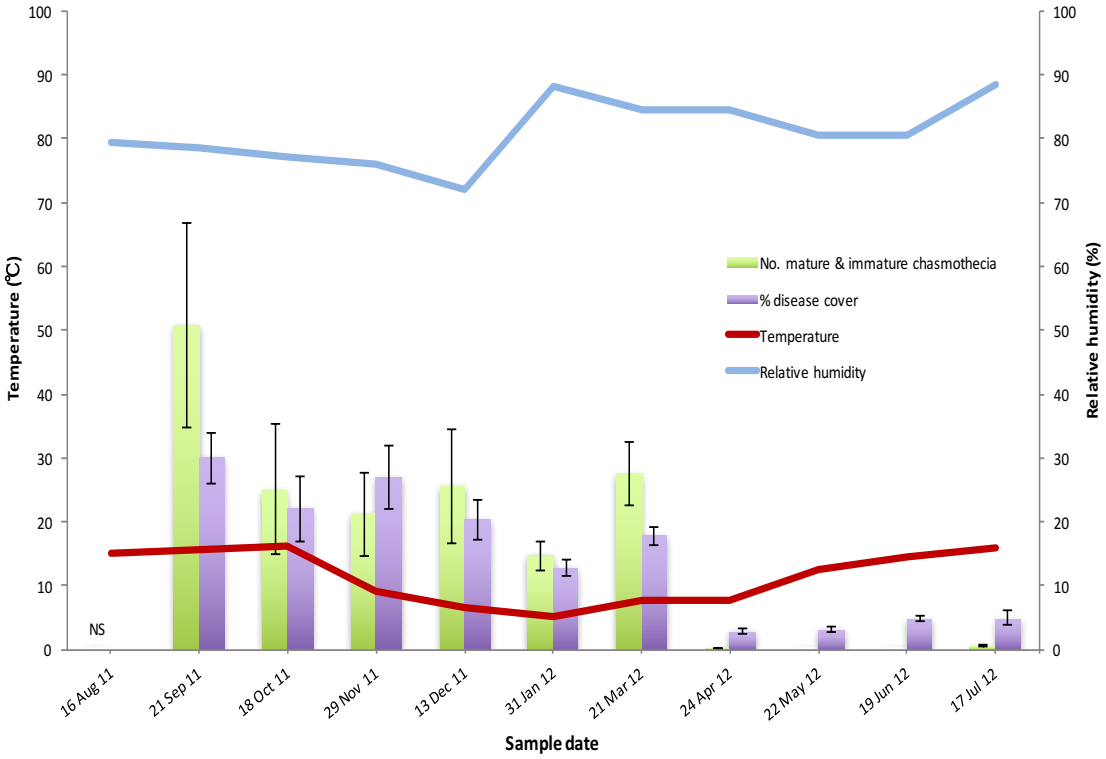


Figure 3.8: The average number of mature and immature chasmothecia per leaflet from 50 strawberry leaflets sampled (cv. Elsanta, Alexandra & Sonata) each month from Diamante field (open field) (8 sprays with fungicide) in Aug 2011 to July 2012. The temperature and relative humidity were measured in Diamante field from Aug 2011 to July 2012 at Maltmas Farm (data= mean monthly temperature and humidity). NS= did not sample. Bars represent standard errors of mean, 4 degrees of freedom.

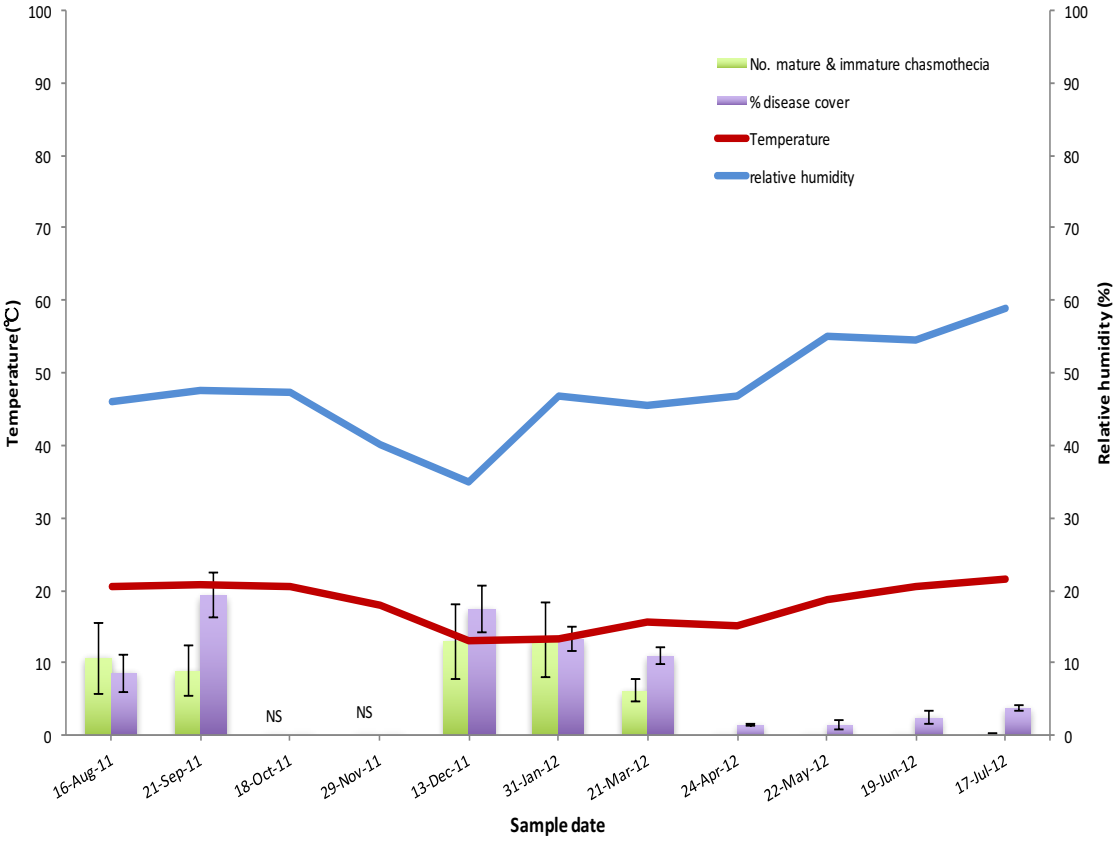


Figure 3.9: The average number of mature and immature chasmothecia per leaflet from 50 strawberry leaflets sampled (cv. Elsanta, Alexandra & Sonata) each month from Ladybird field (polyethylene tunnel) (13 sprays with fungicide) in Aug 2011 to July 2012. The temperature and relative humidity were measured in Ladybird field from Aug 2011 to July 2012 at Maltmas Farm (data= mean monthly temperature and humidity). NS= did not sample. Bars represent standard errors of mean, 4 degrees of freedom.

3.4.2 Assessment of chasmothecial development and maturation in the 2011-2012 field experiment

The cross-sectional area of chasmothecia ranged from 0.004 mm^2 (minimum mean) to 0.009 mm^2 (maximum mean). There was a significant ($P < 0.0001$) correlation between the cross-sectional area of chasmothecia and the numbers of mature chasmothecia in both Diamante field open field (8 sprays with fungicide) and Ladybird field polyethylene tunnel (13 sprays with fungicide), which indicated that the larger cross-sectional area of chasmothecia led to the maturation of chasmothecia (Table 3.5). The correlations between the numbers of mature chasmothecia and the different colour of the shell of the chasmothecia (Sienna, Rust and Dark brick) were significant ($P < 0.005$, $P < 0.0001$) from both Diamante field and Ladybird field. The cross-sectional area of chasmothecia also showed a significant ($P < 0.005$, $P < 0.0001$) correlation with the different colour of the shell of the chasmothecia in the Ladybird field (Table 3.5).

Table 3.5: The results of regression analysis showed the correlation between the numbers of mature chasmothecia with the different colour of the shell of the chasmothecia (Sienna, Rust and Dark brick) and the cross-sectional area of chasmothecia from both Diamante field open field (8 sprays with fungicide) and Ladybird field polyethylene tunnel (13 sprays with fungicide) from August 2011 to April 2012 at Maltmas Farm near Wisbech in Cambridgeshire.

	Sienna shell of chasmothecia		Rust shell of chasmothecia		Dark brick shell of chasmothecia		Cross-sectional area of chasmothecia	
	R	R^2	R	R^2	R	R^2	R	R^2
Diamante field								
No. mature chasmothecia	0.46	0.21a	0.89	0.79b	0.44	0.19a	0.66	0.44b
cross-sectional area of chasmothecia	0.56	0.32c	0.48	0.23a	0.23	0.05d		
Ladybird field								
No. mature chasmothecia	0.63	0.40b	0.84	0.71b	0.47	0.23a	0.90	0.82b
cross-sectional area of chasmothecia	0.53	0.28c	0.78	0.60b	0.49	0.24a		

a: $P < 0.005$, b: $P < 0.0001$, c: $P < 0.0005$ & d: $P < 0.2$

3.4.2.1 Germinating ascospores on strawberry leaflets in the 2011-2012 field experiment

The number of ascospores on leaflets in Diamante field (open field) (8 sprays with fungicide) was greater than that in the Ladybird field (polyethylene tunnel) (13 sprays with fungicide) (Fig. 3.10). The ascospores started to germinate in April in Diamante field; those in the Ladybird field started in May (Fig. 3.11), this indicated that the initial inoculum started earlier in Diamante field than in Ladybird field. This also indicated that a late autumn fungicide spray results in fewer chasmothecia being formed, thus also contributing to a reduction in initial inoculum in the following spring. Moreover, the chasmothecia disappeared during the period April to June because the chasmothecia released the ascospores and dropped off from the leaf. However, there was no significant difference ($P > 0.05$) in the number of ascospores and germinated ascospores between the Diamante field and the Ladybird field.

3.4.3 The development of chasmothecia in the 2012-2013 field experiment

In 2012 - 2013, the results between figure 3.12 and figure 3.13 showed that there was a significant difference between the untreated and fungicides treatment (commercial fungicide application schedule is in Appendix 15) in the numbers of chasmothecia (mature and immature) ($P < 0.001$) and numbers of mature chasmothecia ($P < 0.05$) in the Diamante field. The binomial test also showed there were significantly ($P < 0.05$) greater numbers of chasmothecia (mature and

immature) observed in November 2012 and January 2013 in the untreated compared with the fungicide treatment. Furthermore, in the fungicide treated beds only small numbers of chasmothecia were formed from September to November, which indicated that fewer chasmothecia developed when there were more frequent sprays (Appendix 15). These complement the results of the previous year.

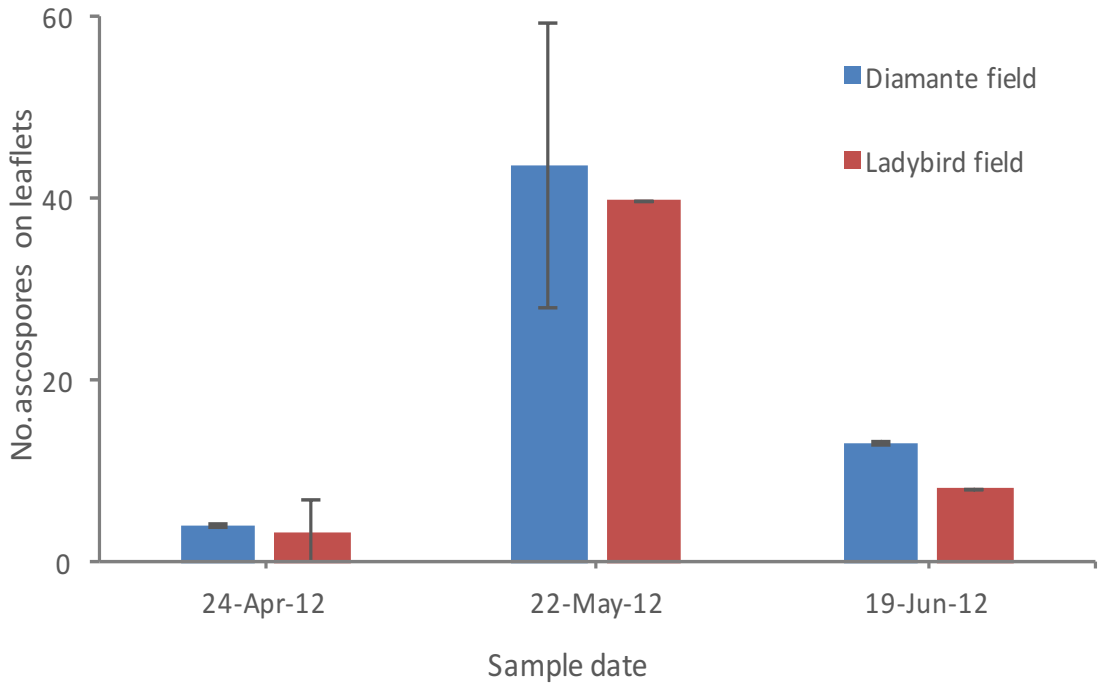


Figure 3.10: The average number of ascospores per leaflet from 20 strawberry leaflets sampled (cv. Elsanta, Alexandra & Sonata) each month from Diamante field (open field) (8 sprays with fungicide) and Ladybird field (polyethylene tunnel) (13 sprays with fungicide) from April to June in 2012 at Maltmas Farm near Wisbech in Cambridgeshire. Bars represent standard errors of mean, 2 degrees of freedom.

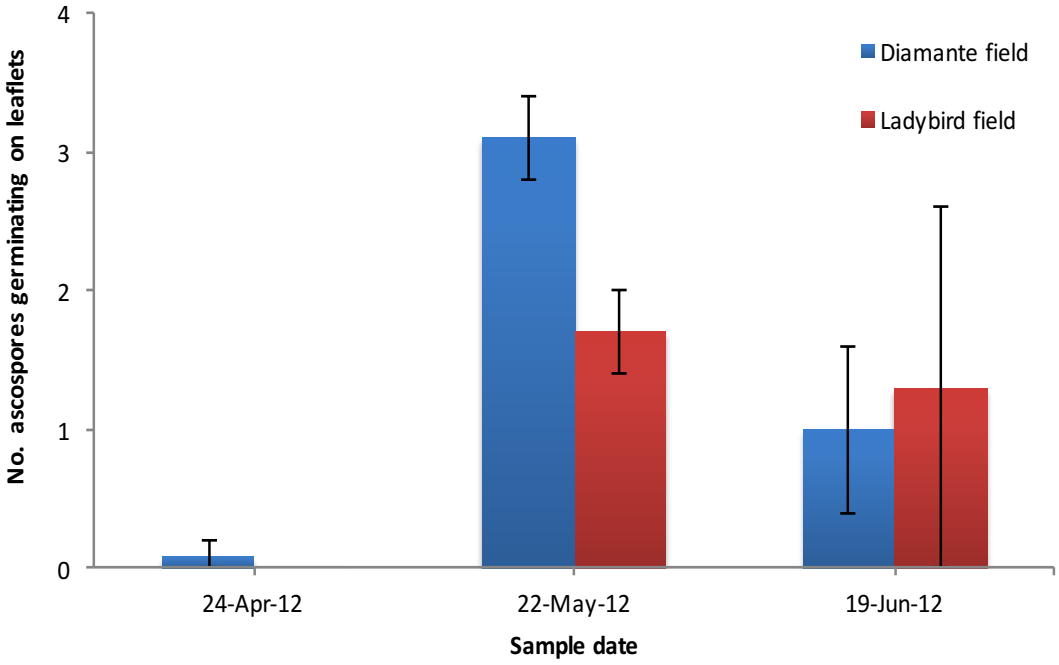


Figure 3.11: The average number of germinating ascospores per leaflet from 20 strawberry leaflets sampled (cv. Elsanta, Alexandra & Sonata) each month from Diamante field (open field) (8 sprays with fungicide) and Ladybird field (polyethylene tunnel) (13 sprays with fungicide) from April to June in 2012. Bars represent standard errors of mean, 2 degrees of freedom.

Moreover, there were significantly greater numbers of mature chasmothecia in October ($P < 0.05$), November ($P < 0.01$) and December ($P < 0.01$) in the untreated compared with the fungicides treatment (Fig. 3.13). There were no mature chasmothecia in the fungicide treatment during September to November (Fig. 3.13).

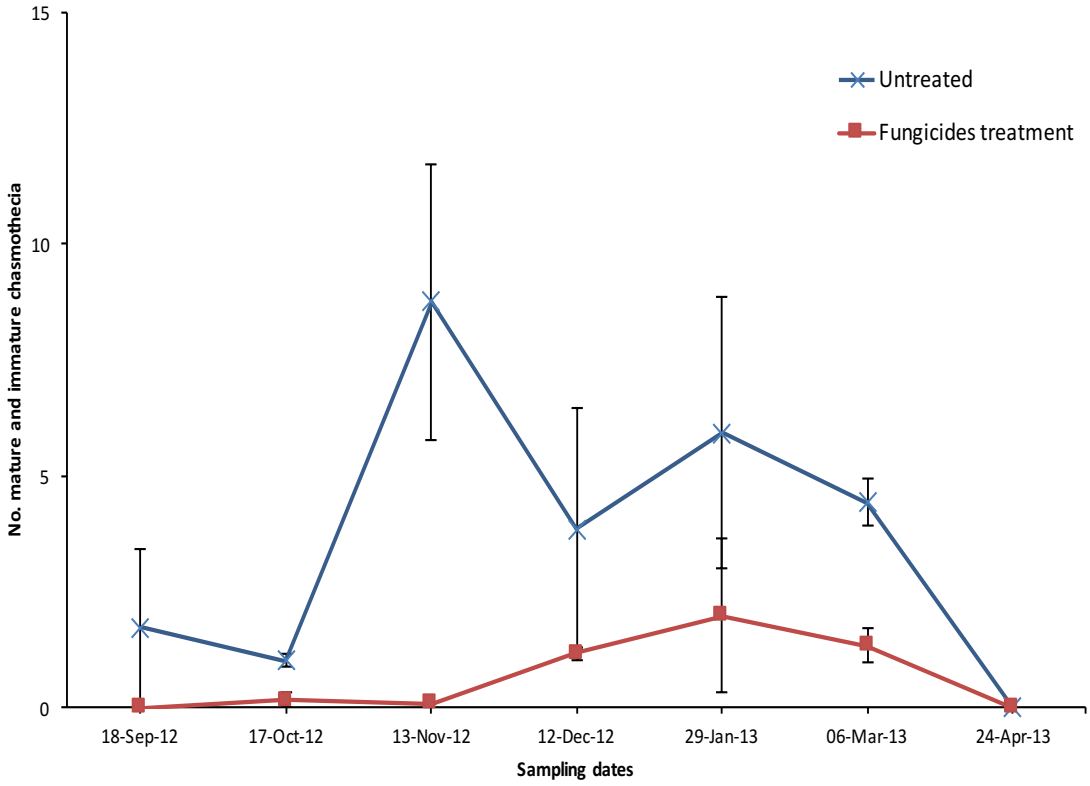


Figure 3.12: The average total number of mature and immature chasmothecia on 50 strawberry leaves (cv. Elegance, Alexandra & Sonata) with the untreated and fungicides treatment ($P < 0.001$) (commercial fungicide) sampled each month in Diamante field (open field) from September 2012 to April 2013 at Maltmas Farm. Bars represent standard errors of mean, 2 degrees of freedom.

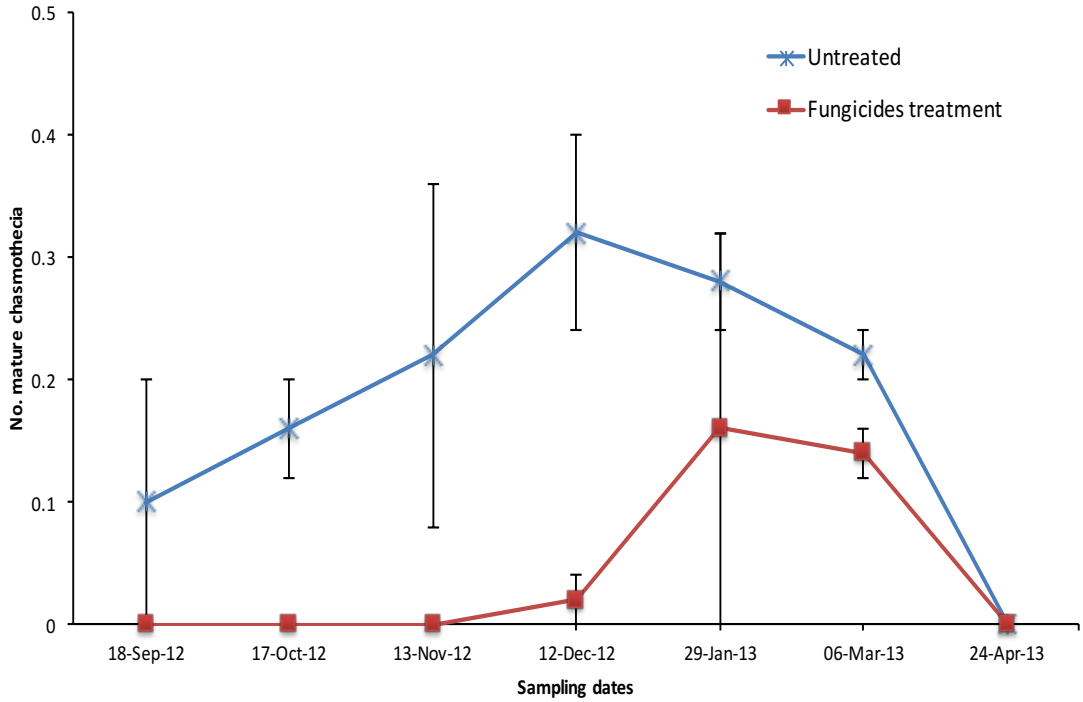


Figure 3.13: The average number of mature chasmothecia on 50 strawberry leaflets (cv. Elegance, Alexandra & Sonata) with the untreated and fungicides treatment ($P < 0.05$) (commercial fungicides) sampled each month in Diamante field (open field) from September 2012 to April 2013 at Maltmas Farm near Wisbech in Cambridgeshire. Bars represent standard errors of mean, 2 degrees of freedom.

The temperature in Diamante field was lower compared to the previous year especially in January and March (from Sep 2012 to April 2013) (Fig. 3.8 & 3.14). The chasmothecia were formed when the temperature was < 20 ºC and humidity > 70% which was slightly different with previous year. The numbers of mature and immature chasmothecia were less compared to the previous year (Fig. 3.8 & 3.14), which was due to the fungicide sprays over the winter or different temperature and humidity.

The percentage of disease cover in the untreated was greater than in the fungicides treated bed especially in November and January, when the temperature was < 20 ºC and humidity > 90% (Fig. 3.15). This indicated that the favourable environmental condition for chasmothecia formation is when the temperature < 20 ºC and humidity > 70%. These complement the results of the previous year and also showed that smaller disease cover of *P. aphanis* resulted in fewer chasmothecia being formed (Fig. 3.15).

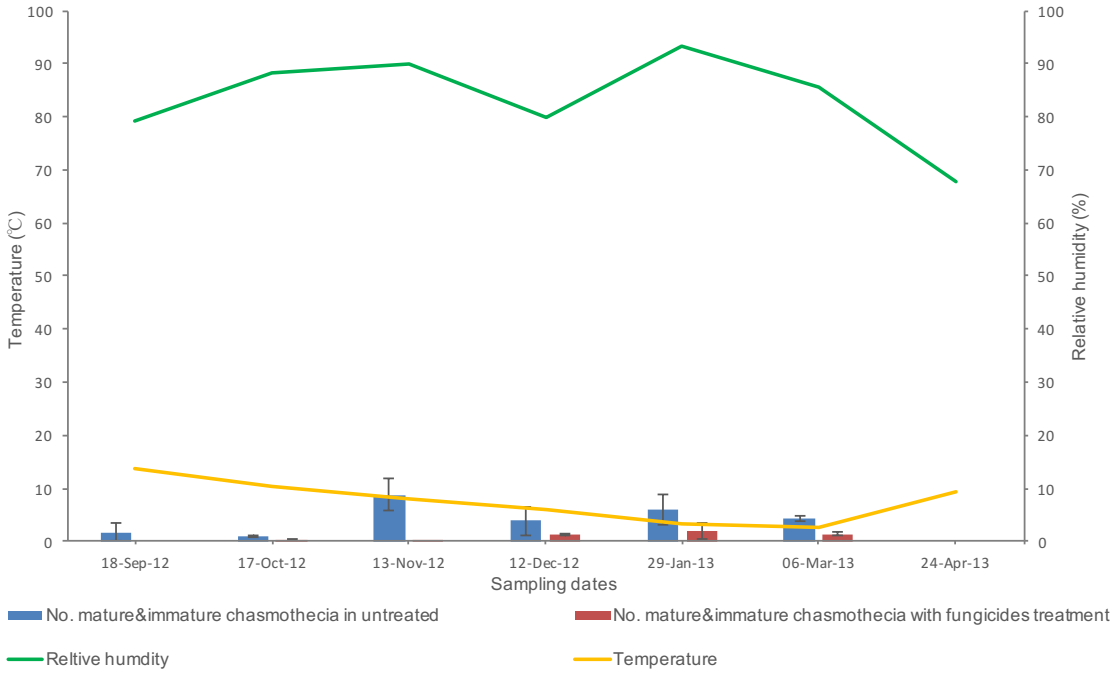


Figure 3.14: The average number of mature and immature chasmothecia per leaflet from 50 strawberry leaves (cv. Elegance, Alexandra & Sonata) with untreated and fungicides treatment, which were sampled each month from Diamante field (open field) from September 2012 to April 2013. The temperature and relative humidity were measured in Diamante field from Sep 2012 to April 2013 at Maltmas Farm. Bars represent standard errors of mean, 2 degrees of freedom.

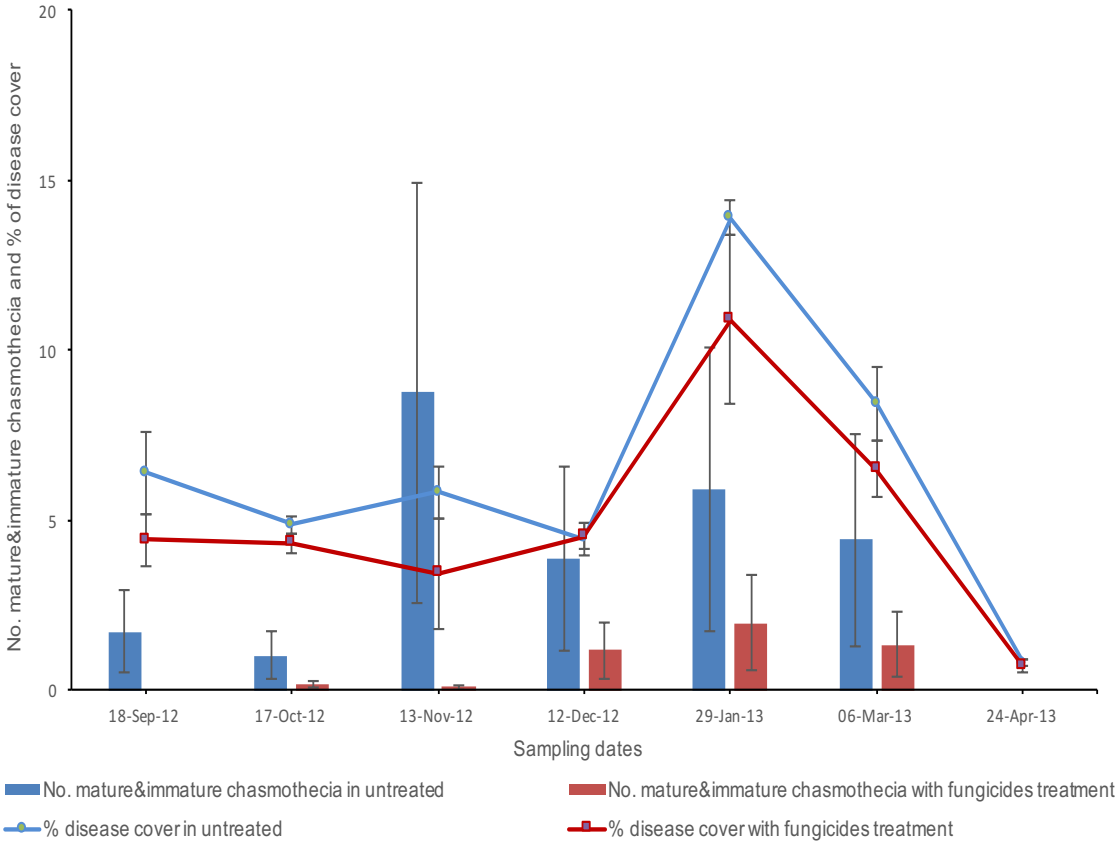


Figure 3.15: The average number of mature and immature chasmothecia per leaflet and percentage of disease cover on 50 strawberry leaves (cv. Elegance, Alexandra & Sonata) in untreated and fungicides treatment, which were sampled each month from Diamante field (open field) from September 2012 to April 2013. Bars represent standard errors of mean, 2 degrees of freedom.

3.4.4 Assessment of chasmothecial development and maturation in the 2012-2013 field experiment

The correlations between the numbers of mature chasmothecia and the different colour of the shell of the chasmothecia (Sienna, Rust and Dark brick) were significant ($P < 0.0005$, $P < 0.0001$) in the fungicides treatment (Table 3.6). The numbers of mature chasmothecia only correlated with the rust shell of the chasmothecia in the untreated ($P < 0.0001$) (Table 3.6). The sienna and dark brick shell of the chasmothecia were not correlated with the number of mature chasmothecia in the untreated (Table 3.6). This demonstrated that the correlations between the colour of the shell of the chasmothecia and the numbers of mature chasmothecia were varied. Therefore, the colour of the shell of the chasmothecia appears to be not important to the maturation of chasmothecia, which complement the data of the previous year.

3.4.4.1 Germinating ascospores on strawberry leaf in the 2012-2013 field experiment

There was no significant difference ($P > 0.1$) between untreated and fungicides treatment. However, the untreated had greater numbers of ascospores compared with the fungicides treatment (Fig. 3.16). The ascospores started germinating in April, which was the same time as the previous year. The dimensions of ascospores were in the range $\leq$ 15-25 $\times$ 10-15 μm, length and width (Fig. 3.17).

Table 3.6: The results of regression analysis showed the correlation between the numbers of mature chasmothecia with the different colour of the shell of the chasmothecia (Sienna, Rust and Dark brick) from the untreated and fungicides treatment from September 2012 to April 2013 at Maltmas Farm near Wisbech in Cambridgeshire.

	Sienna shell of chasmothecia			Rust shell of chasmothecia			Dark brick shell of chasmothecia		
	R	R^2	*P*	R	R^2	*P*	R	R^2	*P*
Untreated									
No. mature chasmothecia	0.17	0.03	> 0.2	0.99	0.98	< 0.0001	0.47	0.22	> 0.05
Fungicides treatment									
No. mature chasmothecia	0.84	0.71	< 0.0005	1	1	< 0.0001			*

* no dark brick shell of the chasmothecia were found

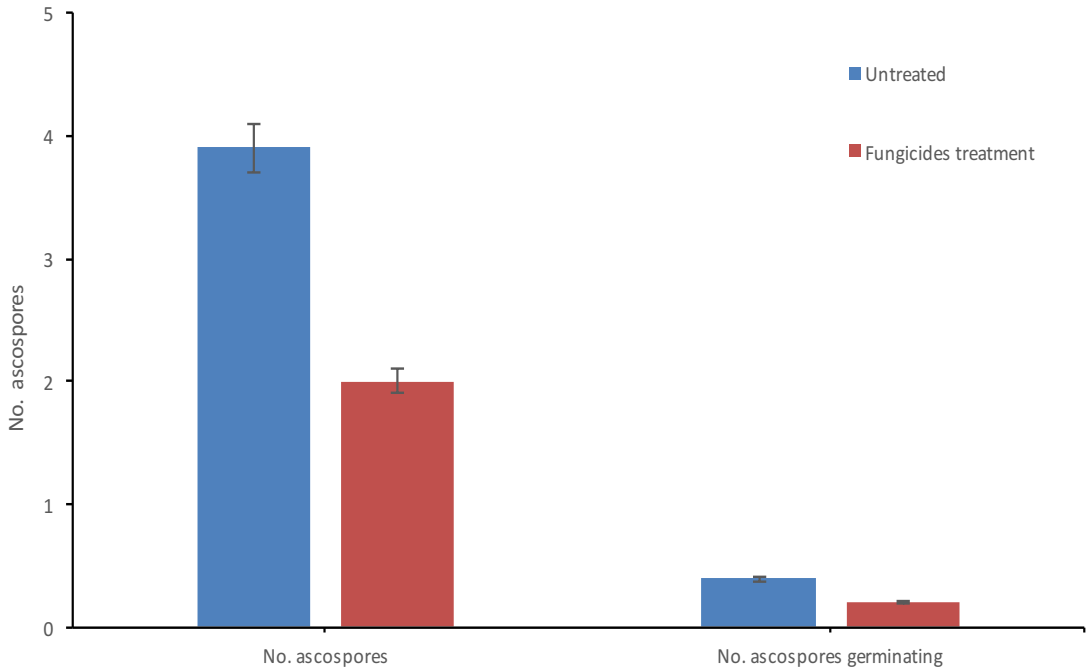


Figure 3.16: The average number of ascospores and ascospores germinating per leaflet with untreated and fungicides treatment in April 2013 at Maltmas Farm. Bars represent standard errors of mean, 2 degrees of freedom.

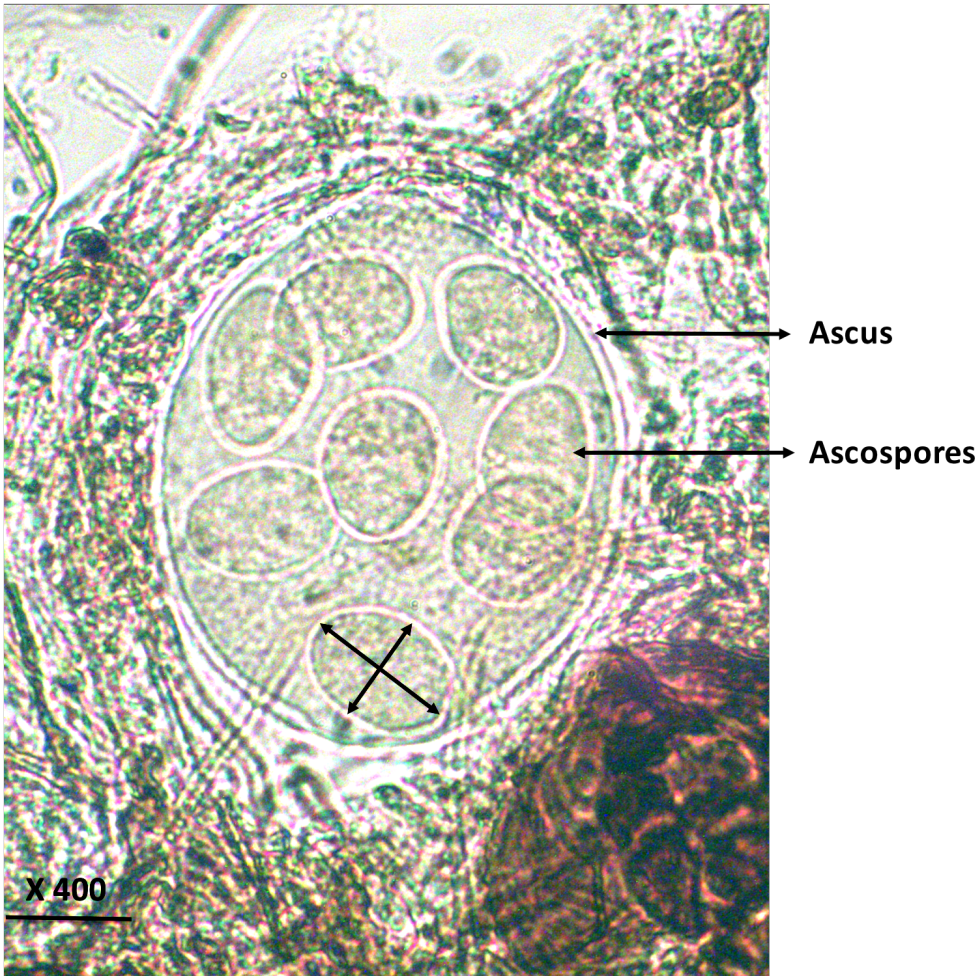


Figure 3.17: One ascus with eight ascospores, the black arrows showed on ascospore was the measurement of length and width (x 40 objective).

3.4.5 **The development of chasmothecia in the 2013-2014 field experiment**

From October 2013 to March 2014, there were no chasmothecia formed in the Amelia field, which might have been due to low percentage disease cover on leaves during May to October (Fig. 3.18). However, a great number of chasmothecia were formed in the Barn field, where the percentage of disease

cover was greater than in the Amelia field (Fig. 3.18). Furthermore, there was a significant correlation ($P < 0.05$) between the percentage of disease cover and the number of mature and immature chasmothecia, which again complement with previous years (Fig. 3.18).

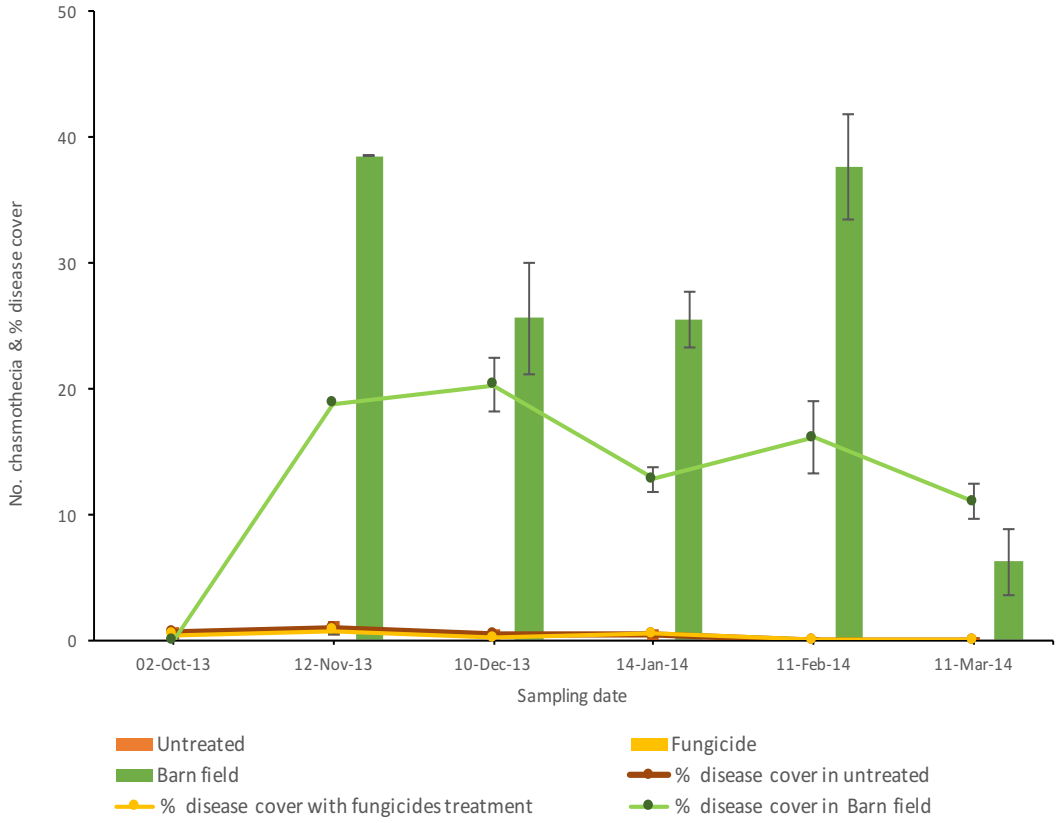


Figure 3.18: The average number of chasmothecia per leaflet from 50 strawberry leaves sampled each month from Amelia field (untreated and fungicide, cv. Sonata, open field) and Barn field (cv. Shelley) (polyethylene tunnel) from October 2013 to March 2014 at Maltmas Farm. Chasmothecia were not found in the Amelia field (untreated and fungicide, cv. Sonata, open field). Bars represent standard errors of mean, 4 degrees of freedom.

The temperatures in 2013 to 2014 were similar to previous years (August 2011-April 2013), but the relative humidity was greater compared to previous years (Fig. 3.19). Thus, the environmental conditions might not be the reason why no chasmothecia not being formed in the Amelia field.

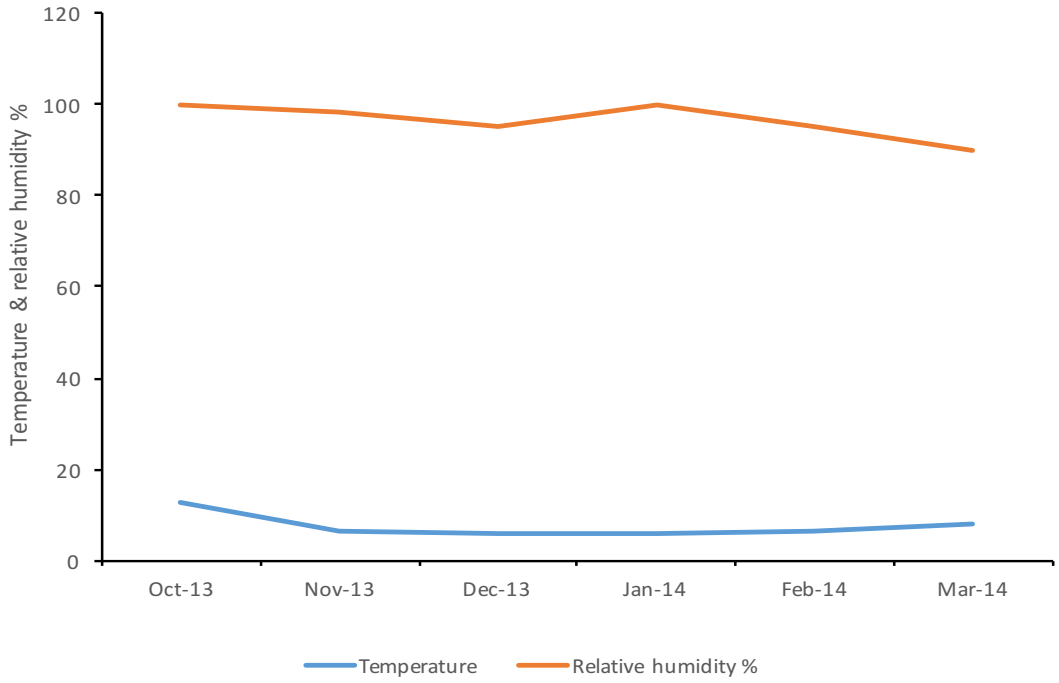


Figure 3.19: Average temperature and percentage of relative humidity from October 2013 to March 2014 at Maltmas Farm.

3.4.6 **Assessment of chasmothecial development and maturation in the 2013-2014 field experiment**

The cross-sectional area of chasmothecia ranged from 0.003 mm^2 (minimum

mean) to 0.009 mm^2 (maximum mean). There was a significant ($P < 0.01$) correlation between the cross-sectional area of chasmothecia and the numbers of mature chasmothecia in the Barn field (polyethylene tunnel) which indicated that the bigger cross-sectional area of chasmothecia led to greater numbers of mature chasmothecia.

3.4.7 Effects of temperature and relative humidity on chasmothecial development

3.4.7.1 Chasmothecial development in damp chamber

The immature chasmothecia took 10/11 days to mature in the damp chamber at room temperature 20 °C and 100% humidity. The chasmothecia were colour-less when they first appeared on leaflets with the ascus not yet formed, then becoming yellowish or orange when the ascus started to form (but no ascospores in ascus), and finally brown (no ascospores in ascus) to dark brown or black when mature (ascospores in ascus) (Fig. 3.20).

Maturity of chasmothecia

The mature chasmothecium contains eight ascospores in the ascus (Fig. 3.21 A). when the black chasmothecia (at ×10 or ×40 objective) were assessed, not all of them contained eight ascospores in ascus, some were deformed and only four

ascospores were formed which indicated black chasmothecia have not always matured (Fig. 3.21). The mature chasmothecia (i.e. black chasmothecia) took 5/6 days to release the ascus at room temperature 20 °C and 100% humidity (Fig. 3.22). Moreover, the chasmothecia showed the natural opening curve on the surface when they start to release the ascus (Fig. 3.23, 3.24 & 3.25). The ascospores started to germinate in March or April (Fig. 3.26).

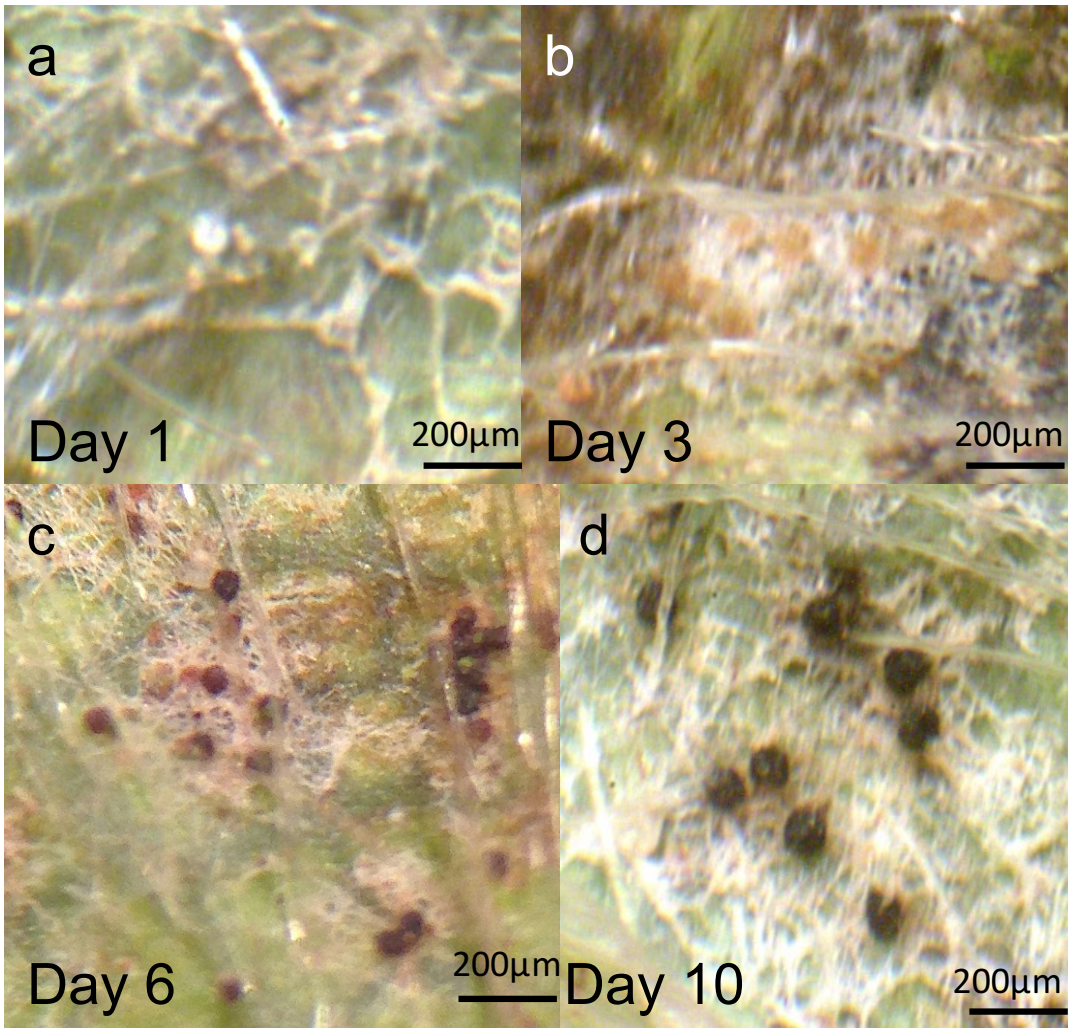


Figure 3.20: The development of chasmothecia (10x3 magnification). a. Immature chasmothecia (colour-less) at day one; b. immature chasmothecia (orange) at day three; c. immature chasmothecia (brown) d. mature chasmothecia (dark brown or black) at day ten.

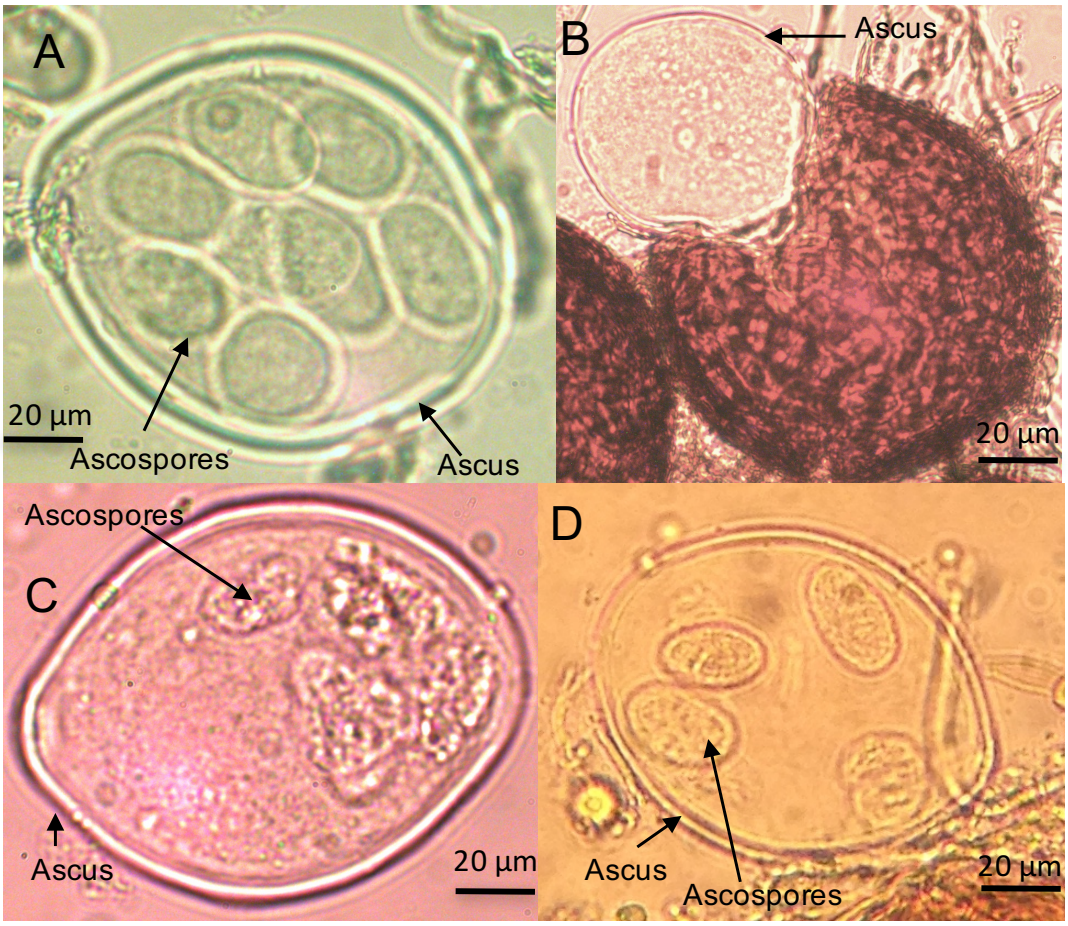


Figure 3.21: Mature chasmothecium and immature chasmothecium with ascus; A) eight ascospores in an ascus; B) ascus without ascospores; C) deformed ascopores in ascus; D) five ascopores in ascus (10x40 magnification).

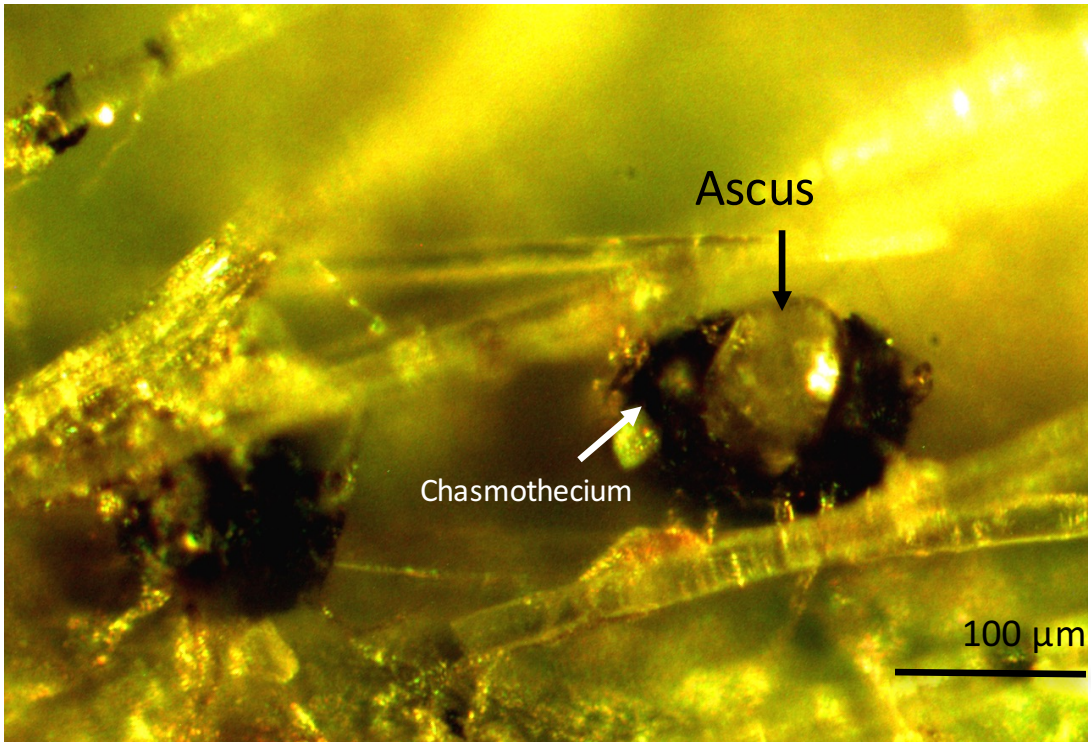


Figure 3.22: One chasmothecium showing release of ascus (10 x10 magnification).

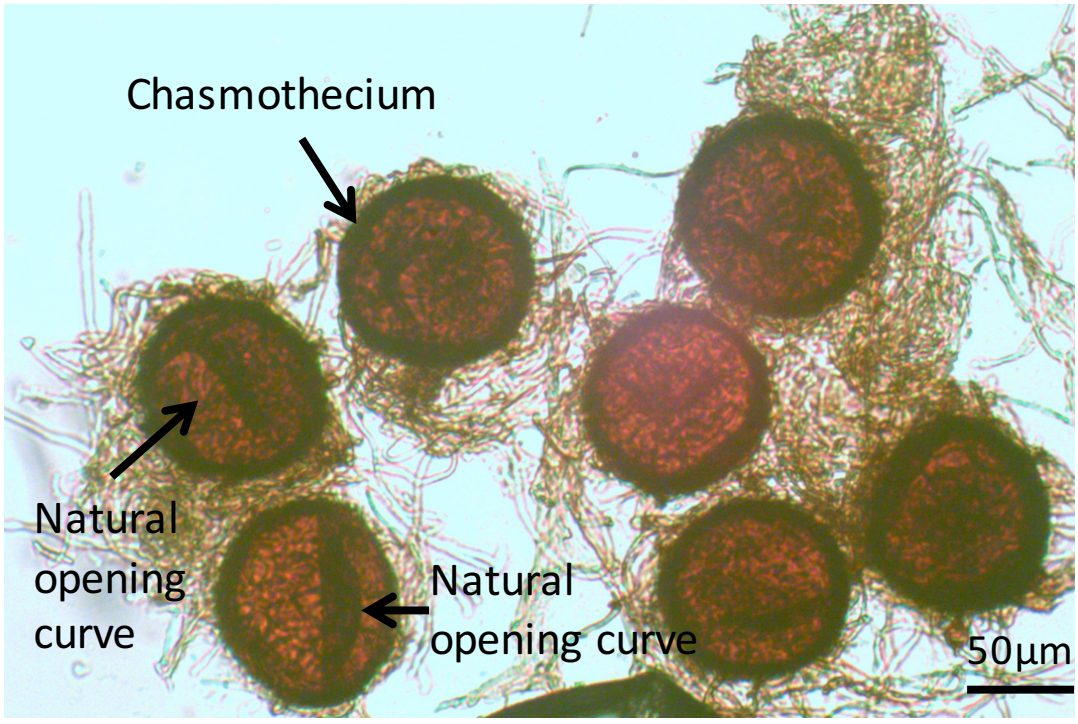


Figure 3.23: Black chasmothecia showed a natural opening curve on the surface (10 x 10 magnification).

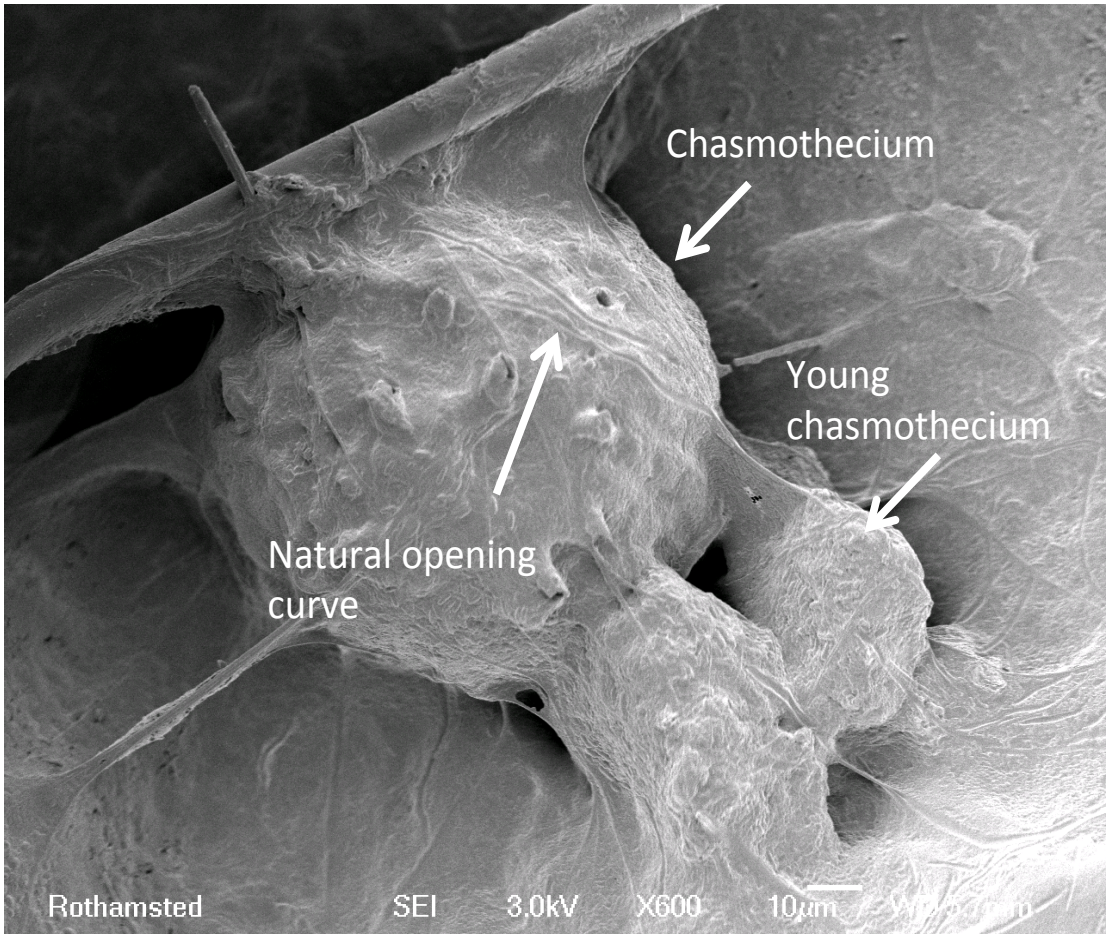


Figure 3.24: SEM image of chasmothecium showing a natural opening curve on the surface with two young chasmothecia by the side.

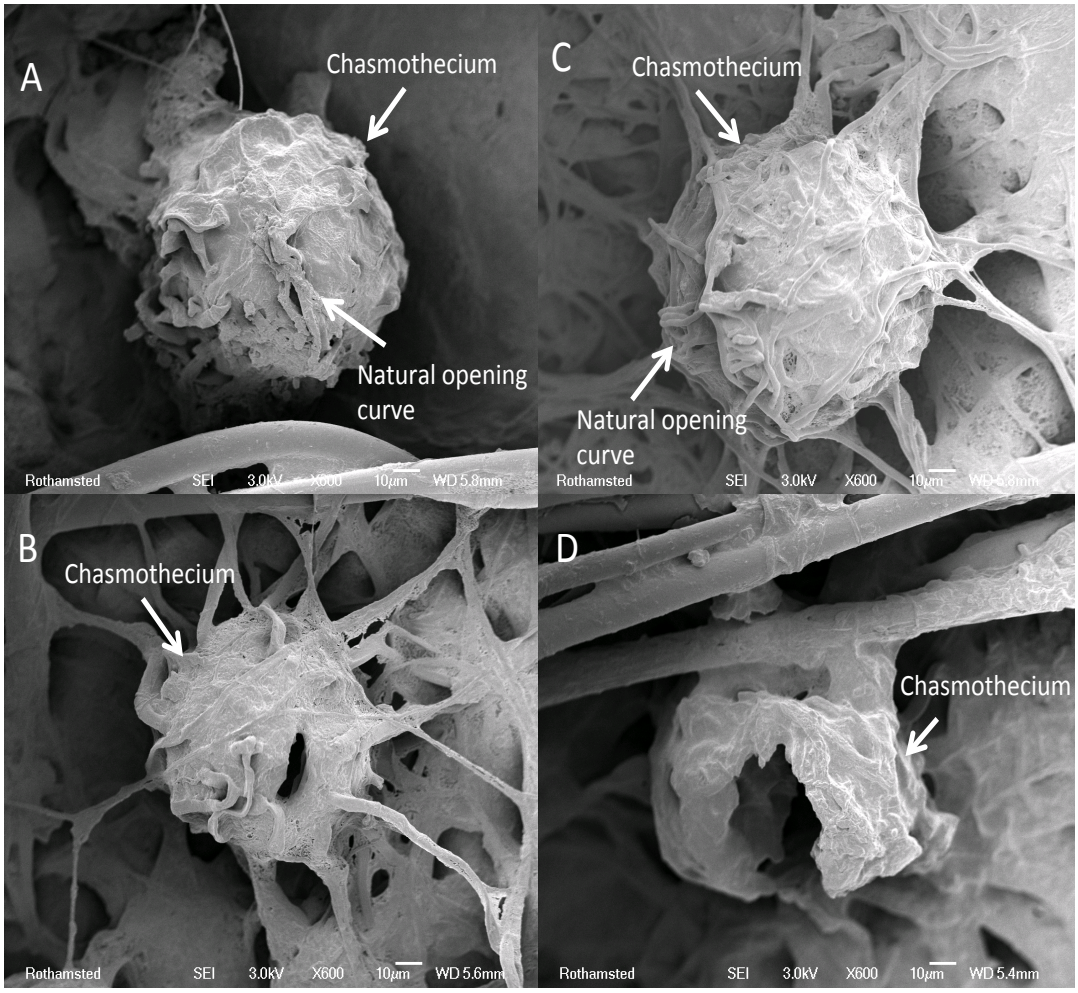


Figure 3.25: SEM image of chasmothecium showing the progress of release of the ascus. A) chasmothecium showing the natural opening curve on the surface; B) the natural opening curve becoming wider on the surface; C) the natural opening curve half open; D) chasmothecium after release of the ascus.

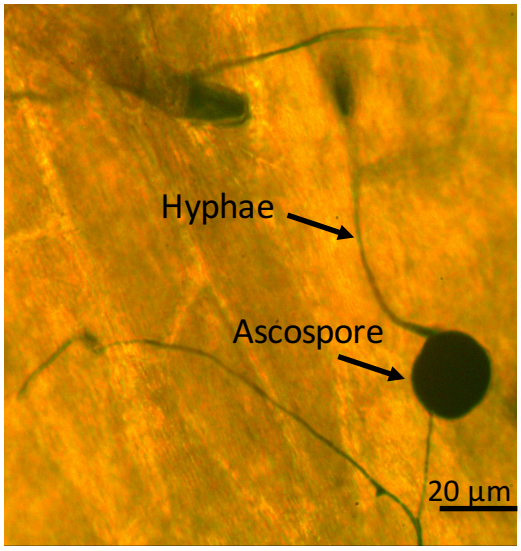


Figure 3.26: Germinating ascospore of *Podosphaera aphanis* on leaf surface in March (10 x 40 magnification).

3.4.7.2 The development of mature chasmothecia at different temperatures

Time series of percentage of the chasmothecia with natural opening curve present

The chasmothecia sample A (kept at -20°C for one year and half) at 15°C, 20°C and 25°C were contaminated with bacteria at day 7, however, it did not show any response before day 7. The sample A did show the natural opening curve on day 17 at 30 °C (Figure 3.30). The chasmothecia sample B (after one month at 4°C) and C (after one week at 4°C) showed the natural opening curve occurring on day 9 at 15°C, 20 °C, 25 °C and 30°C which was significantly different ($P < 0.005$) to sample A (Figure 3.27, Figure 3.28, Figure 3.29 & Figure 3.30). However, there was no significant difference between sample B and C. There was also no

significant difference between each temperature for sample C. Sample B at 15°C showed significantly ($P < 0.05$) greater percentage of chasmothecia with natural opening curve present compared to the temperature at 20°C, which demonstrated the optimal temperature for natural opening curve occurrence is about 15°C or above.

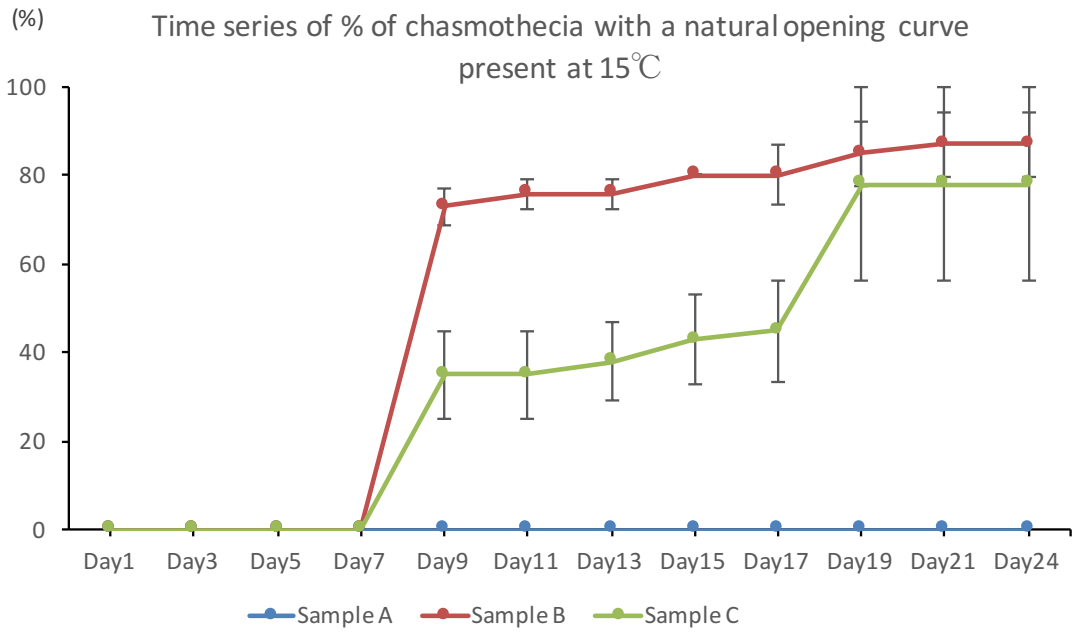


Figure 3.27: The time series of percentage of the chasmothecia with a natural opening curve present was observed at 15°C. Sample A was kept in freezer at -20°C for eighteen months, sample B was kept for one month at 4°C and sample C as kept for one week at 4°C. Sample A was contaminated at day 7. Bars represent standard errors of mean, 2 degrees of freedom.

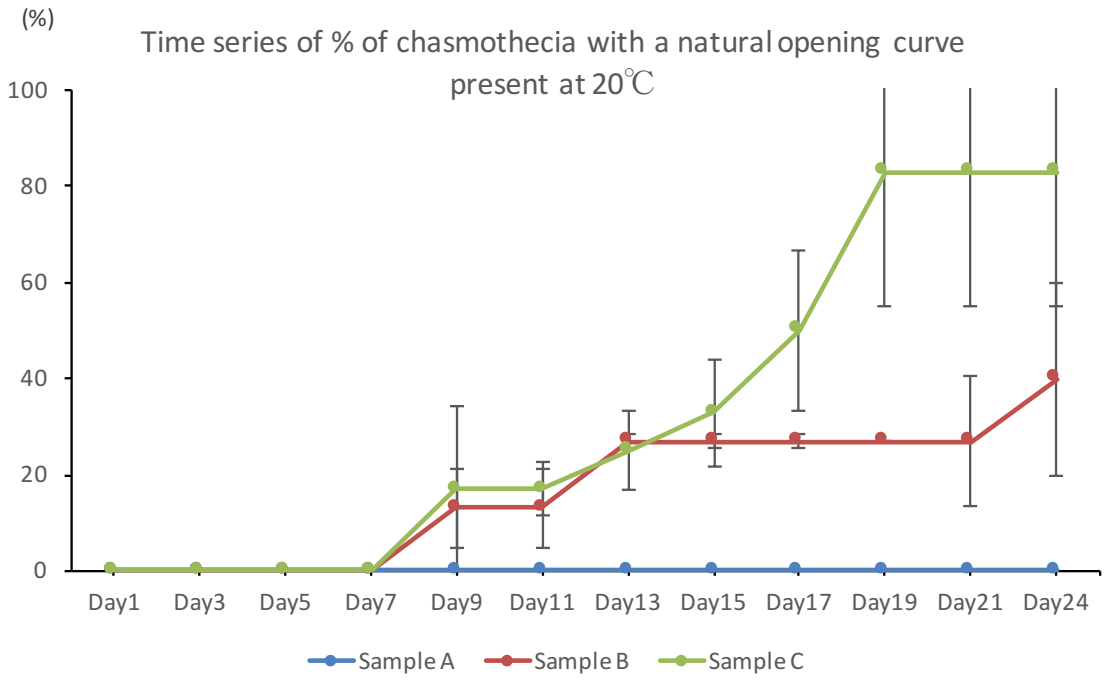


Figure 3.28: The time series of percentage of the chasmothecia with a natural opening curve present was observed at 20°C. Sample A was kept in freezer at -20°C for eighteen months, sample B was kept for one month at 4°C and sample C as kept for one week at 4°C. Sample A was contaminated at day 7. Bars represent standard errors of mean, 2 degrees of freedom.

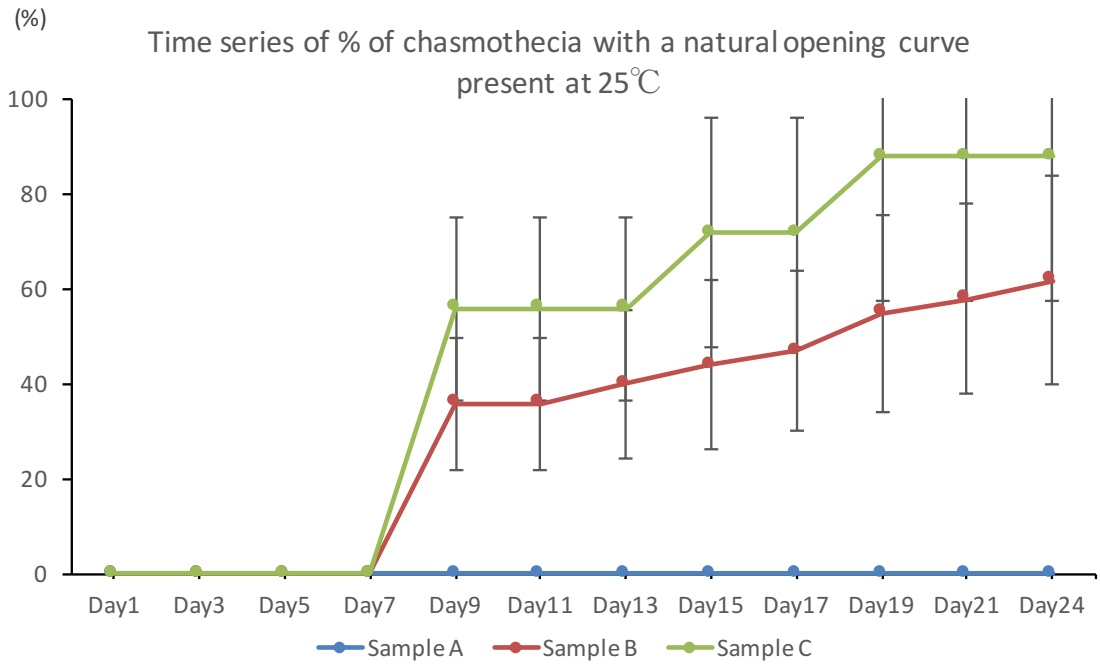


Figure 3.29: The time series of percentage of the chasmothecia with a natural opening curve present was observed at 25°C. Sample A was kept in freezer at -20°C for eighteen months, sample B was kept for one month at 4°C and sample C as kept for one week at 4°C. Sample A was contaminated at day 7. Bars represent standard errors of mean, 2 degrees of freedom.

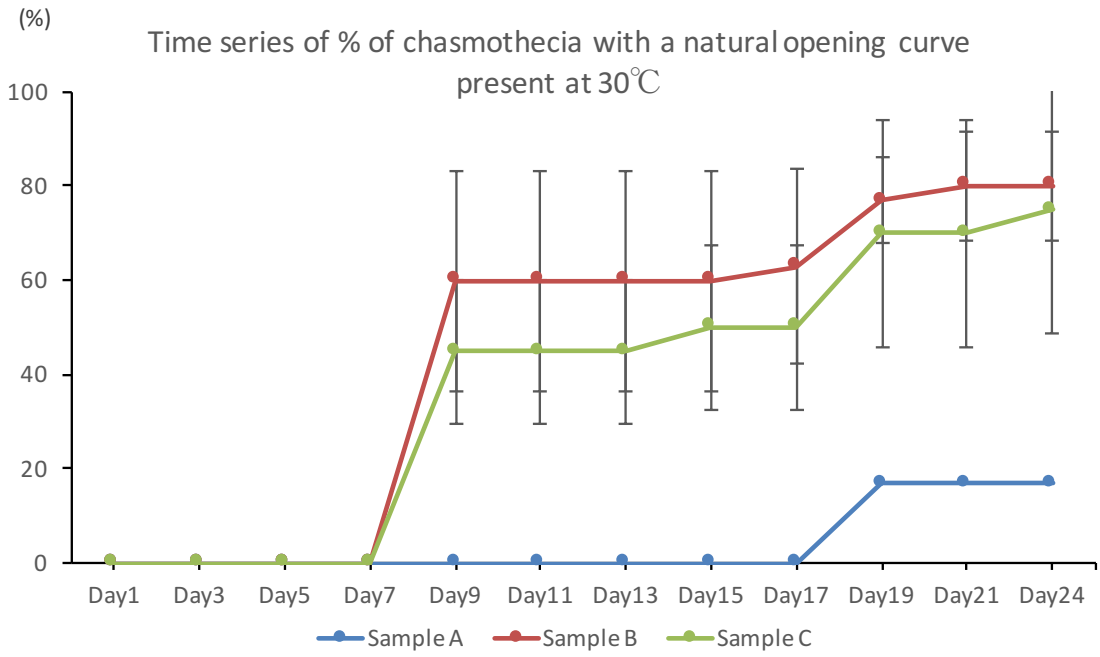


Figure 3.30: The time series of percentage of the chasmothecia with a natural opening curve present was observed at 30°C. Sample A was kept in freezer at -20°C for eighteen months, sample B was kept for one month at 4°C and sample C as kept for one week at 4°C. Sample A was contaminated at day 7. Bars represent standard errors of mean, 2 degrees of freedom.

Time series of percentage of empty chasmothecia

The chasmothecia sample A (kept in freezer at -20°C for one year and half) at 15°C, 20°C and 25°C were contaminated at day 7, however, the sample A showed 20% of empty chasmothecia on day 24 at 30°C (Figure 3.29). The chasmothecia Sample B (after one month at 4°C) showed the percentage of empty chasmothecia (the ascus was released from the chasmothecium) at

15°C, 20 °C, 25 °C and 30°C (Figure 3.31,Figure 3.32,Figure 3.33 &Figure 3.34) but no significant difference between each temperature. The chasmothecia Sample C (after one week at 4°C) showed the percentage of empty chasmothecia at 15°C, 20 °C and 25 °C but not at 30°C and no significant difference between temperatures. Furthermore, there was no significant difference between sample B and C at 15°C and 20°C (Figure 3.31 &Figure 3.32), however, there was significant difference at 25 °C and 30°C (Figure 3.33 & Figure 3.34).

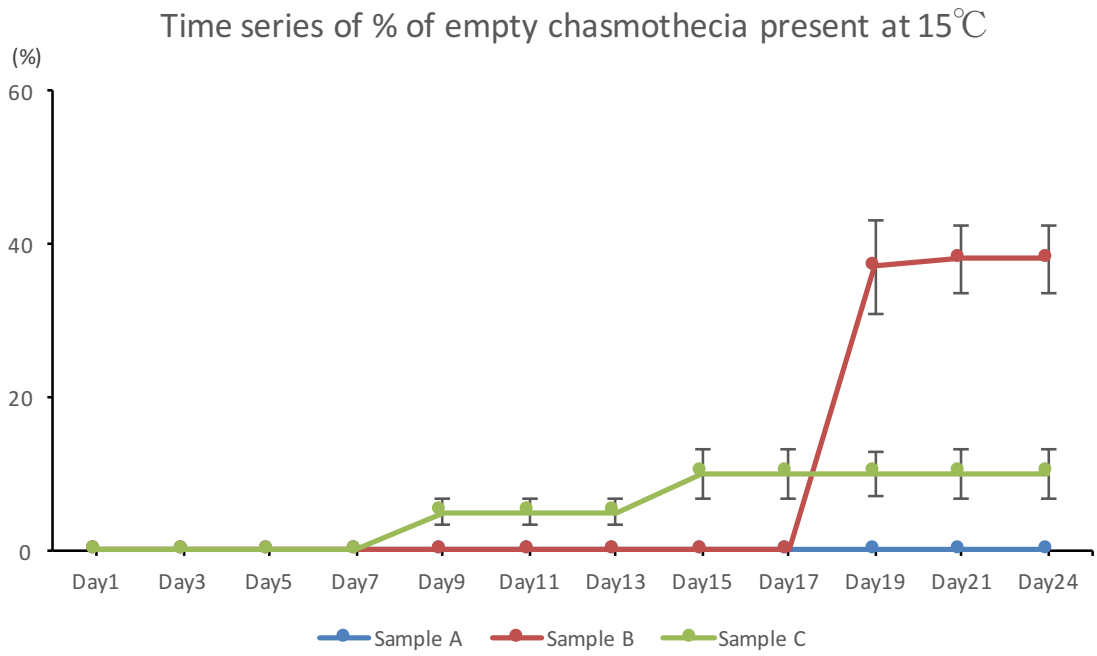


Figure 3.31: The time series of percentage of the empty chasmothecia present was observed at 15°C. Sample A was kept in freezer at -20°C for eighteen months, sample B was kept for one month at 4°C and sample C as kept for one week at 4°C. Sample A was contaminated at day 7. Bars represent standard errors of mean, 2 degrees of freedom.

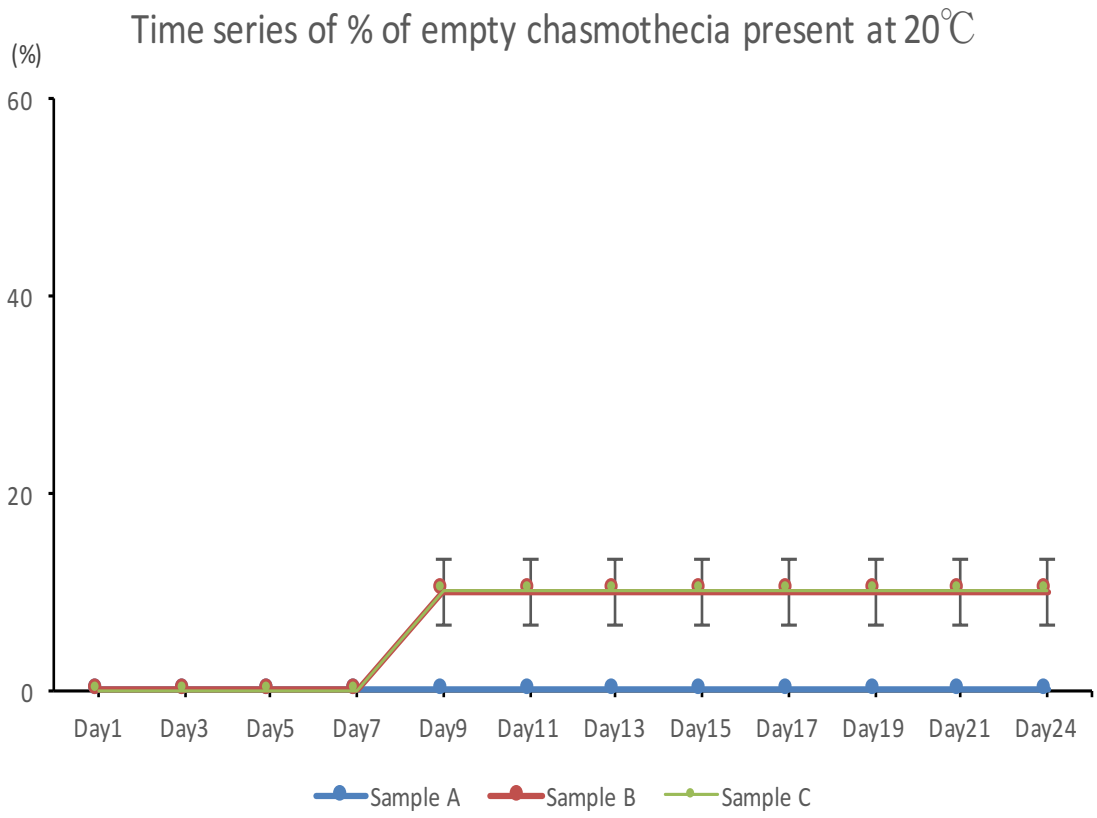


Figure 3.32: The time series of percentage of the empty chasmothecia present wasobserved at 20°C. Sample A was kept in freezer at -20°C for eighteen months, sample B was kept for one month at 4°C and sample C as kept for one week at 4°C. Sample A was contaminated at day 7. Bars represent standard errors of mean, 2 degrees of freedom.

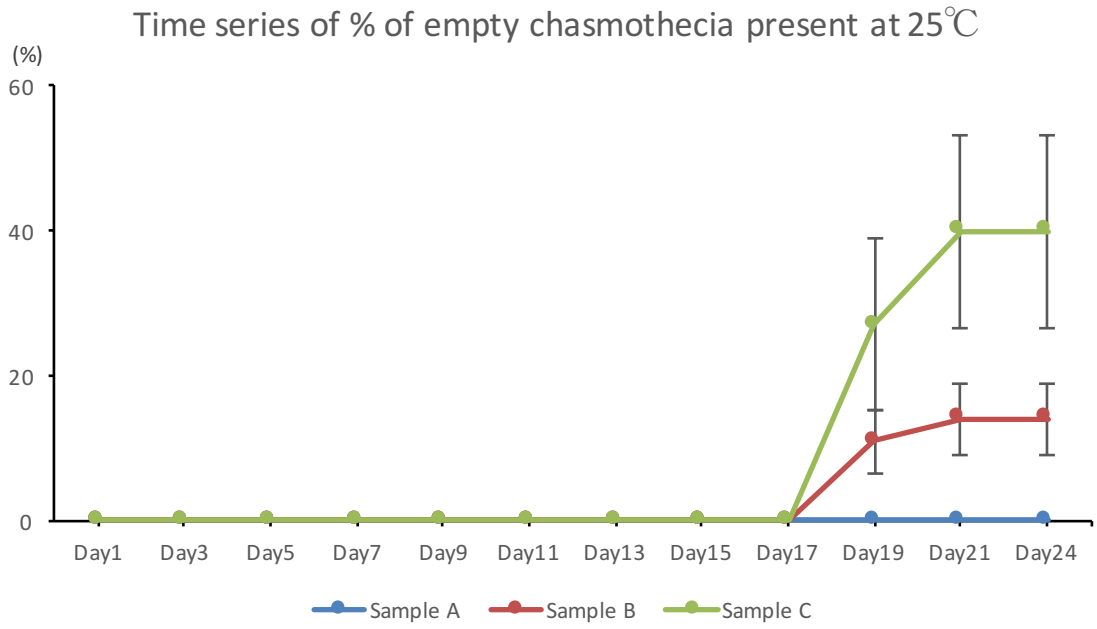


Figure 3.33: The time series of percentage of the empty chasmothecia present was observed at 25°C. Sample A was kept in freezer at -20°C for eighteen months, sample B was kept for one month at 4°C and sample C as kept for one week at 4°C. Sample A was contaminated at day 7. Bars represent standard errors of mean, 2 degrees of freedom.

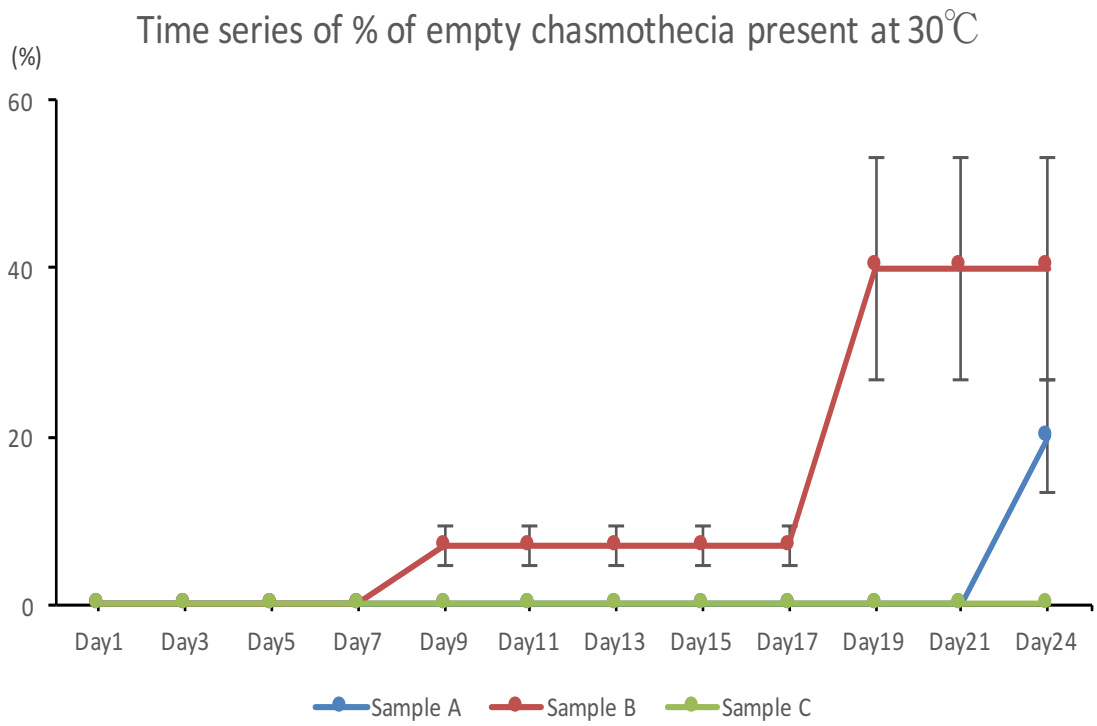


Figure 3.34: The time series of percentage of the empty chasmothecia present was observed at 15°C. Sample A was kept in freezer at -20°C for eighteen months, sample B was kept for one month at 4°C and sample C as kept for one week at 4°C. Sample A was contaminated at day 7. Bars represent standard errors of mean, 2 degrees of freedom.

Time series of percentage of chasmothecia with ascospores germinating

The chasmothecia sample A (kept in freezer at -20°C for one year and half) at 15°C, 20°C and 25°C was contaminated at day 7 and no ascospores germinated at 30°C (Figure 3.38). The chasmothecia Sample B (after one month at 4°C) showed ascospores germinating at 15°C, 25 °C, 30 °C (Figure 3.35, Figure 3.37

& Figure 3.38) but not at 20°C(Figure 3.36) and no significant difference between each temperature. The chasmothecia sample C (after one week at 4°C) showed ascospores germinating at 15°C, 20°C , 25°C and 30°C (Figure 3.35, Figure 3.37, Figure 3.38 & Figure 3.36), no significant difference between each temperature. Moreover, there was no significant difference between Sample B and C between each temperature.

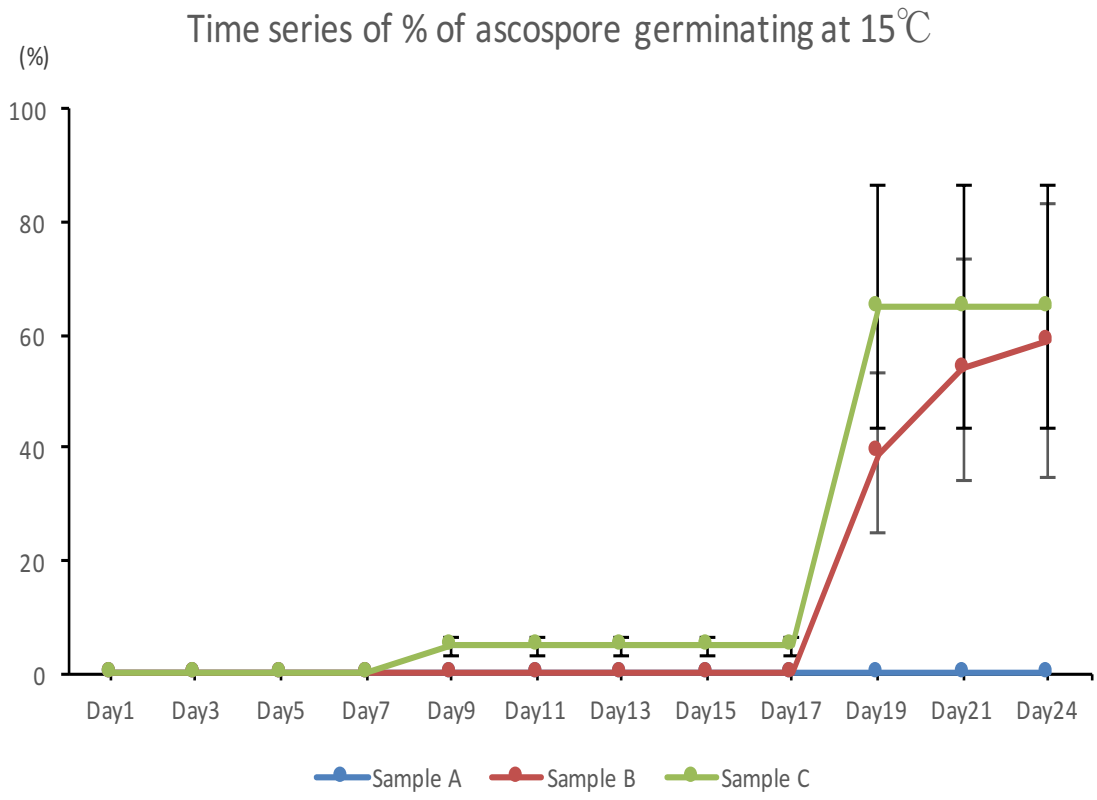


Figure 3.35: The time series of percentage of ascospore germinating was observed at 15°C. Sample A was kept in freezer at -20°C for eighteen months, sample B was kept for one month at 4°C and sample C as kept for one week at 4°C. Sample A was contaminated at day 7. Bars represent standard errors of mean, 2 degrees of freedom.

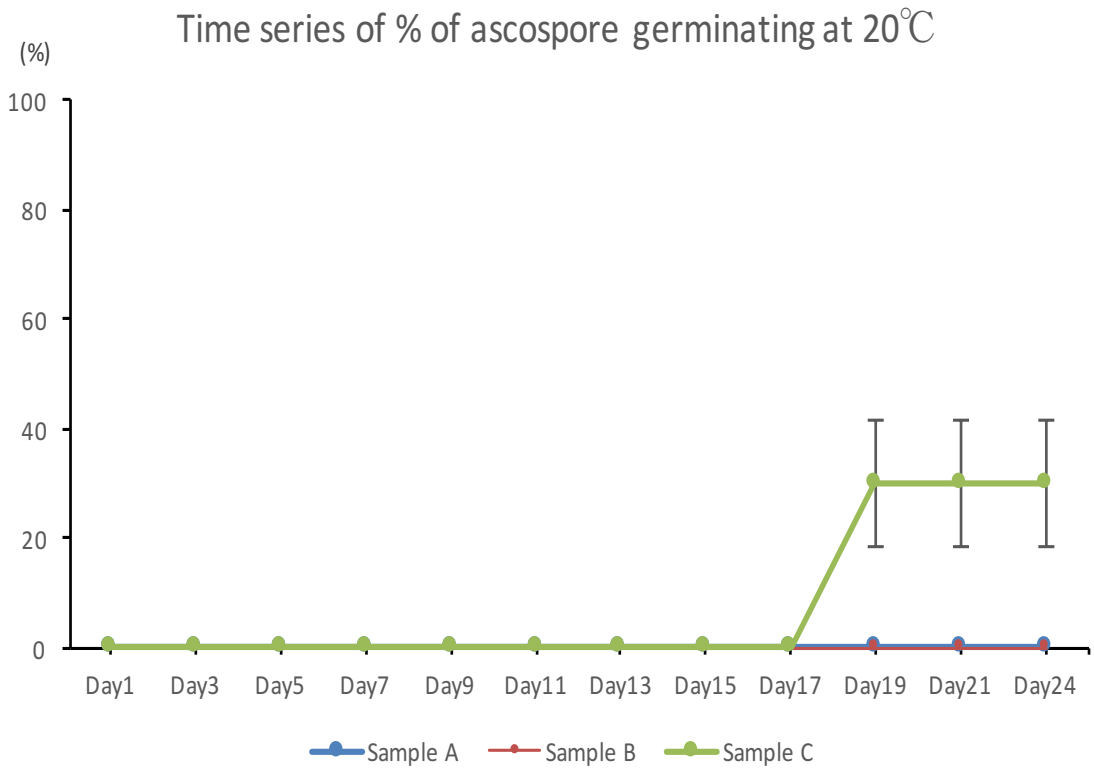


Figure 3.36: The time series of percentage of ascospore germinating was observed at 20°C. Sample A was kept in freezer at -20°C for eighteen months, sample B was kept for one month at 4°C and sample C as kept for one week at 4°C. Sample A was contaminated at day 7. Bars represent standard errors of mean, 2 degrees of freedom.

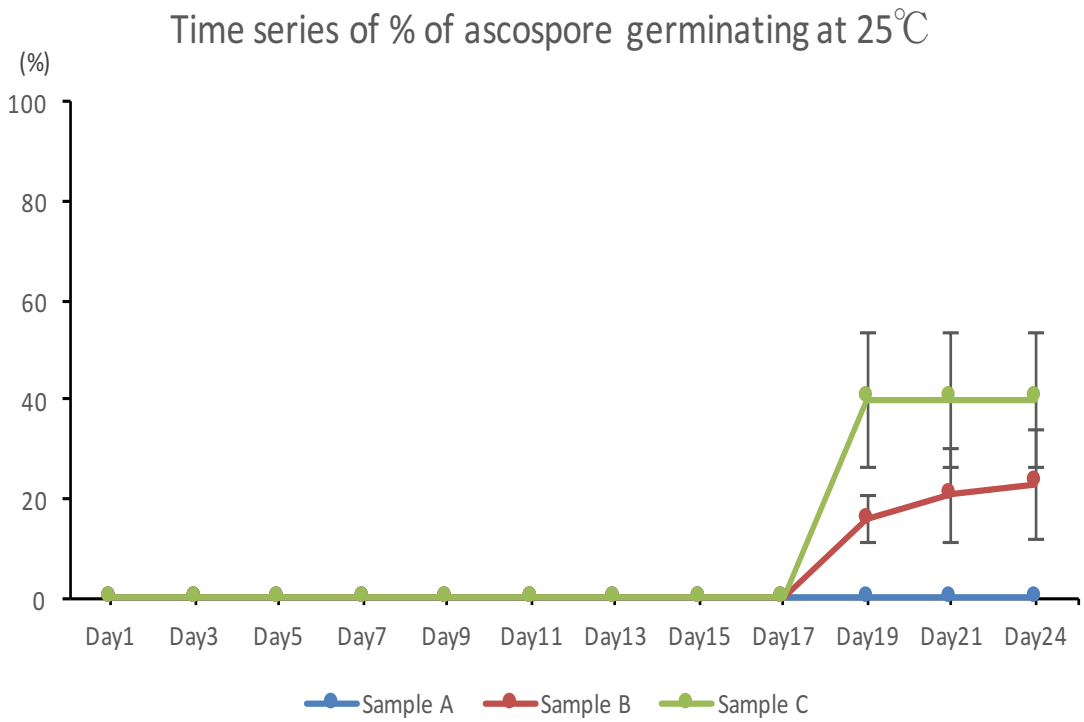


Figure 3.37: The time series of percentage of ascospore germinating was observed at 25°C. Sample A was kept in freezer at -20°C for eighteen months, sample B was kept for one month at 4°C and sample C as kept for one week at 4°C. Sample A was contaminated at day 7. Bars represent standard errors of mean, 2 degrees of freedom.

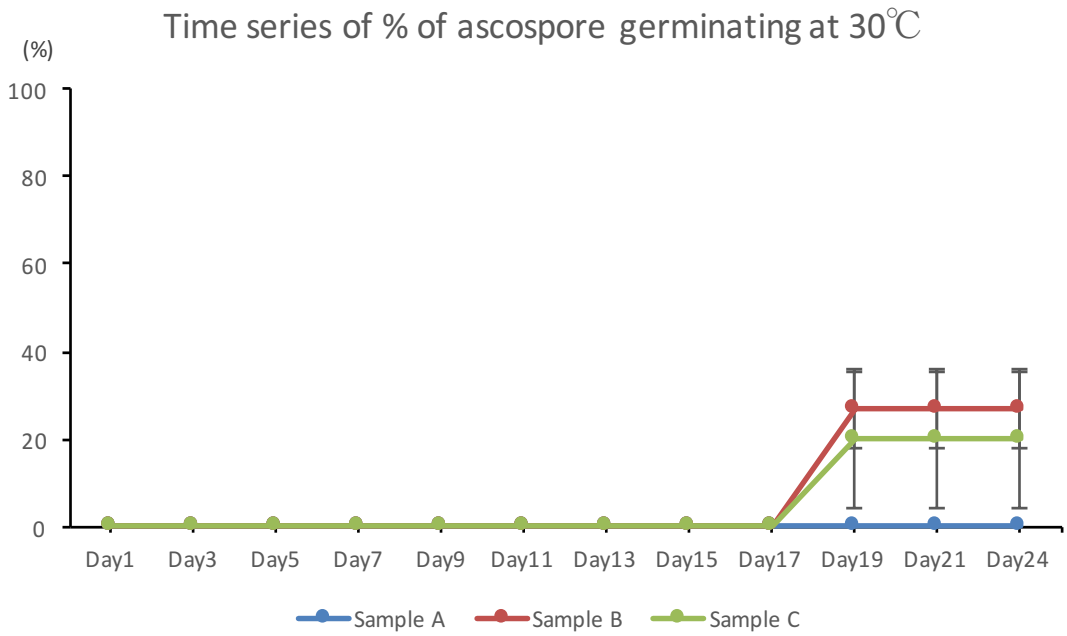


Figure 3.38: The time series of percentage of ascospore germinating was observed at 30°C. Sample A was kept in freezer at -20°C for eighteen months, sample B was kept for one month at 4°C and sample C as kept for one week at 4°C. Sample A was contaminated at day 7. Bars represent standard errors of mean, 2 degrees of freedom.

3.4.7.3 Assessment of diseases on new strawberry plants from propagator

The results from the 2013 UH glasshouse experiment showed that 14% of strawberry mother plants had been infected by *P. aphanis* in July 2013. When runner plants were replanted in March 2014, a month later, 13% of runner plants showed symptoms of powdery mildew. The pre-assessment of plants for the 2013 Si fertigation experiment done in March 2013 showed that 4% of the strawberry plants showed symptoms of powdery mildew. Strawberry leaf spot (*Mycosphaerella fragariae*) was also observed on the pre-assessment of plants in the 2013 Si fertigation experiment (Appendix 10).

3.5 Discussion and Conclusion

Chasmothecia of *P. aphanis* are generally present in overwintering crops. When conditions are unsuitable for mycelia growth, the chasmothecia as sexual overwintering bodies can provide a route for inoculum to survive across strawberry production seasons (Gourley, 1979), but did not completely stop the production of conidia (Asalf et al., 2013). Hoffmann et al. (2012) indicated that applications of fungicides from the start of the growing season to the beginning of the fruit ripening period did not only affect the disease level but also determined the incidence of the autumn leaf infection. In the present study, the results demonstrated that the use of fungicides in late autumn and spring could reduce the number of chasmothecia (mature and immature) and therefore reduce the initial inoculum in the next growing season.

Furthermore, the environmental conditions and mating type have an impact on the chasmothecia forming on strawberry crops. Asalf et al (2013) stated that the conducive temperatures for chasmothecia initiation ranged from 5°C to 13°C. However, Asalf et al (2013) also indicated that the number of chasmothecia were greatly reduced at 20°C to 25°C than 5°C to 13°C. The work here also suggested that the chasmothecia started to form when the temperature was about 20°C or below and started to release the ascus at about 15°C or above. Moreover, mature chasmothecia take days to form visible round black cases on the leaf (Putnicki & Glawe, 2009). The chasmothecial coloration from white to brown to black was faster at a higher temperature than at a lower temperature (Asalf et al., 2013). In

the present study, the immature chasmothecia took 10/11 days to mature in the damp chamber at room temperature 20 °C and 100% humidity.

The development of chasmothecia in 2011-2012 showed that the number of chasmothecia (mature and immature) and the maturity of chasmothecia ($P < 0.01$) were significantly less in Ladybird field (received 12 fungicide sprays) compared to the Diamante field (received 8 fungicide sprays) (Fig. 3.6 & 3.7). The development of chasmothecia in 2012-2013 also showed that the fungicide treated plants had significantly ($P < 0.001$) fewer chasmothecia (mature and immature) and fewer mature chasmothecia ($P < 0.05$) than the untreated (Fig. 3.12 & 3.13). The results in 2011-2012 and 2012-2013 were similar, which indicated that there was a reduction in chasmothecial production with late autumn fungicide sprays.

Moreover, Hoffmann et al. (2012) stated that the level of leaf disease affected the number of chasmothecia formed on the leaves. A good correlation ($P < 0.001$) has been made between the number of chasmothecia (immature and mature) and the percentage of disease cover on leaves in both years (2011-2012 & 2013-2014) (Table 3.4), which demonstrates that the more mycelium on a leaf, the greater the number of chasmothecia and also suggests that a reduction on the percentage of disease cover will have a knock on effect on disease carryover. Therefore, the low disease carried over in 2010 to 2011 **(**Fatema, 2014) had the effect of a lower number of chasmothecia in Ladybird field. There was also a low disease carry over in 2013 (refer to Chapter 4, 4.4.2), which explained why no

chasmothecia formed in 2013-2014 in Amelia field and the mating type also could be one of the reasons for chasmothecia not being formed. In addition, the number of chasmothecia varied between months in Diamante field and Ladybird field. This could be due to the spray dates not being well planned. However, using fungicides in the late autumn and early spring is effective in reducing chasmothecia numbers and maturity, thereby reducing the initial inoculum in the next growing season.

Smith et al. (1988) stated that the deeper the colour of the chasmothecia, the greater the level of maturation. This is in agreement with the present study, where the immature chasmothecia took 10/11 days to mature in the damp chamber, which the colour of the chasmothecia changed from colourless to black. Moreover, the black mature chasmothecia were assessed for maturation, and the colours of the chasmothecial shell were recorded into three colours; Sienna, Rust and Dark brick (Anon, 1969). The correlation between the colours of the chasmothecial shell and the maturity of chasmothecia were random in both 2011-2012 and 2012-2013 (Table 3.4 & 3.5), which indicated that the colours of chasmothecial shell might not be related to the maturity of chasmothecia. However, there was a good correlation ($P < 0.001$) in both years between the maturity of chasmothecia and the cross-sectional area of chasmothecia, which indicated that the larger the chasmothecia, the greater the level of maturation.

Three different storage methods for chasmothecia were assessed for the different stage of release of ascus and ascospore which had been kept in the deep freezer

at -20°C for eighteen months (sample A), in the cold room for one month at 4°C (sample B) and in the cold room for one week at 4°C (sample C). Samples were assessed under different temperature in order to get the optimal temperature and the difference between samples. The results showed that chasmothecia showed the natural opening curve and germinating ascospores on day 7 at 15 °C or above, which correlates with the fact that the asospores were starting to germinate in April to May when the temperature was about 15 °C in the field experiments (2011-2012 & 2012-2013) (Fig. 3.10 & 3.16). Furthermore, the significant discovery in this study was the chasmothecia sample A (kept in deep freeze at -20°C for eighteen months) which still showed the natural opening curve on day 17 and empty chasmothecia on day 24, which suggested that the chasmothecia of *P. aphanis* may be important in cold strawberry-growing regions.

Furthermore, strawberry powdery mildew (*Podosphaera aphanis)* and strawberry leaf spot (*M. fragariae*) were present in strawberries when they came from the propagator, which shows that there are no disease-free plants and the strawberry propagator is the main source of the disease. Chasmothecia are generally present in overwintering strawberries. Therefore, in both cases the use of fungicides can reduce the initial inoculum from these disease sources. To sum up, this study suggests that spraying out of season contributes to disease control. This should encourage farmers to spray out of season. Hence, spraying in late autumn and spring could decrease the spread of the disease.

Chapter 4 – Use of silicon in integrated control of strawberry powdery mildew

4.1 Introduction

This chapter reports the effects of different concentrations of Si nutrient treatment (foliar spray treatment and root treatment) on the morphology and physiology of strawberry plants. It also reports the development of strawberry powdery mildew in the field in order to test the hypothesis that use of Si nutrient can delay the onset of the epidemic of strawberry powdery mildew.

Si nutrient is considered a non-essential nutrient for the majority of plants. Anecdotal evidence indicates that Si uptake by plants can provide many benefits such as improved pest and pathogen resistance, reduction in the effects of biotic and abiotic stresses, enhanced crop growth, heavy-metal tolerance, improved crop quality and yield (Epstein, 1999). However, the effects and benefits of Si absorption vary from species to species. Ma et al. (1989) and Miyake and Takahashi (1986) stated that Si uptake is most important for plant growth during the reproductive phase for rice, tomato and strawberry. Some studies by Kanto et al. (2004), Liang et al. (2005), Kanto et al. (2007), Moyer et al. (2008), Savvas et al. (2009), Shetty et al. (2012) and Dallagnol et al. (2015) cited that Si induced resistance against powdery mildew in wheat, rose, strawberry, melon, cucumber

and gerbera daisy. Field observations by growers suggest that Si and potassium bicarbonate act synergistically to reduce disease development. Moreover, earlier work by Fatema and Hall (2011) showed that a Si nutrient reduced *P. aphanis* ascospore germination early in the season.

Datnoff et al., (2007) indicated that the concentration of Si in plant tissues varies greatly among species. Fatema et al. (2012) suggested that Si accumulation in strawberry plants enhances leaf hair growth on adaxial and abaxial leaf surfaces and has a role in reducing strawberry powdery mildew development.

ºBrix is the ratio of *Total Soluble Solids* (TSS) in a given weight of plant juice which contains mostly sucrose molecules but also small amounts of minerals, amino acids, vitamins, plant hormones, fructose, proteins and other solids (Harrill, 1998). Wei and Lu (2004) demonstrated that the ºBrix was increased in sugarcane with Si fertilizer application compare to the untreated. Moreover, Higher ºBrix led to higher nutrient density and higher fruit quality (Harrill, 1998) (Table 4.1). Miyake & Takahashi (1986) suggested that the strawberry plant translocates Si freely from the roots to the tops. However, strawberry plants are in the Anglospermae (Anglosperms), which are considered as a Si non-accumulator (Figure 4.1). These Si studies suggest two questions: how does Si benefit the strawberry plants and does Si reduce the levels of strawberry powdery mildew development?

Table 4.1: Refractive index of crop juices calibrated in % sucrose or Degree Brix.

Plant	Poor	Average	Good	Excellent
Hot Peppers	4	6	8	10+
Kiwi Fruit	8	12	11	18+
Kumquat	4	6	8	10+
Lemons	4	6	8	12+
Lettuce	4	6	8	10+
Limes	4	6	8	12+
Mangoes	4	6	10	14+
Nectarines	6	12	16	20+
Onions	4	6	8	12+
Onions (sweet)	6	8	10	16+
Onions (green)	6	12	16	20+
Oranges	6	12	16	20+
Papayas	6	10	18	22+
Parsley	4	0	5	10+
Peaches	6	12	16	20+
Peanuts	4	6	8	10+
Pears	6	12	16	20+
Pineapple	12	14	20	24+
Prunes	0	12	16	20+
Potatoes	4	6	10	12+
Raisins	60	70	75	30+
Raspberries	6	8	11	16+
Rutabaga	4	6	10	12+
Squash	6	8	12	16+
Strawberry	6	10	11	16+
Sweet Corn	6	8	18	24+
Sweet Potato	6	8	10	14+
Tomatoes	4	6	7	12+
Tomatoes (cherry)	10	14	16	22+
Turnip	4	6	8	10+
Watermelon	8	12	14	16+

Source: Harrill (1998)

Si is absorbed by plants in the form of uncharged silicic acid, H_4SiO_4, and is ultimately irreversibly precipitated throughout the plant as amorphous silica (SiO_2–nH_2O, silica gel, or phytoliths in higher plants). Therefore, although Si is an abundant element most sources of Si are insoluble and not in a plant-available form. Typical concentrations of silicic acid in soil range from 0.1 to 0.6 mM (Lewin

& Reimann, 1969). Lewin and Reimann (1969) indicated that silica gel is deposited in the epidermal cells, cell walls and xylem of many plant species. Silicic acid may replace or interfere with metabolism of other elements or it may function in the cell similarly to one or others. Moreover, the stimulation of cell wall reinforcement and the cuticle thickness has been found in strawberry, rice and other high plants (Kanto et al., 2004; cited in Dallagnol et al., 2015; Ma & Yamaji, 2006). Okuda and Takahashi also demonstrated that in the interactions between phosphorus and Si in rice, Si seemed to enhance the excessive uptake of phosphorus by rice plants and to promote the translocation of phosphorus into the grain which suggested the role of Si is unique in rice (cited in Lewin & Reimann,1969).

Ma and Yamaji (2006) recorded a gene family that controls Si uptake in rice. A gene family encoding Si transporters has also been identified in the marine diatom *Cylindrotheca fusiformis,* which requires Si as an essential element. Furthermore, Ma and Yamaji introduced one of the diatom Si transporter genes into tobacco but it did not increase Si uptake in tobacco. Thus, Si uptake system in higher plants is different from that in diatoms (Ma & Yamaji, 2006). Ma and Yamaji (2006) also proposed two mechanisms for Si enhanced resistance to diseases; one is that Si acts as a physical barrier because Si is deposited beneath the cuticle to form a Si cuticle double layer. Another mechanism is that soluble Si acts as a modulator of host resistance to pathogens (Ma & Yamaji, 2006). Si studies have shown that plants supplied with Si can enhance phenolic compounds and phytoalexins in response to fungal infection such as by rice blast

or powdery mildew (Ma et al., 2006, Silva et al., 2010 and Dallagnol et al., 2015). Si also enhanced the activity of chitinases, peroxidases and polyphenoloxydases in order to activate some defence mechanisms. Those biochemical responses are only induced by soluble Si, which suggest that soluble Si may play an active role in enhancing host resistance to disease by stimulating some mechanism of the defence reaction (Ma & Yamaji, 2006 ; Ma et al., 2006).

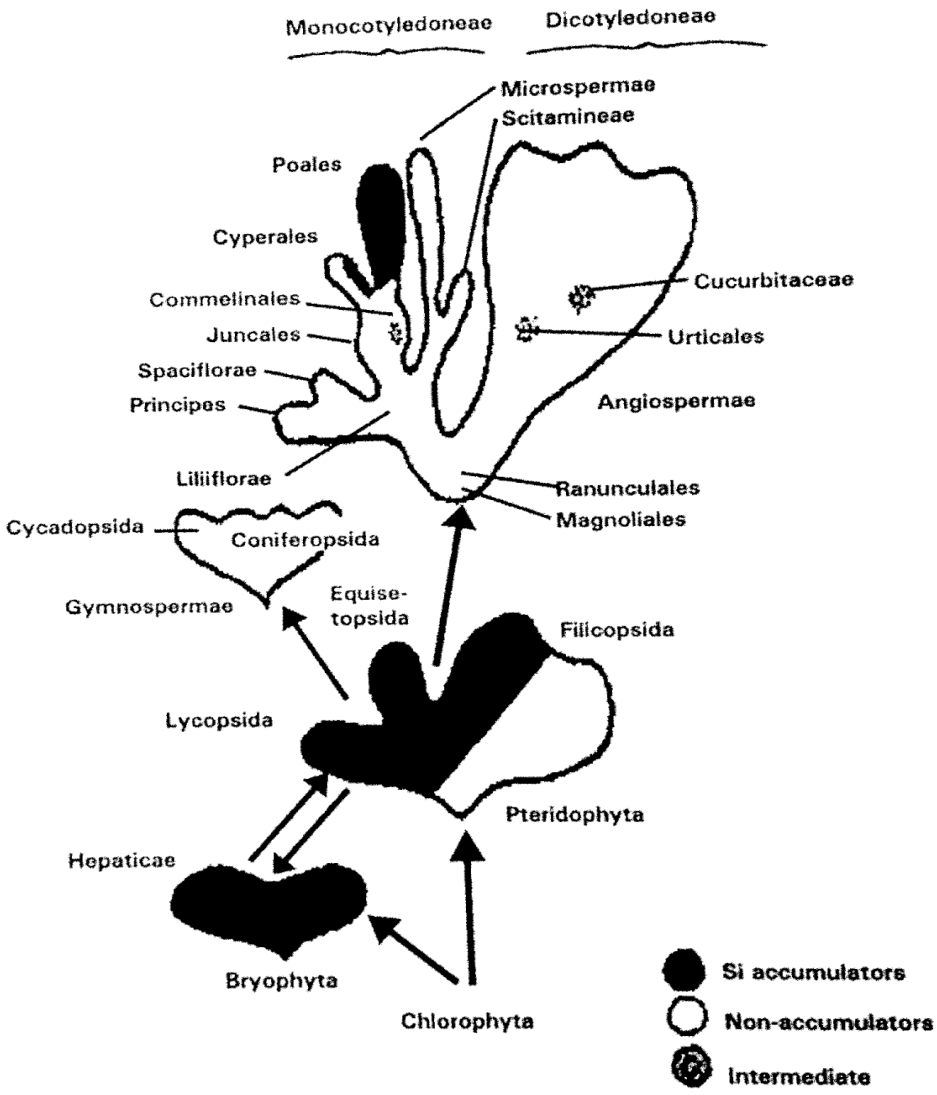


Figure 4.1: Distribution of Si accumulators in plant kingdom

Source: Epstein (1999)

4.2 Aims and objectives

4.2.1 Aims

Investigate the hypothesis that the application of Si nutrient reduces disease susceptibility and changes on morphology and physiology of strawberry plants.

4.2.2 Objectives

1. Follow the build-up and progression of *P*. *aphanis* in the field to Investigate the efficacy of Si nutrient (foliar spray application and root treatment through fertigation tube) in reducing disease susceptibility to strawberry powdery mildew
2. Identify the effects of Si nutrient in changing morphology and physiology of strawberry plants
3. Quantify the Si concentration in Si nutrient treated and untreated strawberry plant tissue and investigate the correlation between the si concertation in plant and disease level
4. Location of Si in strawberry plant tissue

4.3 Method and materials

4.3.1 The effect of Si nutrient (foliar spray and root treatments) on the development of strawberry powdery mildew in field experiments

In July 2012 and July 2013, two Si nutrient (foliar spray treatment) field experiments were conducted (see chapter 2, Table 2.2). The source of Si nutrient was the commercial products SW7 and Sirius, which are composed of the same ingredients (refer to Appendix 5). The commercial product SW7 was used in the 2012 Si nutrient field experiment (foliar spray treatment) and Sirius was used both in the 2013 Si nutrient field experiment (foliar spray treatment) and the 2013 Si nutrient fertigation field experiment (root treatment). The source of potassium bicarbonate (K50) was the commercial product K50 (see 4.3.1.1, Table 4.6).

The Si nutrient was applied by spraying all leaves weekly in the 2012 (in the Diamante field) and 2013 (in the Amelia field) field experiments (foliar spray treatment). The six treatments (0.25% Si nutrient (v/v), at 0.5% Si nutrient (v/v) alone, 0.25% Si nutrient (v/v) plus K 50, 0.5% Si nutrient (v/v) plus K50, K50 alone and control were arranged in a randomized design with three (2012) or two replications (2013), each consisting of a plot containing fifteen to twenty strawberry plants (Figure 4.2 & Figure 4.3). The control treatment consisted of plants sprayed with deionized (DI) water. Fifteen leaves were sampled weekly from each plot (refer to chapter 2, 2.2.1). All samples were assessed for amount

of disease and number of chasmothecia (mature and immature) per leaf; a pre-assessment was done before the first application of the Si nutrient. The concentrations of available Si (extracted with Autoclave Induced Digestion (AID) method refer to 4.3.3.4) were 0.1 mg cm^3 (0.25% (v/v) Si nutrient) and 0.2 mg cm^3 (0.5% (v/v) Si nutrient). Each plot was sprayed with 500 ml Si nutrient on the adaxial surface of the leaves until runoff. A qualified sprayer operator sprayed the 2012 Si nutrient foliar spray field experiment. In the 2013 Si nutrient spray field experiment, handheld manual sprayers were used by author and others to spray the treated plot. Laboratory coat, goggles and gloves were required for spraying Si nutrient on the strawberry plants (Figure 4.4, Risk assessment form in appendix 11). Temperature and humidity were monitored by using two Tinytags (refer to chapter 2, 2.2.3).

The 2013 Si nutrient fertigation field (in Roadside field) experiment (root treatment) was conducted in commercially operated polyethylene tunnels using 10 beds in each of two tunnels with four treatments; untreated, Si nutrient alone, fungicide alone and Si nutrient plus fungicide (Fig. 4.5). The Si nutrient was applied through the fertigation system (root treatment). The control treatment consisted of plants were used tap water to apply through the fertigation system. The Si nutrient was applied as a nutrient at 0.017% Si nutrient (v/v) and the concentration of available Si nutrient was 0.003 mg/cm^3. The four treatments had five replicates, each consisting of (1m length of each coir bag) containing twelve strawberry plants. The Si nutrient was added to the fertigation system twice a week for four months. The strawberry variety (cv. Shelley) planted as an annual

crop and ten old leaves and ten fully expanded new leaves were randomly sampled from two coir bags of each bed weekly for thirteen weeks. All samples were assessed for amount of disease per leaf; a pre-assessment was done before the first application of the Si nutrient. Temperature and humidity were monitored by using two Tinytags (refer to chapter 2, 2.2.3).

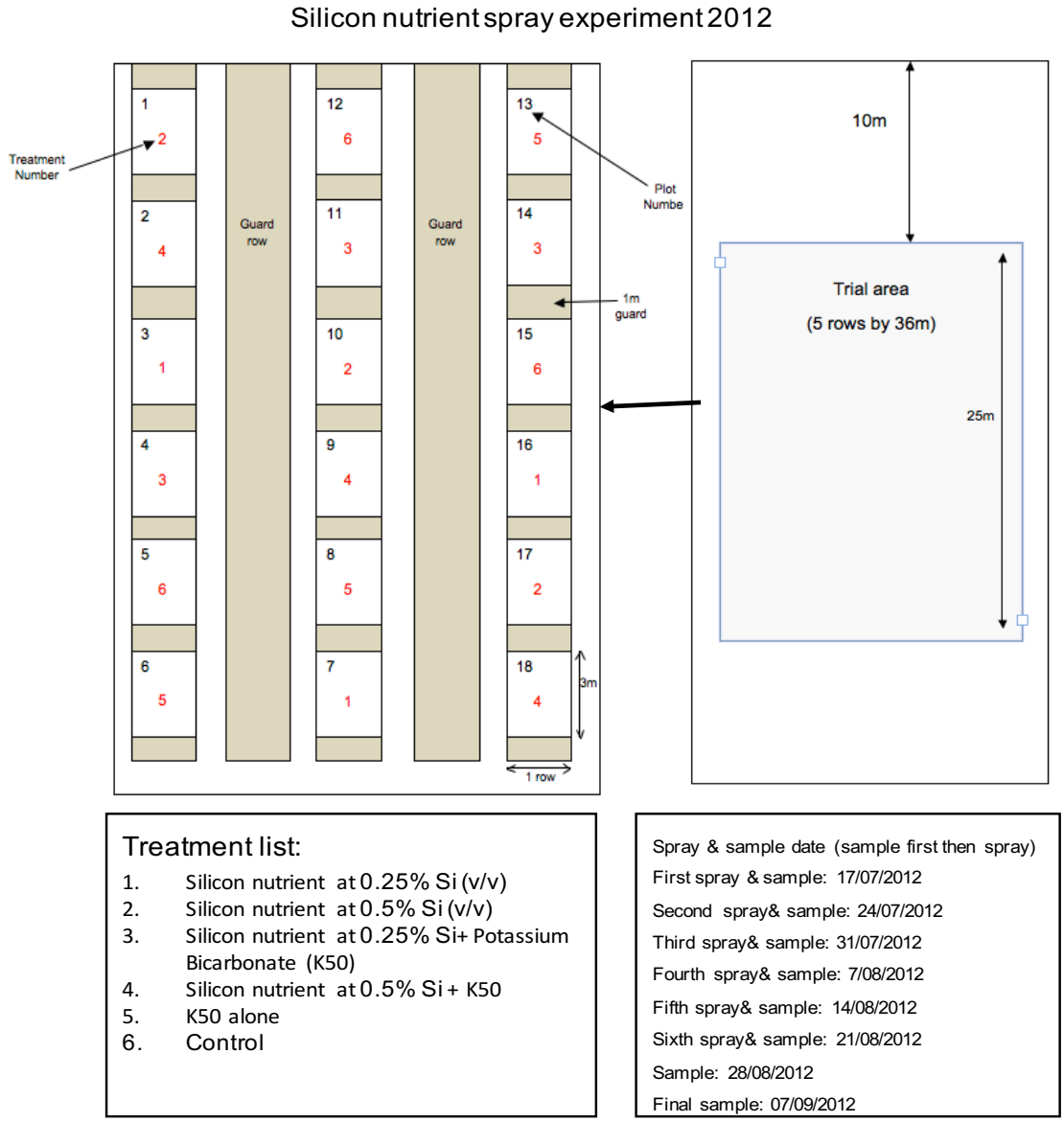
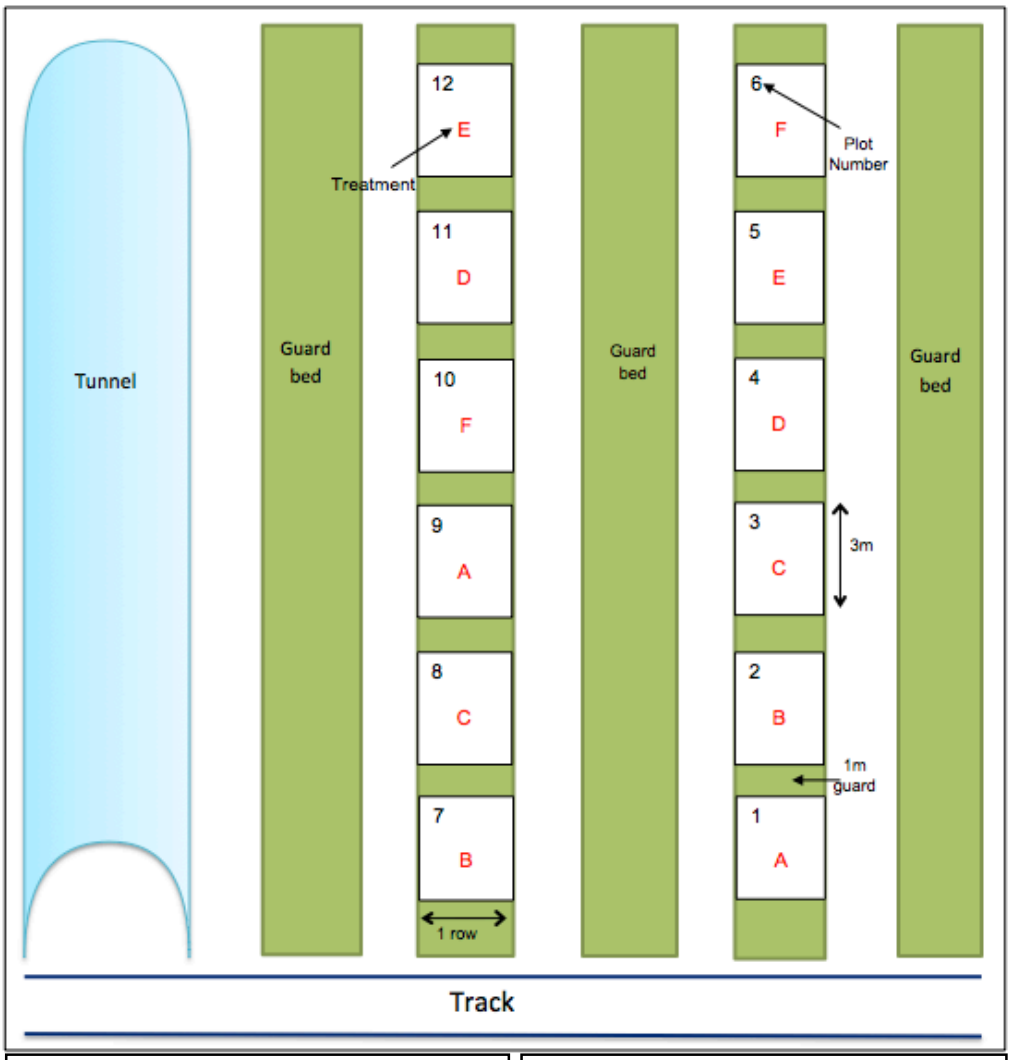


Figure 4.2: Randomized plot design of Si nutrient foliar spray experiment at Maltmas farm in 2012 in Diamante field (open field).

Silicon nutrient spray experiment 2013



Treatment list:

A. Silicon nutrient at 0.25% Si (v/v)

B. Silicon nutrient at 0.5% Si (v/v)

C. Silicon nutrient at 0.25% Si + Potassium Bicarbonate (K50)

D. Silicon nutrient at 0.5% Si + K50

E. K50 alone

F. Control

Spray & sample date (sample first then spray)

First spray & sample: 25/07/2013

Second spray& sample: 31/07/2013

Third spray& sample: 07/08/2013

Fourth spray& sample: 14/08/2013

Fifth spray& sample: 21/08/2013

Sixth spray& sample: 28/08/2013

Sample: 12/09/2013

Final sample: 02/10/2013

Figure 4.3: Randomized plot design of Si nutrient foliar spray experiment at Maltmas farm in 2013 in Amelia field (open field).



Figure 4.4: The author spraying Si nutrient on one strawberry plant in Bayfordbury station 2014.

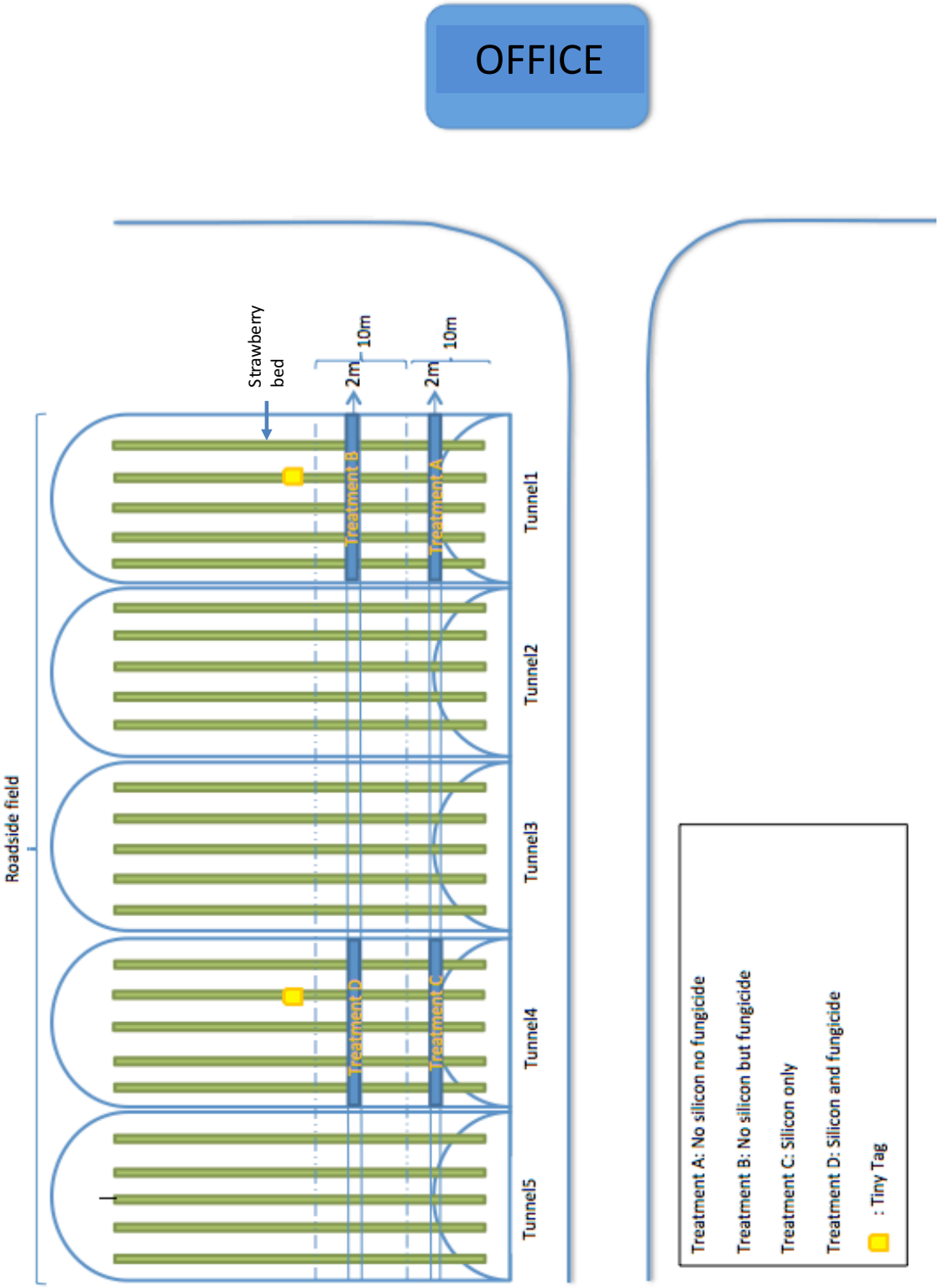


Figure 4.5: The design of Si nutrient fertigation field experiment (root treatment) at Maltmas farm in 2013 in Roadside field (polyethylene tunnels).

4.3.2 The dilutions of different rate of Si nutrient and K50 used in experiments

The dilutions method of Si nutrient for glasshouse and field experiments were all the same, but each experiment may have used different concentration of Si nutrient. The different concentrations of Si nutrient were used on strawberry plants to test the different effect. The recommended Si nutrient application concentration for foliar spray is 0.1% - 0.25% Si nutrient (v/v) on strawberry plants from OMEX (King's Lynn Norfolk, PE34 3JA) (Table 4.2). Thus, the 0.1% - 0.25% Si nutrient (v/v) concentrations were used in experiments; and strawberry grower at Maltmas Farm used the concentration of 0.017% Si nutrient (v/v) (refer to appendix 12) for the 2013 Si nutrient fertigation experiment. The dilutions of Si nutrient for glasshouse and field experiments were worked out from the rate chart of required quantity of Si nutrient per ha (Table 4.3) (Anon, 2014). The following Si nutrient concentrations were used in different experiments; 0.017% Si nutrient (v/v), 0.1% Si nutrient (v/v), 0.25 % Si nutrient (v/v), 0.5 % Si nutrient (v/v), 1.25% Si nutrient (v/v) and 2.5 % Si nutrient (v/v).

The recommended 0.25% Si nutrient (v/v) concentration was used by mixing 500ml Si nutrient with 200L water (Anon, 2014). The dilutions of different concentration of Si nutrient were mixed in the laboratory at UH. Table 4.4 shows the dilutions for different concentration of Si nutrient; 0.017% Si nutrient (v/v), 0.1% Si nutrient (v/v), 0.25 % Si nutrient (v/v), 0.5 % Si nutrient (v/v), 1.25% Si nutrient (v/v) and 2.5 % Si nutrient (v/v).

Table 4.5 shows the exact Si concentration in each Si nutrient concentration were used. The 1 ml original Si nutrient was assessed for Si concentration, which was 74.4 mg/cm^3. The K50 was used together with Si, the recommended dose of K50 was 10ml diluted with 1L DI water (Table 4.6).

Table 4.2: The recommended Si nutrient application rate on different plants

CROP	TIMING	RATE	COMMENTS
Cereals	Any time	0.1%	With foliar nutrients
Oilseed Rape	Any time	0.1%	With foliar nutrients
Lettuce	Any time	0.1% - 0.25%	With CalMax, Vitomex and DP98
Brassicas	Any time	0.1% - 0.25%	With CalMax, Vitomex and DP98
Potatoes	Any time	0.1% - 0.25%	With CalMax and DP98
Strawberry, Raspberry	Any time, but take care to avoid flowering	0.1 - 0.25%	With CalMax and Vitomex
Apples, Pears	Any time	0.1 - 0.25%	With CalMax and Vitomex
Other crops	Any time	0.1 - 0.25%	With any foliar nutrients

Source from: Anon (2014)

Table 4.3: Rate chart of required quantity of Si per ha.

Water volume L/ha	Required quantity of Si per ha		
	0.1%	0.15%	0.25%
200	200 ml	300 ml	500 ml
400	400 ml	600 ml	1 litre
600	600 ml	900 ml	1.5 litres

Source from: Anon (2014)

Table 4.4: The dilutions of different concentrations of Si in laboratory.

Application type	Dilutions (v/v) (Si/H_2O)
1) Root application 0.017 % (v/v)	1: 6000
1) Root and foliar spray application 0.1% (v/v)	1:1000
2) Foliar spray application 0.25% (v/v)	1:400
3) Foliar spray application 0.5% (v/v)	1:200
4) Foliar spray application 1.25% (v/v)	1:80
5) Foliar spray application 2.5% (v/v)	1:40

Table 4.5: The Si concentration in each Si nutrient used in each experiment

Si nutrient	0.017 % (v/v)	0.1 % (v/v)	0.25 % (v/v)	0.5 % (v/v)	1.25 % (v/v)	2.5 % (v/v)
Si concentration (mg/cm^3)	0.00007	0.05	0.1	0.2	0.5	0.8

Table 4.6: The recommended K50 application rate on different plants

CROP	RATE l/ha	RATE ml/100l	APPLICATION DETAILS
Cereals	2.0	200	When deficiency is suspected repeat applications as necessary at 10-14 day intervals
	5.0	500	1 or 2 applications when deficiency is diagnosed
Cut Flowers	3.0	300	3-4 applications during bud initiation and main flowering period at 10-14 day intervals
Fruit	3.0-5.0	300-500	When deficiency is diagnosed or suspected. Repeat as necessary at 10-14 day intervals.
	2.0	200	In spring to assist flower bud opening and set. Omex K50 can benefit skin finish and brix level in fruit.
Potatoes	up to 5.0	500	2-4 applications at bulking at 10-14 day intervals
Salad crops, Leafy crops	2.0	400	When deficiency is diagnosed or suspected. Repeat as necessary at 10-14 day intervals.
Vines	2.0	200	3-5 applications at 10-14 day intervals
Other crops	5.0	1000	When deficiency is diagnosed. Repeat as necessary at 10-14 day intervals.
	2.0	400	When deficiency is suspected. Repeat as necessary at 10-14 day intervals.

Source from: Anon (2014)

4.3.3 **The effects of Si nutrient on strawberry plants**

4.3.3.1 The measurement of Si concentration in strawberry plants

The AID method was used (Elliott & Snyder, 1991) to extract Si from strawberry plants. Strawberry specimens (washed or unwashed leaves and petioles) were dried in the oven at 60°C for 48h then ground using a porcelain mortar and pestle to fine powder. The experiment was carried out in replicates for consistency. 100mg of the ground plant sample was added to an autoclave resistant

polyethylene bottle. The plant samples were moistened with 3ml of 50% H_2O_2 followed by the addition of 3.5ml of 50% NaOH. The bottles were gently vortexed and individually covered with loose fitting caps, placed in a rack and autoclaved at 138Pka at 120°C for 1h. The contents were allowed to cool and were quantitatively transferred to 10ml polycarbonate tubes through coarse filter paper. 1ml of content was taken out from the 10ml polycarbonate tubes by pipette (P1000) and brought to a final volume of 5ml with DI water. Si in dilutions of samples was determined calorimetrically by the following procedure.

To each sample, 0.75ml of 2.5% boric acid was added and mixed by inversion. 0.25ml of ammonium molybdate (54g/L, pH7.0) was added, mixed and left to stand for 5min. 0.125ml of 20% tartaric acid was then added, after which 0.125ml of the reducing solution followed. The reducing solution was made in two parts; A (made by weighing 2g of Na_2SO_3 and 0.4g of 1-amino-2-naphthol-4 sulfonic acid in 25ml DI water) and B (25g of $NaHSO_3$ in 200ml DI water). Parts A and B were combined to a final volume of 250ml with DI. The absorbance was read using a semi-micro non-UV spectrophotometer (CECIL 2000, Cambridge) at 650nm.

A standard curve (Figure 4.1) was prepared to determine the concentration of Si in the strawberry leaves (treated or untreated) using known concentrations of Si. It was carried out for each experiment. The 250mg of 99% silicate powder was used as a known concentration of Si, which was diluted in 10 ml DI water. After serial dilution had been carried out, the concentrations used were 0.00007,

0.0001, 0.0003, 0.0006, 0.001, 0.002, 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5 mg/ml. The absorbance of these known concentrations were used to extrapolate the Si concentrations in the sampled strawberry leaves graphically using a regression line. The concentration was determined mathematically using the equation Y= 1.1833x.

4.3.3.2 Si content calculation

Si (µg/mg) = (C)(V) (d.f) / w

Where C is the concentration of the Si in the strawberry sample in mg /ml; V is the volume of the undiluted sample in ml; W is the sample weight in milligrams; and d.f is the dilution factor, as described below:

d.f = volume of diluted sample in ml/volume of aliquot taken for dilution in ml.

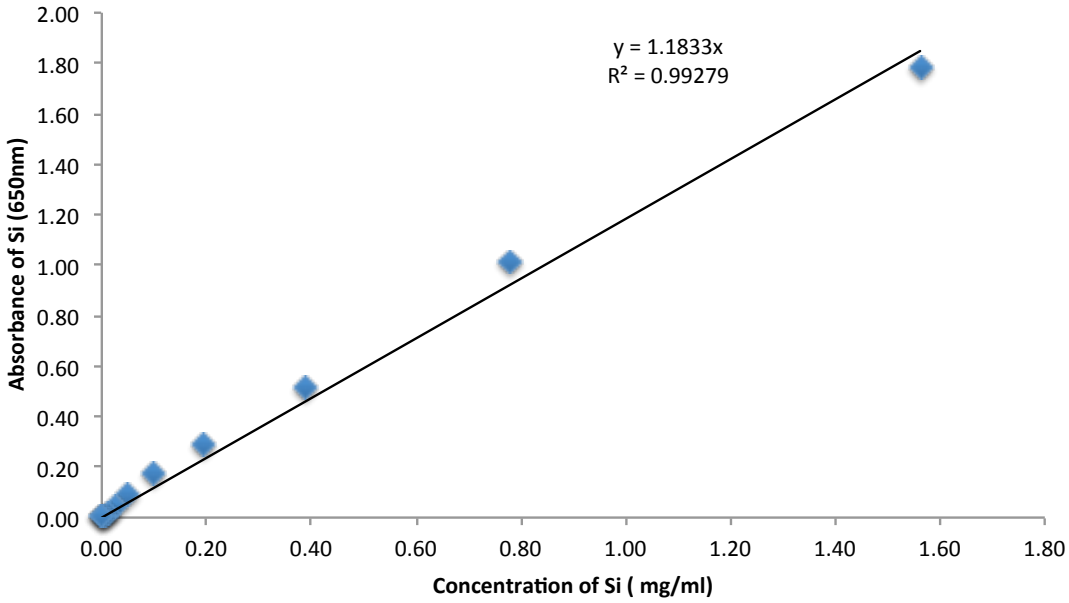


Figure 4.6: The Si concentrations standard curve.

Example:

Part A: strawberry leaf sample absorbance = 0.08

Use equation Y= 1.1833x to calculate the concentration of the Si in the strawberry leaf sample

0.08 = 1.1833x

X = 0.07 mg/ml

C = 0.07 mg/ml

Part B: Use equation Si (µg / mg) = (C)(V) (d.f) / w

Si (µg / mg) = 0.07 mg/ml*6.5ml*6.25ml/100mg

Si = 28 µg / mg

Thus, 100 mg of strawberry leaf sample contains 280 µg of Si.

4.3.3.3 The effects of Si nutrient (foliar spray and root application) on leaf morphology

4.3.3.3.1 Leaf hair measurement

The densities of leaf hairs on adaxial and abaxial surfaces were measured per cm^2 per leaf with six replicates under microscope at 10×3 magnification (Figure 4.7a & b) in the 2011 Si nutrient foliar spray glasshouse and the 2012 Si nutrient foliar spray field experiment. The lengths of leaf hairs were also measured per leaf with ten replications for using the eyepiece graticule at 10×3 magnification (Nikon dissecting microscope).

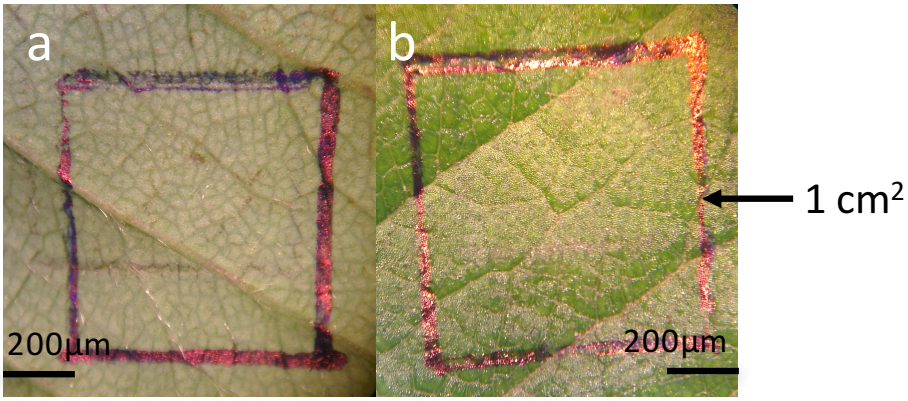


Figure 4.7: The strawberry leaflet surface with one cm^2 areas marked on at 10×3 magnification a) lower leaf surface, b) upper leaf surface.

4.3.3.3.2 Leaf thickness and appearance of wax formation measurement

A cross-section of strawberry leaves was made to observe leaf thickness. The

leaf cross-sections required the following equipment: slides and cover glasses (method refer to chapter 2, 2.3.3), Petri dishes filled with water for dipping, dissection needles, new one-sided razor blades, 1 raw carrot (cut to a few pieces) and the plant material to be sectioned. Cut the leaflet to three strips, put the middle leaf strip onto the center of carrot (carrot was cut out a cross deep gap in the center in order to hold the leaf strip) (Figure 4.8). Respectively, held the carrot to face the razor blade and then used the razor blade in the other hand, cut 6 -10 sections by making back and forth motions. With a wet dissection needle, remove the sections from the edge of the razor blade and place them in a petri dish with water. Inspect the sections and remove any that are too thick. The most transparent ones will be the thinnest. Use the dissection needle to select a few transparent sections and place section onto a slide with one or two drops of fungal mounting fluid to avoid sections dry out, then add on a cover glass. Finally, place the slide under a microscope (Nikon, model YS100) to measure leaf thickness by using the eyepiece graticule at 10×10 magnification.

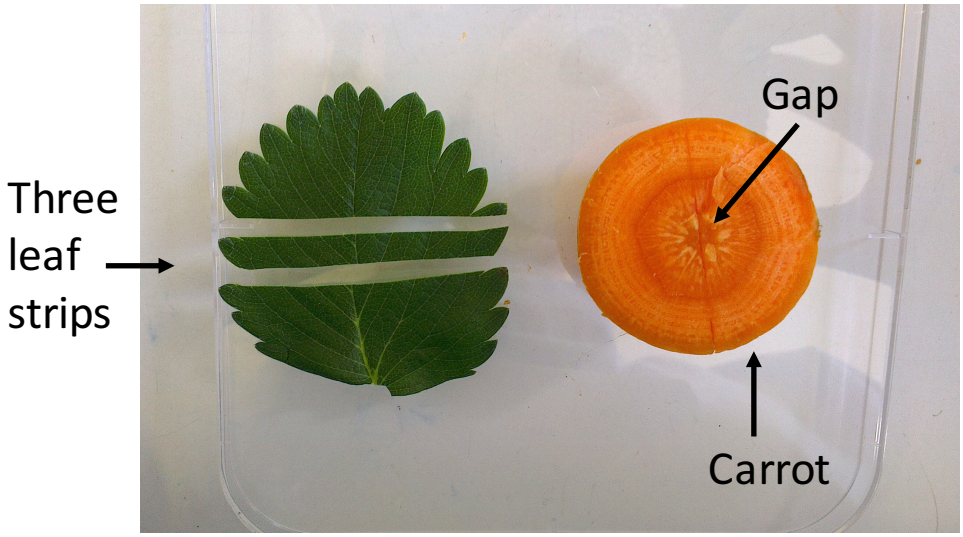


Figure 4.8: Required equipment (leaf sample and carrot) for leaf sectioning.

The appearances of wax on leaf surface and leaf cuticle thickness with different Si nutrient input treatments (foliar spray and root) were observed using SEM (scanning electronic microscopy). Si was detected (in leaf and petiole) in the cryo fracture face using the smart mapping mode or the Point and Identify tool, so an alternative option was chosen to obtain data (Figure 4.9) (for method refer to Appendix 4). Five spectrums were examined for each leaf sample and eight spectrums for petiole samples. Each spectrum line showed different elements in the examined area (Fig. 4.9 below). Then the computer calculated the percentage of each element in the area was examined. The mean of the data from five spectrums were calculated for each sample.

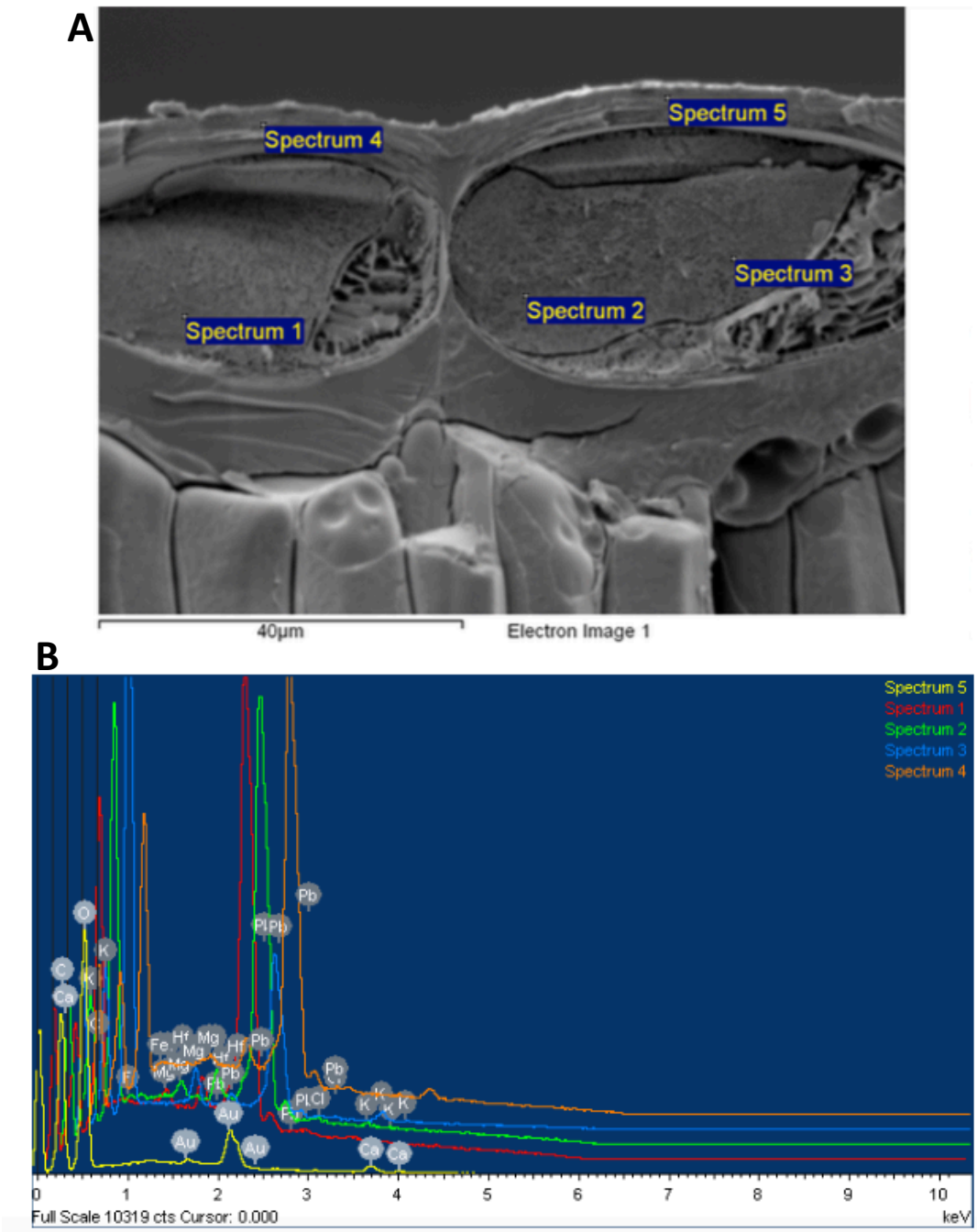


Figure 4.9: A) SEM image of freeze fracture across upper epidermal cells of strawberry leaf, each spectrum was the examined area B) the smart mapping mode showing different elements in each spectrum. Each spectrum line showed different elements in the examined area.

4.3.3.3.3 **The measurements of ºBrix on strawberry plant**

The juice of strawberry leaves, petioles and fruits were examined with the refractometer (Extech RF15 Handheld Brix Refractometer with ATC; 0 to 32%). Samples with different Si nutrient input treatments (foliar spray and root) were examined with four or five replications. A refractometer with a prism, a numeric scale and an eyepiece was used. Samples were squeezed to get some juice out. A pipette was used to drop 2 sample juices onto the prism and a demarcated line was viewed on the scale.

4.3.4 **Glasshouse experiments (2011-2014)**

Two Si nutrient glasshouse experiments were conducted in 2011 and 2014 respectively at UH Bayfordbury station. In 2011, four foliar spray treatments were arranged in a randomized design with five replications (refer to Chapter 2, Table 2.3), each replicate consisting of a single pot containing one strawberry plant. The control treatment consisted of plants sprayed with deionized (DI) water only. The spray application treatments were sprayed with 40 ml Si nutrient for each strawberry plant once every two weeks, which give total a five applications. The Si nutrient was sprayed on the upper surface of the leaves until runoff using a handheld manual sprayer. The density and length of leaf hairs were measured using eyepiece graticule at 10x3 magnification. The Si was extracted from strawberry leaf samples of different Si nutrient treatments to determine Si contents.

In 2014, sixty runner plants were repotted in the UH Bayfordbury glasshouse (Figure 4.10). Six treatments were arranged in a randomized design with ten replications (refer to Chapter 2, Table 2.3), each consisting of a single pot containing one strawberry plant. The control treatment consisted of plants sprayed with DI water only. The spray applications were sprayed weekly for nine applications with 40ml Si nutrient for each strawberry plant. The Si nutrient was sprayed onto the upper surface of the leaves until runoff using a handheld manual sprayer. For root treatments, 40ml Si nutrient were added with measuring cylinder into the compost through four 10cm deep holes around the plants weekly (Figure 4.11). In total, nine applications were made. The Si was extracted weekly from strawberry leaf samples of different Si nutrient treatments to determine Si contents. At the end of experiment, all strawberry plants were sampled to assess biomass.

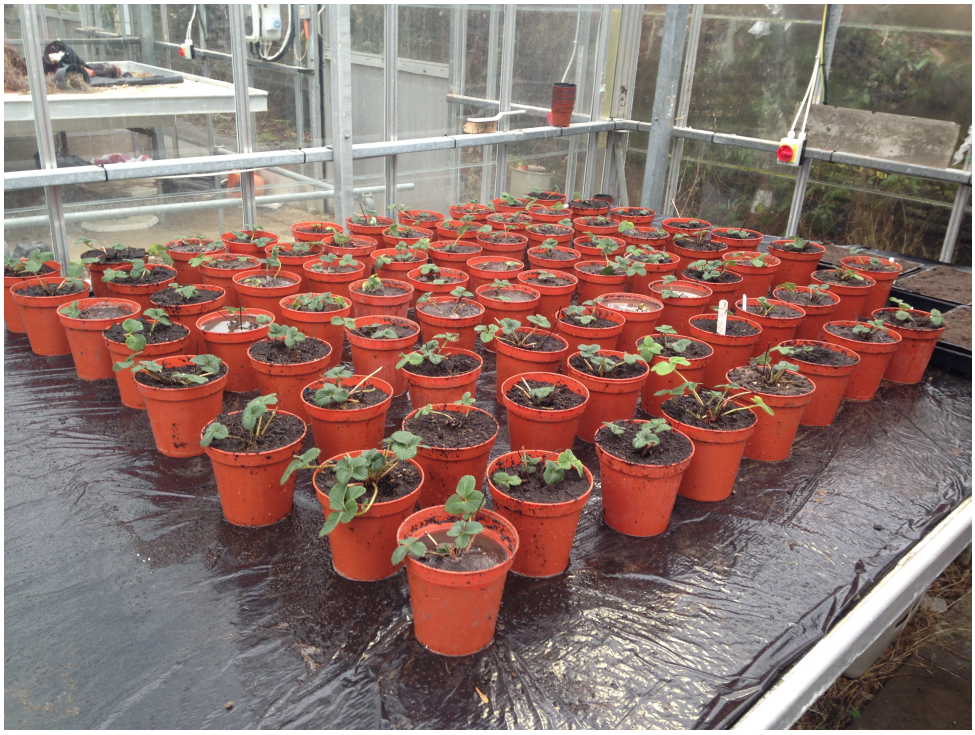


Figure 4.10: Runner plants were repotted and randomly placed on the bench in the UH Bayfordbury glasshouse.



Figure 4.11: One runner plant in a pot with four 10 cm deep holes around it.

4.3.4.1 The effects of different Si nutrient input treatments on the Biomass of strawberry plants

The assessment of biomass of strawberry plants with different Si nutrient input treatments (foliar spray or root application) used samples from the 2014 glasshouse experiment, which with ten replicates. The diameter of the basal stem (cm) (Figure 4.12), the length of highest petioles (cm), the length of roots (cm) and the fresh weights (g) of whole plants, top of the plants, roots, fruits and

runners (Figure 4.13) were measured before the plants were oven dried at 60 °C. After 72 h drying, the weights of dried samples (whole plants, top of the plants, roots, fruits and runners) were measured.



Figure 4.12: A cross-section of basal stem of strawberry plant.

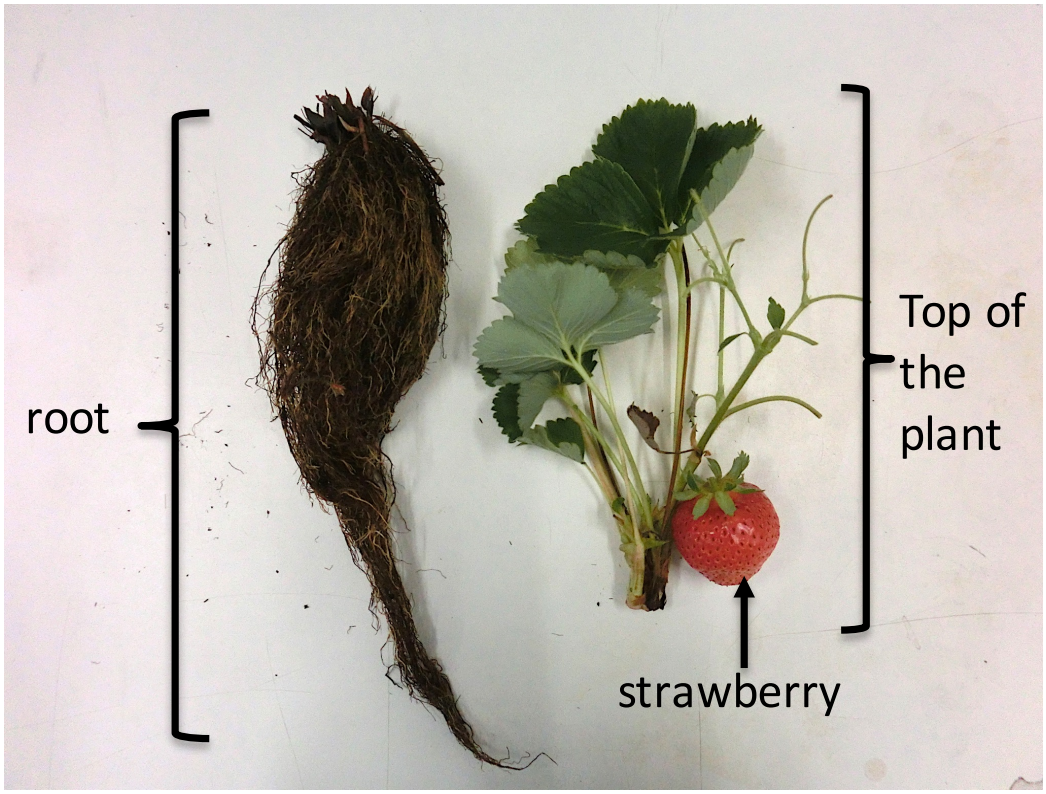


Figure 4.13: A strawberry plant was cut to separate the root and shoot.

4.3.5 Confocal microscopy for localization of Si in strawberry leaves and petioles with and without Si nutrient treatment

The confocal microscope (Nikon eclipse TE 2000-U) was used to study the spatial Si deposition in the strawberry leaves and petioles of plant either with Si nutrient treatment or untreated. For each treatment, ten strawberry leaves and petioles were examined. Prior to examination, cross sections of strawberry leaves and petioles were cut. The cross sections of strawberry specimens were stained for two hours separately in small petri dishes with the basic amine Lyso Tracker Yellow HCK-123 (a fluorescence dye, final concentration 1µM) (Shetty et al.,

2012). After two hours staining, a dissection needle was used to move specimens onto slides and viewed using confocal microscope at 10 x 40 magnification. The wavelength was set at 450 nm. The 'green' fluorescence of the stain was detected using an emission filter of 505–545 nm. The objective used was HC PL APO X 20／0.70 W (Shetty et al., 2012). One stack of 30 images (60 lm) was made for each recording either with different concentrations of Si nutrient treatment (foliar spray and root) or untreated.

4.3.6 **Statistical analysis**

The epidemic progress rate (*r*) of strawberry powdery mildew was calculated by using formula $\ln q = \ln q_{(0)} + rt$ in 2012 and 2013 (foliar spray and root) field experiments, to identify statistical differences between Si nutrient treatments on the rate of disease epidemic (Lucas, 1998). The area under the disease progress curve (AUDPC) (Lucas, 1998) was used to identify statistical differences between Si nutrient treatments on the amount of disease progress in 2012 and 2013 field experiments. AUDPC is estimated as:

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1} \left[(X_i + X_{i+1})/2 \right] (t_{i+1} - t_i).$$

4.4 Results

4.4.1 The effect of Si nutrient (foliar spray treatments) on the development of strawberry powdery mildew in the 2012 field experiment

Treatment of strawberry plants with 0.5%(v/v) Si nutrient resulted in a slowest epidemic progress rate (*r* = 0.002) of strawberry powdery mildew compared to other treatments (Figure 4.14). Treatments with 0.25%(v/v) Si nutrient plus K50 (*r* = 0.004) and 0.5%(v/v) Si nutrient plus K50 (*r* = 0.007) resulted in a slower epidemic compared to the untreated (*r* =0.018). The results of epidemic progress rate did not differ much between treatments K50 alone (*r* = 0.013) and the untreated (Figure 4.14). The Area Under the Disease Progress Curve (AUDPC) (Figure 4.15) showed that the strawberry powdery mildew developed more slowly with 0.5%(v/v) Si nutrient (AUDPC = 44) and 0.5%(v/v) Si nutrient plus K50 (AUDPC = 38) compared to other treatments and untreated (AUDPC = 70). Treatments of K50 alone (AUDPC =53), 0.25%(v/v) Si nutrient plus K50 (AUDPC = 48) and 0.25%(v/v) Si nutrient (AUDPC = 51) resulted in a slower disease development compared to the untreated (Figure 4.15). Both *r* and AUDPC values demonstrated that disease severity was less for the 0.5% Si nutrient (v/v) and 0.5% Si nutrient (v/v) plus k50 Si nutrient treatments than for other treatments. Moreover, the final foliar spray treatments were at day 35, high concentration of Si nutrient and high concentration of Si nutrient plus K50 treatments still hold the disease development between day 42 to 49, which indicated Si nutrient had a subsequent effect of reducing the overwintering stage of the pathogen (chasmothecia) and thus reducing disease carry over from one season to the next (chapter 3).

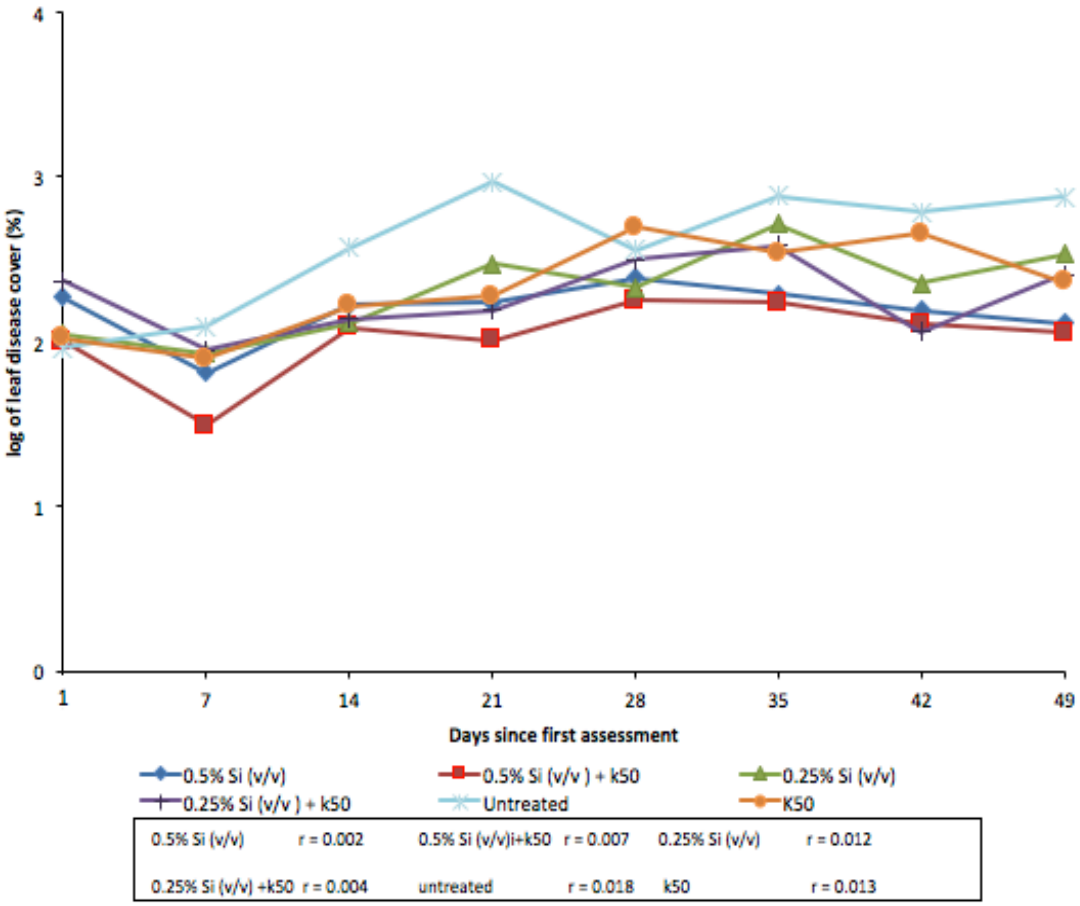


Figure 4.14: The progression of disease severity (*r* = the rate of epidemic development) (as measured by % of disease) over a seven week period for strawberry powdery mildew. There are six test groups, four treated with Si nutrient (foliar spray treatments) and two without. The disease severity was assessed each week. Si nutrient applied weekly from 17 July 2012 (day 1) to 21 August 2012 (day 35) (six applications in total). The first assessment was on 17 July 2012. (cv. Elegance, Alexandra & Sonata).

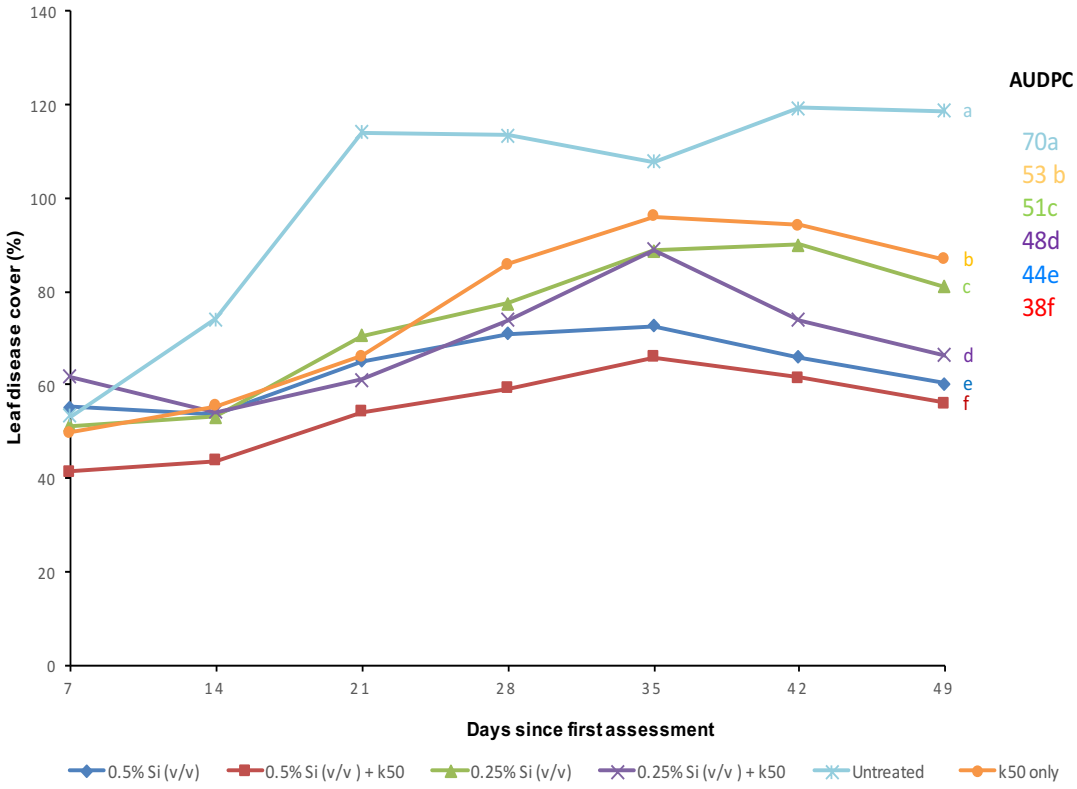
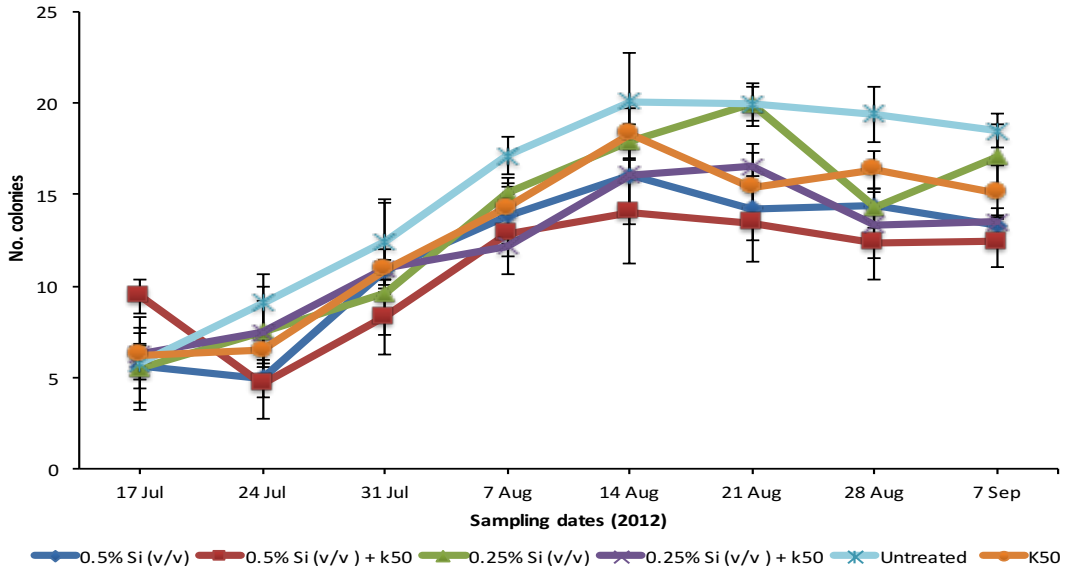


Figure 4.15: The progression of disease severity (AUDPC) (as measured by % of disease) over a seven week period for strawberry powdery mildew. There are six test groups, four treated with Si nutrient (foliar spray treatments) and two without. The disease severity was assessed each week. Si nutrient applied weekly from 17 July 2012 (day 1) to 21 August 2012 (day 35) (six applications in total). The first assessment was on 17 July 2012. (cv. Elegance, Alexandra & Sonata). The data of AUDPC are shown on the right side of this figure, the colour of the number matches the colour of the line which represents each treatments.

The average number of colonies and number of colonies per cm^2 per leaf showed that treatments of strawberry plants with 0.5% Si nutrient (v/v) and 0.5% Si nutrient (v/v) plus K50 resulted in a significant ($P < 0.001$) overall reduction in strawberry powdery mildew severity compared to the untreated (Figure 4.16). Treatments with 0.25 % Si nutrient (v/v), 0.25 % Si nutrient (v/v) plus K50 and K50 alone gave little reduction in strawberry powdery mildew compared to the untreated. There were no significant differences ($P > 0.05$) among 0.25% Si nutrient (v/v), 0.25% Si nutrient (v/v) plus K50 and K50 alone compared to the untreated (Figure 4.16).

Moreover, there was a significant ($P < 0.001$) correction between average number of colonies and average percentage disease cover on abaxial surface from 17 July 2012 to September 2012, which indicated that the greater the percentage disease cover was, the greater the number of colonies was (Figure 4.17).

(a)



(b)

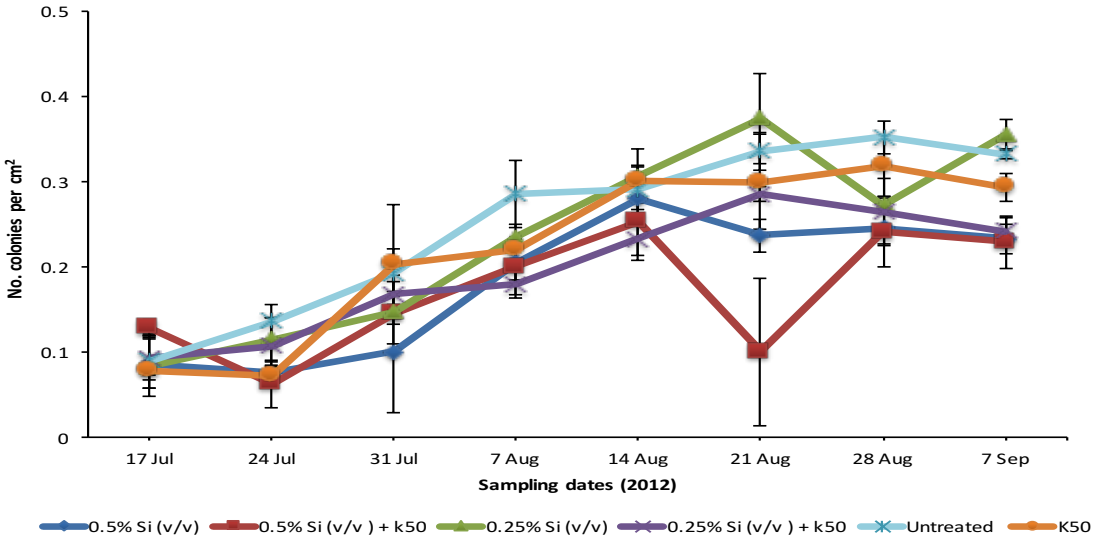


Figure 4.16: The effects of Si nutrient (foliar spray treatments) on the average number of colonies (a) and average number of colonies per cm^2 (b) per leaf of strawberry powdery mildew on abaxial surface. Si nutrient applied weekly from 17 July 2012 (day 1) to 21 August 2012 (day 35) (six applications in total). Bars represent standard error of mean, 2 degrees of freedom. (cv. Elegance, Alexandra & Sonata)

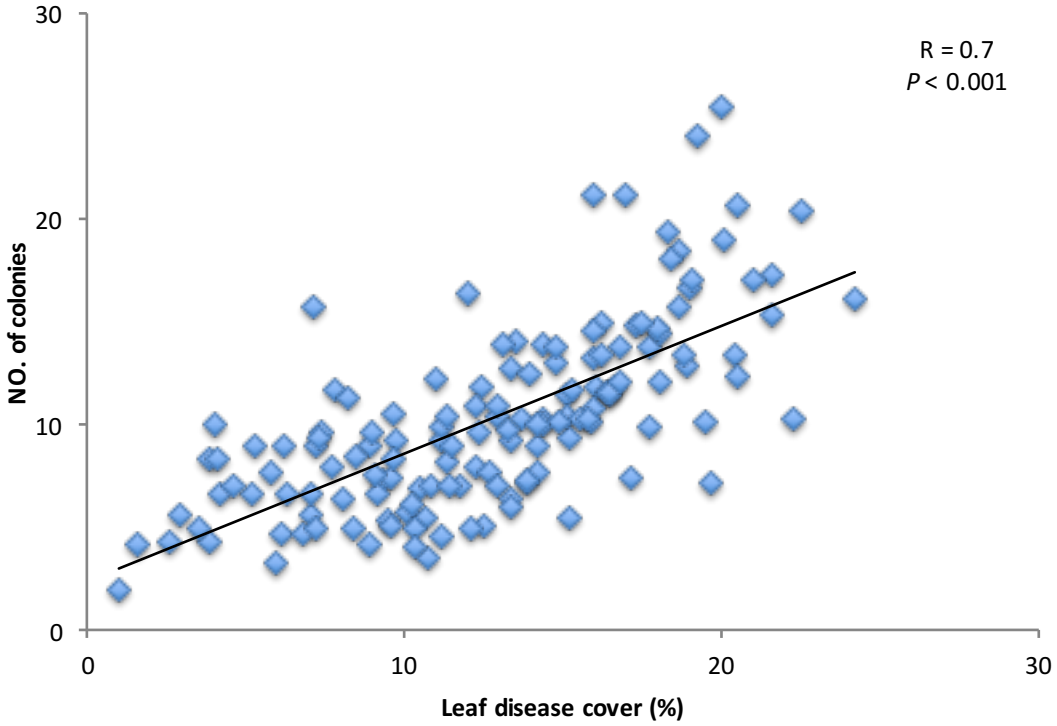


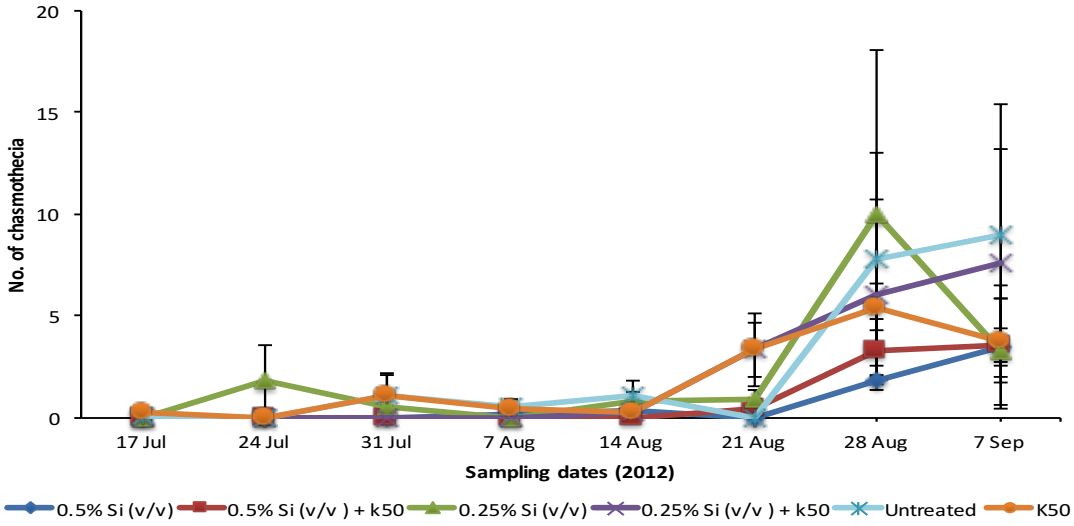
Figure 4.17: The correlation between average number of colonies and average percentage disease cover (P < 0.001) on abaxial surface from 17 July 2012 to 7 September 2012.

4.4.2 The effect of Si nutrient (foliar spray treatments) on chasmothecial development in the 2012 field experiment

There were similar numbers of chasmothecia and chasmothecia per cm^2 in treatments between 17 July 2012 to 21 August 2012 (Figure 4.18). However, after six Si foliar spray applications, treatment of strawberry plants with 0.5% Si nutrient (v/v) and 0.5% Si nutrient (v/v) plus K50 had fewer numbers of chasmothecia and numbers of chasmothecia per cm^2 compared to the untreated (Figure 4.18). Treatment of 0.25% Si nutrient (v/v) and K50 alone had fewer numbers of chasmothecia and chasmothecia per cm^2 compared to the untreated on 7 September 2012 (Figure 4.18). Treatment of 0.25% Si nutrient (v/v) plus K50 had similar numbers of chasmothecia and numbers of chasmothecia per cm^2 compared to the untreated. There were no significant differences between treatments and the untreated ($P > 0.05$). However, the high concentration of Si nutrient worked better than the low concentration of Si nutrient.

In addition, treatment of strawberry plants with 0.5% Si nutrient (v/v) and 0.5% Si nutrient (v/v) plus K50 had fewer numbers of mature chasmothecia compared to the untreated and other treatments (Fig. 4.19). Therefore, the greater reduction in the number of chasmothecia in 0.5% Si nutrient (v/v) and 0.5% Si nutrient (v/v) plus K50 treatments indicated a reduction in the number and maturity of overwintering chasmothecia, thus contributing to a reduction in initial inoculum in the next growing season (see Chapter 3).

(a)



(b)

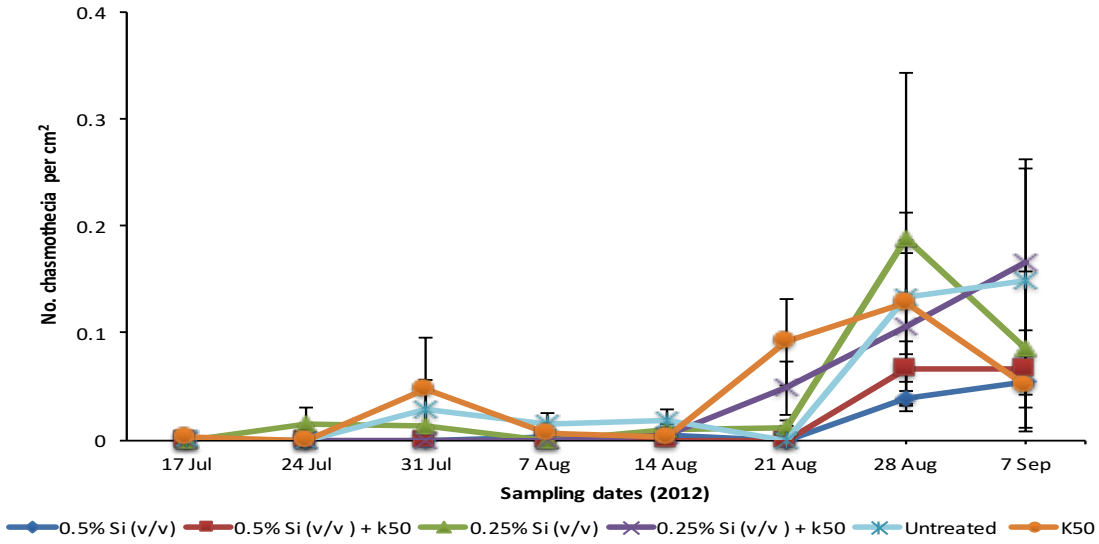


Figure 4.18: The effects of Si nutrient (foliar spray treatments) on the average number of strawberry powdery mildew chasmothecia (a) and average number of chasmothecia per cm^2 on abaxial surface (b) per leaf of on abaxial surface. Si nutrient applied weekly from 17 July 2012 (day 1) to 21 August 2012 (day 35) (six applications in total). Bars represent standard error of mean, 2 degrees of freedom. (cv. Elegance, Alexandra & Sonata).

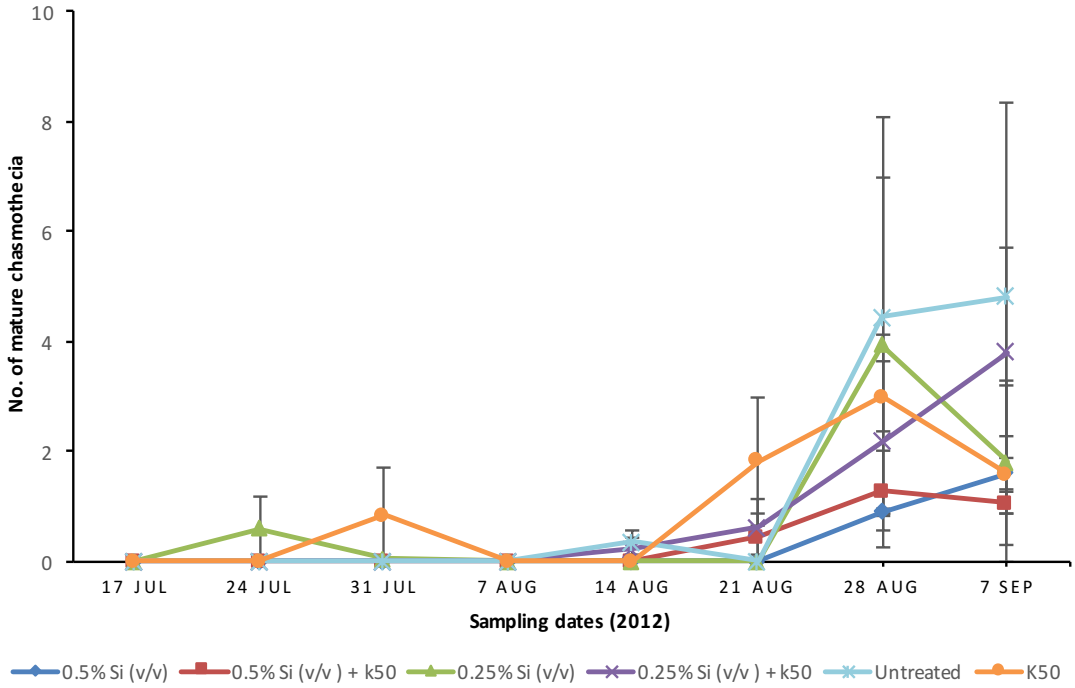


Figure 4.19: The effects of Si nutrient (foliar spray treatments) on the average number of mature chasmothecia per leaf on abaxial surface. Si nutrient applied weekly from 17 July 2012 (day 1) to 21 August 2012 (day 35) (six applications in total). Bars represent standard error of mean, 2 degrees of freedom. (cv. Elegance, Alexandra & Sonata).

There was a significant ($P < 0.001$) correlation between the average number of chasmothecia and the average percentage of disease cover on abaxial surface (Figure 4.20a). In addition, there was also a significant ($P < 0.001$) correlation between the average number of colonies per cm^2 and the average number of chasmothecia per cm^2 on abaxial surface from 17 Jul 2012 to 7 Sep 2012 (Figure 4.20b). Those strong correlations indicated the less disease cover there was, the less the number of chasmothecia formed.

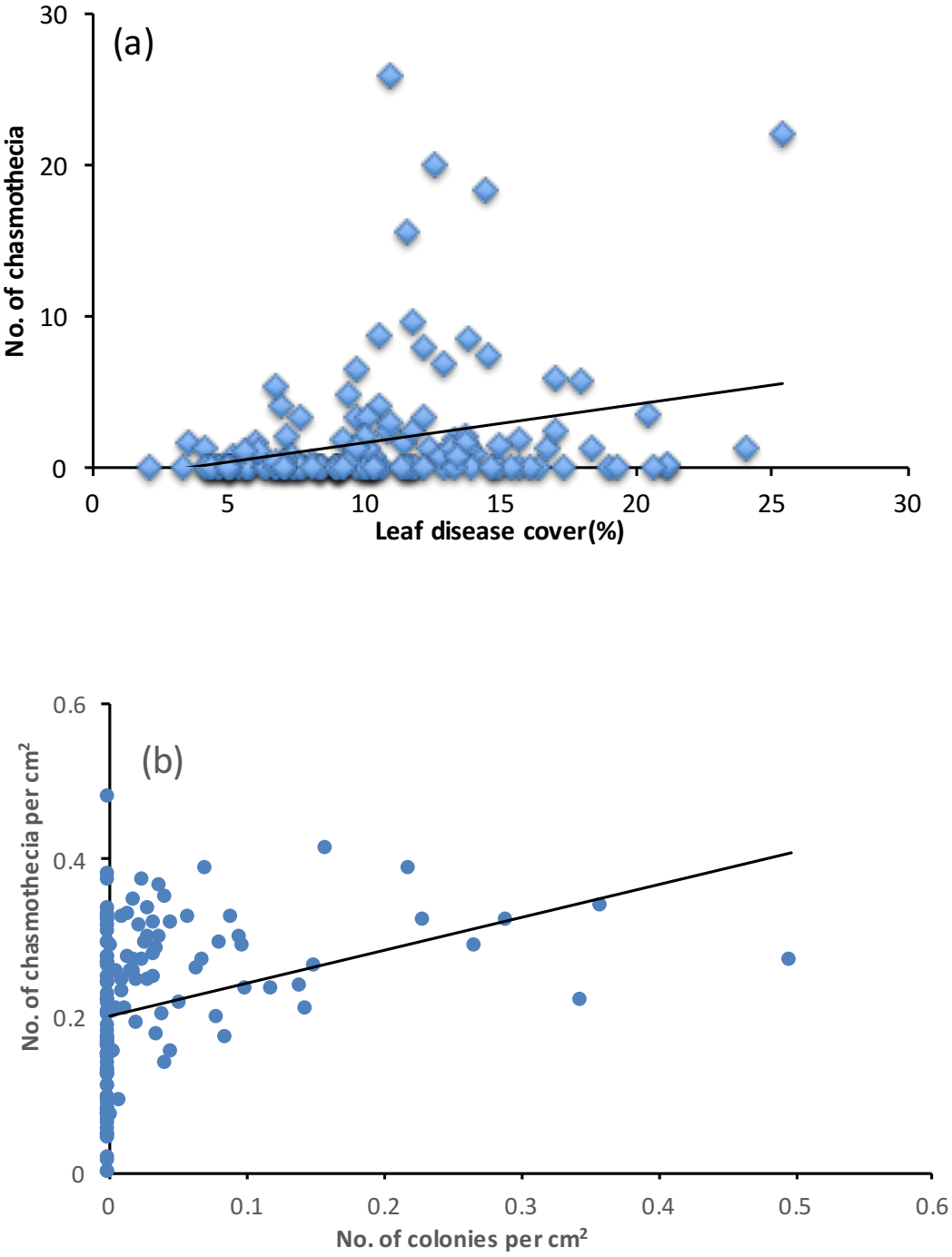


Figure 4.20: The correlation between chasmothecia and disease severity (a) correlation between average number of chasmothecia and average disease cover on abaxial surface ($P < 0.001$) (b) correlation between average number of chasmothecia per cm^2 and average number of colonies per cm^2 on abaxial surface ($P < 0.001$).

4.4.3 The effect of Si nutrient (foliar spray treatments) on inhibiting the development of strawberry powdery mildew in the 2013 field experiment

Treatment of 0.5% Si nutrient (v/v) plus k50 resulted in a slowest epidemic progress rate (*r* = 0.00009) of strawberry powdery mildew compared to other treatments (Figure 4.21). Treatment of 0.5% Si nutrient (v/v) (*r* = 0.0004), 0.25% Si nutrient (v/v) plus k50 (*r* = 0.0002) resulted in a slower epidemic progress rate compared to the untreated (*r* = 0.0012) (Figure 4.21). Treatment 0.25% Si nutrient (v/v) (*r* = 0.0009) and K50 alone (*r* = 0.0009) were also showed lower progress rate (*r*) compared to the untreated (Figure 4.21). The area under the disease progress curve (AUDPC) (Figure 4.22) showed that the strawberry powdery mildew developed most slowly with 0.25%(v/v) plus k50 (AUDPC = 6) and 0.5%(v/v) plus k50 (AUDPC = 4) compared to other treatments. Treatment of 0.5 % Si nutrient (v/v) (AUDPC = 12)resulted in a slower disease development compared to the untreated (AUDPC = 27) (Figure 4.22). Treatment 0.25% Si nutrient (v/v) (AUDPC = 26) and K50 alone (AUDPC = 24) also showed lower disease than the untreated (Figure 4.22). The disease severity varied greatly among treatments at pre-assessment (day 1), both *r* and AUDPC demonstrated that the high concentration of Si nutrient treatment has a greater effect on inhibiting the development of strawberry powdery mildew compared to the low concentration of Si nutrient treatment. Moreover, the final foliar spray treatments were at day 35, high concentration of Si nutrient and high concentration of Si nutrient plus K50 treatments still hold the disease development between day 50

(September) to 70 (October), which complement the result in previous year (2012).

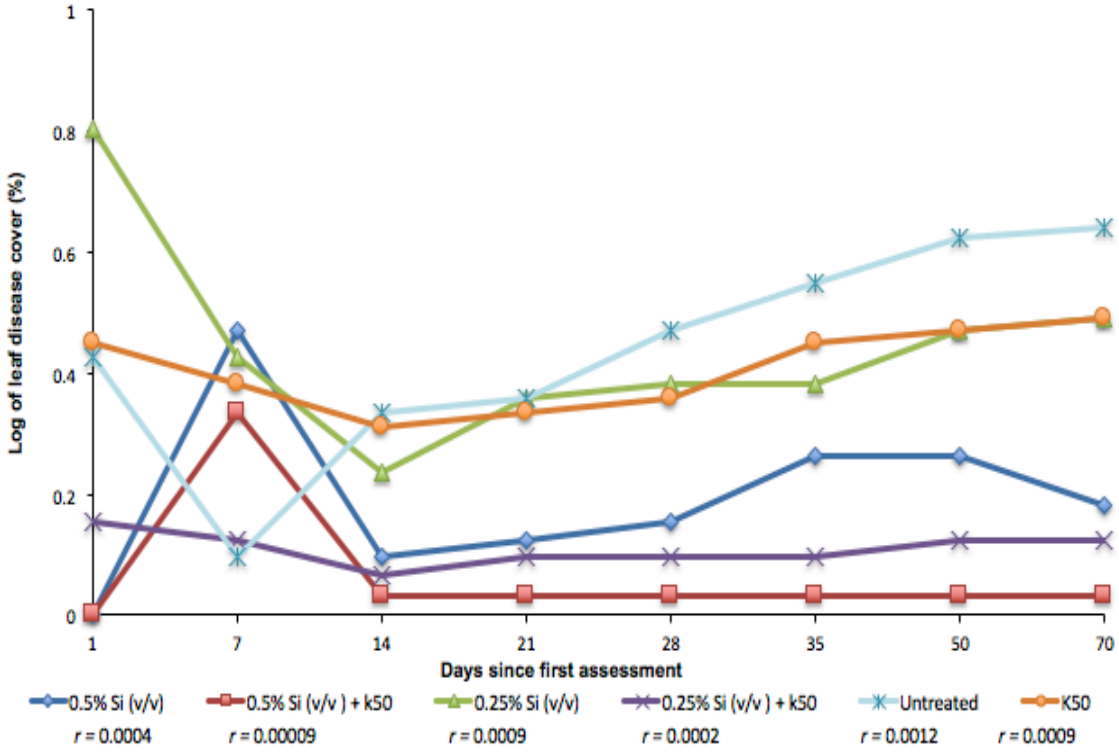


Figure 4.21: The progression of disease severity (*r* = the rate of epidemic development) (as measured by % of disease) over an eight week period for strawberry powdery mildew. There are six test groups, four treated with Si nutrient (foliar spray treatments) and two without. The disease severity was assessed each week. Si nutrient applied weekly from 25 July 2013 (day 1) to 28 August 2013 (day 35) (six applications in total). The first assessment was on 25 July 2013. (cv. Sonata).

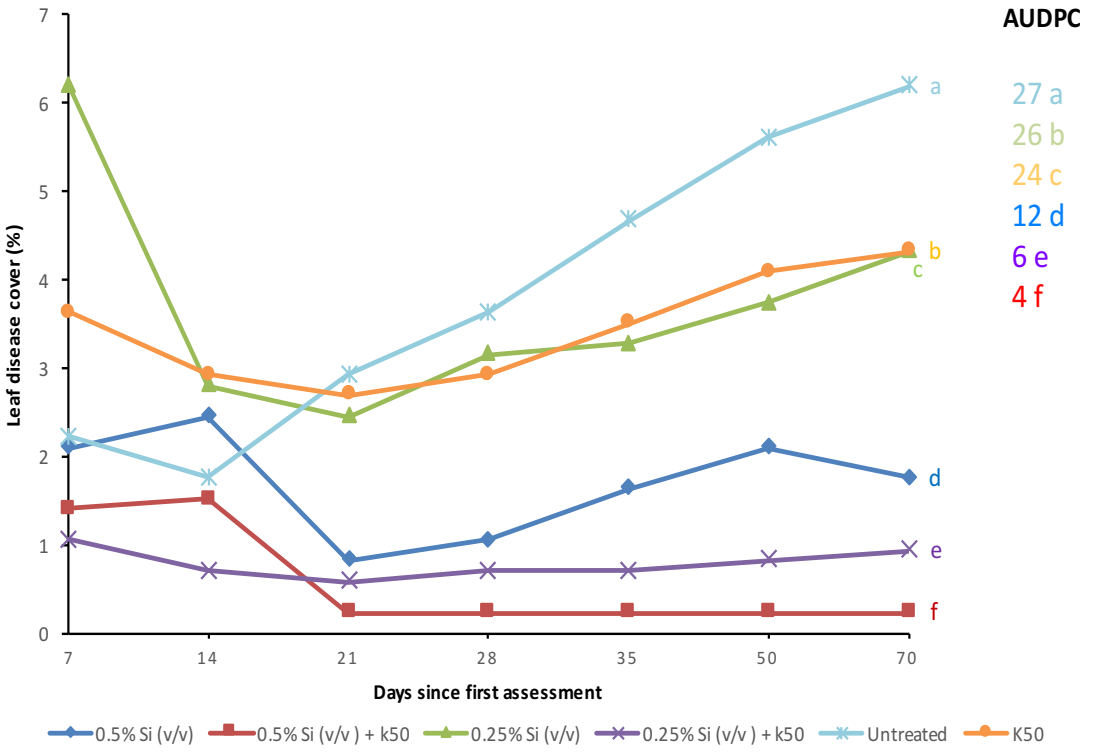


Figure 4.22: The progression of disease severity (AUDPC) (as measured by % of disease) over an eight week period for strawberry powdery mildew. There are six test groups, four treated with Si nutrient (foliar spray treatments) and two without. The disease severity was assessed each week. Si nutrient applied weekly from 25 July 2013 (day 1) to 28 August 2013 (day 35) (six applications in total). The first assessment was on 25 July 2013. (cv. Sonata). The data of AUDPC are shown on the right side of this figure, the colour of the number matches the colour of the line which represents each treatments.

The average number of colonies showed that treatment of 0.5% Si nutrient (v/v), 0.25% Si nutrient (v/v) plus K50 and 0.5% Si nutrient (v/v) plus K50 resulted in a significant ($P < 0.001$) reduction of strawberry powdery mildew compared to the untreated, which again demonstrated that higher concentration of Si nutrient and

higher concentration of Si nutrient plus K50 treatments have a marked effect on inhibiting the disease development (Figure 4.23). Treatments with 0.25% Si nutrient (v/v) and K50 alone gave little reduction of strawberry powdery mildew compared to the untreated. Moreover, there was a significant (P < 0.001) correlation between average number of colonies and average disease cover (%) on abaxial surface from 25 July 2013 to 2 October 2013 which indicated that the larger disease cover was, the greater the number of colonies was (Figure 4.24).

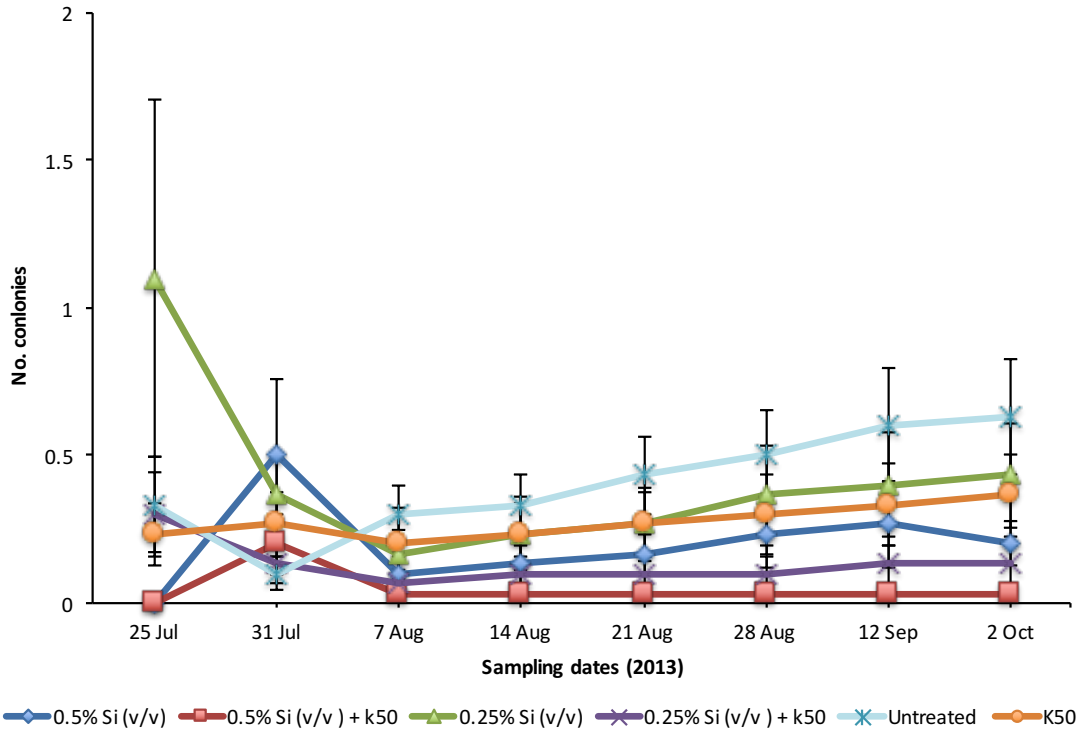


Figure 4.23: The average number of colonies of strawberry powdery mildew per leaf on abixial with different Si nutrient foliar spray treatments. Si nutrient applied weekly from 25 July 2013 (day 1) to 28 August 2013 (day 35) (six applications in total). Bars represent standard error of mean, 1 degrees of freedom. (cv. Sonata).

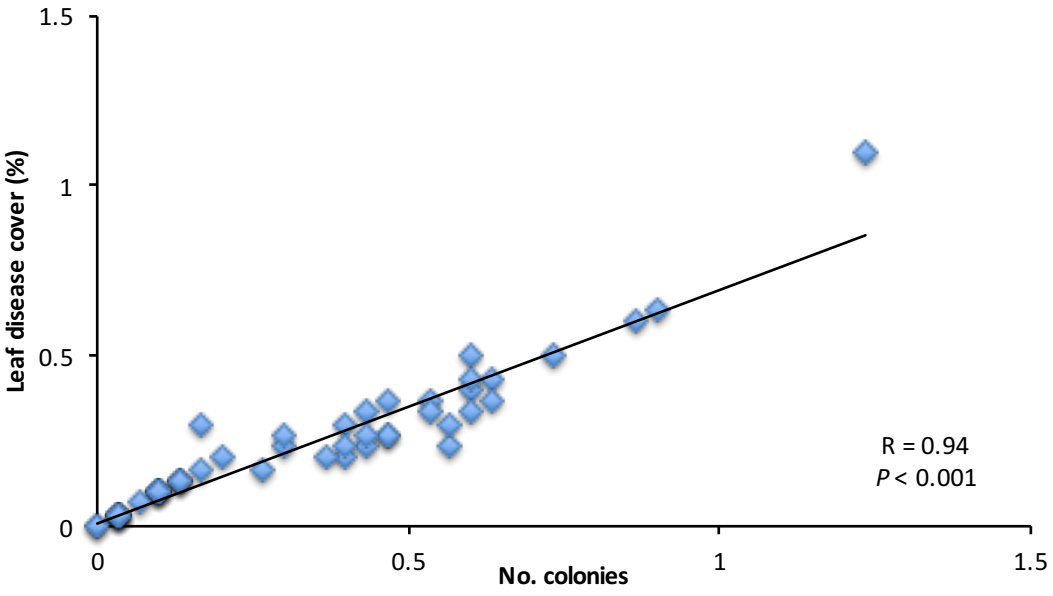


Figure 4.24: The correlation between average percentage of disease cover and average number of colonies ($P < 0.001$) per leaf on abaxial surface from 25 Jul 13 to 02 Oct 13.

4.4.4 The effects of the Si nutrient (foliar spray treatments) on strawberry plants

4.4.4.1 The concentration of Si in strawberry leaves (foliar spray) in the 2012 field experiment

The Si concentration in strawberry leaves was enhanced by the use of Si nutrient, spray with a high concentration (0.5% Si nutrient (v/v)) giving higher level of Si (Figure 4.25). The level of Si was still higher in the strawberry leaves with high concentration (0.5% Si nutrient (v/v)) compared to the untreated after 17 days without Si nutrient foliar spray. Furthermore, there was no significant differences ($P > 0.05$) between each of the treatments.

Moreover, there was a significant (P < 0.001) correlation between the Si concentration in strawberry leaves and the average number of colonies and number of colonies per cm^2, which demonstrated that a greater accumulation of Si in a leaf may result in less disease severity.

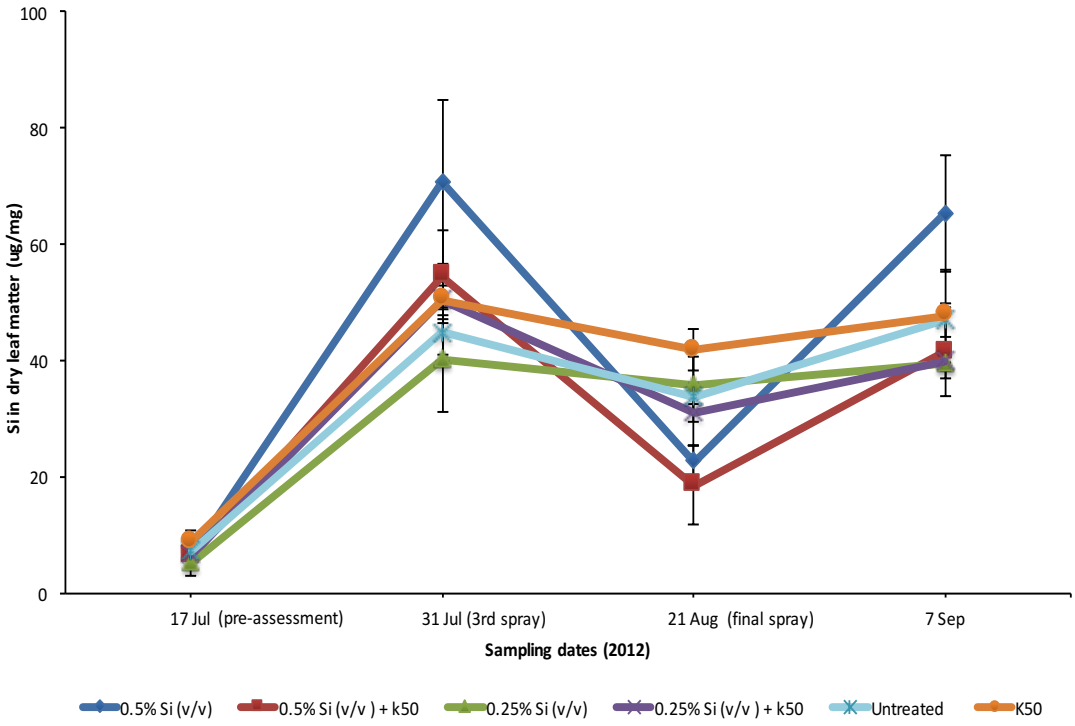


Figure 4.25: The concentrations of Si in strawberry leaves with different concentration of Si nutrient foliar spray treatments applied weekly in the 2012 field experiment. Bars represent standard error of mean, 2 degrees of freedom.

4.4.4.2 The concentration of Si in strawberry leaves (foliar spray) in the 2013 field experiment

The Si concentration in strawberry leaves was increased by Si nutrient spray

treatments, with the high concentration (0.5%(v/v) Si nutrient) giving higher level of Si (Figure 4.26). There were no significant ($P > 0.05$) differences between Si nutrient spray treatments and the untreated. However, the level of Si was still higher in the Si nutrient treated (0.5%(v/v) Si nutrient and 0.25%(v/v) Si nutrient) strawberry leaves compared to the untreated after 35 days without Si nutrient foliar spray (Figure 4.26). Furthermore, the previous year's data demonstrated that more accumulation of Si concentration in the leaf may result in lower disease severity. Therefore, the more Si was, the greater the disease resistance was.

The scanning electron microscope (SEM) Si detection showed the percentage of Si in leaf had a significant ($P < 0.001$) difference between the 0.5% Si nutrient (v/v) and the untreated on 2 October 2013 (Figure 4.27). There was no significant difference ($P > 0.05$) between other treatments and the untreated. Moreover, the Si was detected in strawberry petiole (0.54%) with treatment of 0.5% Si nutrient (v/v). There was no Si detected in strawberry petiole with the untreated. Therefore, the results again demonstrated that the higher concentration of Si nutrient foliar spray treatment resulted in more Si accumulation in plants, which complemented the previous year's data.

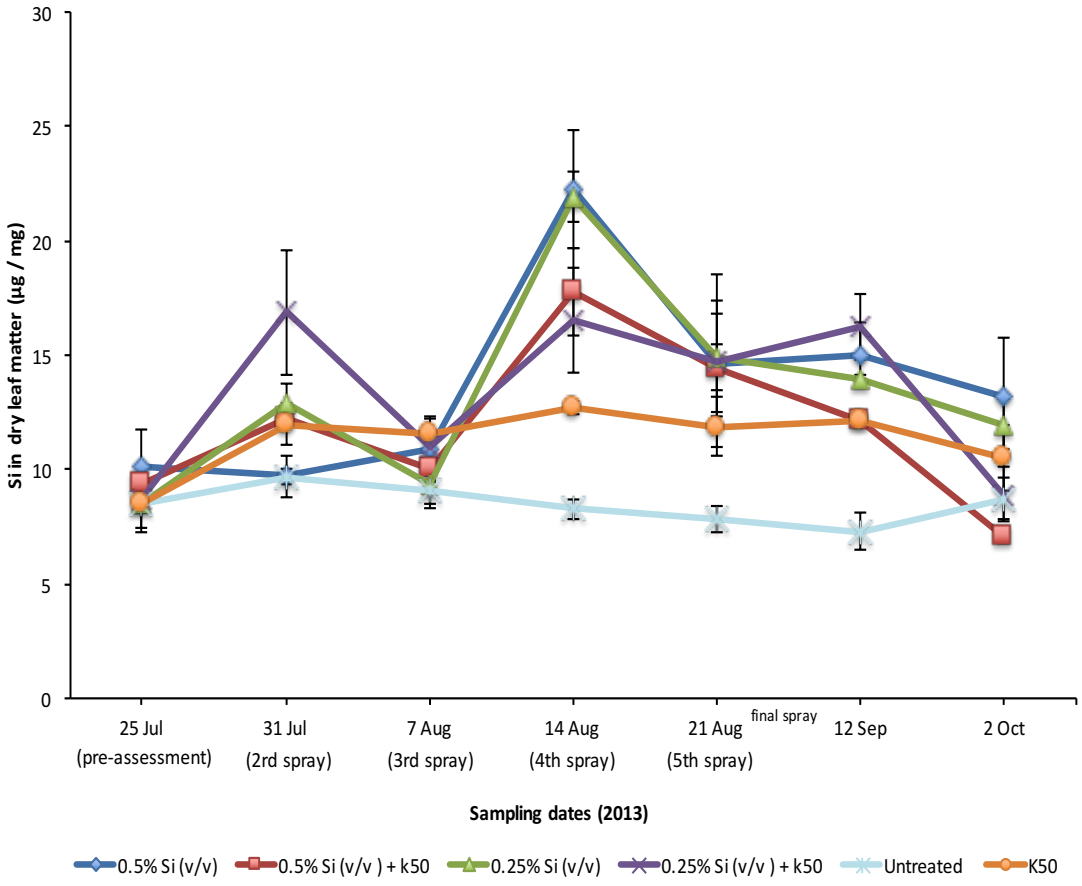


Figure 4.26: The concentrations of Si in strawberry leaves with different concentrations of Si nutrient foliar spray treatments in the 2013 field experiment. Bars represent standard error of mean, 2 degrees of freedom.

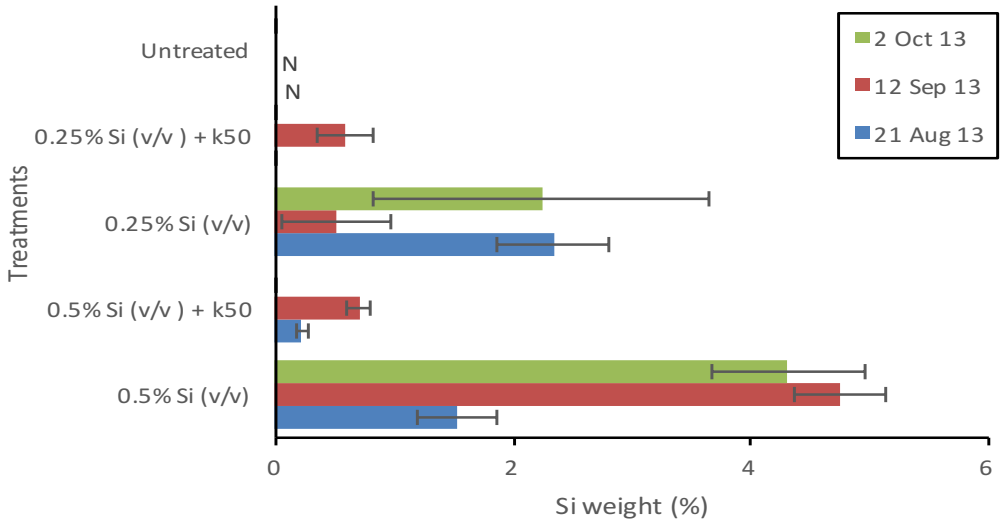


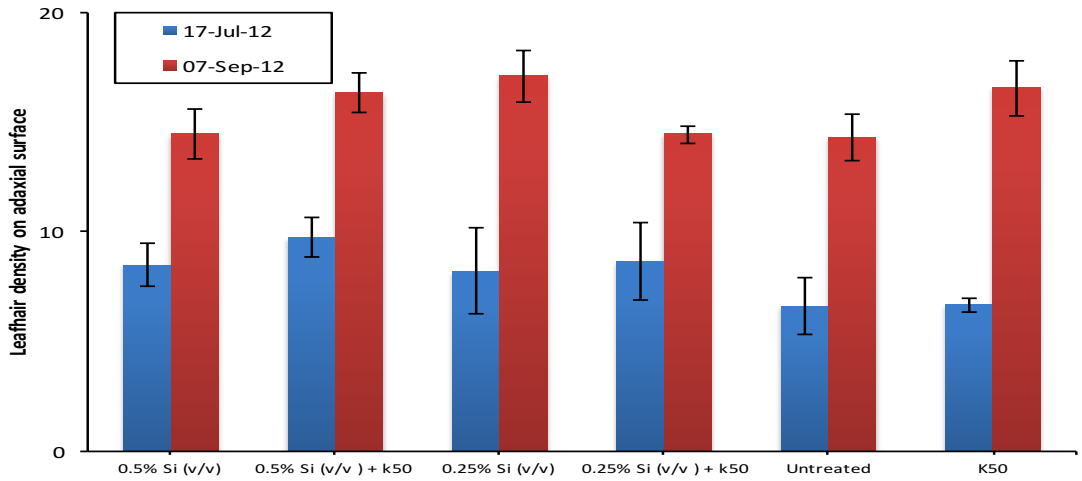
Figure 4.27: The scanning electron microscope (SEM) imaging for Si detection. Si (weight %) was detected in epidermal cells of strawberry leaves with different concentration of Si nutrient foliar spray treatments at three different sampling dates in 2013 field experiment. N = Sample not tested. Bars represent standard error of mean, 2 degrees of freedom.

4.4.4.3 The effects of Si nutrient (foliar spray treatments) on leaf morphology

4.4.4.3.1 **Leaf hair growth (in the 2012 field experiment)**

The average density of strawberry leaf hairs on abaxial and adaxial surface showed that the low concentration of Si nutrient (0.25% Si nutrient (v/v)) gave little increase compared to other treatments on 07 September 2012 (Figure 4.28). However, there were no significant differences ($P > 0.05$) between Si nutrient treatments and the untreated, which indicated that Si nutrient might not have a significant effect on the density of strawberry leaf hair on both abaxial and adaxial surface.

(a)



(b)

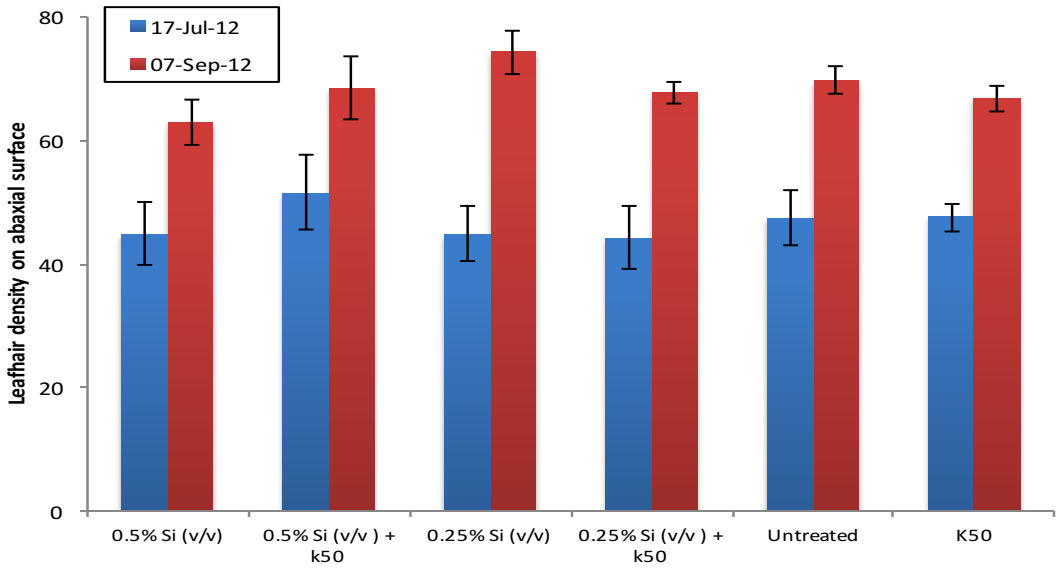


Figure 4.28: The effect of Si nutrient foliar spray treatments on the average density of strawberry leaf hairs on (a) adaxial surface and (b) abaxial surface. Si nutrient applied weekly from 17 July 2012 to 07 September 2012 (six applications in total). The pre-assessment was on 17 July 2012, the final assessment was on 07 September 2012. Bars represent standard error of mean, 2 degrees of freedom. (cv. Elegance, Alexandra & Sonata).

4.4.4.3.2 **Leaf and cuticle thickness (in the 2012 and 2013 field experiments)**

Treatments with 0.25% Si nutrient (v/v), 0.5% Si nutrient (v/v), 0.25% Si nutrient (v/v) plus K50 and 0.5% Si nutrient (v/v) plus K50 showed a significant ($P < 0.05$) increase in the leaf thickness compared to the untreated after five Si nutrient foliar spray treatments in the 2012 field experiment (Figure 4.29). Treatment with K50 alone showed less thickness than the untreated in 2012 experiment. Therefore, the Si nutrient treatments stimulate the plant growth, which strengthen the plant defense mechanism.

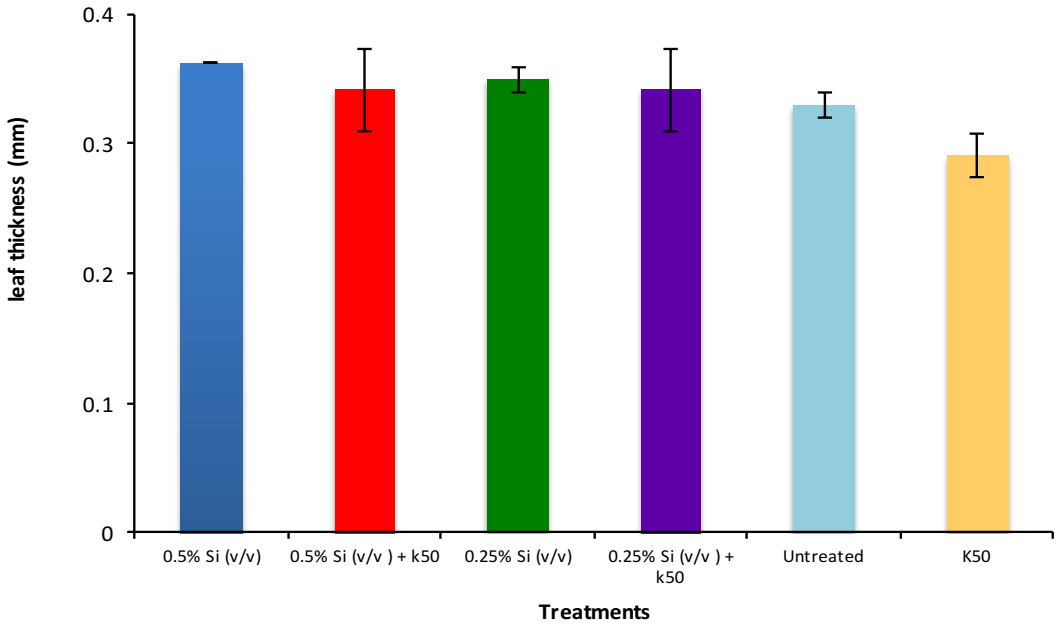
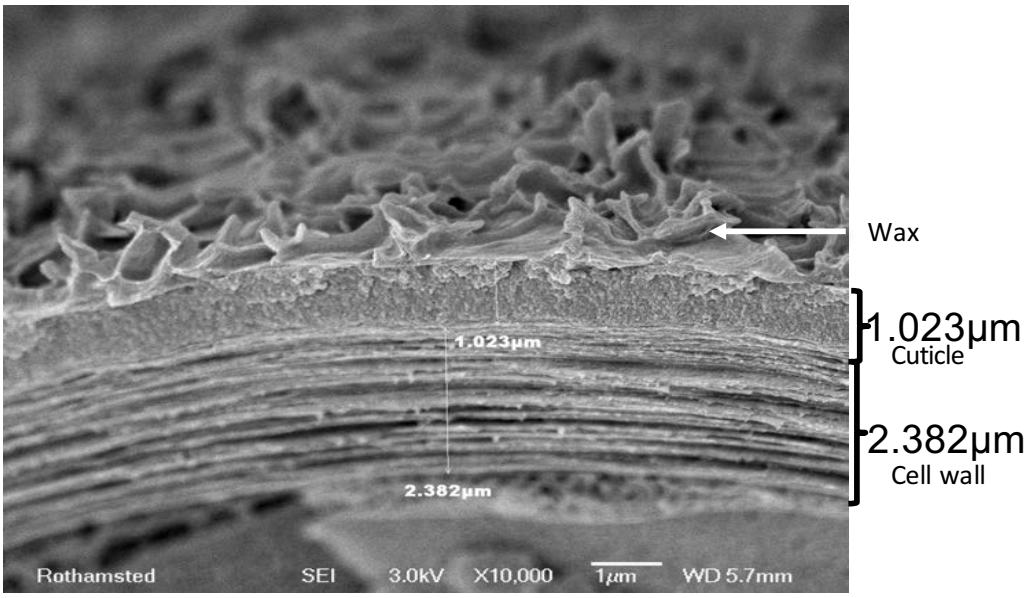


Figure 4.29: The effect of Si nutrient foliar spray treatments on the strawberry leaf thickness in the 2012 field experiment. The leaf samples were treated five times with different concentrations of Si nutrient. Bars represent standard error of mean, 2 degrees of freedom. (cv. Elegance, Alexandra & Sonata).

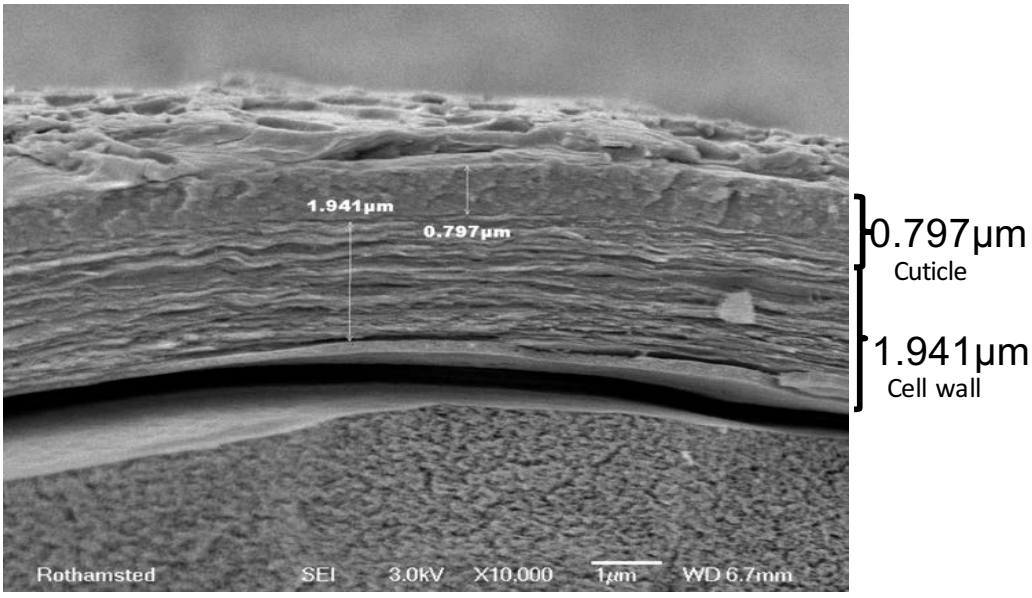
The SEM image of cuticle thickness showed that the 0.5% Si nutrient (v/v) had greater cuticle thickness than the 0.25% Si nutrient (v/v) in the 2013 field experiment (Fig. 4.30). The high concentration of Si nutrient (0.5% Si nutrient (v/v)) showed greater leaf thickness than the low concentration of Si nutrient (0.25% Si nutrient (v/v)) in the previous year data (2012). Therefore, Si nutrient spray treatments had great effect on the leaf and cuticle thickness which result in disease resistance. The untreated from 2013 field experiment was not tested.

4.4.4.3.3 **Appearance of wax formation on adaxial leaf surface (in the 2013 field experiment)**

The SEM image of appearance of wax formation showed that treatment with 0.5% Si nutrient (v/v) formed greater and thicker wax density formation on adaxial leaf surface compared to the 0.25% Si nutrient (v/v) at two different magnification x 5000 (left) x 10,000 (right) in the 2013 field experiment (Figure 4.31). Therefore, the high concentration of Si nutrient foliar spray treatment resulted in accumulated more Si on adaxial surface than the low concentration of Si nutrient, which demonstrated that greater wax density formation could prevent spore penetration. The untreated from the 2013 field experiment was not examined.

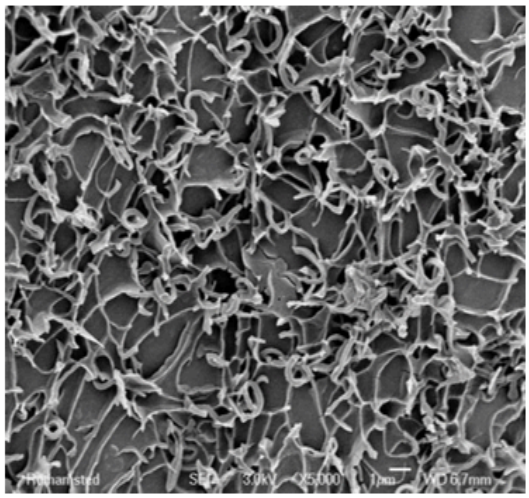


0.5% Si (v/v) adaxial surface (at x 10,000)

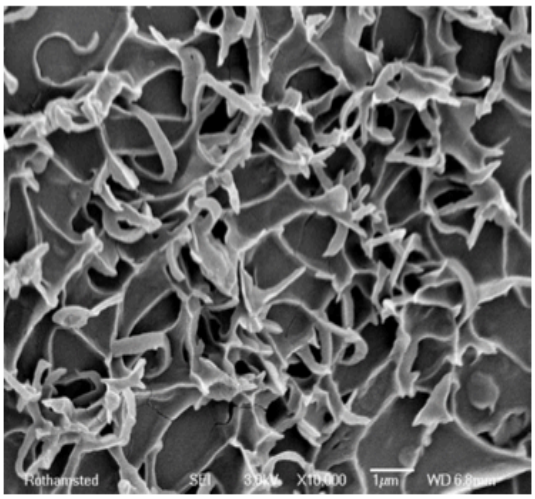


0.25% Si (v/v) adaxial surface (at x 10,000)

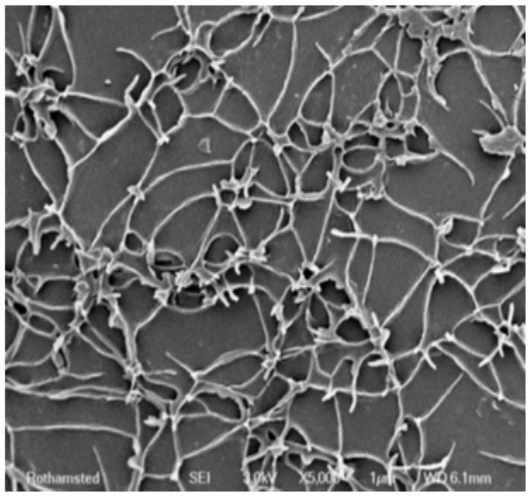
Figure 4.30: The effect of Si nutrient foliar spray treatments on the adaxial surface of strawberry leaf epidermal wall in the 2013 field experiment. The SEM image of strawberry leaf epidermal wall with treatments of 0.25% Si nutrient (v/v) and 0.5% Si nutrient (v/v). The leaf samples were treated with five applications of different concentrations of Si nutrient. (cv. Sonata).



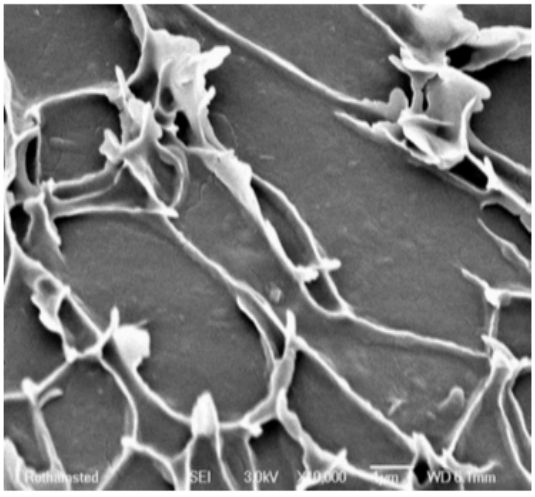
0.5% Si (v/v) adaxial surface (at x 5,000)



0.5% Si (v/v) adaxial surface (at x 10,000)



0.25% Si (v/v) adaxial surface (at x 5,000)



0.25% Si (v/v) adaxial surface (at x 10,000)

Figure 4.31: The effect of Si nutrient foliar spray treatments on the appearance of wax density in the 2013 field experiment. The SEM image showing the wax forming on the adaixal surface of strawberry leaves with 0.25% Si nutrient (v/v) and 0.5% Si nutrient (v/v) foliar spray treatments at two different magnifications.

4.4.5 The effect of Si nutrient (root treatment) on the development of strawberry powdery mildew in the 2013 Si nutrient fertigation field experiment

Treatment of strawberry plants with 0.017% Si nutrient (v/v) plus fungicides (*r* = 0.003) resulted in a slowest epidemic progress rate of strawberry powdery mildew compared to other treatments and delayed the epidemic for five weeks (Figure 4.32). Moreover, the epidemic was reduced at day 97 with 0.017% Si nutrient (v/v) plus fungicide treatment. The fungicides only (*r* = 0.011) and treatment with 0.017% Si nutrient (v/v) only (*r* = 0.019) resulted in a slower epidemic progress rate compared to the untreated (*r* = 0.026). The 0.017% Si nutrient (v/v) only delayed the epidemic for two weeks (Figure 4.32). The Si nutrient plus fungicide inhiabited the development of strawberry powdery mildew better than Si nutrient only or fungicide only.

The AUDPC showed that treatments with 0.017% Si nutrient (v/v) plus fungicides (AUDPC = 0.6) resulted in less amount of disease development compared to other treatments (Figure 4.33). The fungicides only (AUDPC = 1.2) and treatments of 0.017% Si nutrient (v/v) only (AUDPC = 12.8) resulted in a slower disease development compared to the untreated (AUDPC = 60.4) (Figure 4.33). Therefore, both *r* and AUDPC demonstrated that treatment with 0.017% Si nutrient (v/v) plus fungicide have a great effect on inhibiting the development of strawberry powdery mildew and treatment with 0.017% Si nutrient (v/v) alone delayed the strawberry powdery mildew development approximately up to two

weeks. Consequently, the late Si nutrient plus fungicide treatment in autumn had a subsequent effect of reducing the overwintering stage of the pathogen (chasmothecia) and thus reducing disease carry over from one season to the next.

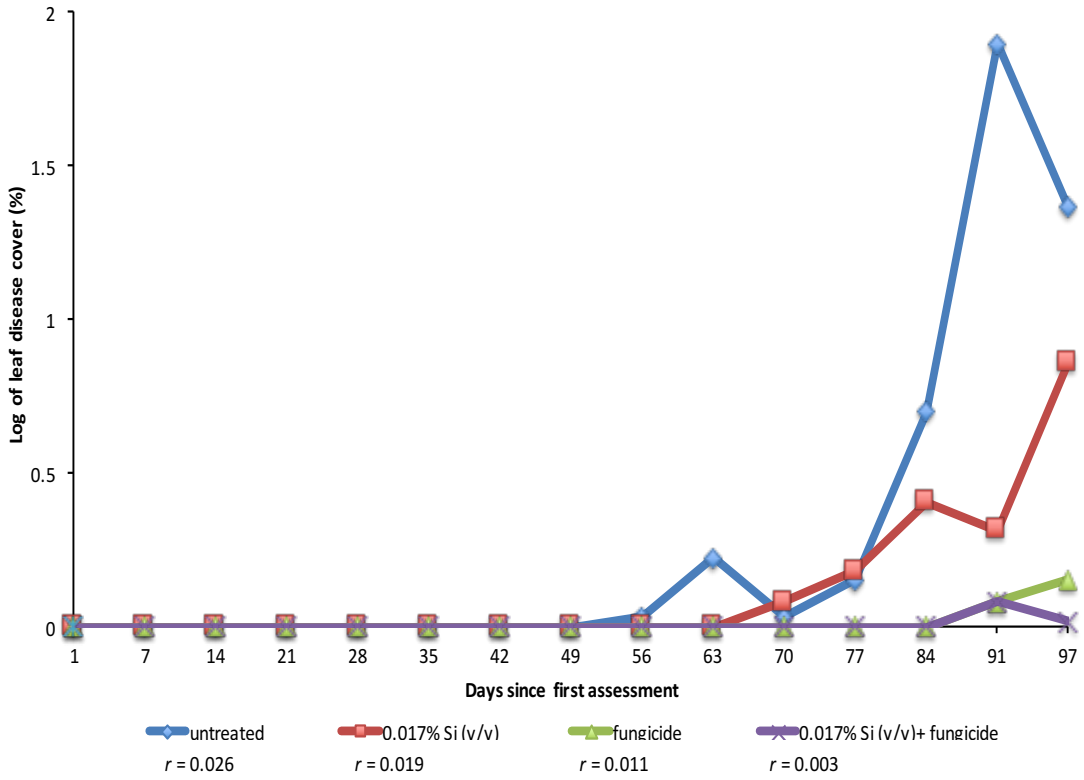


Figure 4.32: The progression of disease severity (*r*) (as measured by % of disease) over a fourteen week period for the strawberry powdery mildew in the 2013 Si nutrient fertigation field experiment (root treatments, polythene tunnel). There are four test groups, two treated with Si nutrient (root treatments) and two without. The disease severity was assessed each week. Si nutrient applied through fertigation tubes twice a week from 24 April 2013 to 31 July 2013. The first assessment was on 24 April 2013. (cv. Shelley).

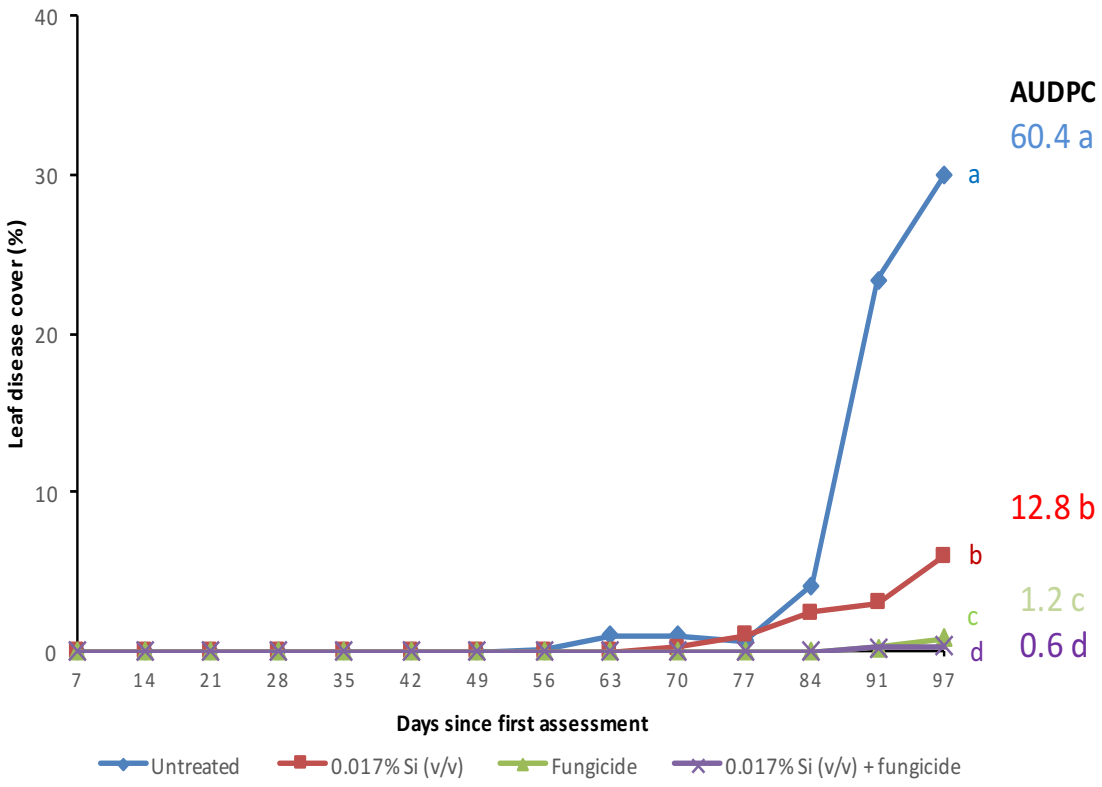


Figure 4.33: The progression of disease severity (AUDPC) (as measured by % of disease) over a fourteen week period for the strawberry powdery mildew in the 2013 Si nutrient fertigation field experiment (root treatments, polythene tunnel). There are four test groups, two treated with Si nutrient (root treatments) and two without. The disease severity was assessed each week. Si nutrient applied through fertigation tubes twice a week from 24 April 2013 to 31 July 2013. The first assessment was on 24 April 2013. (cv. Shelley). The data of AUDPC are shown on the right side of this figure, the colour of the number matches the colour of the line which represents each treatments.

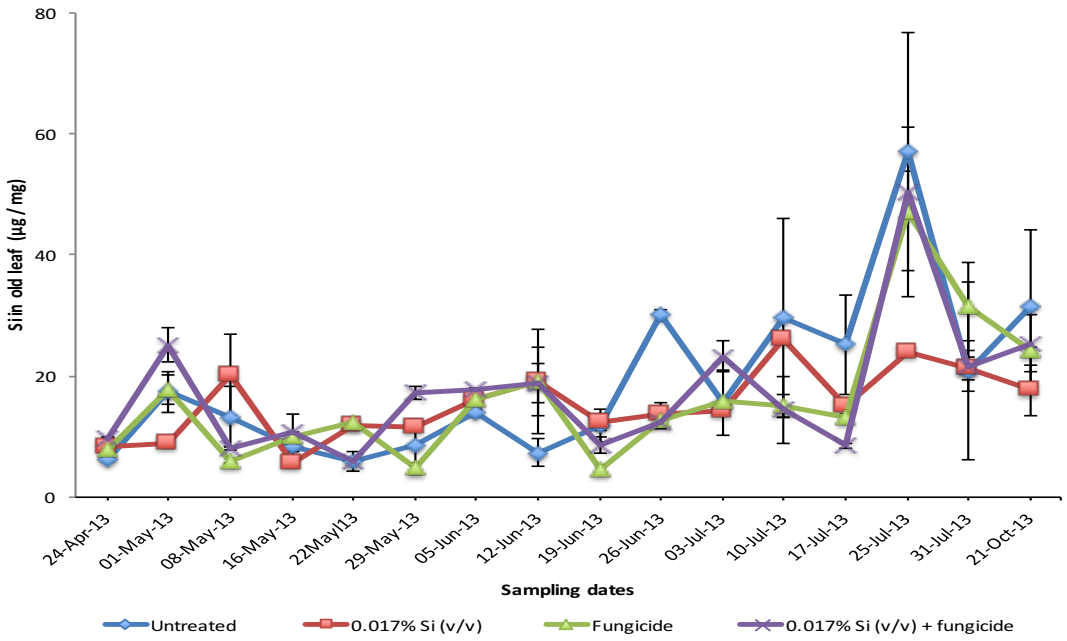
4.4.6 The effects of Si nutrient (root treatments) on strawberry plants in the 2013 Si nutrient fertigation field experiment

4.4.6.1 The concentration of Si in strawberry leaves, petioles, fruits (root treatments)

The Si concentration in old (dark green) strawberry leaves was increased with 0.017% Si nutrient (v/v) only and 0.017% Si nutrient (v/v) plus fungicides treatment from May to June compared to the untreated (Figure 4.34 a). There were no significant ($P > 0.05$) differences between each treatment and the untreated. There was also no significant correlation ($P > 0.05$) between the Si concentration of strawberry leaf and disease severity. The Si concentration in new (new expanded) strawberry leaves was similar between each treatment (Figure 4.34 b). There were no significant ($P > 0.05$) differences between each treatment and the untreated. However, the Si concentration in old strawberry leaves was greater than Si concentration in new strawberry leaves. Therefore, the old strawberry leaf accumulated more Si than the new strawberry leaves.

The Si concentration in old strawberry petioles was increased by 0.017% Si nutrient (v/v) and 0.017% Si nutrient (v/v) plus fungicides treatment and the Si concentration in new strawberry petioles was also increased with treatment 0.017% Si nutrient (v/v) plus fungicides (after 9 root applications) (Figure 4.35).

(a)



(b)

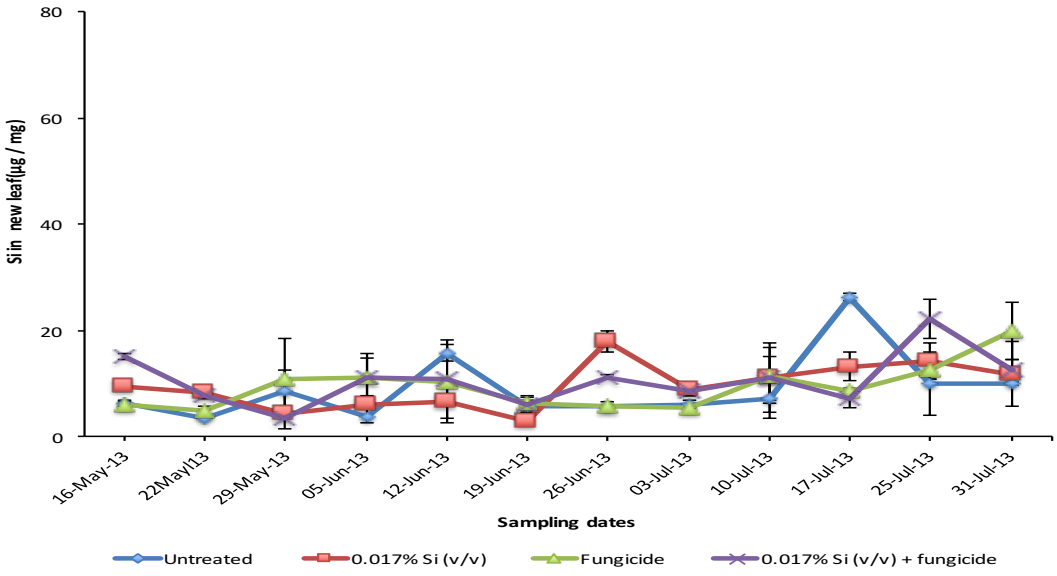


Figure 4.34: The concentration of Si (a) in old (dark green) strawberry leaves and (b) new (newly expanded) strawberry leaves with Si nutrient (root treatments) treatments in the 2013 Si nutrient fertigation field experiment. The final treatment was on 17 July 2013. Bars represent standard error of mean, 4 degrees of freedom. (cv. Shelley).

Furthermore, the Si concentration in old and new strawberry petioles were both increased with 0.017% Si nutrient (v/v) plus fungicide treatment (after 11 root applications) (Figure 4.36).The Si concentration in old petioles were increased after 11 root applications with 0.017% Si nutrient (v/v) plus fungicides compared to 9 root applications (Figure 4.35 & Figure 4.36). There were no significant differences ($P > 0.05$) between each treatment and the untreated. However, new strawberry petioles accumulate less Si compared to the old strawberry petioles, which indicated strawberry plants did accumulate some Si in petioles. Consequently, the small increase in strawberry petioles could enhance the Si concentration in strawberry plants and subsequent effect of increasing the ºBrix in plants.

The Si concentration in ripe strawberry fruits was increased with treatment of 0.017% Si nutrient (v/v) plus fungicides (after 8 root applications) compared to the untreated but no effects on unripe strawberry fruits was observed (no significant differences, $P > 0.05$) (Figure 4.37). The Si concentration in ripe and unripe strawberry fruits was significantly ($P < 0.05$) increased with treatment 0.017% Si nutrient (v/v) compared to the untreated (after 12 root applications) (Figure 4.38). Therefore, this link to the previous data on Si concentration in petioles, which demonstrated that more Si concentration in strawberry petioles could lead to the increasing the Si concentration in strawberry fruits and subsequent effect of increasing º Brix in strawberry fruits.

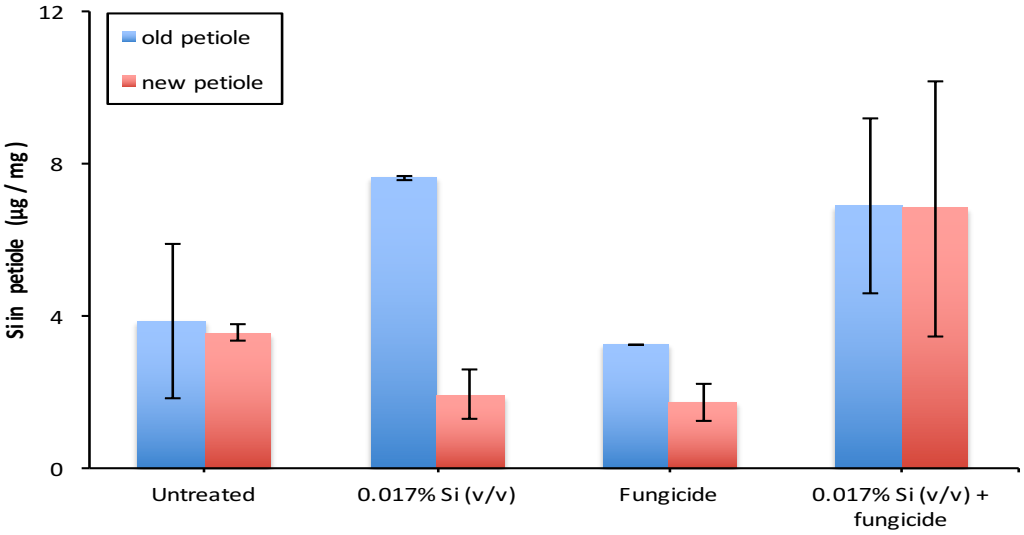


Figure 4.35: The Si concentration in strawberry petioles treated with Si nutrient (root treatments) in the 2013 Si nutrient fertigation field experiment. The samples were collected on 26 June 13 (after 9 root applications). Bars represent standard error of mean, 2 degrees of freedom. (cv. Shelley)

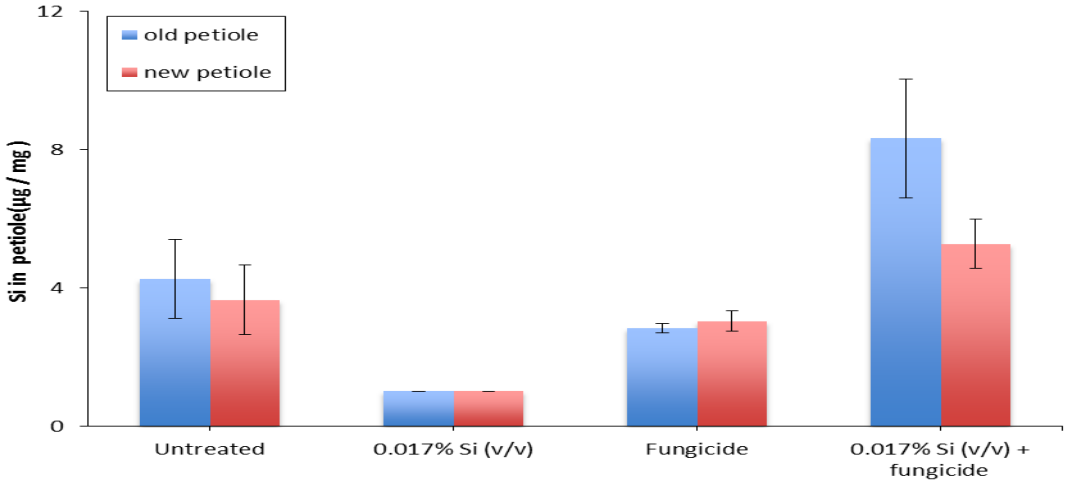


Figure 4.36: The Si concentration in strawberry petioles treated with Si nutrient (root treatments) in the 2013 Si nutrient fertigation field experiment. The samples were collected on 10 July 13 (after 11 root applications). Bars represent standard error of mean, 2 degrees of freedom. (cv. Shelley)

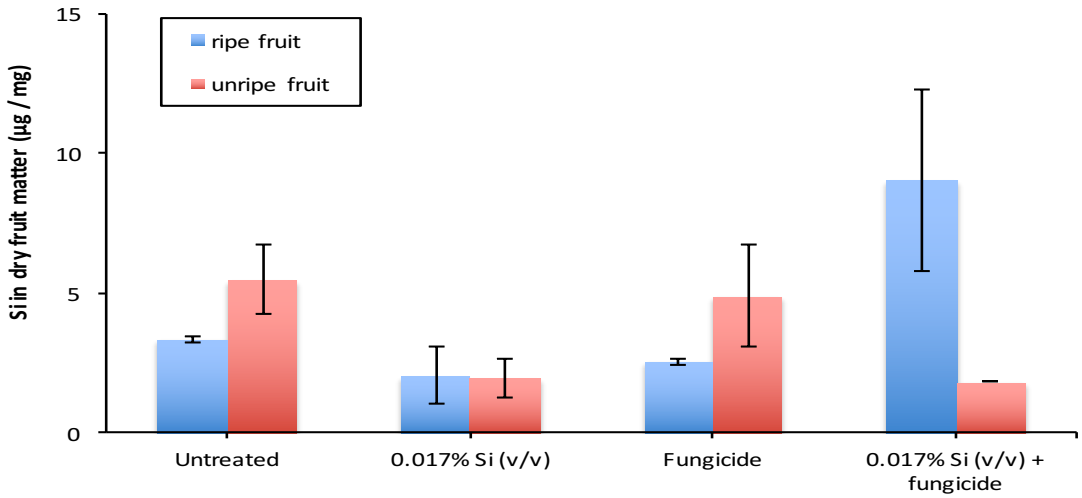


Figure 4.37: The Si concentration in strawberry fruits treated with Si nutrient (root treatments) in the 2013 Si nutrient fertigation field experiment. The samples were collected on 19 June 13 (after 8 root applications). Bars represent standard error of mean, 2 degrees of freedom. (cv. Shelley)

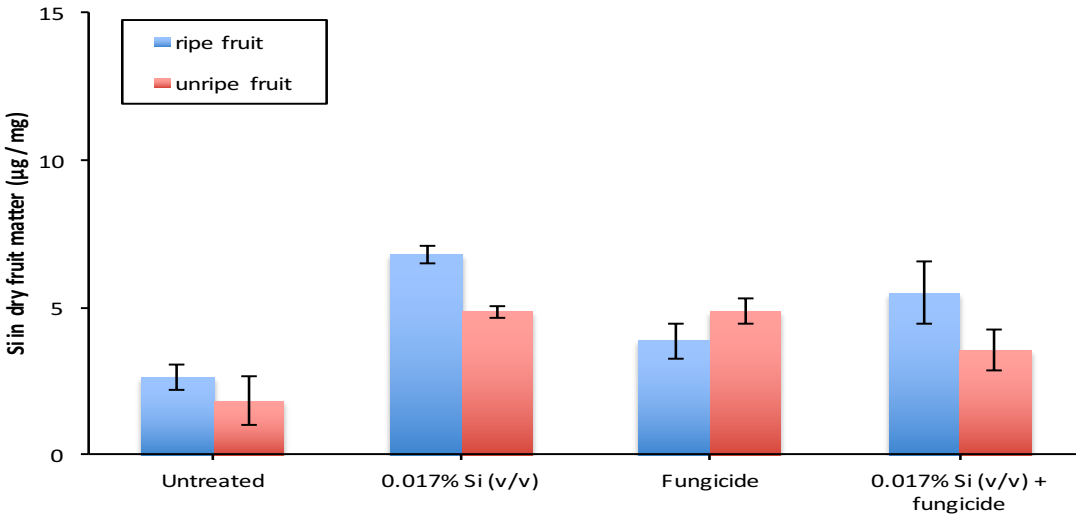


Figure 4.38: The Si concentration in strawberry fruits treated with Si nutrient (root treatments) in the 2013 Si nutrient fertigation field experiment. The samples were collected 25 July 13 (after 12 root applications). Bars represent standard error of mean, 2 degrees of freedom. (cv. Shelley)

The Si concentration in strawberry plants were examined after no Si nutrient application for three months. The Si concentration in strawberry petioles and fruits was still greater than the untreated. The Si concentrations in strawberry petioles were significantly ($P < 0.05$) greater with 0.017% Si nutrient (v/v) treatment compare to the untreated on 21 October 2013 (Figure 4.39). Therefore, the petioles with Si nutrient treated before were still keeping the Si inside even there was no Si nutrient application for three months. This demonstrated that the Si nutrient root treatment could have an extended effect on strawberry plants.

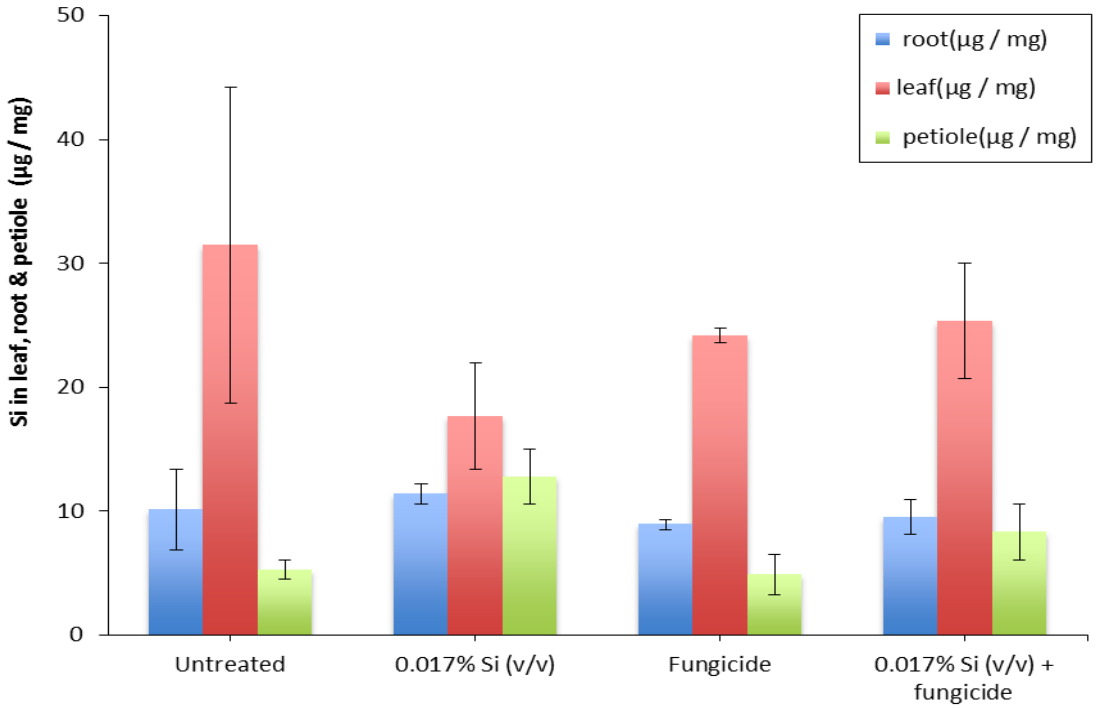


Figure 4.39: The Si concentration in strawberry roots, leaves and petioles treated without Si nutrient application for three months in the 2013 Si nutrient fertigation field experiment. The samples were collected 21 October 2013. Bars represent standard error of mean, 2 degrees of freedom.

4.4.6.2 The Si concentration in the water in the 2013 Si nutrient fertigation field experiment

The Si concentration in water from fertigation tubes was assessed, which was delivered to strawberry plants on three occasions. The Si concentration in water with 0.017% Si nutrient (v/v) and 0.017% Si nutrient (v/v) plus fungicides treatment was greater compared to the untreated on 24 April 2013 (pre-assessment, no significant difference $P > 0.05$) (Figure 4.40). The Si nutrient concentration in water was similar between each treatment on 19 June 2013 (middle of experiment, no significant difference $P > 0.05$). The Si concentration in water was greatly increased in each treatment on 14 August 2013 (final assessment) (Figure 4.40). The Si concentration in water in untreated was similar with the 0.017% Si nutrient (v/v) plus fungicides treatment, which was not expected (Figure 4.40). However, the high Si concentration in water at final assessment explained that the Si concentration in untreated strawberry plants were increased even when there was no Si nutrient application in 2013 Si nutrient fertigation field experiment (root treatment).

4.4.6.3 Si concentration in substrate coir and soil from different field in 2013

The Si concentration in the Ladybird field (soil) and Diamante field (soil from Si nutrient foliar spray experiment bed) were greater than substrate coir (Figure 4.41). The Si concentration in the Roadside field (coir from the untreated bed in

2013 Si nutrient field experiment (root treatment)) was greater than the Roadside field (coir from the 0.017% Si nutrient (v/v) treated bed in the Si nutrient field experiment (root treatment)), which again explained the Si concentration in untreated strawberry plants was greater than the Si nutrient treated plants (Figure 4.41). The Si concentration in unplanted coir was greater than unplanted coir (extracted in water). The amount of Si in unplanted coir (extracted in water) was approximately 25 μg/ml (Figure 4.41), which demonstrated that the available Si for the strawberry plant to uptake was little. Moreover, this confirmed that coir has little Si and soil has more (but may not be bio-available).

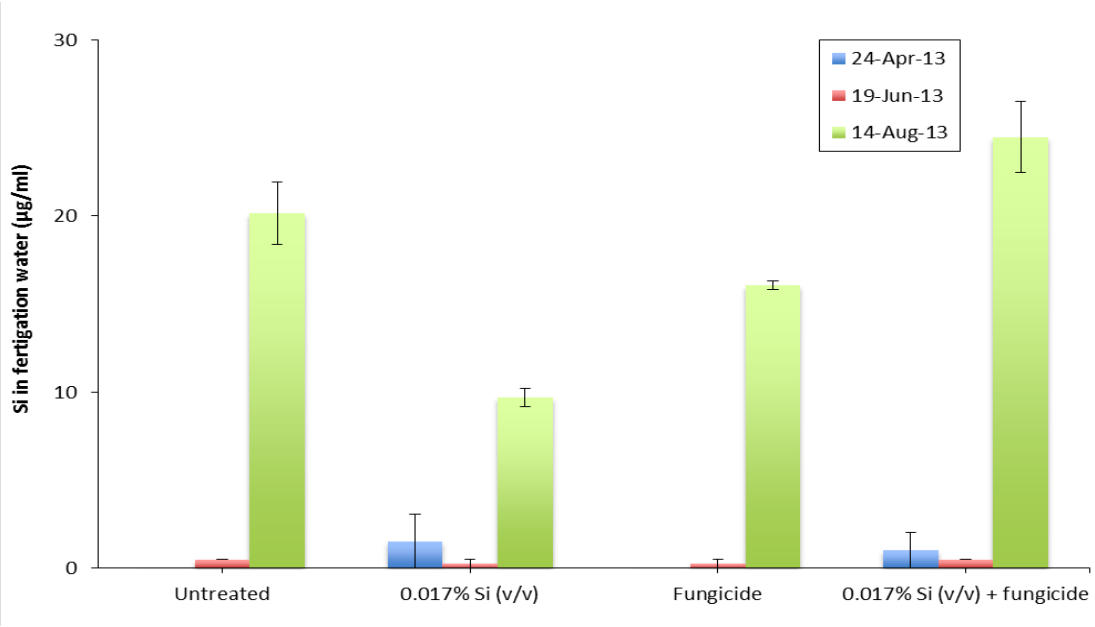


Figure 4.40: The Si concentration in water from fertigation tubes at three different sample dates in the 2013 Si nutrient fertigation field experiment (root treatments) (pre-assessment on 24 April 2013, middle of experiment on 19 June 2013 and final assessment on 14 August 2013). Bars represent standard error of mean, 2 degrees of freedom.

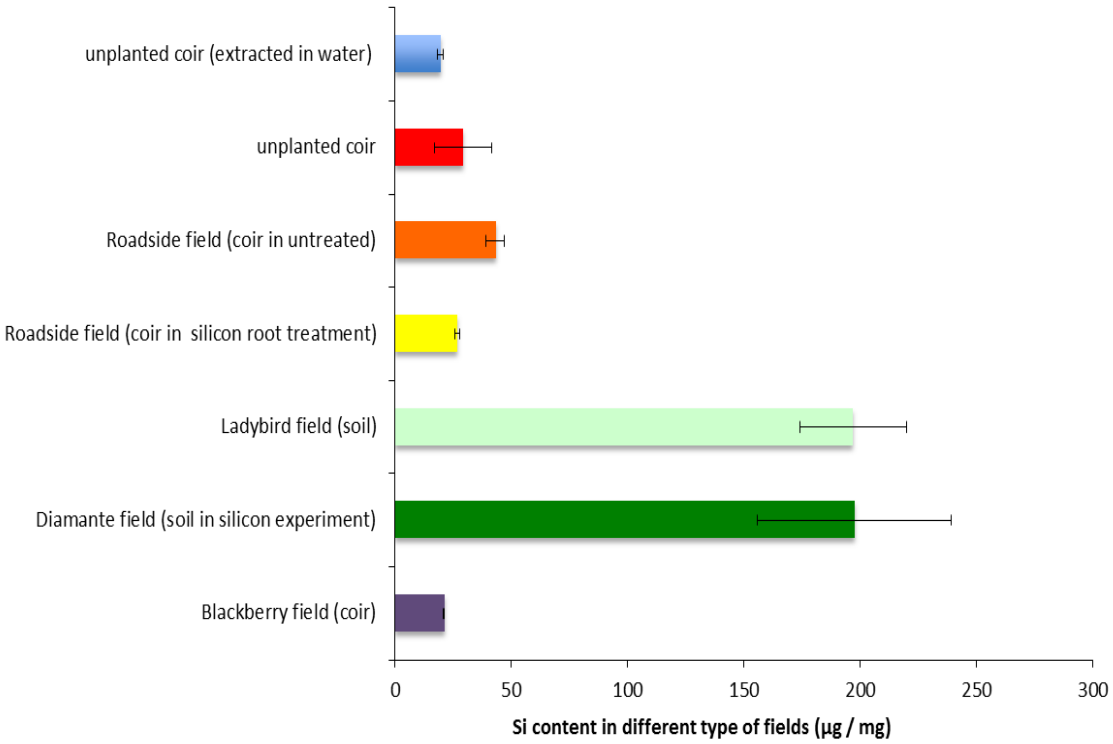


Figure 4.41: The Si concentration in substrate coir and soil in the different strawberry fields in 2013. Bars represent standard error of mean, 2 degrees of freedom.

4.4.7 The effects of Si nutrient (root treatments) on leaf morphology and physiology in the 2013 Si nutrient fertigation field experiment

4.4.7.1 Leaf thickness

The average leaf thickness showed that there was no significant difference among treatments on 24 April 2013 (pre-assessment) (Figure 4.42). The average leaf thickness was increased with 0.017% Si nutrient (v/v) ($P < 0.05$) and 0.017% Si nutrient (v/v) plus fungicide ($P > 0.05$) treatments compare to the untreated on

31 July 2013 (the end of field experiment after 13 root applications). Treatment with fungicide had similar leaf thickness with untreated (Figure 4.42). This indicated that the strawberry leaves were getting thicker with Si nutrient treatment, which complement the results of Si nutrient foliar spray treatment in 2012 and 2013.

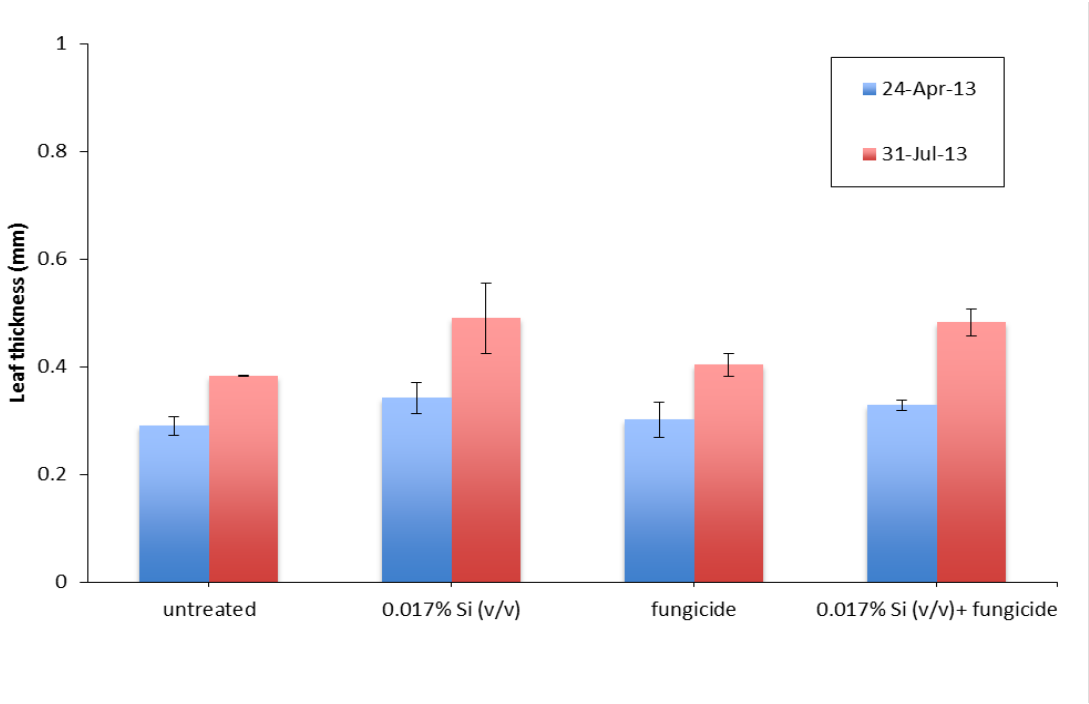


Figure 4.42: The average strawberry leaf thickness with Si nutrient root treatments in the 2013 Si nutrient fertigation field experiment. The leaf samples were sampled on the beginning of the field experiment (pre-assessment) and the end of field experiment (final assessment, after 13 root applications). Bars represent standard error of mean, 2 degrees of freedom. (cv. Shelley).

4.4.7.2 The effects of Si nutrient (root treatments) on ºBrix of different parts of strawberry plants in the 2013 Si nutrient fertigation field experiment

The average Brix° of old (dark green) leaf was significantly ($P < 0.05$) increased with treatments of 0.017% Si nutrient (v/v) and 0.017% Si nutrient (v/v) plus fungicides compared to the untreated (Figure 4.43). There was no significant ($P > 0.05$) increase among treatments in the average °Brix of new (new expanded) leaf (Figure 4.44). Furthermore, there was a significant ($P < 0.01$) correlation between the Si concentration in old and new leaves and the average Brix° of old and new leaves, which indicated the higher level of Si concentration in the strawberry leaf could enhance the °Brix value in the strawberry leaf.

The average °Brix of old and new petioles showed that there was no significant ($P > 0.05$) increase among treatments on 26 June 13 (after 7 root applications) (Figure 4.45). Moreover, the average °Brix of ripe and unripe fruits showed that there was no significant ($P > 0.05$) increase among treatments on 19 June 13 (after 6 times root applications) (Figure 4.46). The average °Brix of ripe fruits was significantly ($P < 0.05$) increased with treatments of 0.017% Si nutrient (v/v) and 0.017% Si nutrient (v/v) plus fungicides compare to the untreated on 25 July 13 (after 11 times root applications) (Figure 4.47). The average Brix° of unripe fruits was significantly ($P < 0.05$) increased with treatment of 0.017% Si nutrient (v/v) plus fungicides compare to the untreated on 25 July 13 (Figure 4.47). Furthermore, there was a significant ($P < 0.01$) correlation between the Si

concentration in ripe fruit and the average °Brix of ripe fruits, which indicated that the high level of Si concentration in fruit could enhance the °Brix value in fruit.

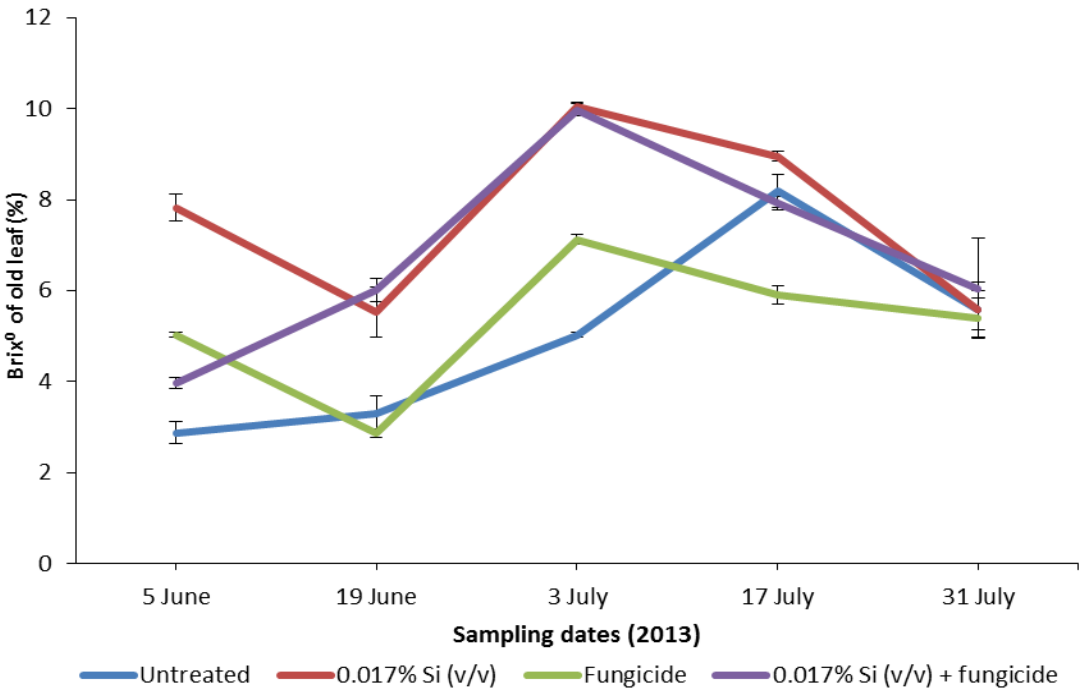


Figure 4.43: The average °Brix value in old (dark green) leaf with Si nutrient root treatments in the 2013 Si nutrient fertigation field experiment from 5 June 2013 (after 4 root applications) to 31 July 2013 (after 13 root applications). Bars represent standard error of mean, 4 degrees of freedom. (cv. Shelley).

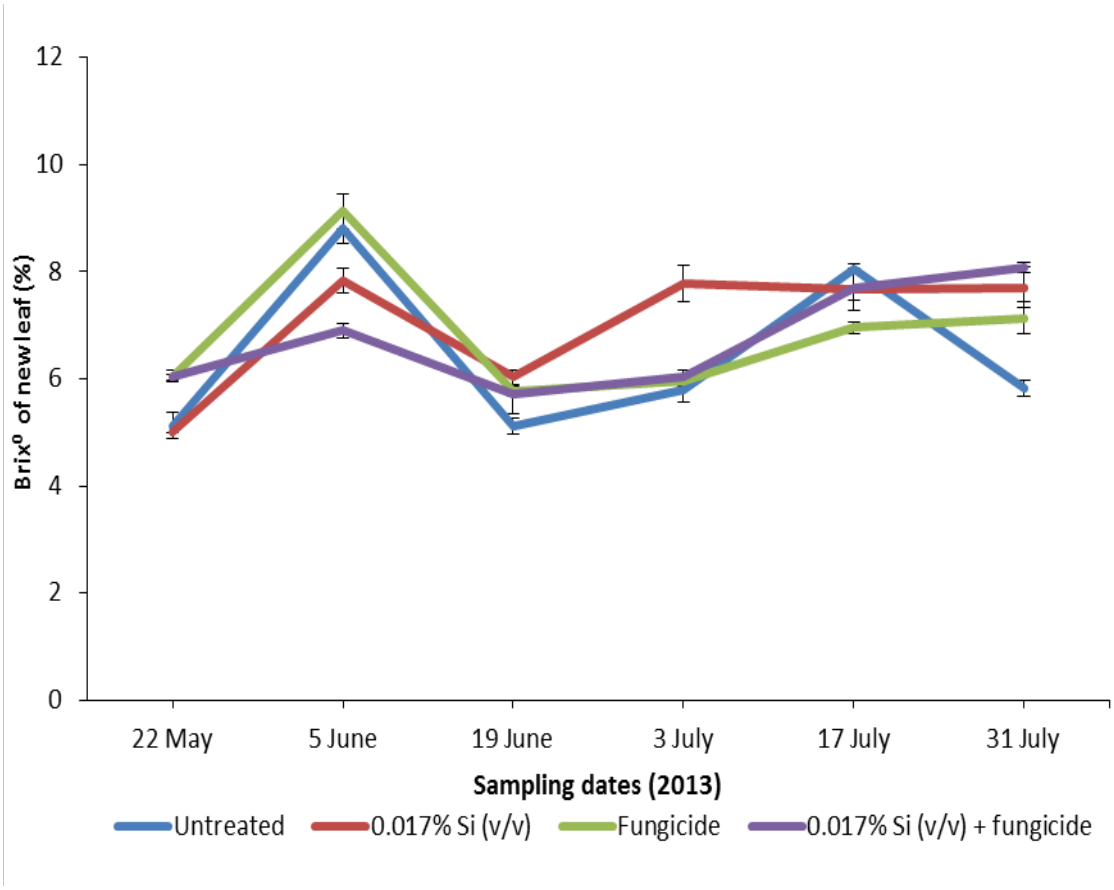


Figure 4.44: The average °Brix value in new (new expanded) leaf with Si nutrient root treatments in the 2013 Si nutrient field experiment from 5 June 2013 (after 4 root applications) to 31 July 2013 (after 13 root applications). Bars represent standard error of mean, 4 degrees of freedom. (cv. Shelley).

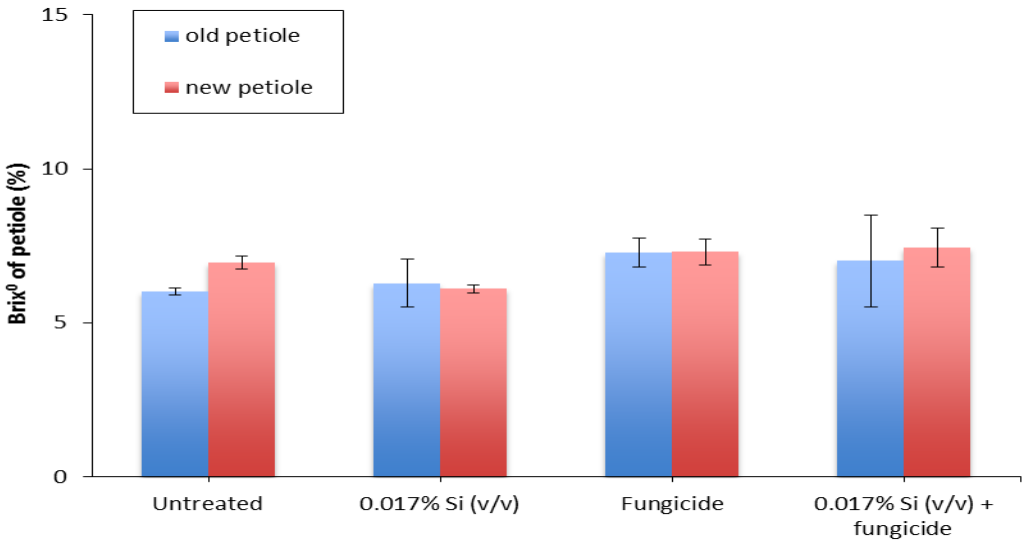


Figure 4.45: The average °Brix value of old and new petiole with Si nutrient root treatments in the 2013 Si nutrient fertigation field experiment. The petioles were sampled on 26 June 13 (after 7 root applications). Bars represent standard error of mean. 4 degrees of freedom. (cv. Shelley).

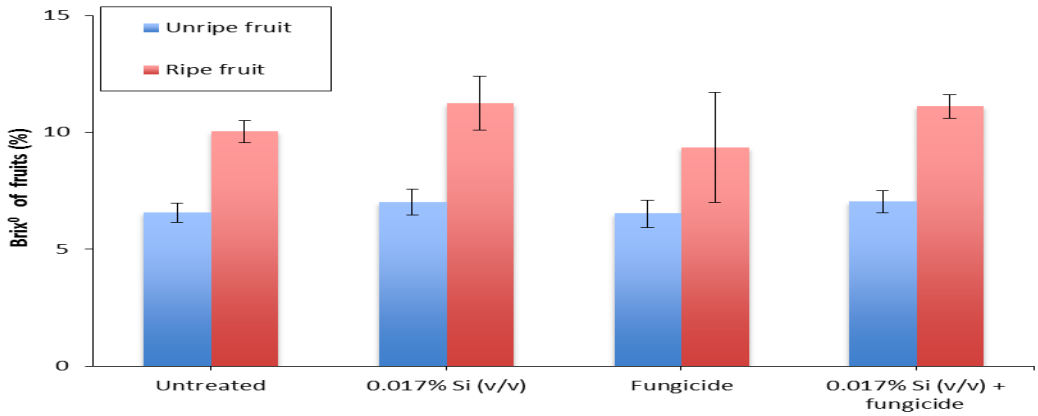


Figure 4.46: The average °Brix value of ripe and unripe fruits with Si nutrient root treatments in the 2013 Si nutrient fertigation field experiment. The fruits were sampled on 19 June 13 (after 6 root applications). Bars represent standard error of mean, 4 degrees of freedom. (cv. Shelley).

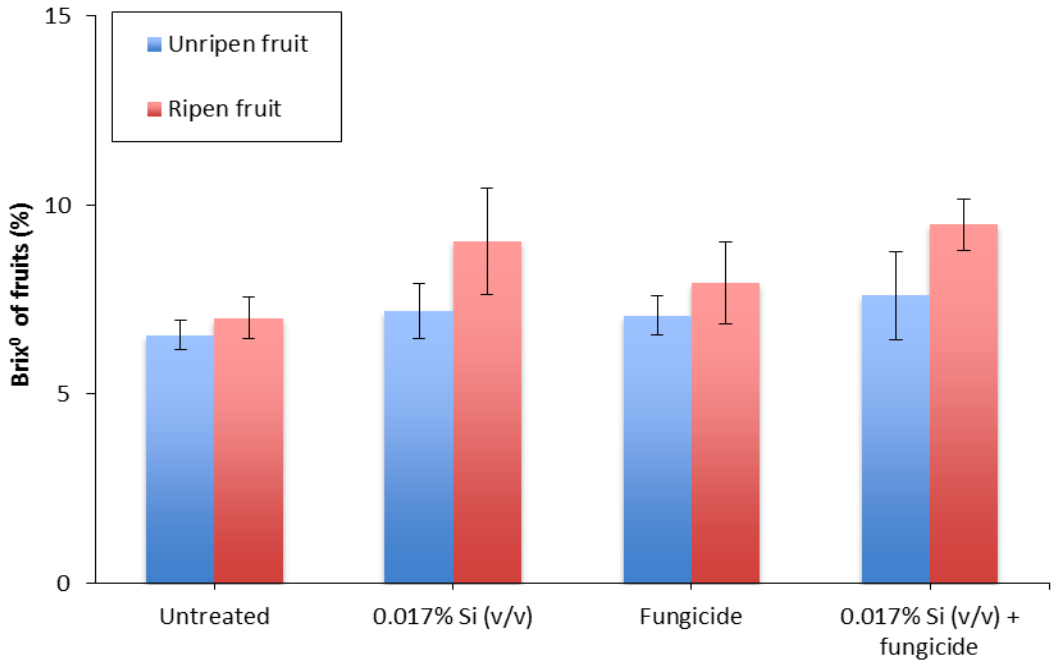


Figure 4.47: The average °Brix value of ripe and unripe fruits with Si nutrient root treatments in the 2013 Si nutrient fertigation field experiment. The fruits were sampled on 25 July 13 (after 11 root applications). Bars represent standard error of mean. 4 degrees of freedom. (cv. Shelley).

4.4.8 **Glasshouse experiments (2011-2014)**

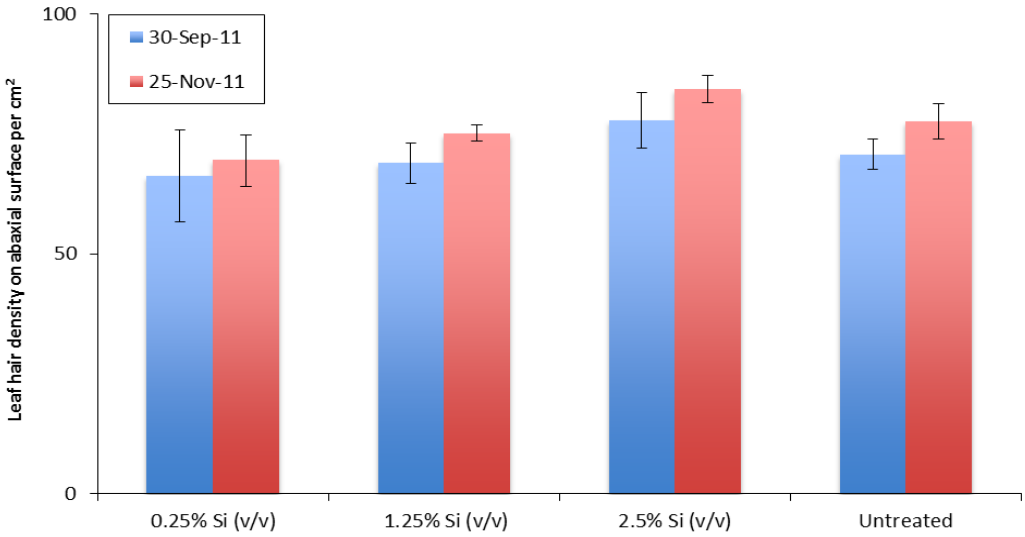
4.4.8.1 The effects of Si nutrient (foliar spray treatments) on leaf hair growth in the 2011 glasshouse experiment

The average density of strawberry leaf hairs per cm^2 on the abaxial surface were significantly (P < 0.05) increased by treatment with 2.5% Si nutrient (v/v) compared to the untreated except for 0.25% Si nutrient (v/v) and 1.25% Si

nutrient (v/v) on 25 November 2011 (final assessment, after 5 foliar spray applications, Figure 4.48 a). The average density of strawberry leaf hair per cm^2 on the adaxial surface also showed that there was a significant ($P < 0.05$) increase with treatments of 1.25% Si nutrient (v/v) and 2.5% Si nutrient (v/v) compared to the untreated on both 30 September 2011 and 25 November 2011 2011 (final assessment, after 5 foliar spray applications, Figure 4.48 b).

The average length of leaf hairs on the adaxial surface were significantly ($P < 0.05$) increased with 1.25% Si nutrient (v/v) treatment compared to the untreated on 25 November 2011 (final assessment, after 5 foliar applications) (Figure 4.49 a). The average length of leaf hairs on the abaxial surface were significantly ($P < 0.05$) increased with 0.25% Si nutrient (v/v), 1.25% Si nutrient (v/v) and 2.5% Si nutrient (v/v) treatments compared to the untreated on 25 November 2011(final assessment, after 5 foliar applications) (Figure 4.49 b).

(a)



(b)

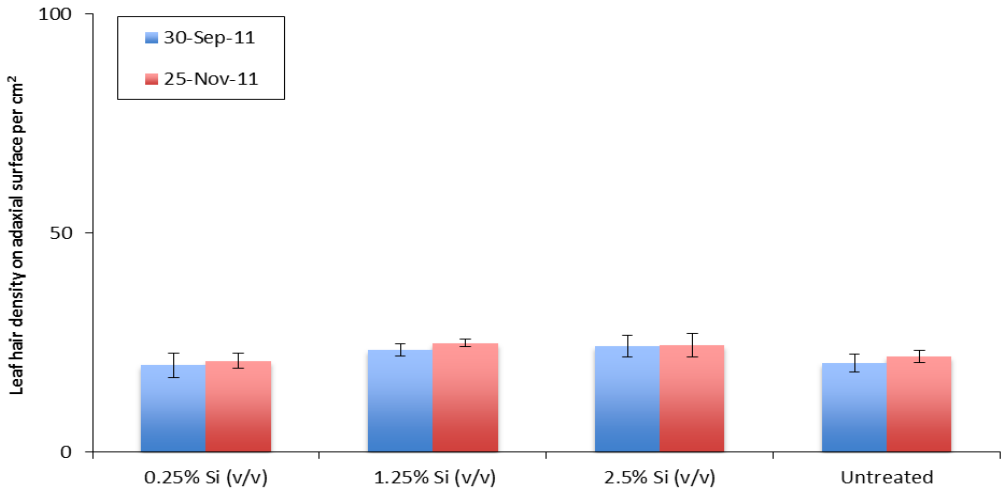
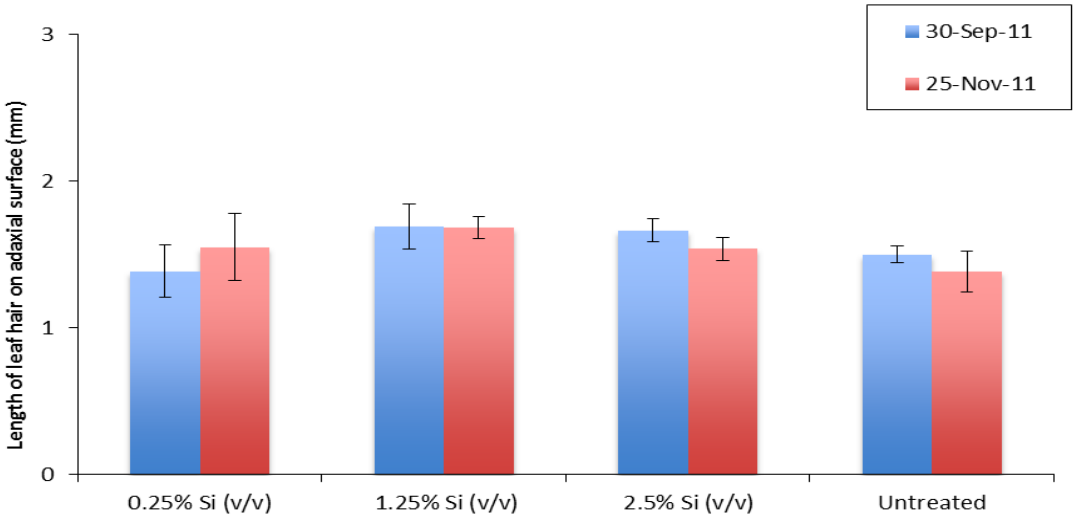


Figure 4.48: The average leaf hair density on (a) abaxial and (b) adaxial surface per cm^2 with different concentration of Si nutrient foliar spray treatments in the 2011 glasshouse experiment. The leaf samples were sampled on 30 September 2011 (pre-assessment) and 25 November 11 (final assessment, after 5 foliar spray applications). Bars represent standard errors of mean, 4 degrees of freedom. (cv. Elsanta).

(a)



(b)

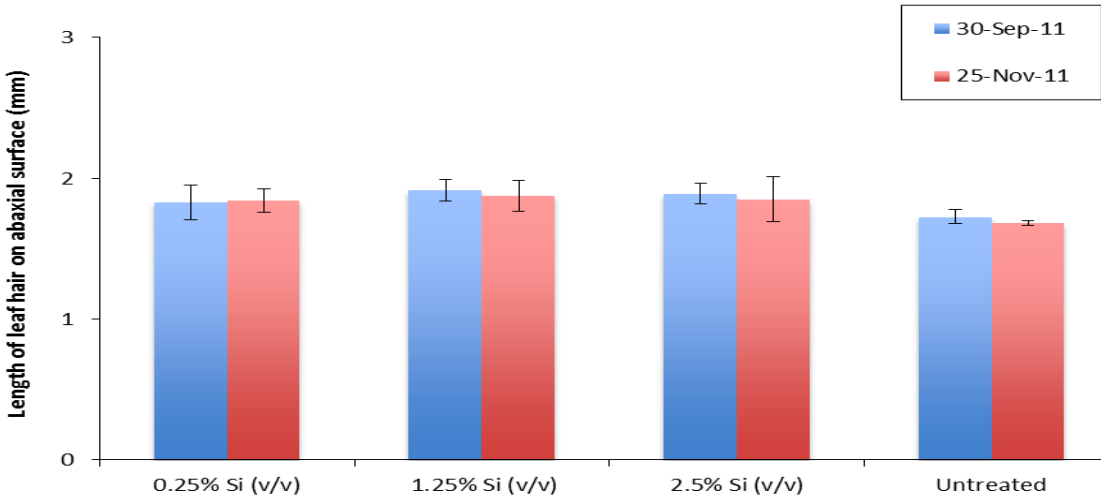


Figure 4.49: The average length of leaf hairs on the (a) adaxial and (b) abaxial surface with different concentration of Si nutrient foliar spray treatments in the 2011 glasshouse experiment. The leaf samples were sampled on 30 September 11 (pre-assessment) and 25 November 11 (final assessment, after 5 foliar spray applications). Bars represent standard errors of mean, 4 degrees of freedom. (cv. Elsanta).

4.4.8.2 The Si concentration in strawberry plants (foliar spray treatments) in the 2011 glasshouse experiment

The Si concentration in strawberry leaves was similar in each treatment on 30 September 11 (pre-assessment). The Si concentration in strawberry leaves was significantly ($P < 0.05$) increased by treatment of 1.25% Si nutrient (v/v) compare to the untreated on 25 November 11 (final assessment, after 5 times foliar applications, Figure 4.50).

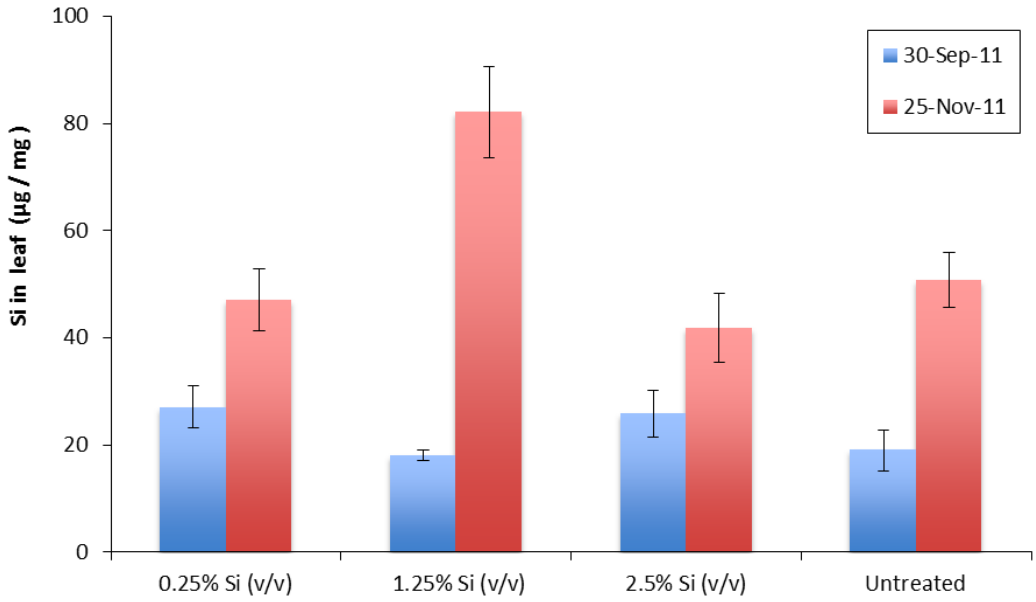


Figure 4.50: The Si concentration in strawberry leaves with different concentration of Si nutrient foliar spray treatments in the 2011 glasshouse experiment. The leaf samples were sampled on 30 September 11 (pre-assessment) and 25 November 11 (final assessment, after 5 foliar spray applications). Bars represent standard errors of mean. 2 degrees of freedom. (cv. Elsanta).

The Si concentration in strawberry unripe fruits was no significant difference between Si nutrient foliar spray treatments and the untreated (Figure 4.51). The Si concentration in strawberry ripe fruits was increased with 0.25% Si nutrient (v/v) treatment compared to the untreated. There was no significant ($P > 0.05$) difference between Si nutrient foliar spray treatments and the untreated.

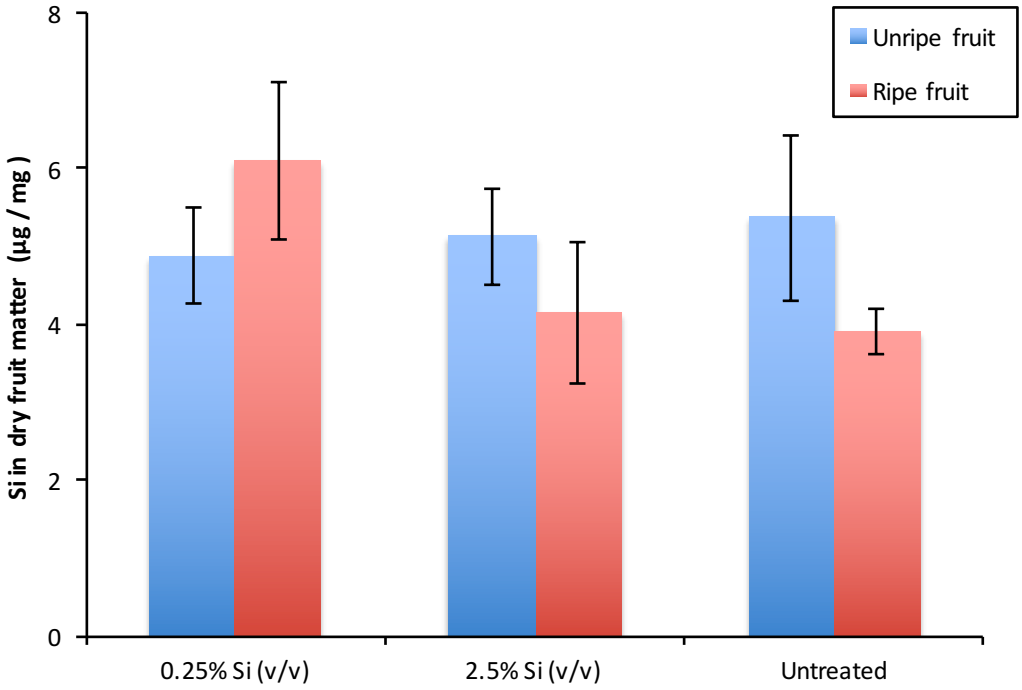


Figure 4.51: The Si concentration in strawberry ripe and unripe fruits with different concentration of Si foliar spray treatments in the 2011 glasshouse experiment. The fruit samples were sampled on 25 November 2011 (final assessment, after 5 foliar spray applications). Bars represent standard errors of mean, 2 degrees of freedom. (cv. Elsanta).

4.4.8.3 The Si concentration in strawberry leaves (foliar spray and root treatments) in the 2014 glasshouse experiment

The Si concentration in rinsed and non-rinsed leaves was both increased by Si nutrient treatments; the Si concentration in non-rinsed leaves was increased with 0.1 % Si nutrient (v/v) compared to other treatments, but there were no significant ($P > 0.05$) difference in rinsed leaves between each treatment (Figure 4.52). The Si concentration in non-rinsed leaves was increased with 0.017 % Si nutrient (v/v) and 0.1 % Si nutrient (v/v) root treatments compared to other treatments (Figure 4.53). The Si nutrient foliar spray treatments appeared to take effect after five times applications in rinsed leaves (9 April 2014). Therefore, Si nutrient root treatments were more effective than Si nutrient foliar spray treatments in increasing Si concentration in strawberry leaves.

4.4.8.4 The effects of Si nutrient (foliar spray and root treatments) on leaf morphology in the 2014 glasshouse experiment

4.4.8.4.1 **Cuticle thickness**

The cuticle thickness of strawberry leaves were greater with Si nutrient treatments compared to the untreated and DI water treated (Figure 4.54). Treatments with 0.017 % Si nutrient (v/v) and 0.1 % Si nutrient (v/v) root applications were thicker than 0.1 % Si nutrient (v/v) and 0.25% Si nutrient (v/v)

foliar applications. This again demonstrated that Si nutrient treatments through the roots were more effective than Si nutrient foliar spray treatments on leaves.

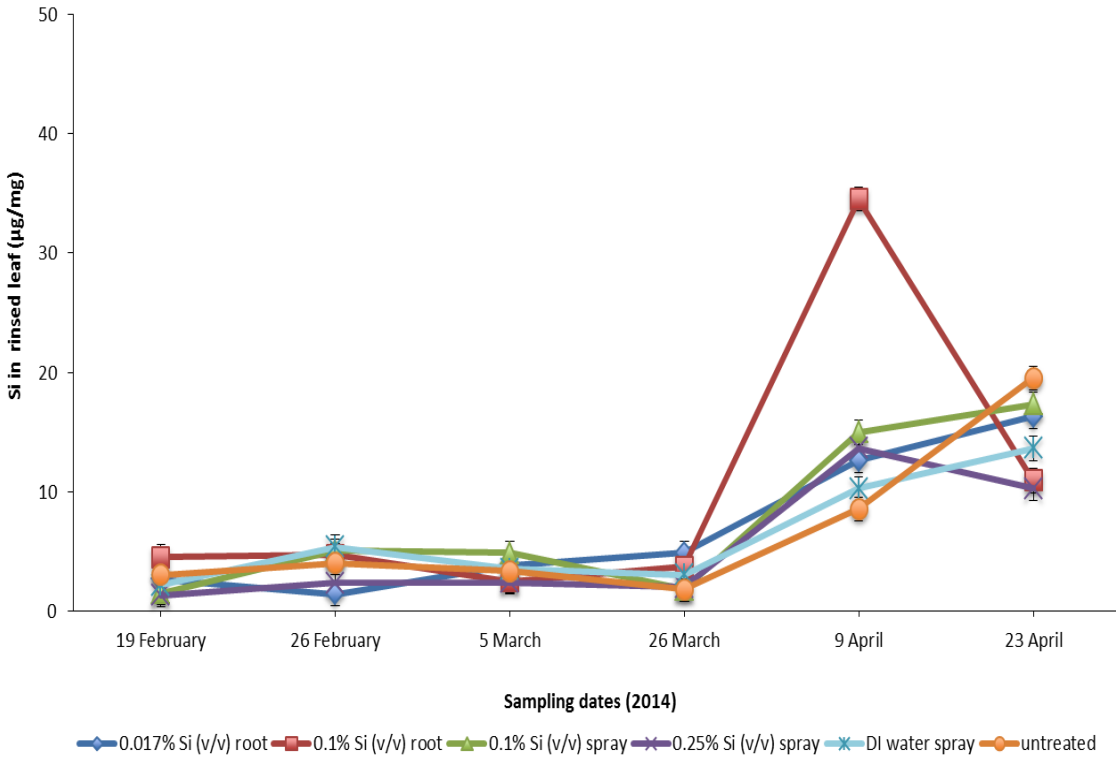


Figure 4.52: The Si concentration in rinsed strawberry leaves with different Si nutrient input treatments (foliar spray and root treatments) in the 2014 glasshouse experiment from 19 February 2014 to 23 April 2014. Bars represent standard errors of mean, 2 degrees of freedom. (cv. Elegance).

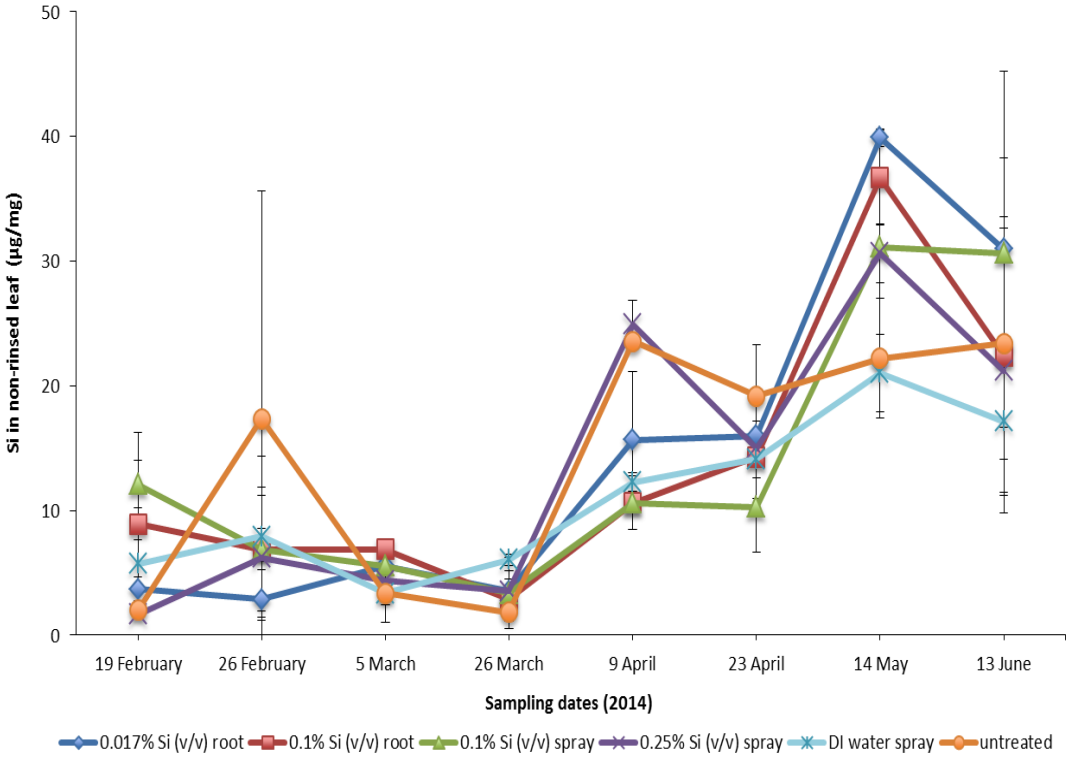


Figure 4.53: The Si concentration in strawberry non-rinsed leaves with different Si nutrient input treatments (foliar spray and root treatments) in the 2014 glasshouse experiment from 19 February 2014 to 23 April 2014. Bars represent standard errors of mean, 2 degrees of freedom. (cv. Elegance).

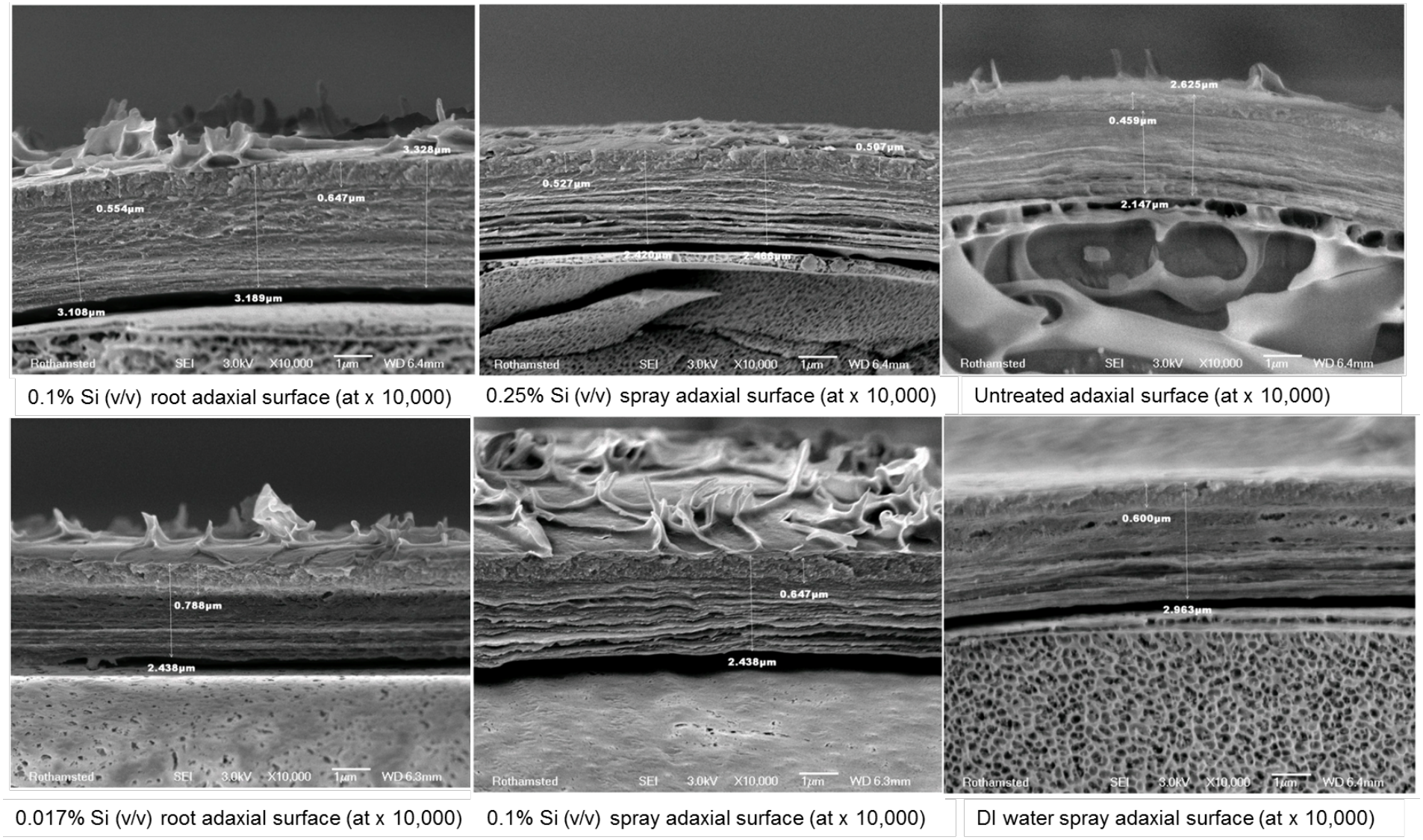


Figure 4.54: Samples of fresh Strawberry leaves treated with different concentration of Si nutrient (foliar spray and root treatment) were observed with SEM for Cuticle thickness.

4.4.8.5 Appearance of wax formation on adaxial surface

The wax formation on strawberry leaves were greater and thicker with Si nutrient treatments compared to the untreated and DI water spray treatment (Figure 4.55). The wax formation on strawberry leaves were greater with 0.017 % Si nutrient (v/v) and 0.1% Si nutrient (v/v) root treatments than 0.1% Si nutrient (v/v) and 0.25% Si nutrient (v/v) foliar spray treatments. Therefore, this again demonstrated that Si nutrient root treatments were more effective than Si nutrient foliar spray treatments on strawberry leaves.

4.4.8.6 The effects of Si nutrient (foliar spray and root treatments) on the biomass of strawberry plants in the 2014 glasshouse experiment

As shown in Table 4.7, the fresh and dry weights of the whole plants, roots, tops and runner with treatments of 0.25% Si nutrient (v/v) foliar spray, 0.1% Si nutrient (v/v) foliar spray and 0.017% Si nutrient (v/v) root treatment were greater than the untreated except 0.1% Si nutrient (v/v) root treatment. Moreover, the fresh and dry weights of the whole plants in the 0.25% Si nutrient (v/v) foliar spray treatment were significantly ($P < 0.05$) greater than the untreated. The fresh and dry weight of the roots showed the same result as the fresh and dry weight of the whole plants. The fruits yield was greater with Si nutrient treatments than the treatments of DI water spray treatment and untreated and the Si nutrient root treatments were slightly greater than the Si nutrient foliar treatments. Furthermore, the plants with Si nutrient treatments were bigger than the treatments of DI water foliar spray application and untreated (Figure 4.56).

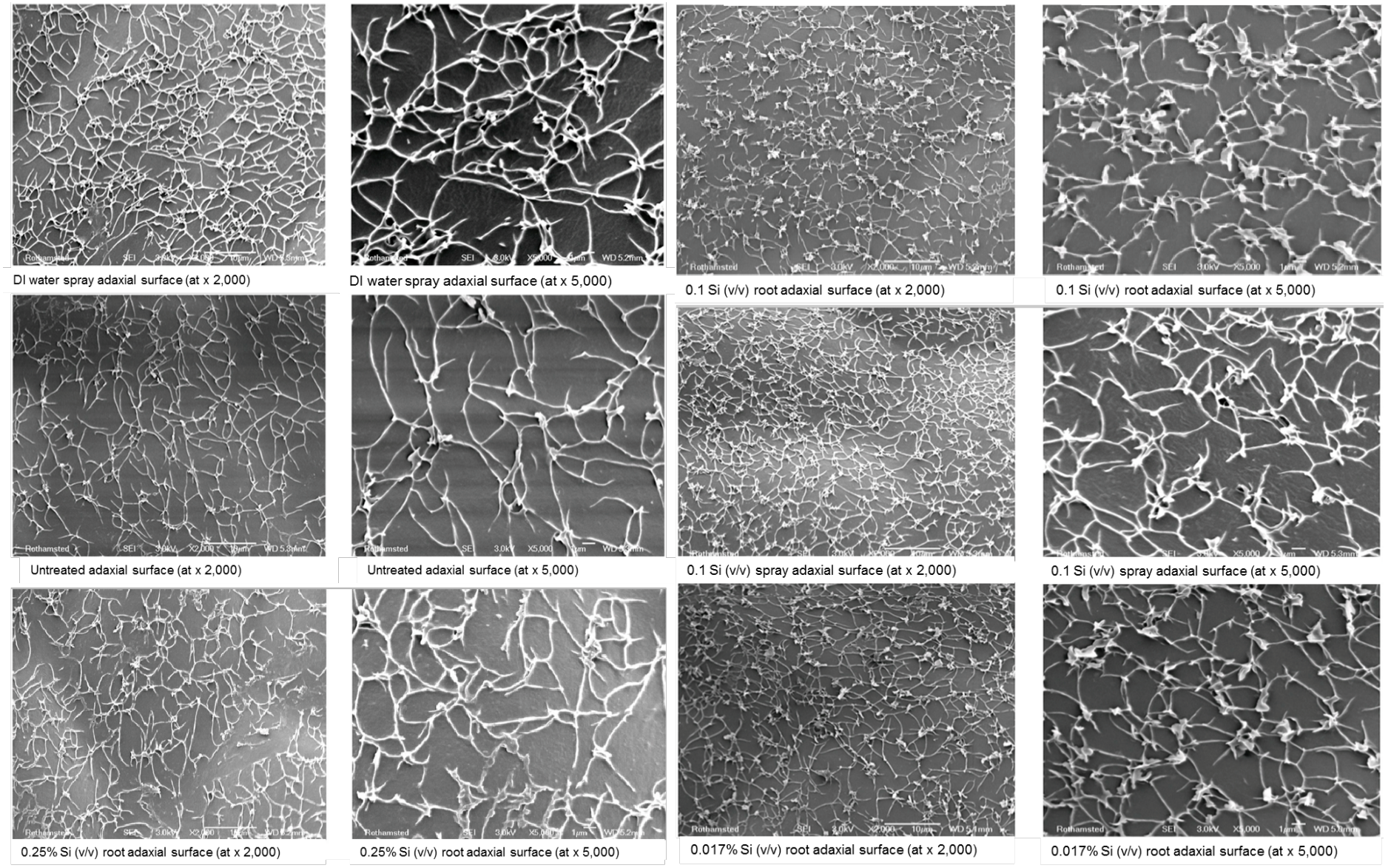


Figure 4.55: Samples of strawberry leaves treated with different concentration of Si nutrient (foliar spray and root treatment) were observed with SEM for formation of wax on adaixal surface at x2,000 and x5,000

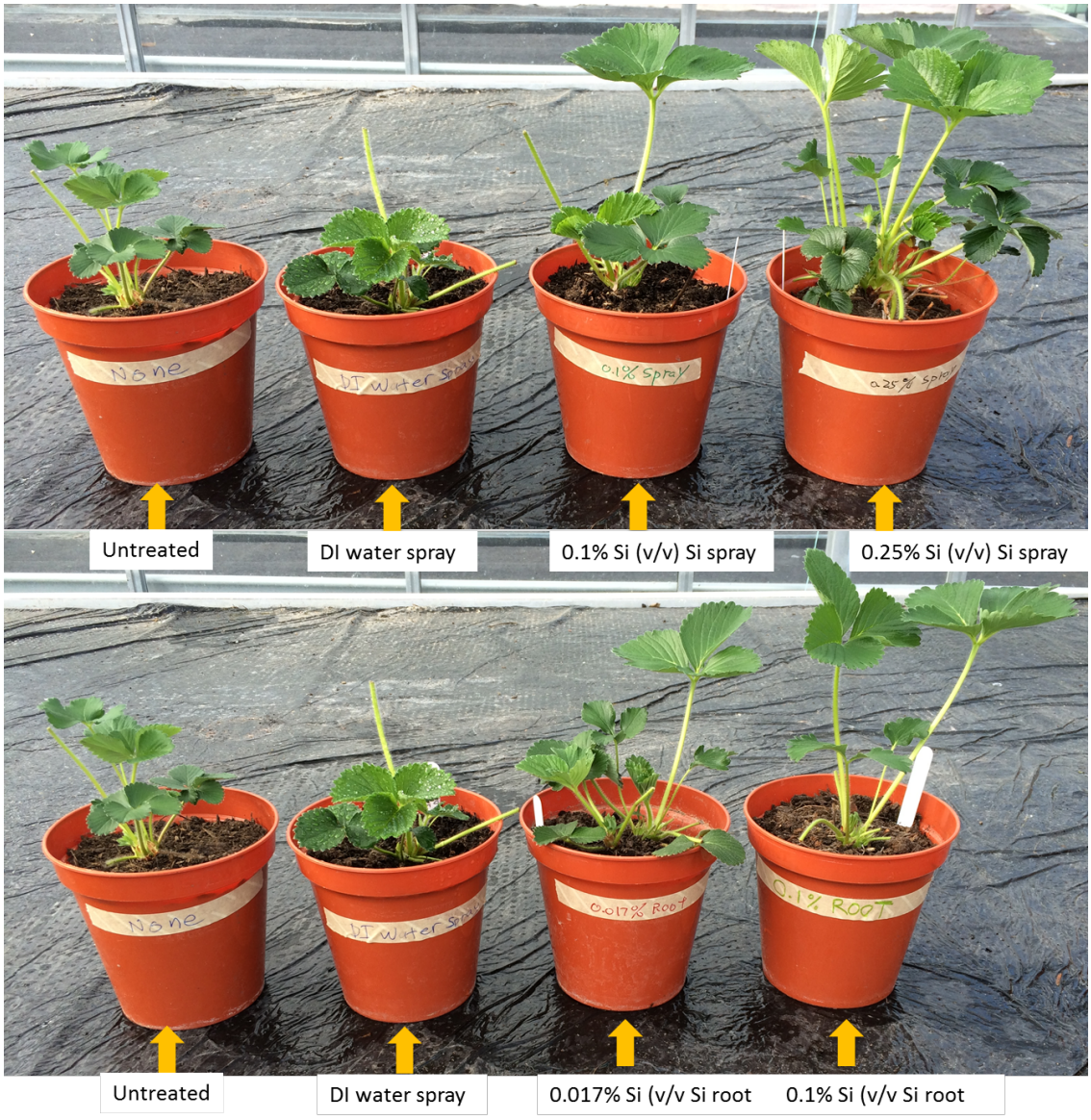


Figure 4.56: The effect of Si nutrient (foliar spray and root treatments) on the growth of strawberry plants after 9 treatments in the 2014 glasshouse experiment.

Table 4.7: Effect of Si nutrient application on the growth and yield of strawberry plants

		Treatments																					
		0.017% Si nutrient (v/v) root			0.1% Si nutrient (v/v) root			0.1% Si nutrient (v/v) foliar			0.25% Si nutrient (v/v) foliar			DI water foliar			Untreated		
whole plant weight	(fresh matter g)	64.6ab	±	20.7	41.4c	±	3	54.2bc	±	8.1	76a	±	20.6	54.9bc	±	10.9	50.3bc	±	18
	(dry matter g)	14.4ab	±	5.7	9.9b	±	1.1	12.7ab	±	2.1	17.5a	±	5.7	14.3ab	±	2.4	12.2b	±	3.9
Top weight	(fresh matter g)	19.6ab	±	6	15.5b	±	2.7	18.7ab	±	3.5	22.7a	±	5.9	17.3ab	±	4.4	17.3ab	±	4.3
	(dry matter g)	5.6ab	±	2.2	4.7b	±	0.9	5.7ab	±	1.2	6.6a	±	1.7	5.4ab	±	1.5	5.3ab	±	1.3
Root weight	(fresh matter g)	38.7ab	±	14.2	18.6c	±	3.4	29.8bc	±	4.5	43.3a	±	12.4	36.4ab	±	6.5	28.1bc	±	9.3
	(dry matter g)	7.9ab	±	3.8	4.4c	±	0.8	6.2bc	±	1.2	10a	±	4.1	8.8ab	±	1.4	5.8bc	±	1.5
Runner weight	(fresh matter g)	0.5a	±	1.2	0.0a	±	0.0	0.0a	±	0.0	0.0a	±	0.0	0.0a	±	0.0	0.9a	±	1.4
	(dry matter g)	0.1a	±	0.3	0.0a	±	0.0	0.0a	±	0.0	0.0a	±	0.0	0.0a	±	0.0	0.2a	±	0.4
Total yield																			
Number of flower		4ab	±	0.5	0.0b	±	0.0	2b	±	0.3	7a	±	0.7	0.0b	±	0.0	0.0b	±	0.0
Number of bud		7a	±	0.7	0.0b	±	0.0	6ab	±	0.9	3ab	±	0.5	2ab	±	0.5	0.0b	±	0.0
Number of fruit		36a	±	3	35a	±	2.9	25a	±	2.1	33a	±	2.6	7a	±	1	16a	±	2.4
Ripe fruit weight	(fresh matter g)	5.8a	±	9.6	7.3a	±	6.1	5.7a	±	7.8	6.4a	±	7.5	1.1a	±	1.6	3.8a	±	6.5
	(dry matter g)	0.8a	±	1.1	0.9a	±	0.7	0.8a	±	0.9	0.9a	±	0.9	0.2a	±	0.4	0.5a	±	0.8
Length of highest petiole	(fresh matter cm)	14.5ab	±	1.8	15.9a	±	1.3	15.5a	±	1.4	14.7ab	±	1.1	13b	±	1.7	15a	±	1.3
Length of root	(fresh matter cm)	23.5b	±	2.5	25.7ab	±	3.1	27.7a	±	1.9	27.1a	±	2.5	26.9a	±	2.4	26.6a	±	1.4
Diameter of basal stem	(fresh matter cm)	1.1bc	±	0.1	1.1bc	±	0.1	1.4a	±	0.1	1.2bc	±	0.1	1.1c	±	0.1	1.1c	±	0.1

± 95% confidence interval. The same letters in the table indicate no significant difference ($P > 0.05$) between each treatment

4.4.9 The location of Si in strawberry leaves and petioles with and without Si treatment in the 2014 glasshouse experiment

Microscope examination of petioles after staining with the fluorescence dye LysoTracker Yellow HCK-123 revealed that in the epidermis, deposition of Si occurred in both Si nutrient treatment and untreated (Figure 4.57). Si deposition in 0.1% Si nutrient (v/v) root treatment and 0.25% Si nutrient (v/v) foliar spray were greater in the epidermis and vascular tissue compare to the untreated.

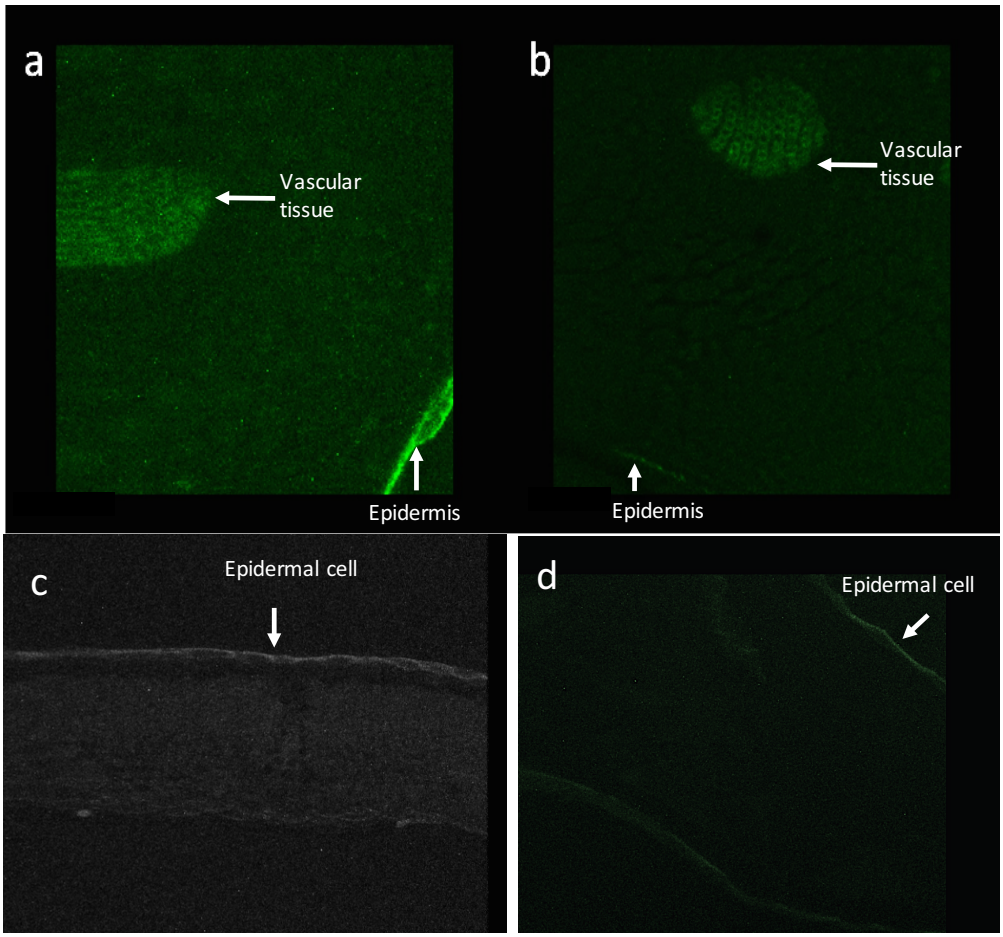


Figure 4.57: Location of Si in a cross section of the leaf and petiole, confocal microscope. a) the fluorescent green shows Si deposition in the vascular tissue and epidermis of the petiole in 0.1% Si nutrient (v/v) root treatment b) little Si deposition in the vascular tissue and epidermis of the petiole in untreated c)) the fluorescent green shows Si deposition in the epidermal cell of the leaf with 0.25% Si nutrient (v/v) foliar spray treatment d) little Si deposition in the epidermal cell of the untreated leaf. Viewed at 10x40 magnification.

4.5 Discussion and conclusion

Si has been known to suppress plant diseases in many plants such as powdery mildew in wheat, rose, strawberry, melon, cucumber and gerbera daisy (Kanto et al., 2004; Liang et al., 2005; Kanto et al., 2007; Moyer et al., 2008; Savvas et al., 2009; Shetty et al., 2012; Dallagnol et al., 2015). In the 1980s, silicate was reported to suppress powdery mildew by Miyake, Takahashi and Besford in the Japan and United Kingdom (cited in Kanto et al., 2004). However, most of the Si studies were carried out in the lab or glasshouse. in contrast, the present Si study was carried out in the field (three field experiments in two years) or in the glasshouse.

In the present study, the results of AUDPC and *r* indicated that high concentration of Si nutrient and high concentration of Si nutrient with K50 foliar spray treatment inhibited the epidemic build-up of *P. aphanis* compared to the untreated plants (Fig. 4.14, 4.15, 4.21 & 4.22). As a foliar spray K50 only gave some disease reduction but K50 with the the high concentration of Si nutrient foliar spray in 2012 gave significant ($P < 0.001$) disease reduction (Fig. 4.16). In 2013, the Si nutrient foliar spray experiment with both high and low concentration of Si nutrient foliar spray with K50 gave greater control of the disease than K50 only (Fig. 4.23), Si nutrient foliar spray only gave better control than K50 only. The results from last two years show that Si nutrient gives greater control than K50 and that Si nutrient enhances the efficacy of K50. Fatema (2014) also indicated that high concentration of Si nutrient foliar spray treatment and high concentration of Si

nutrient plus K50 significantly inhibited the germinating of ascospores of *P. aphanis* on strawberry plants compared to the untreated. Furthermore, the 2013 Si nutrient foliar spray field experiment (Fig. 4.21) had less disease than the 2012 Si nutrient foliar spray field experiment (Fig. 4.15). The 2012 Si nutrient foliar spray field experiment was carried out when *P. aphanis* was well established during a period of high disease pressure in the strawberry field. Whereas, the 2013 Si nutrient foliar spray field experiment had less disease carry over from last year (in Fatema, 2014), even though the start levels of disease were different in 2012 and 2013 and the weather condition were different, Si nutrient foliar spray still reduced the disease level. However, there was less disease reduction when the disease was high at the beginning of the experiment. This was because the disease was well established in the crop before any uptake of Si nutrient. The Si nutrient foliar spray treatments started to inhibit the disease development after four weeks (i.e. after four Si nutrient foliar spray applications) in the 2012 Si nutrient foliar spray field experiment. The 2013 Si nutrient fertigation experiment was partly designed to give Si nutrient treatment before the disease became established. Moyer et al. (2008) also demonstrated that the Si nutrient should be applied before the disease development, which will provide the time for the plant to take up Si nutrient in order to provide protection against disease. Therefore, the earlier Si nutrient foliar spray in spring could provide the time for the plant to take up Si nutrient in order to provide protection against disease, which will have an great effect on inhibiting the development of *P. aphanis* during summer and harvest season in the UK.

The results of AUDPC showed that Si nutrient treatments in the 2013 Si nutrient fertigation (root treatment) field experiment (Fig. 4.33) had less amount of disease compared to the Si nutrient treatments in 2012 (Fig. 4.15) and 2013 (Fig. 4.22) foliar spray field experiment. This demonstrated that Si nutrient used in fertigation as a nutrient feed enhanced the plant defence mechanisms and gives a delay in epidemic build up by up to two weeks. Both Dallagnol et al. (2012 & 2015) and Liang et al. (2005) indicated that the root-applied Si nutrient was more significant at suppressing powdery mildew compared to the foliar-applied Si in melon and cucumber (in greenhouse). Dallagnol et al. (2015) stated that it might be related to differences in the distribution of Si nutrient in plants. In the present study, the Si nutrient was applied through the fertigation tube when the young strawberry plants had just been planted into the coir bag in the 2013 Si nutrient fertigation (root treatment) field experiment. This provided the time for the plants to take up Si nutrient. The Si concentration in plants was greater with the Si nutrient treated compare to the untreated (Fig. 4.26). The leaf cell wall was thicker with the Si nutrient treated compare to the untreated (Fig. 4.29, 4.30 & 4.54), thicker cell wall is a form of resistance to plant disease. Furthermore, Si deposition in Si nutrient treated plants was greater in the epidermis and vascular tissue compared to the untreated (Fig. 4.57). The plants were strengthened and the defence resistance stimulated by Si nutrient taken up through the root at the development of the vegetative stage. Therefore, the root treatment resulted in a greater control of strawberry powdery mildew than the Si nutrient foliar spray treatment. The Si nutrient root treatment with fungicide gave excellent disease reduction but the Si nutrient alone delayed epidemic build up by over two weeks

when compared with the untreated, fungicides only did not control the disease as well (Fig. 4.32). In addition, the commercial fungicides spray (Appendix 15) included Si nutrient foliar sprays [0.25% Si nutrient (v/v)], which demonstrated that if both Si nutrient foliar spray and Si nutrient root treatment are used this gives greater control of strawberry powdery mildew. Liang et al. (2005) indicated that foliar-applied Si can effectively control disease development only via the physical barrier of Si deposited on leaf surfaces, or osmotic effect of the silicate applied, but cannot enhance systemic acquired resistance induced by inoculation. Therefore, Si nutrient applied continuously to roots can enhance defence resistance in response to disease development. Furthermore, the Si nutrient should be applied early in the vegetative growth of the strawberry plant in order to strengthen the plants and pre-inhibit the strawberry powdery mildew development. The results of analysing Si in soil and coir also suggests that it is very important to apply Si nutrient in the fertigation for plants grow in coir.

In the present study, the strawberry plants accumulated more Si compared to the pre-assessment. The Si concentration in strawberry leaves was varied between each Si nutrient treatment in the 2012 and 2013 Si nutrient foliar spray field experiment (Fig. 4.25 & 4.26). There are general factors which could account for this, such as different varieties could have different response to Si, also in large commercial operation there will be uneven spray on crops and delivery in the fertigation system (Fig. 4.40). However, the high concentration of Si nutrient had greater Si accumulation compared to the untreated in the final assessment, which indicated that the Si was absorbed to strawberry plants. Kanto et al. (2004)

indicated there was no significant correlation between the disease severity and the Si concentration in leaves. Whereas, the present study has shown a significant ($P < 0.001$) correlation between the Si concentration in strawberry leaves and the average number of colonies and number of colonies per cm^2 in the 2012 Si nutrient foliar spray field experiment, which demonstrated that a greater accumulation of Si in a leaf may result in less disease severity. The Si concentration in leaves reflected the disease development in the 2013 Si nutrient fertigation field experiment (Fig. 4.34). Liang et al. (2005) also indicated that although the accumulation of Si failed in cucumber, Si still has the effect on inhibiting the disease development. However, the Si did not directly act against infection by strawberry powdery mildew (Kanto et al., 2007), it was absorbed to strawberry plants and subsequently induced physiological changes in strawberry cells. These physiological changes include the chemistry of the cuticle layer changed and cell wall reinforcement, which prevents formation of penetration pegs of strawberry powdery mildew, thus the mildew conidia were inhibited (Kanto et al., 2007). This work suggest that in great reduce susceptibility to strawberry powdery mildew is linked to the increase in wax density and cuticle thickness as the result of Si nutrient application.

The present study showed that the increased leaf thickness (Fig. 4.29 & 4.42), cuticle layer thickness (Fig. 4.30 & 4.54) and wax formation (Fig. 4.31 & 4.55) may be a contributing factor to the disease reduction observed with Si nutrient treatment in the 2012 and 2013 Si nutrient foliar spray field experiment, the 2013 Si nutrient fertigation (root treatment) field experiment and the 2014 Si nutrient

glasshouse experiment. This results of use of Si nutrient (Fig. 4.29, 4.30 & 4.54) demonstrated that the Si nutrient stimulated the thickness of cuticle layer, cell wall and wax formation in strawberry leaves, which inhibited the mildew conidia. Kanto, Maekawa and Aino (2007) also stated that these physiological changes induced the chemistry of the cuticle layer to change, which inhibited the mildew conidia germination and appressorial formation in strawberry leaf. The present study also showed enhancement of the wax density formation on the adaxial surface with both Si nutrient foliar spray and root treatments (Fig. 4.54). The Si nutrient root treatments showed that denser wax formations were formed on the adaxial surface and more phytoliths were formed in the centre compare to the Si nutrient foliar spray treatment (Fig. 4.55). The Si was pushed out through the transpiration process. The formation of papillae has also been found in wheat (cited in Fauteux et al., 2005). Kanto, Maekawa and Aino (2007) also reported that the papillae could prevent the powdery mildew penetration in barley leaves and roses (cited in Kanto et al., 2007; Shetty et al., 2012). Moreover, it has also been shown to enhance the accumulation of phytoalexins in monocotyledonous and dicotyledonous (Fauteux et al., 2005). This study propose that although strawberry is a Si non-accumulator the Si was still absorbed and been used to stimulate the natural defence reaction resulting in enhanced plant disease resistance.

Furthermore, the biomass assessment in the 2014 glasshouse experiment showed that the fruit yield was greater in the Si nutrient root treatment than the untreated and Si nutrient foliar spray treatment (Table 4.4), which again indicated

that the Si nutrient root treatment is more effective than the Si nutrient foliar spray treatment. In addition, the Si nutrient root treatment significantly ($P < 0.05$) increased the ^{0}Brix value in fruits compared to the untreated (Fig. 4.43 & 4.47). Moreover, higher ºBrix values contribute to higher nutrient density and higher fruit quality (Harrill, 1998). Higher ºBrix could led to disease resistance and extend shelf life (Harrill, 1998). There was a significant ($P < 0.01$) correlation between the Si concentration in leaves and ripe fruits and the average °Brix value. This demonstrated that the higher level of Si concentration in the strawberry leaf could enhance their °Brix value in the strawberry leaf, which indicated that the Si nutrient root treatment resulted in better quality fruits compare to the untreated. Therefore, the Si nutrient treatment increased the °Brix value in strawberry leaves and fruits, which enhanced the fruit quality and shelf life and also could enhance the plant disease resistance.

In conclusion, the results of three years Si nutrient field experiments support the hypothesis that use of Si nutrient can delay the onset of the epidemics of strawberry powdery mildew in the field. Moreover, the Si nutrient treatment had a significant effect on the morphology and physiology in the strawberry plants leading to enhanced disease resistance. The Si nutrient root treatment was more effective than the Si nutrient foliar spray treatment. In established over-wintering strawberry crops, the high concentration of Si nutrient should be used form spring onwards whenever fungicides are used. In annual crops, Si nutrient should be used twice a week from planting through out the season. Furthermore, the use of Si nutrient with routine fungicides spray would also help to reduce disease levels.

Chapter 5 – Overall discussion and conclusions

5.1 Discussion

The aims of the project were to: i) investigate the importance of chasmothecia and the chasmothecial development, the sexual, overwintering stage of *P. aphanis* in powdery mildew epidemics on strawberries, investigate the hypothesis that spraying fungicides to decrease numbers of chasmothecia in the crop in the non-harvest season will decrease the amount of *P. aphanis* inoculum in the spring and to investigate the source of disease in plants that came from a propagator, ii) investigate the hypothesis that the application of Si nutrient reduces disease susceptibility and changes on morphology and physiology of strawberry plants. This study has met the aims and hypothesis.

In the present study, three years of chasmothecial development field experiments (2011-2013) demonstrated that the use of fungicides in late autumn and spring significantly ($P < 0.01$) reduced the number of chasmothecia (mature and immature) (Fig. 3.6, 3.12 & 3.18) and the number of germinating ascospores (Fig. 3.16). The significant correlation ($P < 0.001$) between the disease cover and the number of chasmothecia (mature and immature) formed on the strawberry leaf (Chapter 3, Table 3.3) demonstrated that the reduction in the percentage of disease cover resulted in a reduction in the number of chasmothecia (mature and immature) formed in the field. Hoffmann et al. (2012) supported this study and stated that the level of leaf infection altered the number of chasmothecia formed

on the leaves in the laboratory. This study also demonstrated that the epidemic started from germinating ascospores in the spring (Fig. 1.17), which means the chasmothecia are the initial inoculum in *P. aphanis*. Moreover, the numbers of ascospores are reduced by using fungicides (Fig. 3.10 & 3.16). Therefore, the reduction in the number of chasmothecia (mature and immature) formed on strawberry leaves led to a reduction in the initial inoculum in the next growing season which confirm the hypothesis.

Furthermore, the study also showed a significant correlation ($P < 0.0001$ & $P < 0.001$) between the maturity of chasmothecia (i.e. eight ascospores in ascus) and the cross-sectional area of chasmothecia in both years (2011-2012, 2013-2014), which indicated that the larger the chasmothecia, the greater the level of maturation. The larger chasmothecia on dead strawberry leaves were observed to contain eight ascospores in the ascus, whereas, the smaller chasmothecia were less mature. Therefore, the theory is that if the chasmothecia are mature before the leaf dies, the chasmothecia are still alive. Moreover, the significant discovery in this study proved this theory, which was that the chasmothecia sample A (kept in deep freeze at -20°C for eighteen months) still showed the natural opening curve on day 17 (Fig. 3.30) and empty chasmothecia on day 24 (Fig. 3.34), which suggested that the chasmothecia of *P. aphanis* can survive even in cold strawberry-growing regions. Therefore, the application of fungicides from the start of the growing season to the beginning of the fruit ripening period did not only affect the disease level and chasmothecia development but also inhibited the autumn leaf infection and disease carry over to the following spring.

The role of Si in increasing resistance to both biotic (pests and diseases) and abiotic stresses (drought, salinity and heavy metals) in crops has been widely reported (Mburu et al., 2015). However, most of the Si studies were carried out in the laboratory or glasshouse, whereas, the present Si nutrient study was carried out in the field (three field experiments in two years) or in the glasshouse. In the present study, the results of AUDPC and *r* indicated that the high concentration of Si nutrient and high concentration of Si nutrient with K50 foliar spray treatment inhibited the epidemic build-up of *P. aphanis* compared to the untreated in both 2012 and 2013 field experiment (Fig. 4.14, 4.15, 4.21 & 4.22). The results of AUDPC showed that Si nutrient root treatments in the 2013 Si nutrient fertigation field experiment (0.017% (v/v) Si nutrient twice a week, Fig. 4.33) had less disease compared to the Si nutrient foliar spray treatments in the 2012 (Fig. 4.15) and 2013 (Fig. 4.22) field experiment，which demonstrated that the Si nutrient root treatment gave greater control than the Si nutrient foliar spray treatment. The Si nutrient only root treatment gave a delay in epidemic build up by up to two weeks, which demonstrated that Si nutrient used in fertigation as a nutrient feed enhanced the plant defence mechanisms. The Si nutrient root treatment plus fungicides gave excellent disease reduction in the 2013 Si nutrient fertigation field experiment (Fig. 4.32). In addition, the commercial fungicides spray (Appendix 15) included Si nutrient foliar sprays [0.25% Si nutrient (v/v)], which demonstrated that if both Si nutrient foliar spray and Si nutrient root treatment are used this gives greater control of strawberry powdery mildew.

Furthermore, the present study showed a significant ($P < 0.001$) correlation between the Si concentration in strawberry leaves and the average number of colonies and number of colonies per cm^2 in the 2012 Si nutrient foliar spray field experiment. This demonstrated that a greater accumulation of Si in a leaf may result in less disease severity. The Si concentration in leaves reflected the disease development in the 2013 Si nutrient fertigation field experiment (Fig. 4.34). The study also showed increased leaf thickness (Fig. 4.29 & 4.42), cuticle layer thickness (Fig. 4.30 & 4.54) and wax formation (Fig. 4.31 & 4.55) which may be a contributing factor to the disease reduction observed with Si nutrient treatment in the 2012 and 2013 Si nutrient foliar spray field experiment, the 2013 Si nutrient fertigation (root treatment) field experiment and the 2014 Si nutrient glasshouse experiment. These results demonstrated that the Si nutrient stimulated the thickness of the cuticle layer, cell wall and wax formation in strawberry leaves, which inhibited the germination of mildew conidia. The Si nutrient root treatments showed a denser distribution of wax formation were formed on the adaxial surface and more phytoliths were formed in the centre compare to the Si nutrient foliar spray treatment (Fig, 4.55). Kanto, Maekawa and Aino (2007) also reported that the papillae could prevent the powdery mildew penetration in barley leaves and roses (cited in Kanto et al., 2007; Shetty et al., 2012). Furthermore, there was a significant ($P < 0.01$) correlation between the Si concentration in leaves and ripe fruits and the average °Brix value in leaves and ripe fruits. This demonstrated that the higher level of Si concentration in the strawberry leaf could enhance the °Brix value in the strawberry leaves, which indicated that the Si nutrient root treatment resulted in better quality in fruits

compare to the untreated. The Si nutrient treatment increased the Brix° value in strawberry leaves and fruits, which enhanced the fruit quality and also could enhance the plant disease resistance. The results propose that although strawberry is a Si non-accumulator Si was still absorbed and been used by the plants , which result in lower disease level.

Moreover, the present study showed that Si nutrient foliar spray treatment with high concentration of Si nutrient [0.5% (v/v)] and high concentration of Si nutrient plus K50 (potassium bicarbonate) led to fewer chasmothecia per cm^2 on leaves compared to the untreated in the 2012 Si nutrient foliar spray experiment (Chapter 4, Fig. 4.18). The Si nutrient foliar spray experiment demonstrates that the disease control has an ongoing effect by reducing chasmothecial production thus reducing initial inoculum for the following season. In addition, the high concentration of Si nutrient worked better than the low concentration of Si nutrient. Fatema (2014) also indicated that the high concentration of Si nutrient foliar spray treatment significantly inhibited the germinating ascospores of *P. aphanis* on strawberry compared to the untreated. In the 2013 Si nutrient foliar spray experiment, no chasmothecia were observed in the field, which was the same as the 2013-2014 chasmothecial development field experiment. Moreover, there was a significant ($P < 0.001$) correlation between the number of chasmothecia and disease cover on abaxial surface ($P < 0.001$) and a significant ($P < 0.001$) correlation between the number of chasmothecia per cm^2 and number of colonies per cm^2 on abaxial surface (Table 3.3 & Fig. 4.20). Therefore, the reduction in the percentage of disease cover and number of colonies per cm^2

resulted in a reduction of the number of chasmothecia (mature and immature) formed on the strawberry leaf. Consequently, the less disease development in 2013 Si nutrient foliar spray field experiment (Fig. 4.21 & 4.22) resulted in less chasmothecia formed in the field. However, the mating type, different strawberry variety and different environmental condition in the field could be other factors which resulted in chasmothecia not being formed in the field. Nevertheless, the subsequent reduction in formation of chasmothecia in those plots (in the 2012 Si nutrient foliar spray experiment) would reduce the overwintering inoculum by reducing the number of mature chasmothecia, which produce ascospores in the next spring. Therefore, the applications of Si nutrient from the start of the growing season to the beginning of the fruit ripening period not only affected the disease level and chasmothecia formation but also inhibited the autumn chasmothecia development. The use of Si nutrient in the field whether as spray or root application could also reduce the number fungicides used in the growing season. Moreover, this study also found that the strawberry powdery mildew was present on new strawberry plants from the propagators, which showed that the strawberry propagators are probably the main source of the disease.

5.2 Conclusions

To sum up, the chasmothecium has an important role as the initial inoculum in *P. aphanis* epidemic. The three years chasmothecial development field experiments showed that using fungicides in the late autumn and spring is effective in reducing

chasmothecial numbers and maturity. Moreover, the Si nutrient foliar spray and root treatment field experiments demonstrated that the Si nutrient application effectively inhibited the epidemic of *P. aphanis* developing on strawberry plants. The Si nutrient root treatment plus fungicides (include Si nutrient foliar spray) was the most effective treatment in inhibiting the disease development. Thus, using both Si nutrient foliar spray and root treatments in the strawberry field could achieve a great inhibition of the strawberry powdery mildew development. Furthermore, the high concentration of Si nutrient foliar spray treatment also inhibited the chasmothecial development, thus contributing to a reduction in initial inoculum in the next growing season.

The study also indicated that the reduction in the disease development resulted reduction of the chasmothecial development (mature and immature) on strawberry plants. This study suggests that the applications of Si nutrient from the start of the growing season to the beginning of the fruit ripening period did not only affect the disease level but also inhibited the autumn chasmothecial development. This should encourage strawberry famers to use Si nutrient in the early spring and autumn after harvest. Furthermore, this study also suggests the application of Si nutrient should be applied in the vegetative stage of strawberry plant in order to strengthen the plants and pre-inhibit the strawberry powdery mildew development. The use of Si nutrient could facilitate the strawberry propagator to produce healthy runners and less disease in the fields for strawberry growers. Subsequently, less fungicides will be used in the strawberry farm.

5.3 Future work

The prediction system has been shown to have a potential in the strawberry field (Dodgson, 2007) but it is only based on the asexual stage of *P. aphanis.* The data in chapter 3 about the sexual stage could be used to complete the prediction system in order to predict the disease all year around. A comparison should be made between the first part of a field managed by the grower, applying applications as they normally would and the second part of the field where the Si nutrient foliar spray and root applications are applied as and when the prediction system predicts a high risk period. The level of control achieved and the number of Si nutrient applications would be compared to determine the overall reduction in fungicides used.

A few researchers reported that Si could interact with several key components (such as proteins, production of stress hormones and chitinase) of plant stress signalling system ultimately led to induced resistance against pathogenic fungi. The chitinase was identified in strawberry (Khan & Shih, 2004 cited in Kanto et al., 2007). Therefore, the role of Si against pathogens should be investigated further by analyses of pathogenesis-related proteins, enzyme activities in the plant and to evaluate fully the mechanism of resistance.

The possibility that Si could enhance the absorbance of K in plants was reported in early 80s. However, it is not clear the synergistic between potassium bicarbonate and Si. The present study showed that the efficacy of potassium

bicarbonate was significantly improved when used with Si. Therefore, there is a need to investigate the effects of Si on absorbance of potassium bicarbonate in strawberry.

The present study showed that deposition of Si in the epidermis of petiole and vascular tissue with Si root treatment. Further X-ray analyses need to be undertaken to determine the accumulation of Si in leaves and petioles with Si foliar spray and root treatment.

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Appendices

Appendix 1: Strawberry certification scheme from Plant Health Propagation Scheme (PHPS)

Special conditions for Strawberry certification: Foundation, Super Elite, Elite, A, and Approved-Health grades

Inspected for	Trueness to variety, health and vigour. **Approved- Health grade:** Health and vigour only.
Applications	To be submitted through the franchise holder by 1st May. A field plan must be submitted with each application.
Eligible material	**Foundation:** Any material which meets the requirements for Nuclear Stock as specified in the conditions outlined on pages 10-12, or any variety certified F1 or F2 in the previous year. Applications for any variety not listed on page 8 must be accompanied by a varietal description. **Super Elite:** Any variety certified at Foundation grade in the previous year**.** **Elite:** Any variety certified at Foundation or Super Elite grade in the previous year. **A:** Any variety certified at Foundation, Super Elite or Elite grade in the previous year. **Note:** Material entering from other certification schemes must be tested for Xanthomonas fragariae (angular leaf spot) and Xanthomonas arboricola pv.fragariae (bacterial leaf blight) prior to planting. **Approved-Health:** Any material, including plants produced directly from seed, providing it is not a Pedigree variety listed in page 8. All stocks (except plants produced directly from seed) entered for Approved-Health must be tested, prior to planting, for freedom from strawberry blackspot and Xanthomonas fragariae (angular leaf spot) and Xanthomonas arboricola pv. fragariae (bacterial leaf blight), unless parent plants were certified in the previous year. See page 9. If a stock subsequently fails certification due to Verticillium wilt in excess of tolerance, it can enter certification the following year, without additional strawberry blackspot and Xanthomonas testing. Note: Parent stock that has been produced by micropropagation must have been granted an appropriate micropropagation certificate. However, a certificate is not required for material entering at Approved-Health.
Freedom from Quarantine diseases	Crops must not be grown on land known to be infected with the following soil-borne diseases: Rhizomania, strawberry red core or verticillium wilt disease of hops or which is under notice for potato wart disease or potato cyst nematode

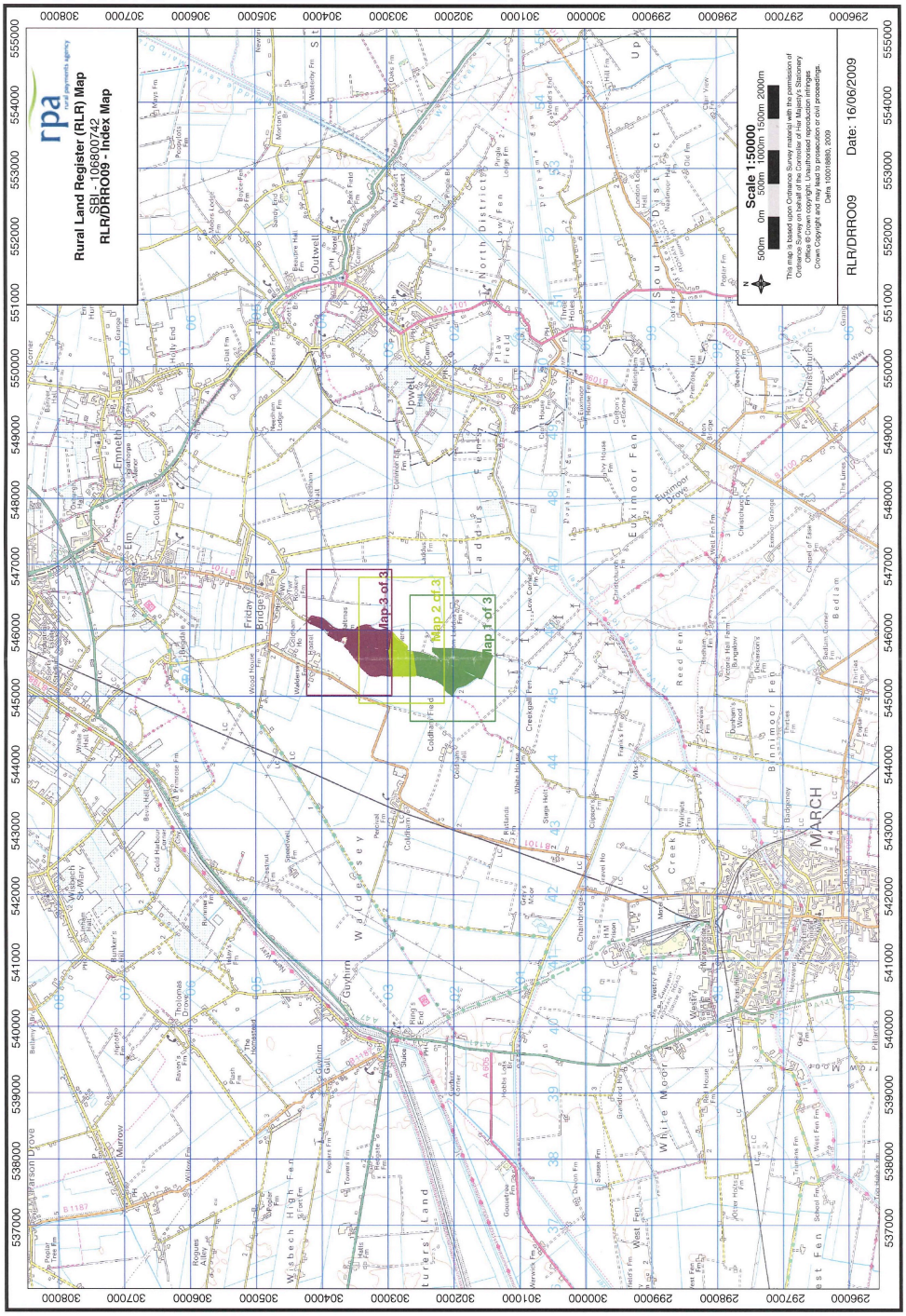
Source: GOV.UK. (2012)

Appendix 2: Biological products and agents with anti-powdery mildew activity

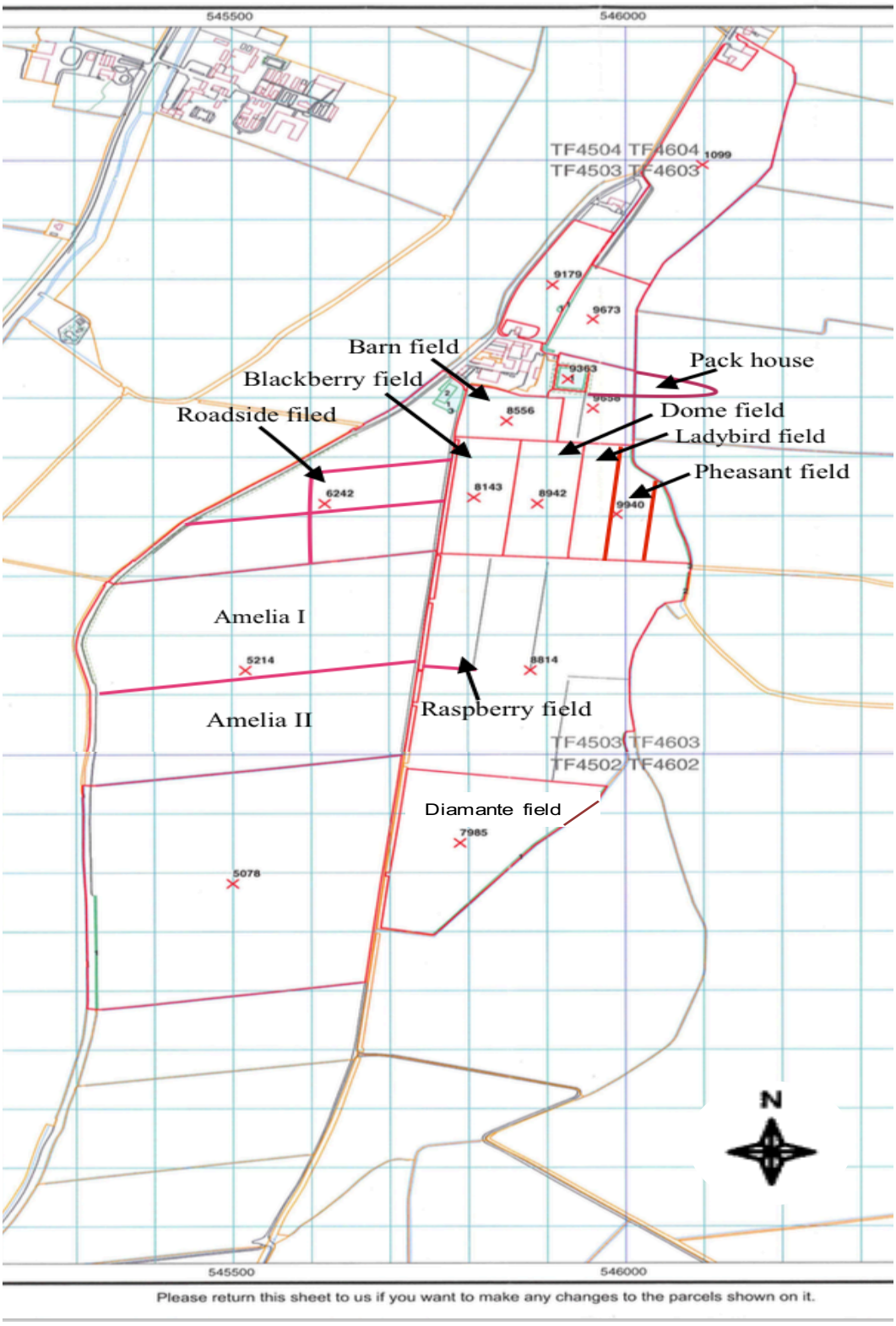
	Products	**Mode of action**	**Reference**
Inorganic products (Synthetic and/natural	Potassium bicarbonate	Fungicide	Cross and Berrie 2006
	Potassium silicate (Kasil)	Fungicide	Borel *et al.*, 2005
	Potassium silicate	Fungicide	Kanto *et al.*, 2007
	Potassium silicate	Induced resistance (IR)	Shetty *et al.*, 2012
	Potassium silicate	Physical IR	Dallagnol *et al.*, 2012
	Baking soda (Sodium bicarbonate)	Fungicide	Horst *et al.*, 1992
	Soluble silicon	Induced resistance (IR)	Belanger *et al.*,1995
	Whitewash or clay	Physical IR	Marco *et al.*, 1994
Organic products (Synthetic and/natural)	Detergents	Protectant/ Fungicide	Cohen *et al.*, 1996
	Antitranspirants	Protectant	Ziv and Hagiladi, 1993
	Oils	Protectant	McGrath and Shishkoff, 2000
	Plant Extracts	IR	Daayf *et al.*, 1995
	Reymoutria sachalinensis		
	(Milsana)		
	Neem kernals	IR	Paisini *et al.*, 1997
	(*Azadirachta indica)*		
Living organism			
(biological)			
Bacterium	*Bacillus subtilis*(Serenade)	Parasitism/antibiosis/IR	Highland, 2000
Fungi	*Ampelomyces quisqualis (AQ-10)*	Parasitism	Sztejnberg *et al.*, 1989
	Verticillium lecanii	Parasitism/antibiosis/	Askary *et al.*, 1998
	Acremonium alternatum	Parasitism	Malathrakis, 1985
	Pseudozyma rugulosa	Antibiosis	Jarvis *et al.*, 1989
	Pseudozyma flocculosa (Sporodex)	Antibiosis	Hajlaoui and Belanger, 1991
Arthropod	*Orthotydeux lambi*	Parasitism	Gadoury *et al.*, 1998

Adapted from: Belanger and Labbe (2002).

Appendix 3: Map A: The overview of Maltmas Farm



Map B: The distribution of different strawberry fields at Maltmas Farm



Appendix 4: The method of taking SEM image



THE ROTHAMSTED CENTRE FOR BIOIMAGING

PROJECT INPUT SUMMARY

Summary of the work carried out by Bioimaging staff on the listed project.

BioImaging Log No.:	2013 012 SLA C
Project Number:	
Project Name:	(External client - Colaboration) Imaging and EDS of Si in Strawberry plants
Sample type:	Detached leaves held at 4°C and uprooted whole plants. (field grown)
Date sample(s) received:	25/11/13
Project completed:	ongoing
Client Name:	Xiaolei Jin (Shelley) Avice Hall. School of Life and Medical Sciences, University of Hertfordshire, College Lane, Hatfield, AL10 9AB

Procedures	Description	Time
Sample preparation	The scanning electron microscope was prepared for cryo operation. Sections of leaves (5 mm x 4mm) were removed using a sterile blade and mounted on cryo stubs using tissue-tek compound. A similar sized section was mounted in a slotted cryo stub for fracturing and microanalysis examination of the fractured surface. Samples were plunged into liquid nitrogen (LN_2) and transferred under vacuum to the cryo prepchamber. The section was fractured using the cold knife mounted in the chamber and contaminating ice was removed from the samples through sublimation. When complete the stage temperature was returned to -150°C and the samples were coated with Au for 60secs. Samples were then transferred to the microscope stage with the temperature maintained at -150°C for examination. After imaging all parameters were adjusted and set up to collect the X-rays for microanalysis. Si was not detected in the cryo fracture face using the smart mapping mode or the Point and Identify tool, so an alternative option was chosen to obtain data. Spectra were obtained from 5 fullframe SOI (adaxial surface) at 1000xs mag for each treatment. Quant optimization was performed during each session to confirm beam stability, exact	0.5hrs

	peak positions to precisely identify individual peaks in the spectrum and resolution.	
Imaging & Analysis	Images and all EDS results were acquired and saved using the on board system and the attached microanalysis system and software.	
Report Data transfer	Results were saved and an electronic copy given to the client. A report was completed and emailed to the client.	
Total Hours	2 days	(16hr)

Signature: Jean Devonshire Date: 28/11/13

Please remember to acknowledge Rothamsted Research - Bioimaging in all related publications. If substantial input has been made in obtaining results and/or analysing data for a publication, please remember to include the appropriate staff member(s) from Bioimaging as co-author(s).

Appendix 5: Silicon nutrient ingredients

SAFETY DATA SHEET

Sirius

Revision 3
Revision date 20-Aug-2009

1. IDENTIFICATION OF THE SUBSTANCE / PREPARATION AND THE COMPANY

Product name Sirius

Company Orion Future Technology Ltd
Henwood House, Henwood. Ashford, Kent, TN24 8DH
info@orionft.com

Telephone Tel:+44 (0) 779 284 3398

Product code qafs474a

2 HAZARDS IDENTIFICATION.

Main hazards Flammable Harmful by inhalation. Irritating to respiratory system. Risk of serious damage to eyes. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

3 . COMPOSITION / INFORMATION ON INGREDIENTS.

Hazardous ingredients

	Conc.	CAS	EINECS	Symbols/Risk phrases
Polyether-modified polysiloxane	1-10%	134180-76-0		Xn;R20 Xn;R21 Xi;R38 Xi;R41 N;R51/53
Ethyl alcohol (Ethanol)	0.5-1%	64-17-5	200-578-6	F; R11
Tetraethyl silicate	70-80%	78-10-4	201-083-8	R10 Xn; R20 Xi; R36/37
Alkyloxypolyethyleneoxyethanol	10-20%	68131-40-8		Xn; R22 Xi; R41

Product Description & Uses A liquid fertiliser.

Product Shelf Life Recommended shelf life 3 years from date of delivery .

4. FIRST AID MEASURES

Skin contact Irritating to skin. Wash off immediately with plenty of soap and water. Seek medical attention if irritation or symptoms persist.

Eye contact Irritating to eyes. Rinse immediately with plenty of water for 15 minutes holding the eyelids open. Seek medical attention. Risk of serious damage to eyes.

Inhalation Move the exposed person to fresh air. Seek medical attention.

Ingestion Ingestion is irritating to the respiratory tract and may cause damage to the central nervous system. DO NOT INDUCE VOMITING. Rinse mouth thoroughly. Drink 1 to 2 glasses of water.

General information If you feel unwell, seek medical advice (show the label where possible). Wash all contaminated clothing before reuse.

5. FIRE FIGHTING MEASURES

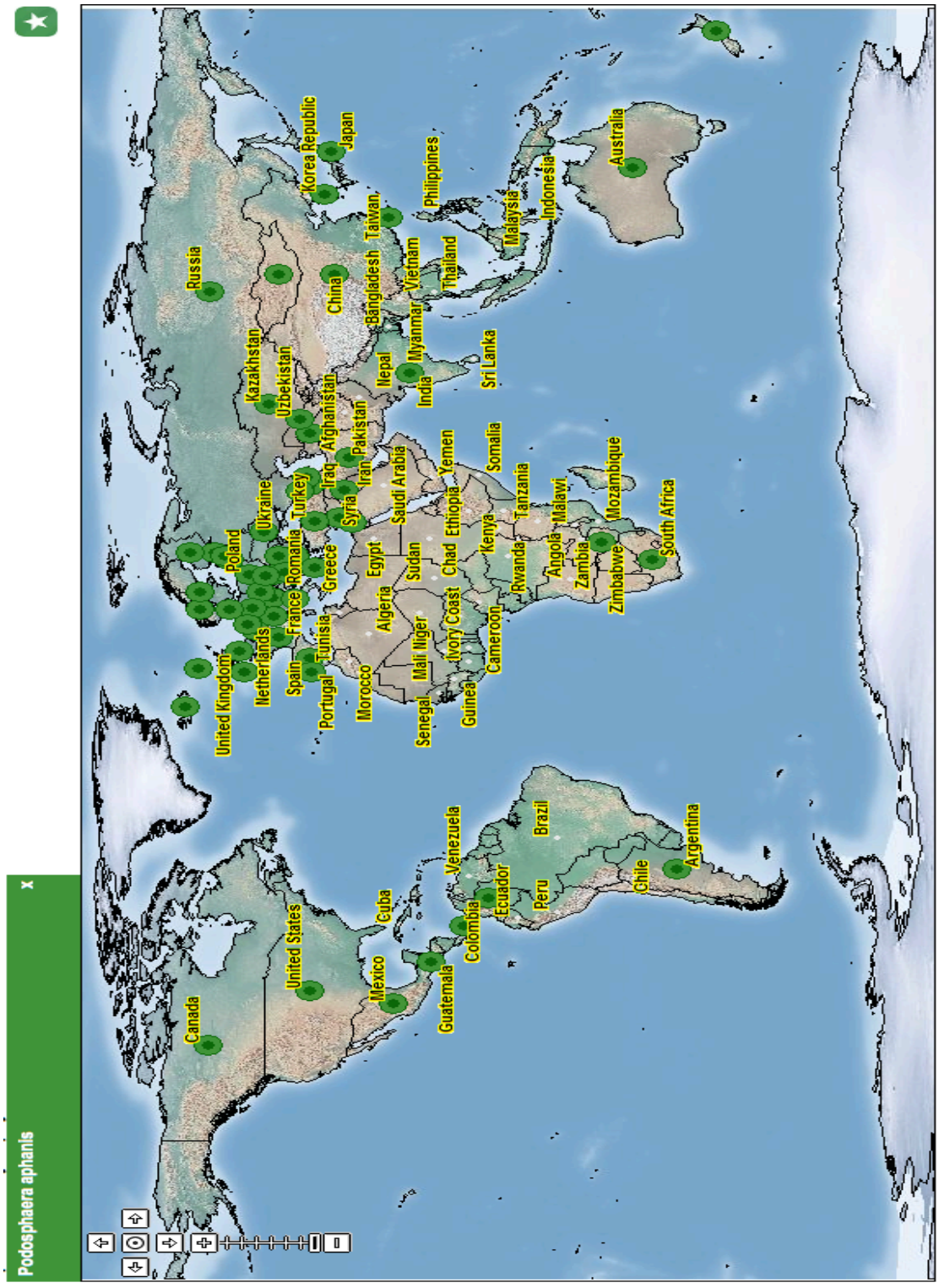
Extinguishing media Use extinguishing media appropriate to the surrounding fire conditions.

Fire hazards Burning produces irritating, toxic and obnoxious fumes.

Protective equipment Wear suitable respiratory equipment when necessary.

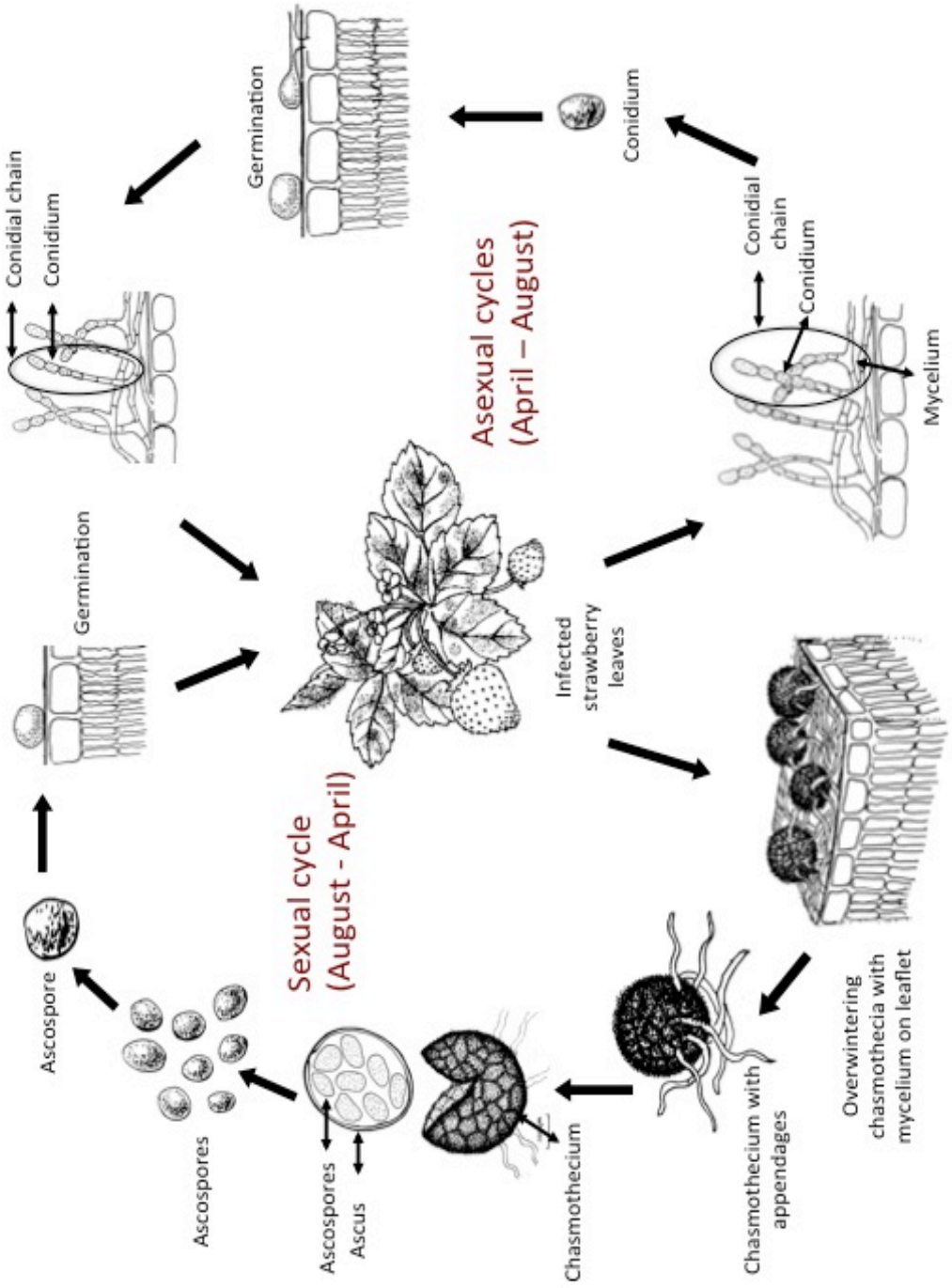
Source: Orion Future Technology LTD

Appendix 6: Word distribution of Strawberry powdery mildew (*Podosphaera aphanis)*



Source from: Plantwise (2015)

Appendix 7: Life cycle of *Podosphaera aphanis*

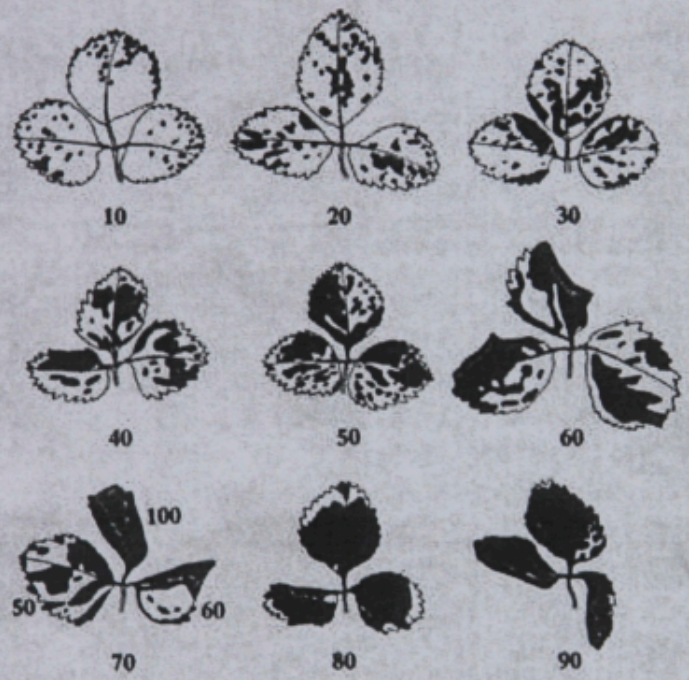


Source: Xiaolei Jin

AGRICULTURAL DEVELOPMENT AND ADVISORY SERVICE

Strawberry Powdery Mildew — Key No. 8·1·1

Sphaerotheca alchemillae Grev.



Percentage of leaf area affected

Black shaded areas represent red/yellow blotches and brown dead tissue

Examine 10 *fully expanded* leaves on one typical plant. Grade these using the diagrams and key. Repeat on 9 further typical plants, giving assessments from 100 leaves. Calculate the mean percentage mildew.

Powdery mildew (%)

- 0 Leaves fully extended, flat and green.
- 5 Slight curling noticeable; mildew found with difficulty.
- 10 Leaves with small red-purple spots. Curling slight. Mildew visible on lower surface.
- 20 Red blotches tending to be confluent. Some browning. Curling obvious from a distance.
- 30 More blotches confluent, with browning becoming more severe. Splitting in centre of larger lesions and curling severe.
- 40 Confluent red and brown blotches. Splitting in centre of larger lesions. Curling now approaching rolling. Leaf becoming brittle.
- 50 Half of leaf area affected and apparently dead.
- 60 Some yellowing in addition to reddening and browning may be present.
- 70 Severe distortion of at least one leaflet.
- 80 Much of leaf affected. Distortion of all leaflets.
- 90 Small marginal areas only remain green.
- 100 Whole leaf red or brown. Severe distortion and very brittle.

Notes:

This key is based on measurements of the reddening and browning symptoms which may be seen on Royal Sovereign at picking time. It is not possible to use the extent of white sporing mycelium as a guide to severity, as this is very difficult to see even when 100% of the lower surface is infected.

PLANT PATHOLOGY LABORATORY
HARPENDEN, HERTS

Ministry of Agriculture, Fisheries and Food
(Publications), Tolcarne Drive, Pinner, Middlesex HA5 2DT

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Appendix 9: Data analysis for chapter 3

Table 1: Binomial test of the average number (mature and immature) of *Podosphaera aphanis* chasmothecia on 50 strawberry leaves sampled each month from Diamante field (open field) (8 sprays with fungicide) and Ladybird field (polyethylene tunnel) (13 sprays with fungicide) from August 2011 to July 2012 at Maltmas Farm near Wisbech in Cambridgeshire

Date	Category	N	Prop	Asymp. Sig. (two- tailed)
21-Sep-11	Diamante field Ladybird field	50 50	0.56 0.24	0.001
29-Nov-11	Diamante field Ladybird field	50 50	0.28 0.20	0.349
13-Dec-11	Diamante field Ladybird field	50 50	0.10 0.04	0.24
31-Jan-12	Diamante field Ladybird field	50 50	0.46 0.28	0.062
21-Mar-12	Diamante field Ladybird field	50 50	0.5 0.2	0.002

24-Apr-12	Diamante field Ladybird field	50 50	0.04 0.02	0.558

Table 2: Binomial test of the average number (mature and immature) of *P. aphanis* chasmothecia on 50 strawberry leaves sampled each month from Diamante field (open field) (8 sprays with fungicide) and Ladybird field (polyethylene tunnel) (13 sprays with fungicide) from September 2012 to March 2013 at Maltmas Farm near Wisbech in Cambridgeshire.

Date	Category	N	Prop	Asymp. Sig. (two- tailed)
18-Sep-12	Untreated Fungicide treated	50 50	0.08 0.00	0.041
17-Oct-12	Untreated Fungicide treated	50 50	0.08 0.04	0.4
13-Nov-12	Untreated Fungicide treated	50 50	0.20 0.04	0.014
12-Dec-12	Untreated Fungicide treated	50 50	0.22 0.12	0.183

29-Jan-13	Untreated Fungicide treated	50 50	0.12 0.10	0.749
06-Mar-13	Untreated Fungicide treated	50 50	0.16 0.04	0.046
Ascospore	Untreated Fungicide treated	10 10	0.3 0.1	0.264
Total	Untreated Fungicide treated	300 300	0.147 0.057	< 0.001

Table 3: The correlation between the numbers of mature chasmothecia and the size of chamothecia in Diamante field and Ladybird field

Sample date	Diamante field	Ladybird field
16/08/11	NS	0.74
21/09/11	0.78	0.44
18/10/11	0.79	NS
29/11/11	0.64	NS
13/12/11	0.45	0.63
31/01/12	0.3	0.71
21/03/12	0.73	0.54

Appendix 10: Assessment of strawberry leaf spot (*Mycosphaerella fragariae)*

Isolation of strawberry leaf spot

The leaf spot pathogen *Mycosphaerella fragariae* is an ascomycete. For isolation from strawberry leaf spots, five small white lesions were cut out from leaves. The small white lesion pieces were surface sterilized by IMS for 2 minutes and subsequently rinsed in sterile distilled water. Then they were dried on sterile paper tissues. The small white lesion pieces were placed on PDA medium and they were incubated at 15°C in darkness. After 3 days, the colonies that developed from diseased tissue were subcultured onto PDA medium and incubated at 15 °C for 3 days in darkness. After 3 days, the colonies were spread on the medium. Five ml distilled water was added to the colony in order to obtain a spore suspension. A haemacytometer was used to count the spore suspension at X 100.

Assessment strawberry leaf spot

Strawberry leaf spot (*Mycosphaerella fragariae*) was also observed on the pre-assessment of plants in the 2013 silicon fertigation experiment and on glasshouse plants, which indicated that those strawberry crowns and plants delivered by the propagator were not disease-free (Figure 1).

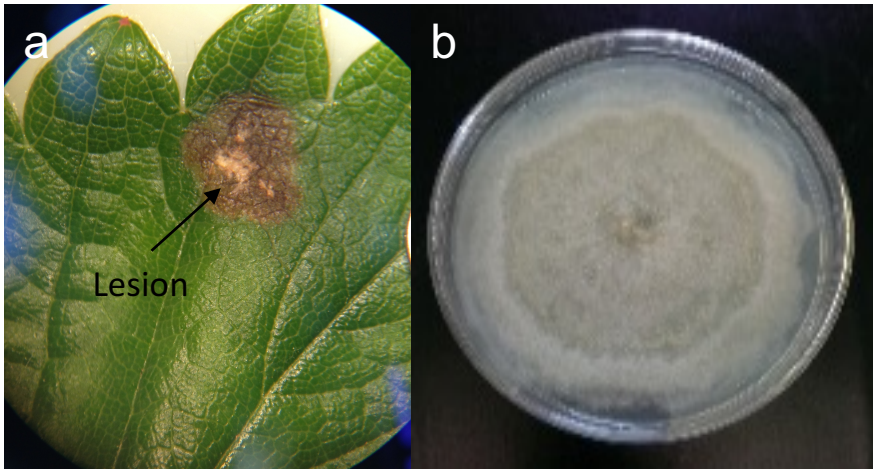

Figure 1: Strawberry leaflet with symptoms of strawberry leaf spot (*Mycosphaerella fragariae*) as it appears in the field and glasshouse. a. the lesion was small and had light centre (Maas, 1998); b. the colonies were spread on PDA medium after 3 days growing on it at 15 °C.

Appendix 11: Risk assessment and CoSHH forms

FACULTY OF HEALTH AND HUMAN SCIENCES
RISK ASSESSMENT

Ref No:	
Date:	
Review Date:	

ACTIVITY INFORMATION	
Name of Assessor/ Contact details	Name: Xiaolei Jin Email address: x.jin3@herts.ac.uk Ext no: 4968
Title of Activity	Field trial at Wisbech
Location of Activity	C.P.Snow Buliding
Description of Activity	1. Travelling to and from field 2. Monthly sampling of leaves with chasmothecia
Personnel Involved	Supervisor: Dr. Avice. M. Hall

TYPES OF HAZARD LIKELY TO BE ENCOUNTERED		
☐ Animal Allergens ☐ Biological Agents (see COSHH) ☐ Chemical Compounds (see CoSHH) ☐ Compressed/liquefied gases ☐ Computers ☐ Electricity ☐ Falling Objects ✓ Farm Machinery ☐ Fire ☐ Glassware Handling	☐ Hand Tools ☐ Ionising Radiation ✓ Office Equipment ☐ Laboratory Equipment ☐ Ladders ☐ Manual Handling ☐ Non-ionising Radiation ☐ Hot or cold extremes ✓ Repetitive Handling ☐ Severe Weather	☐ Sharps ✓ Slips/trips/falls ✓ Stress ✓ Travel ☐ Vacuum systems ☐ Pressure systems ✓ Vehicles ☐ Violence, physical or verbal abuse ☐ Workshop Machinery
The above is not an exhaustive list – all other hazards should be listed here.		

FACULTY OF HEALTH AND HUMAN SCIENCES
RISK ASSESSMENT

HAZARD ASSESSMENT						
Severity of Consequences	Factor	Risk Classification				
No or minor injury/ health disorder Minor Damage or Loss Insignificant Environmental Impact Group 1 Biological agents	1	Trivial (1)	Trivial (2)	Trivial (3)	Trivial (4)	Tolerable (5)
Injury or Health Disorder – resulting in absence up to 3 days Moderate Damage or Loss Moderate Environmental Impact Group 2 Biological agents	2	Trivial (2)	Trivial (4)	Tolerable (6)	Tolerable (8)	Moderate (10)
Injury or Health Disorder – resulting in absence over 3 days Substantial Damage or Loss Serious Environmental Impact Group 3 Biological agents	3	Trivial (3)	Tolerable (6)	Moderate (9)	Moderate (12)	Substantial (15)
Long Term Injury or Sickness – resulting in permanent incapacity Extensive Damage or Loss Major Long Term Environmental Impact	4	Trivial (4)	Tolerable (8)	Moderate (12)	Substantial (16)	Intolerable (20)
Death Serious Structural Damage Environmental Catastrophe Group 4 Biological agents	5	Tolerable (5)	Moderate (10)	Substantial (15)	Intolerable (20)	Intolerable (25)
Note on Risk Classification: 1-4 Trivial 5-7 Tolerable 8-12 Moderate 13-16 Substantial >20 Intolerable	→	1	2	3	4	5
	Likelihood	Almost Impossible	Unlikely – possible exposure every 1-3 years	Harm is possible	Harm is likely to occur	Harm will occur or is very likely to occur.

Please think before printing the attached guidance notes – do you really need them?

FACULTY OF HEALTH AND HUMAN SCIENCES
RISK ASSESSMENT

ASSESSMENT OF RISK CLASSIFICATION			
Hazard	Likelihood Score	Severity Score	Risk Classification
Chances of tripping in the field	2	1	2
Allergy to spores	2	2	4

EFFECT OF RISK CLASSIFICATION	
Risk Classification	Action
Trivial	No further action required. Activity can begin.
Tolerable	No additional controls required. Current controls must be maintained and monitored.
Moderate	Reduce risks if cost effective. Implement new controls over an agreed period.
Substantial	Activity cannot begin without major risk reduction.
Intolerable	Activity must not begin.

RISK CONTROL MEASURES
Is the local code of practice or local rules adequate to control the risks identified? Yes/No If no, list all additional measures required.
Additional Measures:

HEALTH SURVEILLANCE ISSUES	
Persons at Special Risk	
Health Surveillance Measures (including symptoms and signs of exposure)	
Exclusions	

FACULTY OF HEALTH AND HUMAN SCIENCES
RISK ASSESSMENT

SIGNATURES				
	Staff/PhD student/MSc student/Undergraduate	Name (Print)	Signature	Date
Assessor	PhD student	Xiaolei Jin	Shaolei Jin	03/04/2012
Supervisor (if Assessor is a student)	ACADEMIC STAFF	Dr. Avice. M. Hall	Avice M Hall	3.3.12
Local Health and Safety Advisor	TECHNICAL STAFF	DIANA FRANCIS		12/4/12

Please think before printing the attached guidance notes – do you really need them?

FACULTY OF HEALTH AND HUMAN SCIENCES
CoSHH ASSESSMENT

Ref No:	
Date:	
Review Date:	

PROJECT INFORMATION	
Name of Assessor/Contact details	Name: Xiaolei Jin Email address: x.jin3@herts.ac.uk Ext no: 4968
Title of Activity	- Transport the sample leaves to lab - Extraction of silicon - Measure the strawberry leaves
Location of Activity	C.P. Snow, College Lane Campus , Laboratory 2G 165
Description of Activity	- Extract the silicon from strawberry leaves - Observe the leaves length and the density per cm^2 under microscope
Personnel Involved	Student: Xiao Lei Jin, Khin Hsu Hsu San, other students in lab, Technician staffs, cleaning staffs, Security, visitors to lab, supervisor of the project (Avice. M. Hall)

HAZARD CLASSIFICATION

Hazards involved in project.

✓Harmful/Irritant 	□ Corrosive 	□ Radioactive 	□ Biological 
✓Toxic 	✓Health Hazard 	□ Explosive 	□ Oxidising 
✓ Flammable 	□ Hazardous to the Environment 	□ Gas under pressure 	

Severity Classification

Harmful/Irritant 2	Corrosive 3	Radioactive *	Biological **
Toxic 3	Health Hazard 4	Explosive 6	Oxidising 6
Flammable 3	Hazardous to the Environment 4	Gas under pressure 6	

* Radioactive – If any work will involve use of Radioactivity contact the Radiation Protection Supervisor, Janet Evans (ext 4379, j.s.evans@herts.ac.uk) for an accurate severity classification *before* work begins.

** Biological – If any work will involve using biological samples or suspected biohazards contact the Biological Safety Advisor.
For School of Life Sciences contact Di Francis (ext 4527, d.l.francis@herts.ac.uk).
For School of Pharmacy contact James Stanley (ext 4599, j.k.stanley@herts.ac.uk).

Substance Used or Produced.	Concentration or amount used.	Hazard Words and Pictograms	Route of Entry	Severity
1. 50% H_2O_2	3.0 mL	Harmful	Inhalation, ingestion, Eye and skin contact	4
2. 50% NaOH	3.5 mL	Harmful	Inhalation, ingestion, Eye and skin contact	4
3. 2.5 %Boric acid	3 mL	Irritant	Inhalation, Eye and skin contact	2
4. Ammonium molybate	1 mL	Irritant	Ingestion, Eye and skin contact	2
5. 20% Tartaric acid	0.5 mL	Irritant	Inhalation, Eye and skin contact	2
6. Na_2SO_3	2 g	Irritant	Inhalation, ingestion, Eye and skin contact	2
7. 1-amino2-napthol-4 sulfonic acid	0.4 g	Irritant	Inhalation, ingestion, Eye and skin contact	2
8. Sodium by sulphite ($NaHSO_3$)	25 g	Harmful	Inhalation, ingestion, Eye and skin contact	2
9. Strawberry plant		Irritant	Ingestion	1
10. Omex SW7		Flammable	All routes	2
11. Potassium carbonate		Acute toxicity, irritation,	All routes	4

		carcinogenicity		
Information sources (eg MSDS)	Sigma Aldrich			

CONTROL MEASURES
Additional measures to be used over and above local codes of practice and local rules.
• Wearing of lab coat in the laboratory. • Wearing of eye protector. • Wearing of gloves. • Working under the fume cabinet when using toxic materials.
Are alternative less hazardous substances available? If so please list below and state why they cannot be used.
N/A

EMERGENCY AND DISPOSAL PROCEDURES
Measures to be taken in case of Spillage or Uncontrolled release
Solid spill: Without raising any dust, mop up spill with damp tissue paper. Liquid Spill: Absorb spill into tissues.
Disposal Measures
Solids: Send out via yellow bin disposal system. Liquids: Send out via yellow bin disposal system. Solvents: Send out via yellow bin disposal system. Biohazard: Autoclave microbiological wastes.
Measures to be taken in case of Fire
Types of fire extinguisher to be used: Use extinguishing measures that are appropriate to local circumstances and the surrounding environment. Emergency procedure in case of fire: Wear self contained breathing apparatus for fire fighting if necessary. Toxic fumes emitted under fire conditions: Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.
Measures to be taken for First Aid

In contact with eyes: Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

In contact with skin: Wash off with soap and plenty of water. Consult a physician.

If inhaled: If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician

If ingested: Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

Signs and symptoms of exposure: To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Is hospitalisation required? N/A

Persons at Special Risk: N/A

SIGNATURES				
	Staff/PhD student/MSc student/Undergraduate	Name (Print)	Signature	Date
Assessor	PhD student	Xiao Lei Jin		23/02/12
Supervisor (if Assessor is a student)	Staff	Dr. Avice. M. Hall		
Local health and safety advisor	Technician staff	Di Francis		23/2/12

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CoSHH ASSESSMENT

+Ref No:	XJ-1
Date:	5-4-12
Review Date:	One year max

PROJECT INFORMATION	
Name of Assessor/Contact details	Name: Xiaolei Jin Email address: x.jin3@herts.ac.uk Ext no: 4968
Title of Activity	**Observe the strawberry leaves under Microscope**
Location of Activity	C.P. Snow College Lane Gamps Laboratory: 2G165
Description of Activity	**Collecting chasmothecia from leaves** **- Examining the chasmothecia under microscope** **Examining the ascospore germinating**
Personnel Involved	Student: Xiao lei Jin, other students in lab, technical staff, cleaning staff security, visitors to lab, supervisor of project (Dr. Avice.M.Hall)

HAZARD CLASSIFICATION

Hazards involved in project.

☐ Harmful/Irritant 	☐ Corrosive 	☐ Radioactive 	☐ Biological 
☐ Toxic 	☐ Health Hazard 	☐ Explosive 	☐ Oxidising 
☐ Flammable 	☐ Hazardous to the Environment 	☐ Gas under pressure 	

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Severity Classification

Harmful/Irritant 2	Corrosive 3	Radioactive *	Biological **
Toxic 3	Health Hazard 4	Explosive 6	Oxidising 6
Flammable 3	Hazardous to the Environment 4	Gas under pressure 6	

* Radioactive – If any work will involve use of Radioactivity contact the Radiation Protection Supervisor, Janet Evans (ext 4379, j.s.evans@herts.ac.uk) for an accurate severity classification *before* work begins.

** Biological – If any work will involve using biological samples or suspected biohazards contact the Biological Safety Advisor.
For School of Life Sciences contact Di Francis (ext 4527, d.l.francis@herts.ac.uk).
For School of Pharmacy contact James Stanley (ext 4599, j.k.stanley@herts.ac.uk).

Substance Used or Produced.	Concentration or amount used.	Hazard Words and Pictograms	Route of Entry	Severity
Trypan blue (liquid)	0.01% ++++++++++	□ Health Hazard 	**May cause cancer**	4
Fungal mounting fluid	1 drop	□ Harmful/Irritant 	**Ingestion, inhalation**	2
Information sources (eg MSDS)				

CONTROL MEASURES
Additional measures to be used over and above local codes of practice and local rules.
Obtain special instructions before use
Are alternative less hazardous substances available? If so please list below and state why they cannot be used.
No. Safer to buy in liquid, not powder

EMERGENCY AND DISPOSAL PROCEDURES
Measures to be taken in case of Spillage or Uncontrolled release
Solid spill: without raising any dust, mop up spill with tissue paper Liquid Spill: absorb spill onto tissues
Disposal Measures
Solids: COLLECT IN SEALABLE BAG + SEND OUT FOR DISPOSAL VIA YELLOW BIN Liquids: send out via yellow bin disposal system Solvents: " " " " " Biohazard: autoclave microbiological wastes AS LONG AS THEY DO NOT CONTAIN TOXIC MATERIALS!
Measures to be taken in case of Fire
Types of fire extinguisher to be used: use water sprayx, alcohol-resistant foam, dry chemical or carbon dioxide Emergency procedure in case of fire: EVACUATE AREA. INFORM FIRE-FIGHTERS OF NATURE OF CHEMICALS USED Toxic fumes emitted under fire conditions: SPECIAL HAZARDS FROM:. CARBON OXIDES, NITROGEN OXIDES, SULPHUR OXIDES, SODIUM OXIDES
Measures to be taken for First Aid
In contact with eyes: flush eyes with water as a precaution In contact with skin: wash off with soap and plenty of water. Consult a physician. If inhaled: if breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. If ingested: DO NOT GIVE ANYTHING TO DRINK. RINSE OUT MOUTH WITH WATER + SEEK URGENT MEDICAL A.D. Signs and symptoms of exposure: NOT THOROUGHLY INVESTIGATED (ACCORDING TO SIGMA MSDS) Is hospitalisation required? POSSIBLY Persons at Special Risk: ALL PERSONS AT POTENTIAL RISK

SIGNATURES				
	Staff/PhD student/MSc student/Undergraduate	Name (Print)	Signature	Date
Assessor	PhD STUDENT	XIAOLEI JIN		23/04/12
Supervisor (if Assessor is a student)	ACADEMIC STAFF	Avice. M.Hall		
Local health and safety advisor	TECHNICAL STAFF	DIANA FRANCIS		23/4/12

Appendix 12: Dose rate calculation (from grower at Maltmas farm) for the 2013 Si fertigation field experiment from the Maltmas farm

Conventional rate of Si when used as an agricultural wetter is

50 - 100 ml in 100 - 600 L of water. Standard is 100ml Si in 600 L per Ha.

100 ml in 600 L = 0.017% Si (v/v)

It is applied to 12500-meter run of beds containing 40000 - 75000 plants

In Roadside field there were 6 plants per meter

I.e. 100 ml applied to 12500 x 6 = 75000 plants / Ha.

= 0.0013 ml / plant (of product)

The irrigation block is 1860 m long

Therefore, dose of product = 1860/12500 x 100 = 15ml / block.

Appendix 13: Posters

December 2011, The British Society for Plant Pathology (BSPP) presidential meeting

Development and maturation of the chasmothecia of *Podospheara aphanis* on strawberry



Xiao lei Jin and Dr. Avice M Hall (supervisor)
University of Hertfordshire, College Lane, Hatfield, AL10 9AB, UK



Introduction

- Strawberry powdery mildew caused by *Podosphaera aphanis,* is a disease of economic significance (Dodgson, 2008).
- The chasmothecia (figure 1) of *Podosphaera aphanis* appear in late summer and autumn in response to change in environmental conditions.
- The chasmothecia (figure 2) contain one ascus with eight ascospores (Farooq *et al,* 2007 & Gow & Gadd 1995).

Aims

- Follow the development and maturation of the chasmothecia (from August to November 2011)



Figure 1: A leaf showing chasmothecia at x100 magnification source: Xiaolei Jin

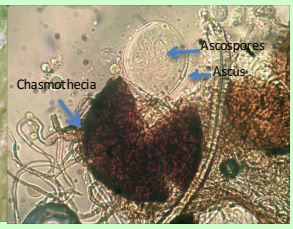


Figure 2: chasmothecia at x400 magnification source: Xiaolei Jin

Results

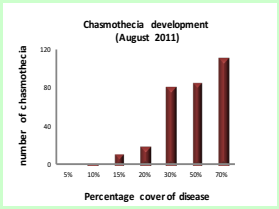


Figure 3: Numbers of the chasmothecia (x 100 magnification) at the different percentage cover of disease and the maturity of the chasmothecia (x400 magnification) in August

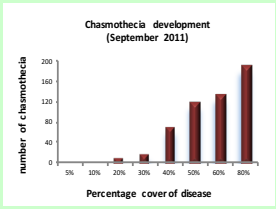


Figure 4: Numbers of the chasmothecia (x 100 magnification) at the different percentage cover of disease and the maturity of the chasmothecia (x400 magnification) in September

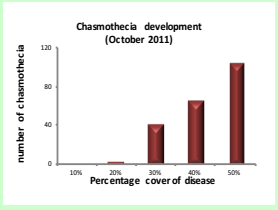


Figure 5: Numbers of the chasmothecia (x 100 magnification) at the different percentage cover of disease and the maturity of the chasmothecia (x400 magnification) in October

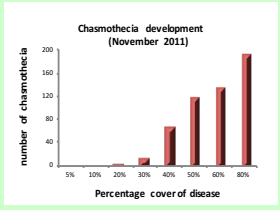


Figure 6: Numbers of the chasmothecia (x 100 magnification) at the different percentage cover of disease and the maturity of the chasmothecia (x400 magnification) in November

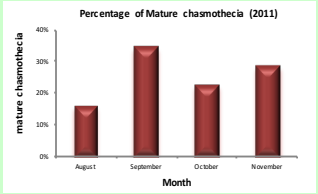


Figure 7: Percentage of mature chasmothecia at different month

Materials and methods

- 60 leaves (Elegance) were randomly sampled from a 180m strawberry bed each month
- All samples were brought back to the lab and assessed for amount of disease and number of the chasmothecia per leaflet
- The chasmothecia were measured using an eye piece graticule at x400 magnification
- 5 chasmothecia were observed under the x400 magnification for maturity per leaflet
- Leaves with immature chasmothecia were placed in a damp chamber at 20 ° C to follow their maturation

Results

- The results show the higher numbers of chasmothecia where the percentage cover of disease is high (figure 3,4, 5,and 6)
- There are no chasmothecia where disease cover is under 10%
- There were more mature chasmothecia in September than in August, October or November (figure 7)
- Immature chasmothecia matured in 10/11 days in the damp chamber at 20 ° C

Discussion

- The highest number of chasmothecia were obtained in September(figure 4) when percentage of disease was high.
- The highest number of matured chasmothecia occurred in September (figure 7) This could be linked to weather conditions , see results in damp chamber.

Future work

- Continue to sample though out the year and monitor the maturity of chasmothecia
- Follow the maturation of chasmothecia in the damp chamber at the different temperature
- Monitor the temperature and humidity in the field though out the year
- This work could then be used to predict the start of epidemics in the spring

Acknowledgement

Thanks for Dr. Avice. M. Hall – project supervisor
Harriet & Henry Duncalfe, Maltmas Farm for providing the field trial

References

1. Dodgson, J., Hall, A and Parker,S. (2008). Control of strawberry powdery mildew under protection. Project SF 62 & SF 62a.
2. Farooq, M., Dodgson, J. & Hall, A (2007). Examination of the morphology of *Podosphaera aphanis* cleistothecia and their role in over wintering of the fungus. *Aspects of Applied Biology*. Vol 92, pp. 671-675
3. Gow, N.A.R. & Gadd, G.W. (1995) *The growing fungus*. Chapman and Hall.

The role of silicon and potassium carbonate in controlling epidemics of *Podospheara aphanis* on strawberry in the field



Xiaolei Jin , Avice M Hall and Bo Liu

School of Life and Medical Sciences, University of Hertfordshire, College Lane, Hatfield, AL10 9AB



Introduction

- Strawberry powdery mildew is caused by *Podosphaera aphanis.* The fungus mycelium is on the plant leaves, stems, shoots, buds; flowers and fruit surfaces (Fig.1), which causes yield loss (Dodgson, 2008).
- Strawberry growers frequently use potassium carbonate to control the disease in a silicon-based wetter in the interval immediately prior to harvest when commercial fungicides cannot be used.

Aim

- To investigate the disease control achieved by the potassium carbonate and the silicon-based wetter, used singly and in combination .



Figure 1: Mycelium on the leaflet and fruit, leaf cupping and red blotch

Materials and methods

- The trial used a randomised block design (Fig.2) with the following 6 treatments each replicated 3 times : untreated, K50 (potassium carbonate) alone, 0.25% (field rate) silicon wetter, 0.25% (field rate) silicon wetter with K50, 0.5% silicon wetter and 0.5% silicon wetter with K50.
- The trial was sprayed each week from 19/07/12 to 24/08/12.
- 15 leaves (cv Elegance) were randomly sampled from each block every week in an open field (Fig.3).
- All samples were brought back to the lab and assessed for amount of disease per leaflet.



Figure 3: Open strawberry field

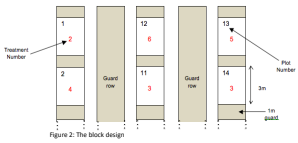


Figure 2: The block design

Results

- The results, show that high rate silicon wetter and K50 together (Fig.4) gave the best control of disease, with low rate silicon wetter and K50 together also giving control.
- The silicon wetter alone at high rate also gave some control of the disease but K50 and silicon wetter at low rate alone gave little control and was similar to the untreated control (Fig.5).
- The pre trial assessment of leaf hair numbers on both lower and upper surface showed that there was no significant (P>0.05) different between untreated and other treatments (Fig.6). At the end of trial (07/09/2012), there was no significant (P>0.05) different between untreated and other treatments on the lower surface. However, at the end of trial (07/09/2012) there were significant (P<0.05) differences between untreated and 0.5%SW7 with K50, 0.25%SW7 and K50 alone on upper surface (Fig.7).

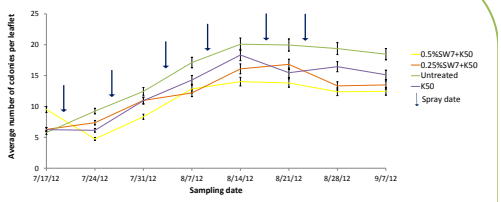


Figure 4: Average number of colonies on leaflets from 18 blocks. The AUDPC for 0.5%SW7+k50 is 540. The AUDPC for 0.25%SW7+k50 is 607. The AUDPC for k50 is 646. The AUDPC for untreated is 772.

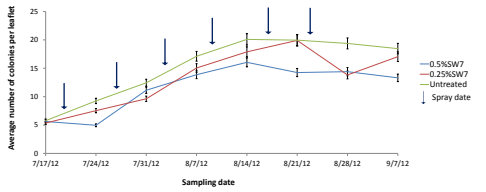


Figure 5: Average number of colonies on leaflets from 18 blocks from 17/07/12 to 07/09/12. The AUDPC for 0.5%SW7 is 588. The AUDPC for 0.25%SW7 is 665. The AUDPC for untreated is 772.

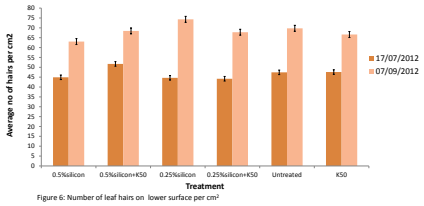


Figure 6: Number of leaf hairs on lower surface per cm²

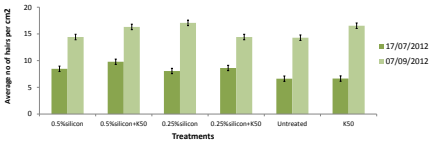


Figure 7: Number of leaf hairs on upper surface per cm²

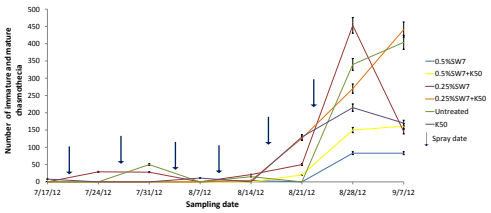


Figure 8: Numbers of mature and immure chasmothecia per leaflets with different treatments.

Discussion

This trial was carried out when the disease was well established during the period of high disease pressure. The results indicated that high rate silicon wetter with K50 and high rate silicon wetter control the epidemic build up after 4 weeks (Fig.4&5), this showed that the efficacy of K50 was improved when used with silicon wetter. The reduction in colonies with 0.5%SW7 with K50 and 0.5%SW7 reduces the number of chasmothecia per leaflet (Fig. 8).

Future work

To analysis the concentration of silicon in leaf. The next field trial will be in 2013, and the aim of the trial will be to investigate the role of an early silicon spray in preventing epidemic build up. A second trial will be to apply the silicon into roots to follow the epidemic build up.

Acknowledgement

Thanks to Harriet & Henry Duncalfe, Maltmas Farm for providing the field trial. Thanks to Gidon Bahiri for the supply of SW7. Thanks to Andy Barker for spraying the crop.

Reference

1. Dodgson, J., Hall, A and Parkes,S. (2008). Control of strawberry powdery mildew under protection. Project SF 62 & SF 62a.

University of Hertfordshire

Integrated control of Strawberry powdery mildew



Xiaolei Jin , Yongju Huang, Bruce D L Fitt, Avice M Hall

School of Life and Medical Sciences, University of Hertfordshire, College Lane, Hatfield, AL10 9AB

Introduction

- Strawberry powdery mildew is caused by *Podosphaera aphanis*. The mycelium is on the plant leaves, stems, shoots, buds, flowers and fruit surfaces (Fig.1), which causes yield loss. Strawberry growers frequently use potassium bicarbonate to control the disease with a silicon nutrient in the interval immediately prior to harvest when commercial fungicides cannot be used.
- The prediction system is based on conidiospore data and constant monitoring of the temperature and humidity in a tunnel but only 'counting' the number of hours when the temperature humidity are correct for disease development.

Aim

- To investigate the disease control achieved by the potassium carbonate and the silicon-based wetter, used singly and in combination.
- To investigate the use of a prediction system to control strawberry powdery mildew.
- To monitor the initiation, development and maturation of chasmothecia in the silicon field trial.

Materials and methods

- The prediction system trial was laid out in an open field. Three beds were sprayed according to the prediction system and two beds were untreated control.
- The silicon field trial used a randomised block design with 6 treatments: untreated, potassium bicarbonate alone, 0.25% (field rate) Silicon nutrient , 0.25% Silicon nutrient with potassium bicarbonate , 0.5% Silicon nutrient and 0.5% Silicon nutrient with potassium bicarbonate. Leaf sample were assessed weekly.

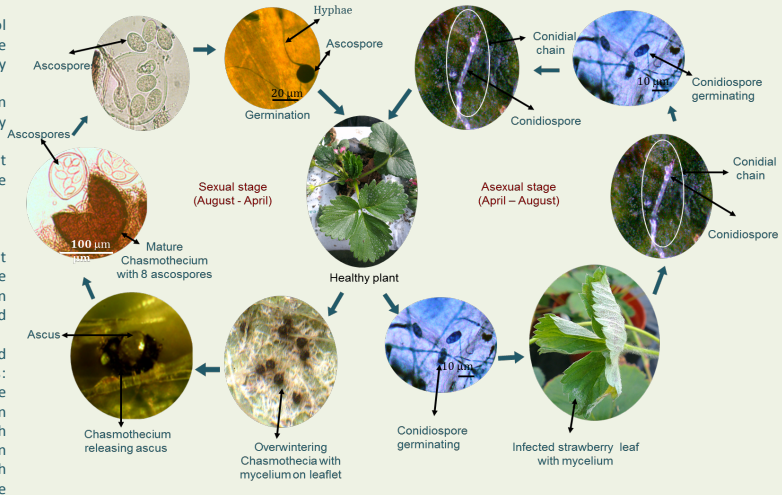


Figure 1: Life cycle of by *Podosphaera aphanis*

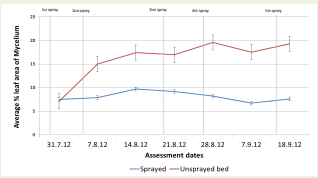


Figure 2: Disease assessment shown by the average % area of leaves covered with colonies (mycelium) on strawberry beds sprayed with fungicide according to the prediction system, and on the untreated control. The AUDPC for the unsprayed is 827.15 and that for the sprayed is 395.70.

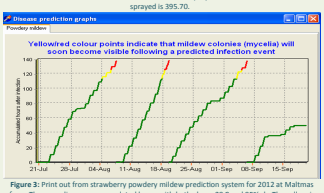


Figure 3: Print out from strawberry powdery mildew prediction system for 2012 at Maltmas farm. The green lines are accumulated hours with both above 20°C and 95% rh. The yellow is the warning period for the grower, having the need to spray, and the need that spores will be germinating and initiating the next round of infection. As soon as the crop is sprayed, the prediction system is reset to zero and the accumulated hours calculation restarts.

Discussion

- This work show that the use of prediction system and silicon nutrient potentially control the epidemic build up.
- The reduction in chasmothecia (Fig.5) which reduces the initial inoculum of germinating ascospores in the spring.

Acknowledgement

Thanks to Harriet & Henry Duncalfe, Maltmas Farm for providing the field trial. Thanks to Gidon Bahiri for the supply of silicon nutrient. Thanks to Andy Barker for spraying the crop. Thanks to Khin Hsu Hsu San for providing the prediction system data. Thanks to Jean for helping to take the Bio-image.

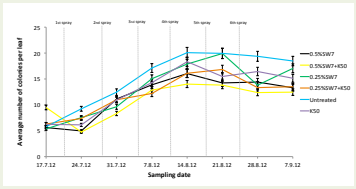


Figure 4: Average number of colonies on leaf from 18 blocks. The AUDPC for 0.5%SW7+potassium bicarbonate is 540, 0.25%SW7+potassium bicarbonate is 607, potassium bicarbonate is 646, 0.5%SW7 is 588, 0.25%SW7 is 665 and untreated is 772.

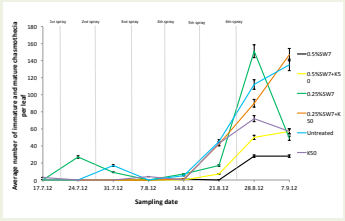


Figure 5: Numbers of mature and immure chasmothecia per leaf with different treatments.

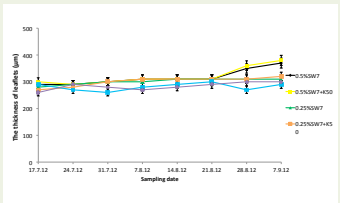


Figure 6: The leaf thickness per leaflets with different treatments.

Results (predication system trial)

- The results showed that the unsprayed bed had a greater percentage of colonies than the sprayed bed (P<0.05), hence the prediction system could control the epidemic (Fig.2&3).

Results (silicon trial)

- The results, show that 0.5% Silicon nutrient and potassium bicarbonate gave the best control of disease (P<0.05)(Fig.4),and 0.5% Silicon nutrient alone (P<0.05), reduces the number of chasmothecia per leaf (Fig.5).
- The Silicon nutrient increases the leaf thickness, the high rate Silicon nutrient alone and with potassium bicarbonate gives the greatest leaf thickness (P<0.05) (Fig.6&7).

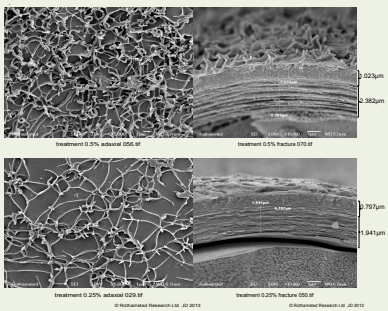


Figure 7: SEM picture of strawberry leaf

Integrated control of Strawberry powdery mildew

Xiaolei Jin , Bruce D L Fitt , Avice M Hall, Yongju Huang
School of Life and Medical Sciences, University of Hertfordshire, College Lane, Hatfield, AL10 9AB





Introduction

Strawberry powdery mildew is caused by *Podosphaera aphanis*. The mycelium on plant leaves, stems, shoots, buds, flowers and fruit surfaces causes yield loss. Chasmothecia (sexual stage) are the survival phase of the fungus, each contains one ascus with eight ascospores (Fig.1). Strawberry growers frequently use potassium carbonate in a silicon-based wetter to control the disease immediately prior to harvest when commercial fungicides cannot be used. Strawberry powdery mildew is polycyclic requiring 144 hours for conidial germination to sporulation. These parameters have been used to make a prediction system.

Aim

- To investigate the disease control achieved by the potassium carbonate and silicon-based wetter, used singly and in combination.
- To test the use of the rule based prediction system to control strawberry powdery mildew.
- To monitor the initiation, development and maturation of chasmothecia in the silicon field trial.

Materials and methods

- The silicon trial used a randomised block design with 6 treatments each replicated 3 times : untreated, K50 (potassium carbonate) alone, 0.25% (field rate) silicon wetter, 0.25% (field rate) silicon wetter with K50, 0.5% silicon wetter and 0.5% silicon wetter with K50. The trial was sprayed each week from 19 July to 24 August 2012. 15 leaves (cv Elegance) were randomly sampled from each block weekly in an open field at Maltmas Farm.
- The prediction system trial was laid out in an open field. Three beds were sprayed according to the prediction system and two beds were untreated controls. Ten leaves (cv Elegance) were taken weekly for assessment from each ten metre stretch from 31 July to 18 September 2012.

Results (silicon trial)

- The results, show that 0.5% silicon wetter and K50 together (Fig.2) gave the best control of disease (P<0.05), with 0.25% silicon wetter and K50 together and high rate silicon alone also giving control (P>0.05). The K50 alone and silicon wetter at low rate alone gave little control and were similar to the untreated control(Fig.2).
- The reduction in colonies with 0.5% silicon wetter with K50, and 0.5% silicon wetter alone (P<0.05), also reduces the number of chasmothecia developing per leaf (Fig. 3).
- The silicon wetter increase the leaf thickness, the high rate silicon wetter alone and with K50 gave a greater leaf thickness than other treatments (P<0.05), therefore silicon wetter enhances the plant disease resistance (Fig. 4).

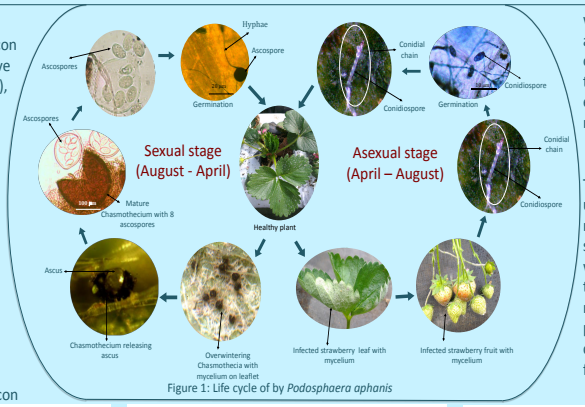


Figure 1: Life cycle of by *Podosphaera aphanis*

Results (prediction trial)

The results show that the unsprayed beds had a greater number of colonies than the sprayed beds (P<0.05) (Fig.5, 6), which indicates that the use of the prediction system during the main growing season enables precision use of fungicides, so control could be achieved with fewer fungicidal sprays.

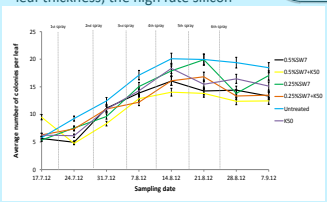


Figure 2: Average number of colonies on leaf from 18 blocks. The AUDPC for 0.5%SW7+k50 is 540, 0.25%SW7+k50 is 607, k50 is 646, 0.5%SW7 is 588, 0.25%SW7 is 665 and untreated is 772.

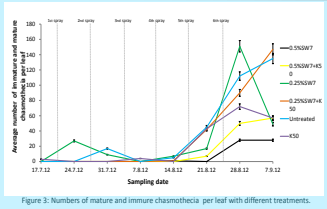


Figure 3: Numbers of mature and immure chasmothecia per leaf with different treatments.

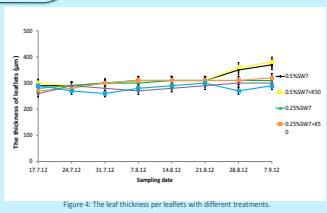


Figure 4: The leaf thickness per leaflets with different treatments.

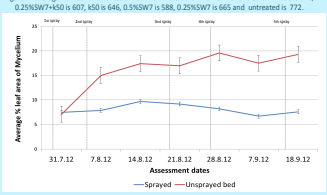


Figure 5: Disease assessment shown by the average % area of leaves covered with colonies (mycelium) on strawberry beds sprayed with fungicide according to the prediction system, and on the untreated control. The AUDPC for the unsprayed is 827.15 and that for the sprayed is 395.70.

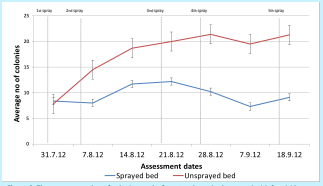


Figure 6: The average number of colonies per leaf on strawberry beds sprayed with fungicide according to the prediction system, and on the untreated control. . The AUDPC for the unsprayed is 903.50 and that for the sprayed is 466.10.

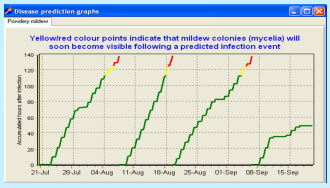


Figure 7: Print out from strawberry powdery mildew prediction system for 2012 at Maltmas farm. The green lines are accumulated hours with both above 20 C and 95% rh. The yellow is the warning period for the grower, having the need to spray, and the need that spores will be germinating and initiating the next round of infection. As soon as the crop is sprayed, the prediction system is reset to zero and the accumulated hours calculation restarts.

Discussion

- This trial was carried out when the disease was well established during a period of high disease pressure. The results indicated that high rate silicon wetter with K50 and high rate silicon wetter control the epidemic build up after 4 weeks. This showed that the efficacy of K50 was improved when used with silicon wetter. The reduction in chasmothecia would reduce the initial inoculum by reducing the number of mature chasmothecia which produce ascospores in the next spring.
- The prediction trial shows the prediction system will control the epidemic build up

Future work

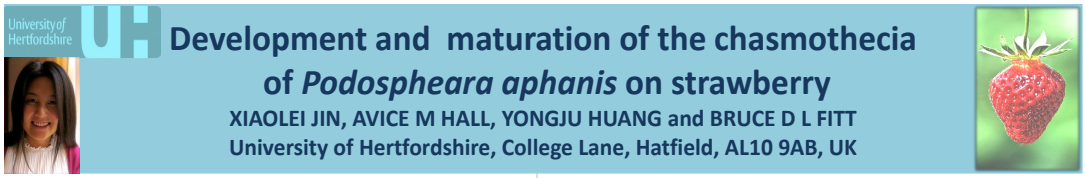
To analyse the data of silicon trial in 2013 which been applied into using the fertigation system roots and on leaf. Analysis leaves for silicon uptake and deposition using bio-imaging and electron microscope.

Acknowledgement

Thanks to Harriet & Henry Duncalfe, Maltmas Farm for providing the field trial. Thanks to Gidon Bahiri for the supply of SW7. Thanks to Andy Barker for spraying the crop. Thanks to Khin Hsu Hsu San for helping the prediction system data. Thanks to Dr. X.Xu, EMR for providing a 'famer friendly' version of the prediction system.

e-mail: x.jin3@herts.ac.uk

December 2013, BSPP presidential meeting. The poster has been awarded the J Colhoun prize (as attached).



Development and maturation of the chasmothecia of *Podospheara aphanis* on strawberry

XIAOLEI JIN, AVICE M HALL, YONGJU HUANG and BRUCE D L FITT
University of Hertfordshire, College Lane, Hatfield, AL10 9AB, UK

Introduction

❖ Strawberry powdery mildew caused by *Podosphaera aphanis*, is a disease of economic significance.

❖The chasmothecia of *Podosphaera aphanis* appear on leaves in late summer and autumn in response to change in environmental conditions (figure 1).

❖ The chasmothecia (figure 1) contain one ascus with eight ascospores.

Aim

❖ To follow the development and maturation of the chasmothecia under different spray regimes.

Materials and methods

❖ The 2011 trial use commercial fungicide in the intercrop period from Aug 2011 to Aug 2012 in two strawberry fields (Ladybird & Diamante).

❖The 2012 silicon trial used a randomised block design with 6 treatments untreated, potassium bicarbonate alone, 0.25% (field rate) silicon nutrient, 0.25% silicon nutrient with potassium bicarbonate , 0.5% silicon nutrient and 0.5% silicon nutrient with potassium bicarbonate .

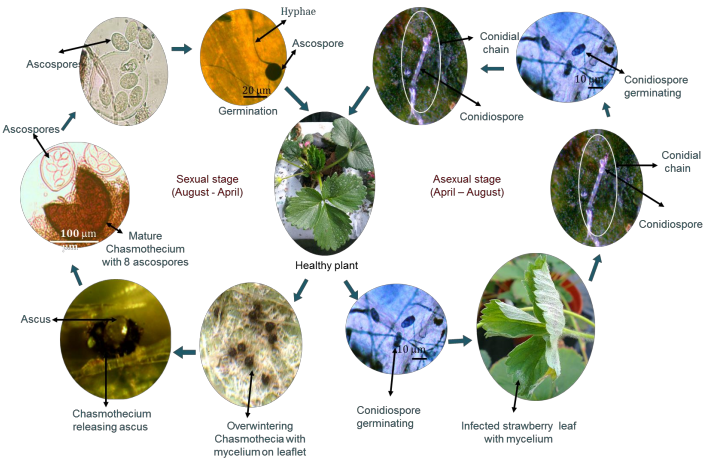


Figure 1: Life cycle of by *Podosphaera aphanis*

Results

❖The results showed that there were greater numbers of mature chasmothecia in Sep 2011 and Mar 2012 in Diamante field (less frequently sprayed) than Ladybird field(frequently sprayed) (Fig. 2).

❖There was a relationship between the amount of mycelium on a leaflet, and the number of chasmothecia (Fig. 3).

❖The 0.5% silicon nutrient with potassium bicarbonate , and 0.5% silicon nutrient alone (P<0.05), reduces the number of chasmothecia per leaf (Fig. 4)

Discussion

❖The 2011 commercial fungicide trial demonstrates that using fungicides in the autumn and spring is effective in reducing chasmothecial maturity thereby reducing the initial inoculum (Fig. 2).

❖The 2012 silicon trial demonstrates reduction in chasmothecia (Fig.4) which reduces the initial inoculum of germinating ascospores in the spring.

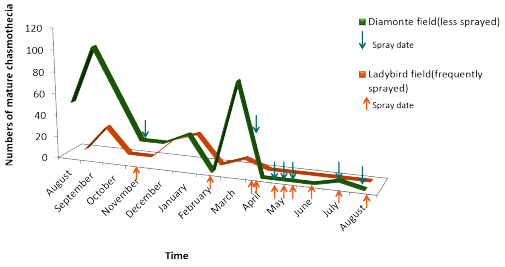


Figure 2: The number of mature chasmothecia each month in Diamante field (less sprayed) and Ladybird field (frequently sprayed) 2011 August to 2012 August:

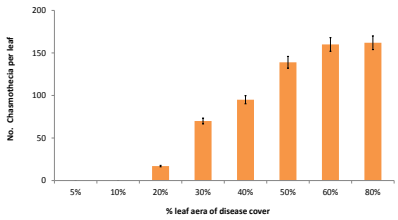


Figure 3 : The number of chasmothecia on 50 leaflets in Dec/2011 in Diamante field

Future work

❖ Continue to sample though out the year to monitor the maturity of chasmothecia and the temperature and humidity in the field (under fleece & mulch). This work could then be used to predict the start of epidemics in the spring.

Acknowledgement

Thanks to Harriet & Henry Duncalfe, Maltmas Farm for providing the field sites. Thanks to Gidon Bahiri for the supply of silicon nutrient. Thanks to Andy Barker for spraying the crop.

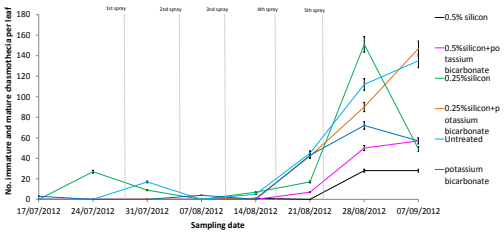


Figure 4: Numbers of mature and immure chasmothecia per leaf with different treatments.


BSPP
British Society for Plant Pathology
Competition Award
This is to certify that
Xiaolei Jin
has been awarded the J Colhoun prize
for best student poster at the
BSPP Presidential Meeting 2013
Michael Shaw
BSPP President

Factors contributing to the holistic control of strawberry powdery mildew



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School of Life and Medical Sciences, University of Hertfordshire, College Lane, Hatfield, AL10 9AB

Introduction

Strawberry powdery mildew is caused by *Podosphaera aphanis*. The mycelium is on the plant leaves, stems, shoots, buds, flowers and fruit surfaces (Fig. 1), which causes yield loss. Strawberry growers frequently use potassium bicarbonate to control the disease with a silicon nutrient in the interval immediately prior to harvest when commercial fungicides cannot be used.

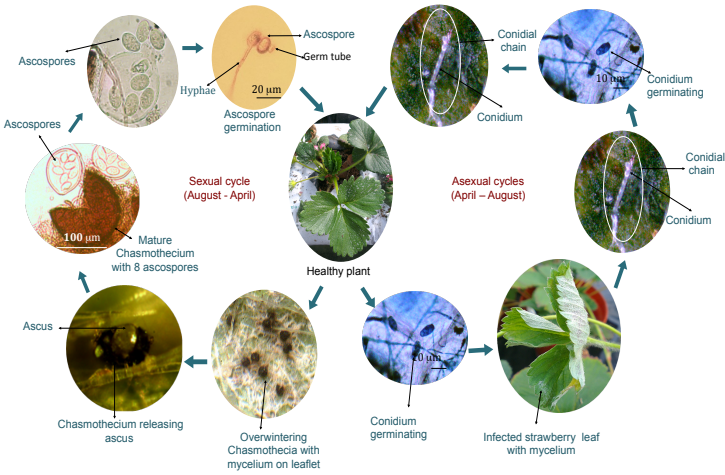

Figure 1: Life cycle of *Podosphaera aphanis* (strawberry powdery mildew). The asexual conidiospores develop and are released from the top of the conidial chain. These germinate and produce the initial infection. Production of conidia is key to creating the secondary cycle of infection and the build up of the epidemic. In the autumn the mycelia begin to produce chasmothecia to survive inclement conditions and begin the cycle again the following spring when disease conducive conditions occur. Chasmothecium contain one ascus and eight ascospores. Germinating ascospores produce germ tubes, which penetrate the epidermis of the leaves where haustoria are formed inside the cells and mycelium starts to grow.

Aim

- To investigate the disease control achieved by the potassium bicarbonate and the silicon nutrient, used singly and in combination. And to monitor the initiation, development and maturation of chasmothecia in the silicon field trial.
- To investigate the disease reduction achieved by applying silicon nutrient into fertigation tubes.
- To investigate the effect on the plant after applying silicon nutrient (leaf thickness, wax and brix).

Materials and methods

- The silicon spray field trial used a randomised block design with 6 treatments: untreated, potassium bicarbonate alone, 0.25% (field rate) Silicon nutrient , 0.25% Silicon nutrient with potassium bicarbonate , 0.5% Silicon nutrient and 0.5% Silicon nutrient with potassium bicarbonate. Leaf sample were assessed weekly.
- The silicon fertigation trial was done in polythene tunnel. The treatments were Untreated, Silicon nutrient alone (at a rate of 0.0017%), Fungicide (commercial) alone and Silicon nutrient plus fungicide. The trial received silicon nutrient twice a week.
- The leaf thickness were measured weekly.
- ° Brix of fruit have been measured.
- SEM images of leaf thickness and wax were taken in the Rothamsted research centre.

Results (silicon spray trial 2012)

- The results, show that 0.5% Silicon nutrient and potassium bicarbonate gave the best control of disease (P<0.05)(Fig.2),and 0.5% Silicon nutrient alone (P<0.05), reduces the number of chasmothecia per leaf (data not shown).
- The Silicon nutrient increases the leaf thickness, the high rate Silicon nutrient alone and with potassium bicarbonate gives the greatest leaf thickness (P<0.05) (Fig.3&4).

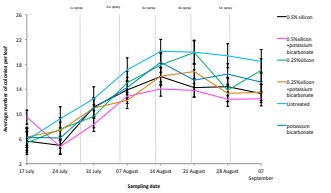

Figure 2: Average number of colonies on leaf on 6 treatment. The AUDPC for 0.5%SW7+potassium bicarbonate is 540, 0.25%SW7+potassium bicarbonate is 607, potassium bicarbonate is 646, 0.5%SW7 is 588, 0.25%SW7 is 665 and untreated is 772.

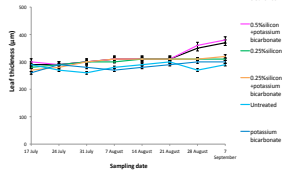

Figure 3: The 2012 field trials from 17/07/12-07/09/12. The leaf thickness per leaflet with different treatments. Si concentrations at high level 0.5%silicon nutrient and 0.5%silicon nutrient with potassium bicarbonate gave a greater leaf thickness than other treatments (P<0.05).

Results (silicon fertigation trial 2013)

- The results of the silicon fertigation trial indicated that silicon nutrient in the fertigation tubes plus commercial fungicide applications gave the greatest disease reduction. The silicon nutrient alone did give some control of *P. aphanis* (P>0.05) (Fig. 5) and it appeared to delay the onset of the epidemic by 12 days.
- The results of ° Brix (the ratio of *Total Soluble Solids* (TSS) to water in solution) of fruit indicated that treatments with silicon in increased the ° Brix in ripe fruits (Fig. 6) .

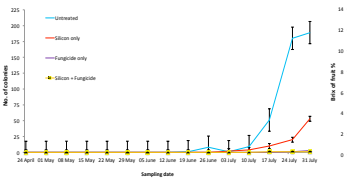

Figure 5: 2013 polythene tunnel fertigation trial. Development of the surface covered by active powdery mildew colonies on the leaves of strawberry plant during 24/04/13-31/07/13. The silicon plus fungicide and fungicide only give significant reduction (P<0.05) compare with silicon only (P>0.05).

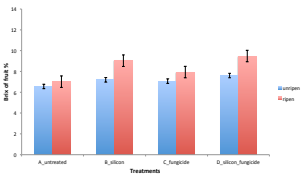

Figure 6: Effects of different treatments on ° Brix of unripen and unripen fruit

Results of SEM images (silicon spray trial 2012)

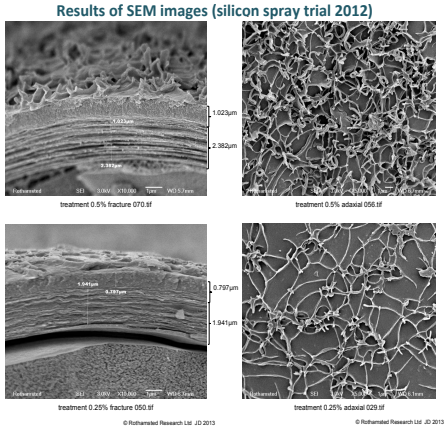

Figure 4: SEM picture of strawberry leaf

Discussion

- The results of the silicon spray trial 2012, showed that the high rate of silicon alone, and the high rate of silicon with potassium bicarbonate reduced the epidemic build up over 2---3 weeks. (The trial also showed that silicon accumulated in the plants over the trial period, though the results are not shown here) the accumulation of silicon in the plant also led to greater leaf thickness and greater density of wax on the leaf surface.
- This showed that the use of the silicon nutrient increased the efficacy of potassium bicarbonate.
- The silicon fertigation trial in 2013 in the polythene tunnel demonstrated that silicon nutrient, used in fertigation pipes as a nutrient feed increases the ° Brix of ripe fruit and gives a delay in epidemic build up of 12 days (Fig.5).

Acknowledgement

Thanks to Harriet & Henry Duncalfe, Maltmas Farm for providing the field trial. Thanks to Gidon Bahiri Orionft for the supply of silicon nutrient. Thanks to Andy Barker for spraying the crop. Thanks to Jean Devonshire for taking the Bio-image. Author e-mail: x.jin3@herts.ac.uk

Effects of fungicide and silicon nutrient on control of strawberry powdery mildew



Xiaolei Jin , Yongju Huang, Bruce D L Fitt, Avice M Hall
School of Life and Medical Sciences, University of Hertfordshire, College Lane, Hatfield, AL10 9AB, UK

Introduction

- Strawberry powdery mildew caused by *Podosphaera aphanis* has the potential to cause > 20% yield loss. The source of disease could come from propagators.
- Chasmothecia are the overwintering sexual structures of *Podosphaera aphanis* that appear on leaves in late summer and autumn (Fig. 1).
- Strawberry growers frequently use a silicon nutrient (Si) to reduce the disease in the period before harvest when fungicides cannot be used.

Aim

- To investigate the source of disease in plants that came from a propagator.
- To study effects of fungicide and silicon nutrient (Si), used singly or in combination, on control of strawberry powdery mildew.

Materials and methods

- Strawberry crowns and runners from a propagator were assessed for powdery mildew in a glasshouse. A Pre-assessment was also done before the Si fertigation experiment.
- Effects of fungicides on development of *P. aphanis* chasmothecia were studied in a field experiment (open field).
- The Si fertigation experiment included four treatments; 1) Untreated, 2) 0.017% Si (v/v), 3) fungicide and 4) 0.017% Si (v/v) plus fungicide (in polythene tunnels). Si was applied through fertigation tubes twice a week.

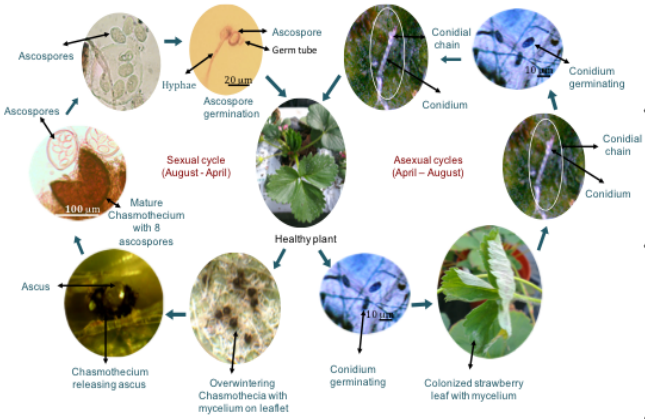

Figure 1: Life cycle of *Podospheara aphanis* (strawberry powdery mildew). Asexual conidiospores released from the conidial chain germinate and produce the initial infection. Production of conidia is key to the secondary cycle of infection and development of epidemic. In autumn the mycelia begin to produce chasmothecia to survive the winter. Chasmothecium contain one ascus and eight ascospores Germinating ascospores produce germ tubes, which penetrate the epidermis of the leaves where haustoria are formed inside the cells.

Effects of fungicide and silicon nutrient on development of strawberry powdery mildew (polythene tunnel)

- The Si fertigation experiment indicated that 0.017% Si (v/v) plus commercial fungicide applications gave the greatest disease control ($P < 0.05$). The 0.017% Si (v/v) alone gave some control of *P. aphanis* ($P > 0.05$) and delayed onset of the epidemic by 12 days (Fig. 3) .
- Si treatment increased leaf thickness (Fig. 4) and wax density (Fig. 5).

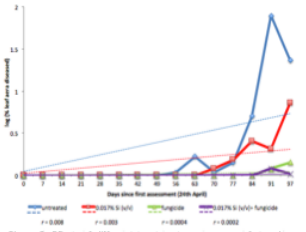

Figure 3: Effects of different treatments on progress of strawberry powdery mildew epidemic in polythene tunnels from April to July 2013 at Maltmas Farm (Wisbech, Cambridgeshire).

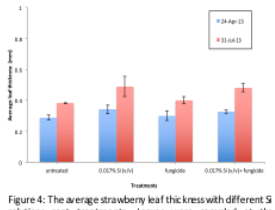

Figure 4: The average strawberry leaf thickness with different Si solutions root treatments. Leaves were sampled at the beginning (pre-assessment) and end of field experiment (after 13 root applications). Bars represent standard errors of mean, degree of freedom 4.

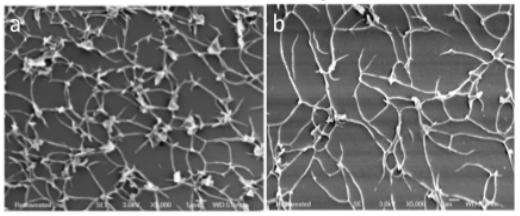

Figure 5: Density of wax on adaxial surface of strawberry leaf; a) 0.017% Si (v/v) alone and b) untreated.

0.017% Si adaxial surface (at x 5,000)

Untreated adaxial surface (at x 5,000)

Results

Development of powdery mildew on strawberry plants from the propagator

- The glasshouse test showed that 14% of strawberry crowns were infected by *Podosphaera aphanis* in July 2013. The runner plants were replanted in March 2014. In April 2014, 13% of runner plants had symptoms of powdery mildew.
- In pre-assessment of plants for the Si fertigation experiment, 4% of the strawberry plants had symptoms of powdery mildew, indicating that strawberry plants delivered by the propagator were not disease-free when they were planted.

Effects of fungicide on development of chasmothecia (field experiment)

Fungicide treatment significantly decreased number of chasmothecia (mature plus immature) ($P < 0.001$) in the open field from September 2012 to April 2013 (Fig. 2).

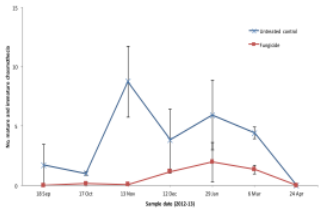

Figure 2: The mean number of chasmothecia (mature plus immature) on 50 strawberry leaves sampled from untreated and fungicide treated beds in open field ($P < 0.001$) from September 2012 to April 2013 at Maltmas Farm (Wisbech, Cambridgeshire). Bars represent standard errors of mean, degree of freedom 2.

Discussion

- This study demonstrated that *Podospheara aphanis* is present in strawberries when they come from the propagator. Chasmothecia are generally present in overwintering crops. In both cases, the use of fungicides can reduce the initial inoculum from these sources (Fig 2).
- The Si fertigation experiment demonstrated that silicon nutrient enhanced plant defence by increasing leaf thickness (Fig. 4) and wax density (Fig. 5) and delaying onset of the epidemic by 12 days (Fig. 3) .

Acknowledgement

Thanks to Harriet & Henry Durcalfe, Maltmas Farm for providing the field trial. Thanks to Gidon Bahiri Orionft for the supply of silicon nutrient. Thanks to Jean Devonshire for taking the Bio-image.

Author e-mail: x.jin3@herts.ac.uk

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Crop Protection in Southern Britain

Development and maturation of the chasmothecia of *Podospheara aphanis* on strawberry

By XIAOLEI JIN, AVICE M HALL, YONGJU HUANG and BRUCE D L FITT

School of Life and Medical Sciences, University of Hertfordshire, College Lane, Hatfield AL10 9AB, UK
Corresponding Author Email: x.jin3@herts.ac.uk

Summary

Strawberry powdery mildew (causal agent *Podospheara aphanis*) can cause serious epidemics on strawberries grown in polythene tunnels in the UK. This study shows a greater number of the chasmothecia in October 2011 and March 2012 in the field that was less frequently sprayed with fungicides. The field sprayed more frequently with fungicides had a smaller total number of chasmothecia and fewer mature chasmothecia. There was a significant difference ($P < 0.05$) between the two fields in March. In December, January and March, the chasmothecia still appeared on leaves in both fields, which shows that the chasmothecia had overwintered. In addition, work followed the changes during maturation and the time required for maturation at a constant temperature and humidity. During maturation the chasmothecia changed from white to black as the ascus developed. This process took 10–11 days in a damp chamber at a constant temperature and humidity.

Key words: Strawberry powdery mildew, *Podospheara aphanis*, chasmothecia

Introduction

Strawberry powdery mildew is caused by *Podosphaera aphanis*. The disease affects leaves, flowers and fruits, thus reducing strawberry yield, both in quantity and quality. The strawberry plants are in the ground for three winters and four harvests. The survival structures of strawberry powdery mildew are chasmothecia, which overwinter. The mature chasmothecia contain one ascus with eight ascospores inside (Fig. 1) (Glawe, 2008). The chasmothecia of *Podosphaera aphanis* appear in late summer and autumn in response to changes in environmental conditions (Dodgson *et al*., 2008). Work reported here followed the development of chasmothecia from August 2011 to August 2012 and quantified the number and maturity of the chasmothecia. Comparison was made between two fields (Ladybird field & Diamante field) receiving routine control measures: one (Ladybird field) more frequently sprayed with fungicide (under polythene tunnel) and the other (Diamante field) less frequently sprayed with fungicide (open field). The aim of this research was to follow the development and maturation of the chasmothecia throughout the year under normal farm conditions and to investigate the use of routine fungicide sprays during the winter to control powdery mildew.

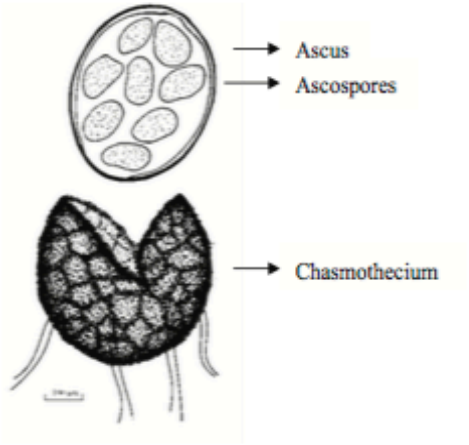

Fig. 1. One mature chasmothecium of *Podosphaera aphanis* with one ascus and eight ascospores inside.

Materials and Methods

Fieldwork methods

Strawberry leaves were collected from two fields, one (Ladybird field) more frequently sprayed with fungicide (under polythene tunnel) and the other (Diamante field) less frequently sprayed with fungicide (open field) at Maltmas Farm near Wisbech. The Ladybird field was covered with fleece in October and November 2011 and again covered with fleece and mulch in March and April 2012. The tunnels were covered with polythene from March 2012 until after harvest (June–August) to enable early harvest. The Diamante field was not covered with polythene, fleece or mulch. Ten leaves (variety Elegance) were randomly sampled from each 30 m length of a 150 m strawberry bed (50 leaves per assessment) and taken to the laboratory for assessment of chasmothecia maturation. All samples were placed in the sample bags and brought back to the laboratory and kept in a cold room (4°C) until assessed.

Laboratory work methods

To assess the amount of disease and the number of chasmothecia per leaflet, the leaflets were placed under a stereomicroscope at ×10 or ×30 magnifications to identify leaves with or without chasmothecia. All leaflets on the 50 leaves collected were examined in the laboratory. Leaflets with white immature chasmothecia were placed in a damp chamber at 20°C to assess their maturation. The leaflets were observed every 2 days to assess the colour change and maturity.

The chasmothecia were selected from 50 leaflets. Five chasmothecia were selected from each leaflet with chasmothecia, then placed on a slide with one drop of fungal mounting fluid (lactic acid and glycerol) and covered by a cover slip. Five chasmothecia per leaflet were observed under ×100 or ×400 magnifications for maturity, number of ascospores in the ascus and colour. The chasmothecia were measured for length and width using an eyepiece graticule at ×100 magnification.

To observe germinating ascospores, leaflets were cleared in 80% denatured alcohol for 48 hours. Then leaflets were placed in Petri dishes and submerged in 0.1% trypan blue. Leaflets were left to stain for 24 h in the laboratory (20°C), then washed with water and cut into two or four strips. The strips were placed on slides, lower surface up. The assessments were done using the microscope at ×100 or ×400 magnification (Nikon, model YS100).

Results

The results showed that there were greater numbers of chasmothecia and more mature chasmothecia in October 2011 and March 2012 in Diamante field (Table 1) than in Ladybird field (Table 2).

Table 1. *The average number of chasmothecia (immature and mature) and numbers of mature chasmothecia per 50 leaflets each month in Diamante field August 2011 to August 2012*

Sample date	No of chasmothecia	No of mature chasmothecia	Fungicide spray date
August	98	51	No spray
September	104	104	No spray
October	197	63	No spray
November	55	22	8 Nov 2011
December	158	22	14 Dec 2011
January	137	32	No spray
February	No sample	No sample	No spray
March	214	84	No spray
April	1	0	10 Apr 2012
May	0	0	3, 5, 11 May 2012
June	0	0	11 June 2012
July	7	5	10 July 2012
August	11	1	2, 8, 17 August 2012

Table 2. *The average number of chasmothecia (immature and mature) and numbers of mature chasmothecia per 50 leaflets each month in Ladybird field August2011 to August2012*

Sample date	No of chasmothecia	No of mature chasmothecia	Fungicide spray date
August	No sample	No sample	No spray
September	99	24	No spray
October	No sample	No sample	No spray
November	No sample	No sample	8 Nov 2011
December	80	19	14 Dec 2011
January	102	26	No spray
February	No sample	No sample	18 Feb 2012
March	45	8	No spray
April	0	0	10, 30 Apr 2012
May	0	0	8, 11, 28 May 2012
June	0	0	5 June 2012
July	1	0	10 July 2012
August	11	0	2, 8, 17 August 2012

The chasmothecia were colourless when they first appeared on the leaves but were placed in the damp chamber after they turned white. The white immature chasmothecia were mature after 10/11 days in a damp chamber. The chasmothecia were colourless when they first appeared on leaflets, and then became yellowish or orange, and finally brown or dark brown to black when

mature. The areas of the chasmothecia were calculated by equation $A = \pi r^2$. Therefore the areas of chasmothecia were from 11.3 mm^2 to 2.8 mm^2. The ascospores started to germinate in March (Table 3). Table 4 shows that there was a relationship between the amount of mycelium on a leaflet, and the number of chasmothecia.

Table 3. *The numbers of ascospores germinating on 10 leaflets from March 2012 to June 2012*

Time	Diamante field	Ladybird field
March	0	1
April	3	2
May	179	120
June	21	18

Table 4. *The number of chasmothecia (immature and mature) on 50 leaflets in December 2011 (Percentage leaf area covered with mycelium and red blotches) in Diamante field*

% leaf area with mycelium	No of chasmothecia
5	0
10	0
20	17
30	70
40	95
50	139
60	160
80	162

Discussion

Strawberry powdery mildew is a major disease, which is caused by *P. aphanis* (Dodgson *et al*., 2008). The fungus mycelium is on the leaf surface, therefore all or most of the mycelium is exposed. Plant leaves, stems, and shoots, buds, flowers and fruit surfaces can all be infected. A large number of conidiospores are produced from the conidia and these conidia are the asexual fruiting body which forms visible white powder (Glawe, 2008). When conditions are unsuitable for mycelia growth, the chasmothecia as sexual overwintering bodies can provide a route for inoculum to survive across strawberry production seasons (Gourley, 1979). The chasmothecia form visible round black cases on the leaf (Putnicki & Glawe, 2009).

Table 1 show that the Diamante field produced both high numbers of chasmothecia and high numbers of mature chasmothecia. Table 2 shows that the Ladybird field had a lower number of chasmothecia and also fewer mature chasmothecia. This demonstrates that using fungicides in the autumn and spring is effective in reducing chasmothecial numbers and maturity thereby reducing the initial inoculum. Table 3 shows that the epidemic starts from germinating ascospores and that the numbers of germinating ascospores are reduced by using fungicides. Hoffmann *et al*. (2012) indicated that applications of fungicides from the start of the growing season to the beginning of the fruit ripening period did not only affect the disease level on the clusters but also determined the incidence of the autumn leaf infection. Furthermore the epidemic could have been expected to develop faster under the polythene tunnels, as the environmental conditions in the tunnel were more conducive to epidemic build up (Dodgson *et al*., 2008). Table 4 demonstrates that the more mycelium on a leaf, the greater the number of chasmothecia. Hoffmann *et al*. (2012) also stated that

the level of leaf infection altered the number of chasmothecia formed on the leaves. Fig. 1 shows the appearance of mature chasmothecia which contain one ascus with eight ascospores inside. The length of time to development is 10/11days in a damp chamber at a constant temperature and humidity. Smith *et al.* (1988) stated that the deeper the colour of the chasmothecia, the greater the level of maturation. However this study suggests that orange, red and dark red chasmothecia can also be mature (under a Nikon, model YS100 at ×100 or ×400 magnification). The study also shows the large areas of chasmothecia are 11.304 mm^2, the smallest are 2.826 mm^2. Thus, this study suggests the larger the chasmothecia, the greater the level of maturation. To sum up, this work suggests that spraying out of season could control the disease spread. This should encourage famers to spray out of season.

Acknowledgments

I would like to thank Harriet and Henry Duncalfe, Maltmas Farm for providing the field trial.

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Aspects of Applied Biology **119**, *2013*
Fruits and Roots: A Celebration and Forward Look

The role of chasmothecia in the initiation of epidemics of powdery mildew (*Podospheara aphanis*) and the role of silicon in controlling the epidemics on strawberry

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Summary

Strawberries are grown worldwide and in many countries they are grown as a perennial crop. Strawberry powdery mildew, caused by *Podospheara aphanis*, has the potential to cause over 20% yield loss, particularly where strawberries are grown under cover. This work shows that to achieve disease control with reduced use of conventional fungicides, disease control needs to be holistic, involving fungicide sprays in the intercrop period to reduce the number of mature chasmothecia, which reduces the initial ascospore inoculum in the spring. However, the use of silicon, either as a spray or as a nutrient applied through the roots produced physiological changes in the plant including increased leaf thickness, which increases strawberry resistance against *P. aphanis*. The integrated use of all these control strategies suppresses disease development so that control is achieved with less use of conventional fungicides.

Key words: Strawberry powdery mildew, *Podospheara aphanis*, chasmothecia, silicon

Introduction

Strawberry powdery mildew is caused by *Podosphaera aphanis* (Braun, 1982). The disease affects leaves, flowers and fruits, thus reducing strawberry yield, both in quantity and quality. The strawberry plants are usually in the ground for three winters and four harvests, either in an open field or under a polythene tunnel. Some varieties are grown as annuals in UK. When the environmental conditions are conducive to disease development, a large number of asexual conidia (Fig. 1) are produced (Glawe, 2008). The survival structures of strawberry powdery mildew are chasmothecia, which overwinter on strawberry plants. A mature chasmothecium contains one ascus with eight ascospores (Fig. 1). The chasmothcia are formed in August and September as the day-length and temperature decrease.

Strawberry growers frequently use sprays of potassium carbonate (K50) in a silicon-based wetter (Sirius) in the period immediately before harvest for controlling disease development. Anecdotal observations suggest that the silicon wetter and the K50 act synergistically to reduce disease development. Recent literature (Fatema *et al*., 2012) also suggests that silicon is accumulated in plants and has a role in reducing disease development. Earlier work by Fatema & Hall (2011) showed that a silicon wetter as a foliar spray increased leaf hair length and density and reduced

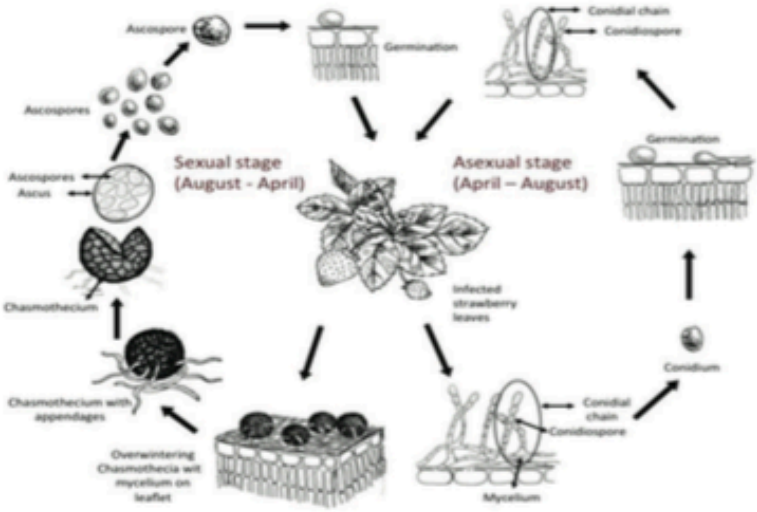

Fig. 1. Life cycle of *Podospheara aphanis* on strawberry. Source from: Xiaolei Jin (author).

P. aphanis ascospore germination early in the season. The 2012 field trial reported here aimed to investigate disease control by using a foliar spray of potassium carbonate plus silicon-based wetter, either applied singly or in combination. The 2013 trial tested the hypothesis that the silicon wetter, used as a nutrient, taken up through the roots from the fertigation tubes, would delay the onset of the epidemic of strawberry powdery mildew.

Materials and Methods

The 2012 trial was in an open field (unprotected) using strawberry cultivar Elegance. The trial used a randomised block design with six spray treatments; untreated, K50 (potassium carbonate) alone, 0.25% (field rate) silicon wetter (Sirius) alone, 0.25% silicon wetter with K50, 0.5% silicon wetter alone and 0.5% silicon wetter with K50, each replicated three times. The trial received spray treatments every week from 19 July to 24 August 2012. The size of each plot was 3 m × 1 m, and 1 m guard row in between. Each week, 15 leaves were randomly sampled from each plot and assessed for disease development. The 2013 trial was done in commercially operated polythene tunnels using 10 beds in each of two tunnels with four treatments; untreated, silicon wetter alone, fungicide alone and silicon wetter plus fungicide. The silicon wetter was applied as a nutrient (15 mL, i.e. a rate of 0.0017%) and added to the fertigation system twice a week .The strawberry cultivar 'Katherine' was planted as an annual crop and 10 old leaves and 10 fully expanded new leaves were randomly sampled from two metre lengths of each plots each week for 13 weeks. All samples were assessed for amount of disease per leaf.

In the 2012 trial, each leaf was placed under a stereomicroscope at ×10 or ×30 magnification to count the number of strawberry mildew colonies per leaf and to identify leaves with or without chasmothecia. Five chasmothecia were selected from each of infected leaflets, then placed on a slide with one drop of fungal mounting fluid (lactic acid plus glycerol) covered by a cover slip, and observed under ×100 or ×400 magnification for maturity, colour and number of ascospores in the ascus. The chasmothecia were measured for length and width using an eyepiece graticule at ×100 or ×400 magnification. Ten leaflets were selected from each treatment for transverse section, and were measured for width using an eyepiece graticule at ×100 magnification. In the 2013 trial, leaves were taken back to the laboratory for disease assessment (colony number).

Results

The results of the 2012 field trial, show that 0.5% silicon wetter and K50 together (Fig. 2) gave the best control of disease (P<0.05), with 0.25% silicon wetter and K50 together and high rate silicon alone also giving control (P>0.05). The K50 alone and silicon wetter at low rate alone give little control of the disease (Fig. 2). The reduction in colonies with 0.5% silicon wetter with K50, and 0.5% silicon wetter alone (P<0.05), also reduces the number of chasmothecia developing per leaf (Fig. 3). The silicon wetter increased the leaf thickness; the high rate silicon wetter alone and with K50 gave a greater leaf thickness than other treatments (P<0.05), (Fig.4). Where colony numbers were reduced by the high rate of silicon; this led to a reduction in the numbers of chasmothecia formed (Fig. 3).

The results of the 2013 fertigation trial indicted that silicon in the fertigation plus on farm fungicide applications gave the greatest disease reduction. The silicon alone did give a little control of *P. aphanis* compared with untreated (P>0.05)(Fig. 5). The untreated control had the greatest number of colonies per leaf when compared to the silicon plus fungicide and fungicide alone (P<0.05). The silicon in the fertigation tubes appeared to delay the onset of the epidemic by 9/10 days.

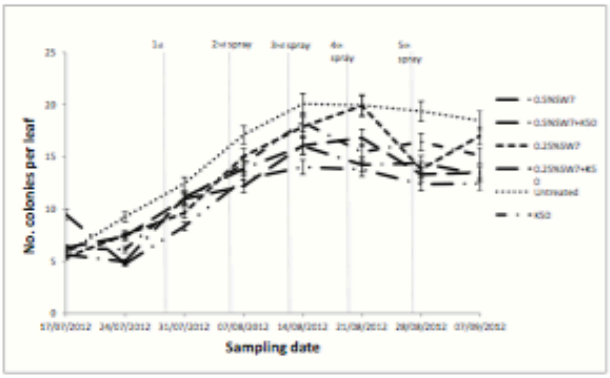

Fig. 2. 2012 spray field trial. Development of the surface covered by active powdery mildew colonies on the leaves of strawberry plant during 17 July 12–07 September 12. Si concentrations at high level 0.5%SW7 and 0.5%SW7 with K50 give significant control (P<0.05) compare with other treatments.

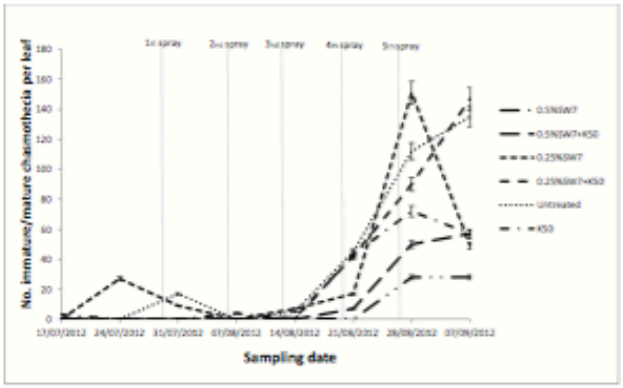

Fig. 3. 2012 field trial from 17 July 12–07 September 12. Numbers of mature and immure chasmothecia per leaf with different treatments. Si concentrations at high level 0.5%SW7 and 0.5%SW7 with K50 give significant control (P<0.05) compare with other treatments.

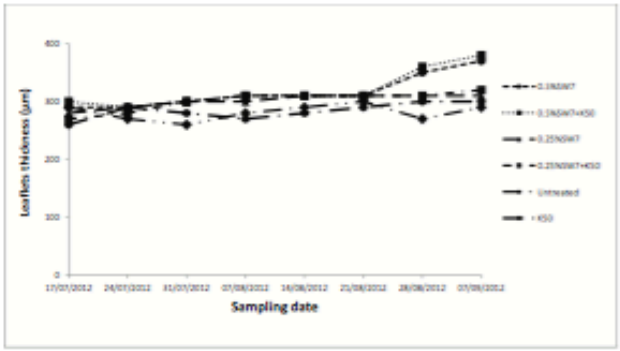

Fig. 4. 2012 field trials from 17 July 12–07 September 12. The leaf thickness per leaflet with different treatments. Si concentrations at high level 0.5%SW7 and 0.5%SW7 with K50 gave a greater leaf thickness than other treatments (P<0.05).

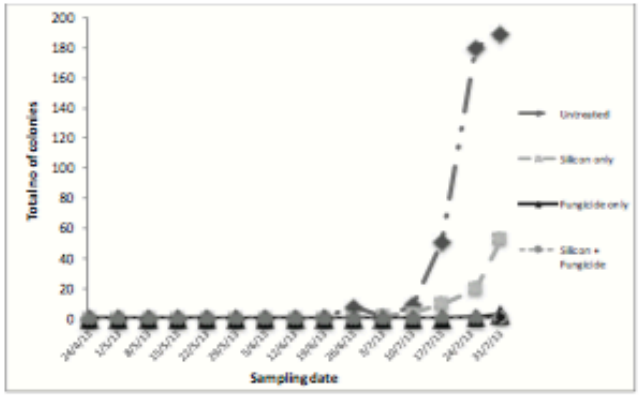

Fig. 5. 2013 polythene tunnel fertigation trial. Development of the surface covered by active powdery mildew colonies on the leaves of strawberry plant during 24 April 13–31 July 13. The silicon plus fungicide and fungicide only give significant control (P<0.05) compare with silicon only (P>0.05).

Discussion

The 2012 silicon spray trial was carried out when *P. aphanis* was well established during a period of high disease pressure. The results indicated that high rate silicon wetter with K50 and high rate silicon wetter alone controlled the epidemic build-up of *P. aphanis* after 4 weeks. This showed that the efficacy of K50 was significantly improved when used with silicon wetter. The subsequent reduction in formation of chasmothecia in those plots would reduce the overwintering inoculum by reducing the number of mature chasmothecia, which produce ascospores in the next spring. The increased leaf thickness may be a contributing factor to the disease reduction observed with the higher levels of silicon application (and silicon accumulation not reported here). Thus the 2012 trial demonstrates that the silicon wetter does have effect improving the performance of the K50 and that the disease control has an ongoing effect by reducing chasmothecial production thus reducing initial inoculum for the following season (Hoffman *et al.*, 2012). The 2013 silicon trial in the polythene tunnel demonstrates that silicon wetter, used in fertigation as a nutrient feed enhances the plant defence mechanisms and gives a delay in epidemic build up by just over a week.

Acknowledgments

The authors thank Harriet & Henry Duncalfe, Maltmas Farm for providing the 2012 field trial and 2013 fertigation trial. We thank Gidon Bahiri for the supply of the silicon wetter and to Andy Barker for spraying the 2012 trial.

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Aspects of Applied Biology **127**, *2014*
Crop Production in Southern Britain: Precision Decisions for Profitable Cropping

Factors contributing to the holistic control of strawberry powdery mildew

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Summary

A holistic approach to the control of strawberry powdery mildew (*Podospheara aphanis*) is important, as the disease is never absent from the crop. Work reported here shows that the disease is present in the crop when new plants are bought in from a propagator and when older plants have been in the growing medium for one or more growing seasons. Control measures used in one growing season reduced the disease carry-over, thus reducing the initial inoculum in the following season. The use of a late autumn fungicide spray or a fungicide spray before fleece use in spring reduced the number and maturity of overwintering chasmothecia, thus also contributing to a reduction in initial inoculum. The role of a silicon nutrient in suppressing powdery mildew development is also reported.

Key words: Strawberry powdery mildew, *Podospheara aphanis*, chasmothecia, fungicide spray

Introduction

Strawberry powdery mildew is caused by *Podosphaera aphanis* (Braun, 1982). The disease affects leaves, flowers and fruits, thus reducing strawberry yield, both in quantity and quality. Strawberry plants are usually in the ground for three winters and four harvests, either in an open field or under a polyethylene tunnel. Some varieties are grown as annuals in UK. When environmental conditions are conducive to disease development, a large number of asexual conidia (Fig. 1) are produced (Glawe, 2008). The strawberry powdery mildew survival structures are chasmothecia, which overwinter on strawberry plants. A mature chasmothecium contains one ascus with eight ascospores (Fig. 1). In the UK, the chasmothcia are formed in August and September as the day-length and temperature decrease. Work reported here followed the development of chasmothecia from April 2011 to March 2012 and quantified the number and maturity of the chasmothecia. Comparison was made between two fields (Ladybird field & Diamante field) receiving routine control measures: one (Ladybird field) received 10 sprays with fungicides (under polyethylene tunnel) and the other (Diamante field) received seven sprays with fungicides (open field). The aims of this research were to record the amount of disease occurring on plants brought into the farm for planting and to follow the development and maturation of the chasmothecia throughout the year under normal farm conditions and to investigate the impact of routine fungicide sprays during the winter to control powdery mildew. In addition the effect of using a silicon nutrient was assessed.

Strawberry growers frequently use sprays of potassium bicarbonate in a silicon nutrient in the period immediately before harvest for controlling development of some diseases. Anecdotal

observations suggest that silicon nutrient and potassium bicarbonate act synergistically to reduce disease development. Recent literature (Fatema *et al.*, 2012) also suggests that silicon is accumulated in plants and has a role in reducing disease development. Earlier work by Fatema & Hall (2011) showed that a silicon nutrient reduced *P. aphanis* ascospore germination early in the season. This paper reports a 2013 trial testing the hypothesis that use of silicon nutrient, taken up through the roots from the fertigation tube, delayed the onset of the epidemic of strawberry powdery mildew.

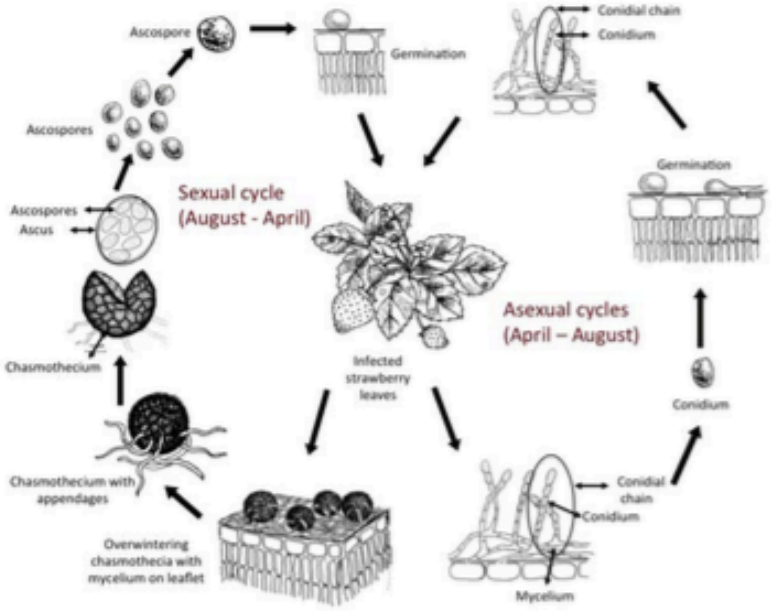

Fig. 1. Life cycle of *Podospheara aphanis* (strawberry powdery mildew). The asexual conidiospores develop and are released from the top of the conidial chain. These germinate and produce the initial infection. Production of conidia is key to creating the secondary cycle of infection and the build up of the epidemic. In the autumn the mycelia begin to produce chasmothecia to survive inclement conditions and begin the cycle again the following spring when disease conducive conditions occur. Chasmothecium contain one ascus and eight ascospores. Germinating ascospores produce germ tubes, which penetrate the epidermis of the leaves where haustoria are formed inside the cells and mycelium starts to grow.
Source from: Xiaolei Jin (author).

Materials and Methods

Development of powdery mildew on strawberry plants from the propagator

The 2013 glasshouse trial was done in the University of Hertfordshire (UH) glasshouse. The strawberry crowns (cv. Elegance) were bought from a propagator at Maltmas Farm and were planted in the glasshouse in February 2013. These plants were monitored weekly for 4 months for powdery mildew development. The runners were potted on and disconnected from the parent plants. All plants were observed for powdery mildew weekly. The plants for the fertigation trial were assessed for strawberry powdery mildew on arrival at the growers.

Effects of different fertigation treatments on development of strawberry powdery mildew, 2013

The 2013 fertigation trial was done in commercially operated polyethylene tunnels using 10 beds in each of two tunnels with four treatments; untreated, silicon nutrient alone, fungicide alone and

silicon nutrient plus fungicide. The silicon nutrient was applied as a nutrient (15 mL per 200 L, i.e. a rate of 0.0017%) and added to the fertigation system twice a week for 4 months. The strawberry cultivar UH5 planted as an annual crop and 10 old leaves and 10 fully expanded new leaves were randomly sampled from 2m lengths of each plot each week for 13 weeks. All samples were assessed for amount of disease per leaf; a pre-assessment was done before the first application of the silicon nutrient.

Effects of different fungicide treatments on development of Podospheara aphanis chasmothecia

In the 2011–2012 field trial, strawberry leaves were collected from two fields; Ladybird field under polyethylene tunnel received more than 10 sprays with fungicides and Diamante field open field received seven sprays with fungicides at Maltmas Farm near Wisbech in Cambridgeshire. Ten leaves (cv. Elegance) were randomly sampled from each 30 m length of a 150m strawberry bed (50 leaves per assessment) and taken to the laboratory for assessment of chasmothecia maturation. All samples were placed in sample bags and kept in a cold room (4°C) until they were assessed. Five chasmothecia were selected from each leaflet with chasmothecia, then placed on a slide with one drop of fungal mounting fluid (lactic acid plus glycerol) and covered by a cover slip. Five chasmothecia per leaflet were observed under ×100 or ×400 magnification for maturity, number of ascospores in the ascus and colour.

Results

Development of powdery mildew on strawberry plants from the propagator

The results of the 2013 UH glasshouse trial shows that strawberry mother plants had been infected 14% by *Podospheara aphanis* naturally in July 2013. The runner plants had been replanted in March 2014. A month later, 13% of runner plants showed symptoms of powdery mildew. The pre-assessment of plants for the 2013 fertigation trial carried out in March 2013, showed that 4% of the strawberry plants showed symptoms of powdery mildew, which indicated that those strawberry crowns delivered by the propagator were not disease-free.

Effects of different fertigation treatments on development of strawberry powdery mildew, 2013

The results of the 2013 fertigation trial indicated that the untreated control showed the epidemic build up from 24 April to 31 July 2013 (Fig. 2). The silicon nutrient alone gave some suppression of *P. aphanis* (P>0.05) (Fig. 2) and it appeared to delay the onset of the epidemic by 9 to10 days when compared to the untreated plots. The silicon nutrient and commercial fungicide applications together gave the good control of the epidemic. There is an indication that, at the end of the trial the silicon and fungicide treatment gave slightly better control than the fungicide alone.

Effects of different fungicide treatments on development of Podospheara aphanis chasmothecia

The results showed that there were greater numbers of mature chasmothecia in September 2011 and March 2012 in Diamante field, which had received seven fungicide sprays, compared with Ladybird field, which had received 10 fungicide sprays (Fig. 3). This result indicated that fewer chasmothecia developed when there was more frequent spraying. This is linked to the results shown in Fig. 4, which shows that smaller colonies of *P. aphanis* result in fewer chasmothcia being formed. Other work (not shown) also indicates that a late autumn fungicide spray results in fewer chasmothecia being formed, thus also contributing to a reduction in initial inoculum the following spring. There was a good correlation between the amount of mycelium on a leaflet and the number of chasmothecia (Fig. 4).

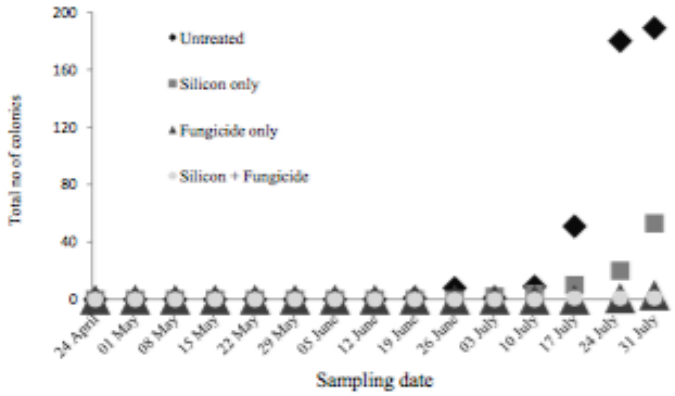

Fig. 2. Graph of epidemic buildup on the four treatments showing number of powdery mildew colonies on the leaves of strawberry plant during the period 24 April to 31 July in the 2013 polyethylene tunnel silicon nutrient fertigation trial.

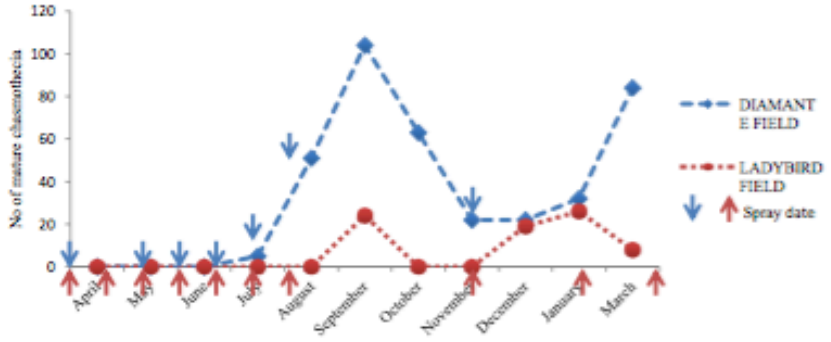

Fig. 3. The number of mature *P.aphanis* chasmothecia on 50 strawberry leaves sampled each month from Diamante field open field (seven sprays with fungicide) and Ladybird field polyethylene tunnel (10 sprays with fungicide) from April 2011 to August 2012 at Maltmas Farm near Wisbech in Cambridgeshire.

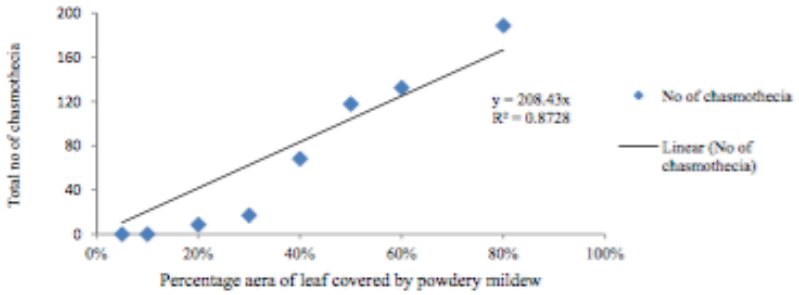

Fig. 4. The number of chasmothecia (immature and mature) on 50 leaves in Sep 2011 correlated with colony size. Percentage area covered with mycelium in Diamante field. $R^2 = 0.872$ which indicated that there was a good correlation between the amount of mycelium on a leaflet and the number of chasmothecia.

Discussion

This study demonstrated that *Podospheara aphanis* is present in a crop when they come from the propagator, which shows there are no disease-free. Chasmothecia are generally present in overwintering crops. In both cases the use of fungicides can reduce the initial inoculum from these sources (Fig 3). The 2013 silicon trial in the tunnel demonstrates that silicon nutrient, as a nutrient feed in fertigation appears enhance the plant defence mechanisms and reduces the build up of the epidemic of powdery mildew. The silicon nutrient alone gave some disease reduction when compared with the untreated control (Fig. 2). By the end of the trial the silicon and fungicide treatment gave slightly better control than the fungicide alone but the effect is small.

Fig. 4 shows that chasmothecia production is linked to colony size and so suggests that any control method, which reduces colony size, will have a knock on effect on disease carryover. This demonstrates that using fungicides in the autumn and spring is effective in reducing chasmothecia numbers and maturity thereby reducing the initial inoculum. Hoffmann *et al.* (2012) indicated that applications of fungicides from the start of the growing season to the beginning of the fruit ripening period did not only affect the disease level but also determined the incidence of the autumn leaf infection. Fig. 4 demonstrates that the more mycelium on a leaf, the greater the number of chasmothiecia. Hoffmann *et al.* (2012) also stated that the level of leaf disease affected the number of chasmothecia formed on the leaves. Hence, this work suggests that spraying in autumn could decrease the spread of the disease. The use of the silicon nutrient is also valuable in reducing epidemic build-up.

Acknowledgments

The authors thank Harriet & Henry Duncalfe, Maltmas Farm for providing the field trials. We thank Gidon Bahiri (OrionFT) for the supply of the silicon nutrient.

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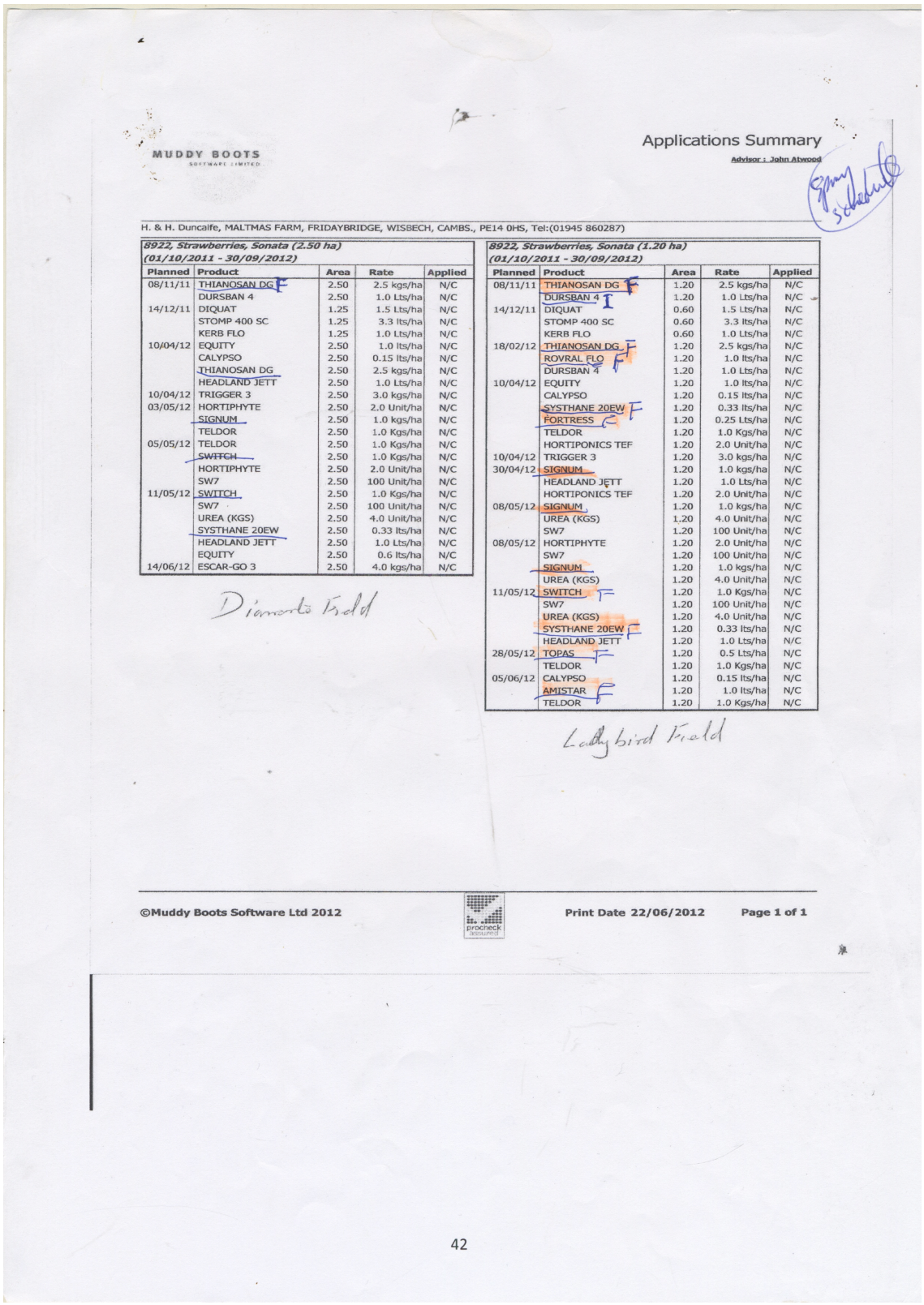
Appendix 15: Maltmas farm fungicides schedule for the Diamante, Ladybird, Amelia, Roadside and Barn fields from 2011-2014



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Applications Summary

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H. & H. Duncalfe, MALTMAS FARM, FRIDAYBRIDGE, WISBECH, CAMBS., PE14 0HS, Tel:(01945 860287)

8922, Strawberries, Sonata (2.50 ha)
(01/10/2011 - 30/09/2012)

Planned	Product	Area	Rate	Applied
08/11/11	THIANOSAN DG	2.50	2.5 kgs/ha	N/C
	DURSBAN 4	2.50	1.0 Lts/ha	N/C
14/12/11	DIQUAT	1.25	1.5 Lts/ha	N/C
	STOMP 400 SC	1.25	3.3 lts/ha	N/C
	KERB FLO	1.25	1.0 Lts/ha	N/C
10/04/12	EQUITY	2.50	1.0 lts/ha	N/C
	CALYPSO	2.50	0.15 lts/ha	N/C
	THIANOSAN DG	2.50	2.5 kgs/ha	N/C
	HEADLAND JETT	2.50	1.0 Lts/ha	N/C
10/04/12	TRIGGER 3	2.50	3.0 kgs/ha	N/C
03/05/12	HORTIPHYTE	2.50	2.0 Unit/ha	N/C
	SIGNUM	2.50	1.0 kgs/ha	N/C
	TELDOR	2.50	1.0 Kgs/ha	N/C
05/05/12	TELDOR	2.50	1.0 Kgs/ha	N/C
	SWITCH	2.50	1.0 Kgs/ha	N/C
	HORTIPHYTE	2.50	2.0 Unit/ha	N/C
	SW7	2.50	100 Unit/ha	N/C
11/05/12	SWITCH	2.50	1.0 Kgs/ha	N/C
	SW7	2.50	100 Unit/ha	N/C
	UREA (KGS)	2.50	4.0 Unit/ha	N/C
	SYSTHANE 20EW	2.50	0.33 lts/ha	N/C
	HEADLAND JETT	2.50	1.0 Lts/ha	N/C
	EQUITY	2.50	0.6 lts/ha	N/C
14/06/12	ESCAR-GO 3	2.50	4.0 kgs/ha	N/C

Diamante Field

8922, Strawberries, Sonata (1.20 ha)
(01/10/2011 - 30/09/2012)

Planned	Product	Area	Rate	Applied
08/11/11	THIANOSAN DG	1.20	2.5 kgs/ha	N/C
	DURSBAN 4	1.20	1.0 Lts/ha	N/C
14/12/11	DIQUAT	0.60	1.5 Lts/ha	N/C
	STOMP 400 SC	0.60	3.3 lts/ha	N/C
	KERB FLO	0.60	1.0 Lts/ha	N/C
18/02/12	THIANOSAN DG	1.20	2.5 kgs/ha	N/C
	ROVRAL FLO	1.20	1.0 lts/ha	N/C
	DURSBAN 4	1.20	1.0 Lts/ha	N/C
10/04/12	EQUITY	1.20	1.0 lts/ha	N/C
	CALYPSO	1.20	0.15 lts/ha	N/C
	SYSTHANE 20EW	1.20	0.33 lts/ha	N/C
	FORTRESS	1.20	0.25 Lts/ha	N/C
	TELDOR	1.20	1.0 Kgs/ha	N/C
	HORTIPONICS TEF	1.20	2.0 Unit/ha	N/C
10/04/12	TRIGGER 3	1.20	3.0 kgs/ha	N/C
30/04/12	SIGNUM	1.20	1.0 kgs/ha	N/C
	HEADLAND JETT	1.20	1.0 Lts/ha	N/C
	HORTIPONICS TEF	1.20	2.0 Unit/ha	N/C
08/05/12	SIGNUM	1.20	1.0 kgs/ha	N/C
	UREA (KGS)	1.20	4.0 Unit/ha	N/C
	SW7	1.20	100 Unit/ha	N/C
08/05/12	HORTIPHYTE	1.20	2.0 Unit/ha	N/C
	SW7	1.20	100 Unit/ha	N/C
	SIGNUM	1.20	1.0 kgs/ha	N/C
	UREA (KGS)	1.20	4.0 Unit/ha	N/C
11/05/12	SWITCH	1.20	1.0 Kgs/ha	N/C
	SW7	1.20	100 Unit/ha	N/C
	UREA (KGS)	1.20	4.0 Unit/ha	N/C
	SYSTHANE 20EW	1.20	0.33 lts/ha	N/C
	HEADLAND JETT	1.20	1.0 Lts/ha	N/C
28/05/12	TOPAS	1.20	0.5 Lts/ha	N/C
	TELDOR	1.20	1.0 Kgs/ha	N/C
05/06/12	CALYPSO	1.20	0.15 lts/ha	N/C
	AMISTAR	1.20	1.0 lts/ha	N/C
	TELDOR	1.20	1.0 Kgs/ha	N/C

Ladybird Field

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8922, Strawberries, Sonata (1.20 ha)
(01/10/2011 - 30/09/2012)

Planned	Product	Area	Rate	Applied
10/07/12	SYSTHANE 20EW	1.20	0.33 lts/ha	N/C
	FORTRESS	1.20	0.25 Lts/ha	N/C
02/08/12	HARVEST	0.60	3.0 lts/ha	N/C
08/08/12	ESCAR-GO 3	1.20	4.0 kgs/ha	N/C
17/08/12	FORTRESS	1.20	0.25 Lts/ha	N/C
	SYSTHANE 20EW	1.20	0.33 lts/ha	N/C
	EQUITY insect	1.20	0.6 lts/ha	N/C
11/09/12	CORBEL	1.20	1.0 lts/ha	N/C
	FORTRESS	1.20	0.25 Lts/ha	N/C
18/09/12	FUSILADE MAX	1.20	1.5 lts/ha	N/C
18/09/12	HARVEST	0.60	3.0 lts/ha	N/C
	VENZAR FLOWABLE	0.60	2.0 Lts/ha	N/C

lady bird

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8922, Strawberries, Sonata (2.50 ha)
(01/10/2011 - 30/09/2012)

Planned	Product	Area	Rate	Applied
02/08/12	HARVEST	1.25	3.0 lts/ha	N/C
08/08/12	ESCAR-GO 3	2.50	4.0 kgs/ha	N/C
17/08/12	FORTRESS	2.50	0.25 Lts/ha	N/C
	SYSTHANE 20EW	2.50	0.33 lts/ha	N/C
	EQUITY	2.50	0.6 lts/ha	N/C
11/09/12	CORBEL	2.50	1.0 lts/ha	N/C
	FORTRESS	2.50	0.25 Lts/ha	N/C
18/09/12	HARVEST	1.25	3.0 lts/ha	N/C
	VENZAR FLOWABLE	1.25	2.0 Lts/ha	N/C

- I.R. Herbicide
- Slug Killer

} I R Herbicides

Take fleece off & till 14/06/12

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8922, Strawberries, Fenella (2.50 ha)
(01/10/2012 - 30/09/2013)

Planned	Product	Area	Rate	Applied
08/11/12	DEVRINOL	2.50	5.0 lts/ha	N/C
15/01/13	DIQUAT	2.50	2.0 Lts/ha	N/C
	SHARK	2.50	0.25 lts/ha	N/C
	STOMP 400 SC	2.50	3.3 lts/ha	N/C

I R. Herbicides

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8922, Strawberries, Sonata (2.50 ha)
(01/10/2011 - 30/09/2012) Diamante field

Planned	Product	Area	Rate	Applied
10/07/12	SYSTHANE 20EW	2.50	0.33 lts/ha	N/C
	FORTRESS	2.50	0.25 Lts/ha	N/C
02/08/12	HARVEST	1.25	3.0 lts/ha	N/C
08/08/12	ESCAR-GO 3	2.50	4.0 kgs/ha	N/C
17/08/12	FORTRESS	2.50	0.25 Lts/ha	N/C
	SYSTHANE 20EW	2.50	0.33 lts/ha	N/C
	EQUITY	2.50	0.6 lts/ha	N/C
11/09/12	CORBEL	2.50	1.0 lts/ha	N/C
	FORTRESS	2.50	0.25 Lts/ha	N/C
18/09/12	HARVEST	1.25	3.0 lts/ha	N/C
	VENZAR FLOWABLE	1.25	2.0 Lts/ha	N/C

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Roadside

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6343, Strawberries, Jubilee (0.50 ha)
(01/10/2012 - 30/09/2013)

Ro adside field

Planned	Product	Area	Rate	Applied
15/01/13	SHARK	0.50	0.33 lts/ha	N/C
	DIQUAT	0.50	1.5 Lts/ha	N/C
15/01/13	DIQUAT	0.50	2.0 Lts/ha	N/C
	SHARK	0.50	0.25 lts/ha	N/C
	STOMP 400 SC	0.50	3.3 lts/ha	N/C
09/04/13	THIANOSAN DG	0.50	2.5 kgs/ha	N/C
	ROVRAL WG	0.50	1.0 kgs/ha	N/C
	FORTRESS	0.50	0.25 Lts/ha	N/C
	HEADLAND JETT	0.50	1.0 Lts/ha	N/C
	EQUITY	0.50	1.0 lts/ha	N/C
	FENOMENAL	0.50	4.0 kgs/ha	N/C
19/04/13	UREA (KGS)	0.50	3.0 Units/ha	N/C
	MAXICROP TRIPLE	0.50	2.0 Lts/ha	N/C
	HEADLAND JETT	0.50	1.0 Lts/ha	N/C
25/04/13	HORTIPHYTE	0.50	2.0 Units/ha	N/C
	UREA (KGS)	0.50	3.0 Units/ha	N/C
	ROVRAL WG	0.50	0.75 kgs/ha	N/C
30/04/13	SIGNUM	0.50	1.5 kgs/ha	N/C
	HEADLAND JETT	0.50	1.0 Lts/ha	N/C
16/05/13	APOLLO 50 SC	0.50	0.1 lts/ha	N/C
	DYNAMEC	0.50	250 mls/ha	N/C
	ROVRAL WG	0.50	0.75 kgs/ha	N/C
	SYSTHANE 20EW	0.50	0.33 lts/ha	N/C
	HORTIPHYTE	0.50	2.0 Units/ha	N/C
23/05/13	AMISTAR	0.50	1.0 lts/ha	N/C
	HORTIPHYTE	0.50	2.0 Units/ha	N/C
29/05/13	SIGNUM	0.50	1.5 kgs/ha	N/C
	HORTIPHYTE	0.50	2.0 Units/ha	N/C
06/06/13	TELDOR	0.50	0.75 Kgs/ha	N/C
	CALYPSO	0.50	0.15 lts/ha	N/C
14/06/13	TELDOR	0.50	0.75 Kgs/ha	N/C
	SW7	0.50	100 Units/ha	N/C
21/06/13	TELDOR	0.50	0.75 Kgs/ha	N/C
	K50	0.50	4.0 Units/ha	N/C
27/06/13	NATURALIS-L	0.50	1.0 lts/ha	N/C
01/07/13	TELDOR	0.50	0.75 Kgs/ha	N/C
	K50	0.50	4.0 Units/ha	N/C
	SW7	0.50	100 Units/ha	N/C
	HORTIPHYTE	0.50	2.0 Units/ha	N/C
09/07/13	NATURALIS-L	0.50	1.0 lts/ha	N/C
	SW7	0.50	100 Units/ha	N/C
09/07/13	NATURALIS-L	0.50	1.0 lts/ha	N/C
	K50	0.50	4.0 Units/ha	N/C
	SW7	0.50	100 Units/ha	N/C
23/07/13	SW7	0.50	100 Units/ha	N/C
	K50	0.50	4.0 Units/ha	N/C
27/07/13	NATURALIS-L	0.50	1.0 lts/ha	N/C
	SW7	0.50	100 Units/ha	N/C

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5214, Strawberries, Sonata (3.30 ha) Amelia Field
(01/10/2013 - 30/09/2014)

Planned	Product	Area	Rate	Applied
04/03/14	DIQUAT	0.33	3.000 lt	N/C
	SHARK	0.33	0.250 lt	N/C
27/03/14	THIANOSAN DG	3.30	2.500 kg	N/C
	ROVRAL WG	3.30	0.750 kg	N/C
	EQUITY	3.30	0.500 lt	N/C
	CALYPSO	3.30	0.250 lt	N/C
	DIQUAT	0.66	3.000 lt	N/C
	SHARK	0.66	0.250 lt	N/C
	VENZAR FLOWABLE	0.66	2.000 lt	N/C
	ESCAR-GO 3	3.30	4.000 kg	N/C
07/04/14	FENOMENAL	3.30	2.000 kg	N/C
17/04/14	ESCAR-GO 3	3.30	4.000 kg	N/C
18/04/14	BIO 20	3.30	3.000 lt	N/C
22/04/14	SIGNUM	3.30	1.000 kg	N/C
	HEADLAND JETT	3.30	1.000 lt	N/C
	EQUITY	3.30	1.000 lt	N/C
	SW7	3.30	100.000 unit	N/C
01/05/14	SIGNUM	3.30	1.000 kg	N/C
	HEADLAND JETT	3.30	1.000 lt	N/C
	UREA (KGS)	3.30	4.000 unit	N/C
	EQUITY	3.30	0.750 lt	N/C
16/05/14	SCALA	3.30	2.000 lt	N/C
	FORTRESS	3.30	0.250 lt	N/C
	UREA (KGS)	3.30	4.000 unit	N/C
	BIO 20	3.30	2.000 lt	N/C
	THIANOSAN DG	3.30	2.500 kg	N/C
	HEADLAND JETT	3.30	1.000 lt	N/C
22/05/14	SIGNUM	3.30	1.000 kg	N/C
	SW7	3.30	100.000 unit	N/C
	UREA (KGS)	3.30	4.000 unit	N/C
	BIO 20	3.30	2.000 lt	N/C
	HORTIPHYTE	3.30	2.000 unit	N/C
27/05/14	SW7	3.30	100.000 unit	N/C
	BIO 20	3.30	2.000 lt	N/C
	UREA (KGS)	3.30	4.000 unit	N/C
	SWITCH	3.30	1.000 kg	N/C
03/06/14	SW7	3.30	100.000 unit	N/C
	BIO 20	3.30	2.000 lt	N/C
	UREA (KGS)	3.30	4.000 unit	N/C
	TELDOR	3.30	1.000 kg	N/C
09/06/14	SW7	3.30	100.000 unit	N/C
	BIO 20	3.30	2.000 lt	N/C
	UREA (KGS)	3.30	4.000 unit	N/C
	TELDOR	3.30	1.000 kg	N/C
09/06/14	K50	3.30	4.000 unit	N/C
18/06/14	TELDOR	3.30	1.000 kg	N/C
	UREA (KGS)	3.30	4.000 unit	N/C
01/07/14	CORBEL	3.30	1.000 lt	N/C
	FORTRESS	3.30	0.250 lt	N/C
22/07/14	CORBEL	3.30	1.000 lt	N/C
	FORTRESS	3.30	0.250 lt	N/C
	HORTIPHYTE	3.30	2.000 unit	N/C
08/08/14	CORBEL	3.30	1.000 lt	N/C
	FORTRESS	3.30	0.250 lt	N/C
27/08/14	DIQUAT	1.65	3.000 lt	N/C
	SW7	1.65	100.000 unit	N/C
	DIQUAT	3.30	3.000 lt	N/C

8922, Strawberries, RUMBA (0.40 ha) Barn Field.
(01/10/2013 - 30/09/2014)

Planned	Product	Area	Rate	Applied
17/02/14	THIANOSAN DG	0.40	2.500 kg	N/C
	ROVRAL WG	0.40	0.750 kg	N/C
	EQUITY	0.40	1.000 lt	N/C
	FENOMENAL	0.40	2.000 kg	N/C
04/03/14	DIQUAT	0.04	3.000 lt	N/C
	SHARK	0.04	0.250 lt	N/C
27/03/14	ESCAR-GO 3	0.40	4.000 kg	N/C
17/04/14	ESCAR-GO 3	0.40	4.000 kg	N/C
18/04/14	BIO 20	0.40	3.000 lt	N/C
22/04/14	BIO 20	0.40	3.000 lt	N/C
	SIGNUM	0.40	1.000 kg	N/C
01/05/14	SIGNUM	0.40	0.750 kg	N/C
	HEADLAND JETT	0.40	1.000 lt	N/C
	UREA (KGS)	0.40	3.000 unit	N/C
09/05/14	TELDOR	0.40	1.000 kg	N/C
	BIO 20	0.40	2.000 lt	N/C
	SW7	0.40	100.000 unit	N/C
	NIMROD	0.40	1.000 lt	N/C
20/05/14	TELDOR	0.40	1.250 kg	N/C
	BIO 20	0.40	2.000 lt	N/C
	UREA (KGS)	0.40	4.000 unit	N/C
22/05/14	TELDOR	0.40	0.750 kg	N/C
	SW7	0.40	400.000 unit	N/C
	UREA (KGS)	0.40	4.000 unit	N/C
	BIO 20	0.40	2.000 lt	N/C
09/08/14	SYSTHANE 20EW	0.40	0.330 lt	N/C
	FORTRESS	0.40	0.250 lt	N/C
27/08/14	DIQUAT	0.20	3.000 lt	N/C
	SW7	0.20	100.000 unit	N/C
	DIQUAT	0.40	3.000 lt	N/C

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