The benefits of using silicon nutrient on strawberries including reducing epidemics of strawberry powdery mildew *(Podosphaera aphanis)*

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Abstract

Powdery mildew of strawberry plants caused by the fungus *Podosphaera aphanis* is a significant fungal disease of protected strawberry crops in the UK, causing yield losses between 20 to 70% of crop potential. At 20% losses, this can contribute to an industry volume of 23,100 tonnes, estimated at a market value of £56.8 million. Although growers frequently limit the spread of strawberry powdery mildew by a weekly to fortnightly application of fungicides (April to October), it has become more prominent in recent years. This research aimed to investigate four key areas. Firstly, the effects of the silicon delivered through a fertigation system on the development of strawberry powdery mildew (*Podosphaera aphanis*) disease levels in different strawberry plant cultivars. Secondly, examine the amounts and pattern of distribution of silicon in leaves, leaf petioles and roots of strawberry plants growing in glasshouse and field experiments. Thirdly, evaluate °Brix levels of silicon-treated strawberry plant fruits and leaf petioles with the untreated control, and lastly, measure growth parameters of strawberry plants in the absence and presence of silicon in a glasshouse hydroponic experiment.

In this thesis, silicon fertigation field experiments were set up on a commercial strawberry farm to evaluate the effects of silicon in reducing levels of disease of *Podosphaera aphanis* throughout the growing season. Results from this study (chapter three) revealed that the application of silicon as a nutrient reduced levels of strawberry powdery mildew in 2016. The lowest disease levels ($P<0.05$) occurred in Malling Centenary strawberry crops that received silicon twice-a-week with fungicides (AUDPC, 410) and without fungicides (AUDPC, 375) compared with the untreated control (AUDPC, 3423). Results from this experiment showed that the addition of silicon delayed the rise in disease levels by 29 days in the silicon twice-a-week treatment with and without fungicides compared with the untreated control. Disease level assessments carried out in 2017 and 2018 field experiments using the cultivar Amesti showed low levels of disease were only found in the untreated control plot compared to all other treatments in 2016.

A silicon deposition experiment was conducted on strawberry plants in a glasshouse (chapter four) in 2017. The results revealed that high amounts of silicon were deposited in the upper and lower cuticle, epidermis, palisade layer, and vein of the leaf (fluorescence intensity,7.2cps) of silicon-treated plants compared with the untreated control (fluorescence intensity, 2.2cps) ($P<0.05$). In the leaf petiole, more silicon was found in the upper and lower cuticle, epidermis and xylem (fluorescence intensity, 7.7cps) of silicon-treated plants compared with the untreated control (fluorescence intensity, 1.9cps) ($P<0.05$). In the roots, more silicon was found deposited mainly in the xylem (fluorescence intensity, 11.6cps) of silicon-treated plants compared with the untreated (fluorescence intensity, 1.2cps) ($P<0.05$). Results from a fertigation field experiment in 2017 also found that more silicon was laid down regularly in the upper and lower cuticle, epidermis and palisade layer of the leaves (fluorescence intensity,

19.4cps) of silicon-treated plants compared with the untreated (fluorescence intensity, 7.9cps) ($P<0.05$). In the leaf petiole, more silicon was found in the xylem (fluorescence intensity, 16.7cps) of silicon-treated plants compared with the untreated (fluorescence intensity, 10.2cps) ($P<0.05$). In the roots, silicon was found in the xylem (fluorescence intensity, 8.4cps) of silicon-treated plants compared with the untreated (fluorescence intensity, 6.0cps) ($P<0.05$). The 2018 deposition field experiment showed that more silicon was laid down in the upper and lower cuticle, epidermis, and palisade layer of the leaves (fluorescence intensity, 4.98cps) of silicon-treated plants compared with the untreated (fluorescence intensity, 2.2cps) ($P<0.05$). In the leaf petiole, silicon was found mainly in the xylem (fluorescence intensity, 3.72cps) of both silicon-treated and untreated plants (fluorescence intensity, 1.98cps) ($P>0.05$). In the roots, silicon was also mainly found in the xylem (4.97cps) of silicon-treated and untreated plants (1.76cps) ($P>0.05$). The hypothesis for chapter four is that the silicon can enhance the passive defence pathway of strawberry plants and is absorbed regularly in this manner.

Chapter five assessed strawberry plants grown hydroponically in Hoagland's solution to measure growth parameters between silicon-treated and untreated plants. This experiment revealed that the plants treated with silicon had significantly increased ($P<0.05$) numbers of leaves, runners and fruits compared with the untreated control. No significant differences ($P>0.05$) were found in the experiment's chlorophyll contents of strawberry leaves. These results suggested that silicon improved the quality of strawberry plants (treated with silicon) in a hydroponic glasshouse experiment by enhancing these growth parameters. This thesis demonstrates that the use of silicon via fertigation not only reduces the severity of strawberry powdery mildew (*Podosphaera aphanis*) and but has some additional benefits in strawberry production. Therefore, it is recommended that growers incorporate silicon nutrient to manage strawberry production, including strawberry powdery mildew disease control.

Acknowledgements

Two roads diverged in a yellow wood,
And sorry I could not travel both
And be one traveller, long I stood
And looked down one as far as I could
To where it bent in the undergrowth.

Then took the other, as just as fair,
And having perhaps the better claim,
Because it was grassy and wanted wear.
Though as for that, the passing there
Had worn them really about the same,

And both that morning equally lay
In leaves, no step had trodden black.
Oh, I kept the first for another day.
Yet knowing how way leads on to way,
I doubted if I should ever come back.

I shall be telling this with a sigh
Somewhere ages and ages hence:
Two roads diverged in a wood, and I—
I took the one less travelled by,
And that has made all the difference.
(Robert Frost)

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1 GENERAL INTRODUCTION

CHAPTER 2 MATERIAL AND METHODS

CHAPTER 5 MEASUREMENT OF STRAWBERRY PLANT GROWTH
PARAMETERS AND QUALITY IN RELATION TO SILICON

LIST OF TABLES

CHAPTER 4 SILICON DEPOSITION IN STRAWBERRY PLANTS

CHAPTER 5 MEASUREMENT OF STRAWBERRY PLANT GROWTH PARAMETERS AND QUALITY IN RELATION TO SILICON

CONFERENCE COMMUNICATIONS

BSPP presidential conference–meeting - Poster presentation Title: Reducing disease levels of strawberry powdery mildew using a silicon nutrient to give reduced susceptibility to *Podosphaera aphanis*) – St Hugh's College, Oxford, UK. 11-13th September 2016. Asiana, I., Hall A.M., Davies K.

Crop protection in Southern–Britain - Poster presentation, Title: Reducing disease levels of strawberry powdery mildew using a silicon nutrient to give reduced susceptibility to *Podosphaera aphanis* – Peterborough, UK, 15 -16th February 2017. Asiana, I., Hall A.M., Davies K.

Lunch time seminar – Oral presentation; Title: Reducing disease levels of strawberry powdery mildew using a silicon nutrient to give reduced susceptibility to *Podosphaera aphanis.* Asiana, I., Hall A.M., Davies K. 12th June 2017, University of Hertfordshire, college lane, UK.

BSPP presidential conference–meeting - Poster presentation, Title: Reducing disease levels of strawberry powdery mildew using a silicon nutrient to give reduced susceptibility to *Podosphaera aphanis*) – The University of Nottingham, UK 11- 13th September 2017. Asiana, I., Hall A.M., Davies K.

Lunch time seminar – Oral presentation; Title: Reducing disease levels of strawberry powdery mildew using a silicon nutrient to give reduced susceptibility to *Podosphaera aphanis.* Asiana, I., Hall A.M., Davies K. 12th January 2018, University of Hertfordshire, college lane, UK.

LMS Research Day- LMS Research Day; Oral presentation; Title: Silicon deposition in strawberry plants. Asiana, I., Hall A.M., Davies K. 16th April 2018, University of Hertfordshire, college lane.

ICPP International Congress of Plant Pathology conference meeting; Poster presentation; Silicon deposition in strawberry plants; Asiana, I., Hall A.M., Davies K, Boston Massachusetts, The United States 29 July – 3rd August 2018.

Berry Gardens –Research - Poster Competition (Annual Technical Conference) – Prize winner of poster competition 24th November 2018 – Title: The deposition of silicon linked to the reduction in susceptibility to strawberry powdery mildew; Asiana, I., Hall A.M., Davies K.

BSPP presidential conference meeting- Poster presentation, Title: Are strawberries ever deficient in silicon?; University of Warwick 10th – 11th December 2018. Asiana, I., Hall A.M., Davies K.

BSPP presidential conference meeting: **Oral presentation**; Title: How does a silicon nutrient enhance the passive defence pathway of strawberries to improve disease control?; Asiana, I., Hall A.M., Davies K. University of West England, Bristol, UK. 2nd – 3rd September 2019. PH Gregory competition.

Lunch time seminar – Oral presentation; Title: The benefits of using silicon nutrient on strawberries, including reducing disease levels of powdery mildew (*Podosphaera aphanis*). Asiana, I., Hall A.M., Davies K. November 11th 2019.

Lunch time seminar – Oral presentation; Title: How does a silicon nutrient enhance the passive defence pathway of strawberries to improve disease control. Asiana, I., Hall A.M., Davies K University of Hertfordshire, College lane. November 14th 2019.

CHAPTER 1 – GENERAL INTRODUCTION

1.1.   Structure of the thesis

Chapter one provides general information about the thesis, focusing on worldwide strawberry production. It covered the health and nutrition benefits of strawberries (section 1.2), a general introduction to strawberry production worldwide (section 1.3), British growing systems (section 1.4), the strawberry plant (section 1.9) and strawberry plant breeding (section 1.10). This chapter introduces *Erysiphales* (section 1.13) and strawberry powdery mildew *Podosphaera aphanis* (Wallr.). Chapter two provides the general material and methods, including farm description (section 2.1), laboratory (section 2.5) and glasshouse experiment methods (section 2.6). Chapter three provides an introduction to the chapter (section 3.1), rationale, aims, objectives and hypothesis (section 3.2.1 to 3.2.4), material and methods used in the chapter (section 3.3), results (section 3.4), discussion (section 3.8) and conclusion (section 3.9). Chapter four provides an introduction to the chapter (section 4.1), rationale, aim, objectives and hypothesis of the chapter (section 4.2.1 to 4.2.4), material and methods (section 4.3), results (section 4.4), discussion (section 4.5) and conclusion (section 4.6). Chapter five provides the introduction to the chapter (section 5.1), rationale, aim, objectives and hypothesis of the chapter (section 5.2 to 5.5), material and methods (section 5.6), results (section 5.8), discussion (section 5.9) and conclusion (section 5.10). Finally, chapter six provides the overall discussion and conclusion of chapters in the whole thesis divided into sections (6.1 to 6.3), conclusion (section 6.4) and future work (section 6.5).

Previously, Jin (2015) conducted experiments to determine the efficacy of silicon nutrient in reducing strawberry powdery mildew. In the study, silicon was applied through foliar sprays and root treatments via a fertigation system once-a-week only. The work reported by Jin (2015) also explored silicon concentration in fertigation water in a 1-year experiment and localization in a glasshouse experiment alone.

The work conducted in commercial field tunnels in this thesis compared the use of silicon in reducing levels of disease of strawberry powdery mildew for 3 consecutive years. Firstly, in 2016, a field experiment was conducted to evaluate the differences in disease reduction between strawberry crops applied with both silicon once-a-week and twice-a-week. Following the 2016 field experiment, silicon was applied twice-a-week only in the 2017 and 2018 field experiments as it showed a higher reduction in disease levels than the once-a-week silicon application in 2016. The silicon contents in strawberry leaves and fertigation water were assessed for 3 years (2016 to 2018). Furthermore, a deposition experiment was set up for the quantification analysis of silicon deposits in plant tissues (leaves, petioles and roots) in a glasshouse and two field experiments (2017 and 2018). Lastly, strawberry plant growth parameters were quantified between silicon-treated and untreated strawberry plants in a hydroponic glasshouse experiment in 2018.

1.2.   Health and nutrition benefits of strawberries

Strawberries are beneficial to human health and nutrition. Researchers have found that their vitamin C content produces 30% of strawberries' total antioxidant capacity (TAC) (Li et al.,2019). Vitamin C is essential to a healthy immune system as it stimulates the white blood cells, which form a defence against infections. According to Danielsen (2018), research on the antioxidant content of strawberries has provided evidence about their ability to lower the risk of cardiovascular disease. Recent studies have shown that improved blood sugar regulation is a health benefit associated with strawberry consumption, mainly after meals (Brown-Paul, 2016).

Researchers have discovered an enhanced regulation of insulin and blood sugar levels in connection with strawberry intake (Foito et al., 2018). Alongside vitamin C, strawberries provide other vital antioxidants and anti-inflammatory nutrients. Strawberries are an excellent source of manganese (29%), a mineral that plays a key antioxidant role as a cofactor for the enzymes superoxide dismutase (Kumar et al., 2019).

Dai (2018) reported that strawberries are not a high-fat food but contain seeds and serve as a good source of omega-3 fatty acid alpha-linolenic acid. Strawberries also offer significant quantities of other nutrients such as folate (vitamin B9), vitamin B6, calcium, iron, copper, phosphorus, potassium, traces of selenium and dietary fibre. Joint inflammation, such as arthritis and gout, can be controlled by the regular consumption of strawberries (Legard, 2018).

The presence of nutrients such as vitamin C, folate, anthocyanins, ellagic acid and ellagitannins in strawberries helps prevent the growth and progression of cancer cells drastically and reduces the chances of tumour formation (Turgut and Cakmakci, 2018). A study has shown that strawberries produce fat-burning and metabolic hormones such as adiponectin and leptin, aiding weight loss. Calvano et al. (2019) pointed out that strawberries' potassium and magnesium are nutrients that effectively control blood pressure.

Strawberries contain a large amount of dietary fibre, which is essential in promoting healthy digestion (Morris and Sistrunk, 2018). Strawberries have high levels of phenolic antioxidants, with levels 2 to 11 times higher than other fruits (Zitouni et al., 2020). The health benefits of strawberries arise from consuming 1 to 2 servings a day (50 grams per serving) and, when consumed daily, contribute to supporting health (Strawberryplants, 2020).

1.3. Strawberry production worldwide

Strawberries (*Fragaria ananassa*) are a popular fruit in many parts of the world. They are widely grown and globally cultivated for their fruits and are best known for their distinctive succulent nature, bright red colour, texture and aroma (Cockerton et al., 2018). Strawberries are either fresh or used to prepare various foods such as jams, juices, milkshakes, ice cream and pies. According to Simpson (2018), the garden strawberry was first propagated during the 1750s in Brittany, France, through crossbreeding *Fragaria virginiana* from the eastern regions of North America and *Fragaria chiloensis* from Chile, which was brought to Europe in 1714. The figures below include 1.1 (a) Worldwide production and yield of strawberries and (b) Top 10 strawberry-producing countries.
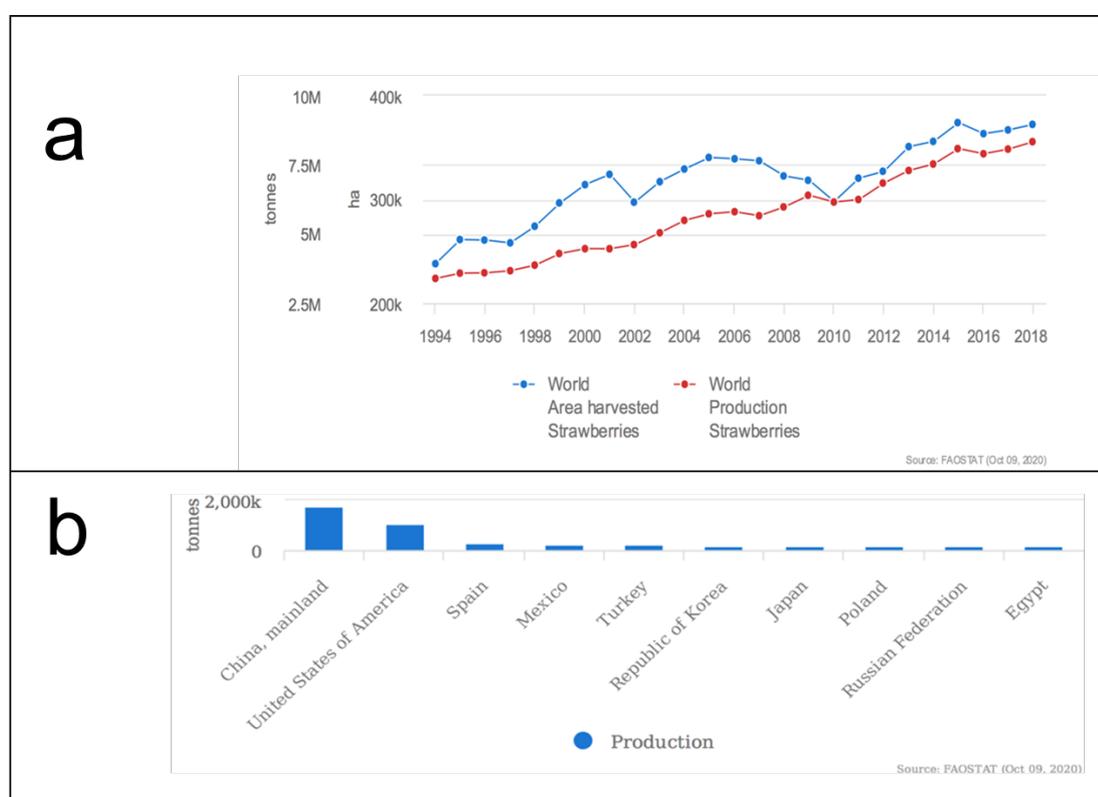
Figure 1.1. (a) Worldwide production and yield of strawberries (b) Top 10 strawberry-producing countries Source: FAOSTAT, 2020.

In figure 1.1a, the blue dotted line on the graph indicates the area harvested strawberries, and the red dotted line indicates production in tonnes. In figure 1.1(b), the bar chart represents the production of strawberries by countries in tonnes. The worldwide production of strawberries is estimated at around 8.3 million tonnes per annum (figure 1.1a and b) (FAOSTAT, 2020); From the graphs displayed, China is the world's leading producer of strawberries with 2,964,263 tonnes of yearly production (figure 1.2a and table 1.1) (FAOSTAT, 2020). The second largest producer of strawberries is the United States, with 1,296,272 tonnes yearly production of the world production of strawberries (figure 1.2b) (FAOSTAT, 2020). Spain is third in world production with 366,161 tonnes yearly (figure 1.2c) after the United States, followed

by Mexico with 658,436 tonnes produced yearly (1.2d) (FAOSTAT, 2020). The production, harvest and yield of strawberries produced are summarised in table 1.1. Below (figure 1.2 a to d) are the 4 top strawberry-producing countries in the world according to FAOSTAT (2020). In each of the graphs, the blue dotted lines indicate the area of harvested strawberries, and the red dotted lines indicate the production of strawberries in tonnes between 1994 to 2018.

Table 1.1 below is a list of 11 strawberry-producing countries. The 4 top strawberry producers are also shown in the table below. China is the largest producer. The United States is the second largest producer, Spain is the third, and Mexico is the fourth largest producer.

Table 1. 1 World strawberry-producing countries

Country	Production tonnes	Production per Capita (kg)	Harvested Area (Ha)	Yield (Kg/Ha)
China	2,964,263	2.7	111,132	26,869
United States of America	1,296,272	4.3	190,919	66,876
Spain	366,161	7.8	7,032	47,648
Mexico	658,436	3.8	13,850	42,219
Turkey	440,968	5.1	16,102	26,904
South Korea	213,054	3.8	5,658	30,906
Japan	163,486	1.3	5,259	29,432
Poland	196,972	5.1	50,600	3,893
Russian Federation	195,578	1.3	29,520	47,833
Egypt	362,639	4.8	8,880	46,566
Germany	143,221	1.7	14,299	10,016

Adapted from FAOSTAT (2020) and Atlasbig (2018).
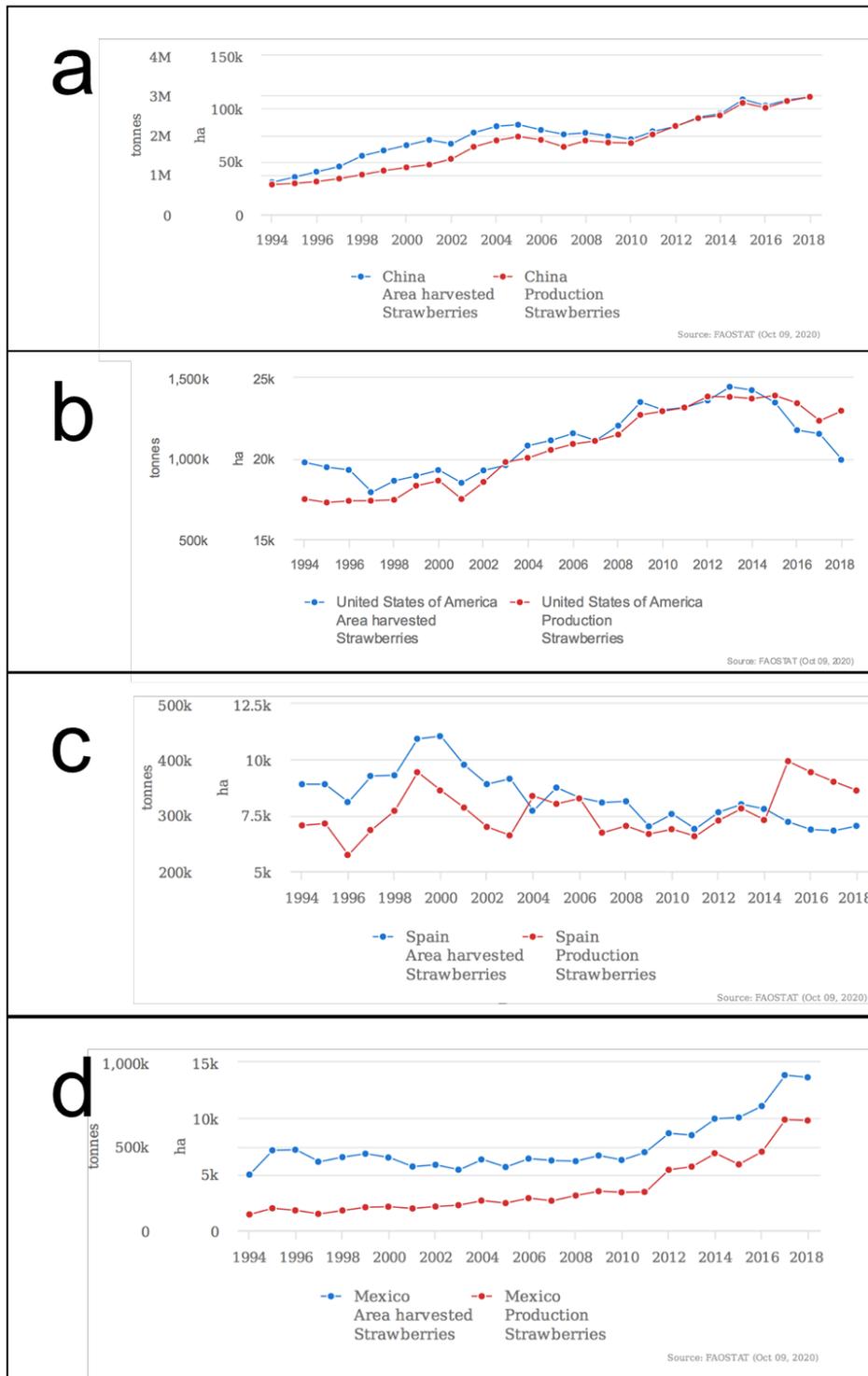
Figure 1.2. Production and yield quantities of strawberries. (a) Production and yield quantities of strawberries in China (b) Production and yield quantities of strawberries in the United States of America (c) Production and yield quantities of strawberries in Spain (d) Production and yield quantities of strawberries in Mexico. The blue line indicates the area of harvested strawberries, and the red line is produced from 1994 to 2018. Source: (FAOSTAT, 2020).

Over the last 20 years, the UK season for strawberry production has increased from more than six weeks to more than seven months (Orde and Sideman, 2019). This has been achieved through polythene tunnels, increased Everbearing varieties (table 1.2), advanced growing systems using coir on raised soil beds, and table-top with automatic fertigation systems (Forcelini & Peres, 2018). Strawberry cultivation in the UK expanded very quickly towards the end of the 19th century and, by 1924, after a short drop in production during the First World War, reached its peak at 13,000Ha (Calleja, Ilbery and Mills, 2012).

The strawberry plant can be classified as a June bearer or an Ever bearer (day-neutral). Everbearing strawberries, also known as perpetual fruiting types, will produce crops multiple times during the growing season (Simpson, 2018). Junebearing strawberries usually produce a harvest in June, which can be further classified into early season, midseason and late session. (Kumar, 2018). The differences and characteristics of these two types are explained further in table 1.2 below. Growers grow the June bearers as an early crop, and the Ever bearers are a continuous crop. Production output has increased by introducing new varieties and growing systems, improving crop productivity and fruit quality (Simpson, 2018). Table 1.2 below is a short list showing the characteristics of June bearers and Ever bearers of strawberries.

Table 1.2. Characteristics of June bearers and Ever bearer strawberries.

June bearers (day length)	Ever bearers (day-neutral)
Develop flower buds in late summer	Planted in February/March (covered with fleece until April) and produce branch crowns and buds throughout the season. However, a higher temperature may inhibit the initiation.
Begin fruiting in June, lasting up to 3-4 weeks.	Begin fruiting in mid-May or June, lasting for 10-12 weeks.
Harvest is once a year	Harvest is twice a year: One in spring and one in late summer or early autumn.
They produce large fruits and heavy cropping over two to three weeks. Produce more runners.	They produce smaller fruits and less heavy cropping and produce fewer runners.

According to the FAOSTAT (2020), The UK produces 131,639 tonnes of strawberries yearly (figure 1.3). The UK produces approximately 80% of the UK demand for strawberries compared to only 20% 20 years ago (Anderson, Rogers and Hoover, 2019). Farminguk (2019) reported that the value of the berry industry had pushed over £1.4 billion since it now has a quarter of the market share of all fruit grown in the UK.

The graph below (figure 1.3) shows the trend of strawberries produced (tonnes) and harvested (hectares) between 1994 to 2018 in the UK alone. In 1994, there was a decline in the areas harvested. However, production is showing a gradual increase over time.
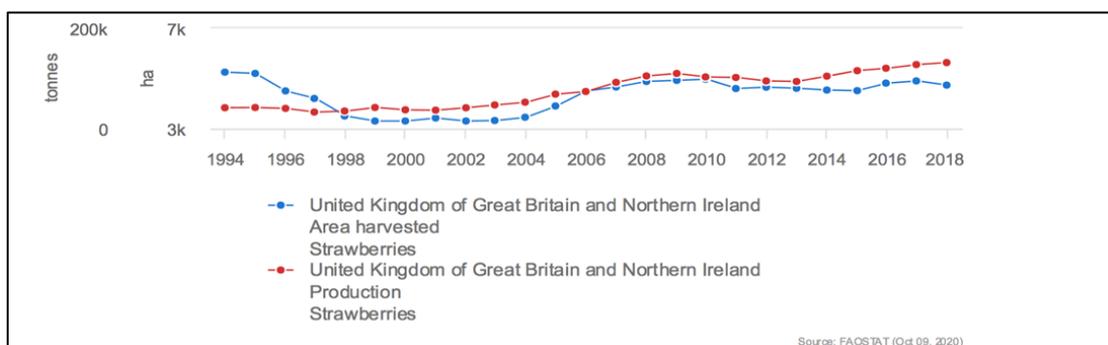
Figure 1.3. Production and yield quantities of strawberries in the United Kingdom. Source: FAOSTAT (2020).

The UK strawberry season begins from April to May and up till October however, in the colder temperatures (winters), it grows and harvests strawberries in polythene tunnels and glasshouses and sources strawberries from countries listed in table 1.3 below. The UK production of strawberries is from April to October, Spain produces strawberries from January to April, Morocco produces strawberries at its peak season from December to April, Egypt produces strawberries from November to April, and Israel produces strawberries from November to February.

Table 1.3. Source of UK strawberries and country of strawberry imports.

Country	Jan	Feb	March	April	May	June	July	August	Sep	Oct	Nov	Dec
UK				🍓	🍓	🍓	🍓	🍓	🍓	🍓		
Spain	🍓	🍓	🍓	🍓								
Morocco	🍓	🍓	🍓	🍓								🍓
Egypt	🍓	🍓	🍓	🍓							🍓	🍓
Israel	🍓	🍓									🍓	🍓

Source: Sampson and Kirk (2016).

There is a variation in cultivation systems for strawberry production in the UK (Whitehouse et al., 2016). They include the growth medium (soil, coir, substrate), crop variety (June bearer, Ever bearer), planting time (spring planted, summer planted), years of cropping (1 to 3) and polythene tunnels and glasshouses (Campos, 2018). Table 1.4 below is a classification of strawberry growing systems used by growers in the UK. British growing systems for strawberry production are classified by: crop protection

(polythene tunnels or glasshouse), growing medium (soil, peat, Hoagland's or coir), variety of crop (June bearers, Ever bearers) and planting time (grown in spring, summer).

Table 1.4. Types of strawberry production in the UK

Classification	Growing system
Protection	Protected crops (i.e., polythene tunnels, glasshouse)
	Unprotected crops (i.e., open fields)
Growth medium	Soil grown
	Substrate (i.e., coir) grown crops
Growth methods	Bags and troughs on raised beds
	Bags and troughs on tabletops
Planting time	Spring planted
	Summer planted
Crop variety	June bearer
	Ever bearer

Source: Husaini and Neri (2016)

1.4. British growing systems

Strawberries are grown in a wide range of production systems to produce the objective of producing high yields of quality fruit with sufficient flexibility to meet market demand and labour availability. Twitchen (2018) mentioned that polythene tunnels enable fruit growers to extend the strawberry fruit season from May to mid-autumn. Brown-Paul (2016) suggested that through polythene tunnels, there is guaranteed fruit quality produced by protecting the crops from rain and other environmental conditions. There has been a reduction in crop wastage, improvements in yields and control in labour costs through the use of the polythene tunnels (Allen et al., 2015).

Polythene tunnels (figures 1.6 to 1.8) shield crops from rain, decreasing the need to spray with fungicides to avoid diseases, including *Botrytis,* also known as grey mould (Durner, 2018). Claire et al. (2018) mentioned that the conditions under the polythene tunnel encourage the development of powdery mildew. They also create an environment suitable for natural pest control in which a pest is used to attack another in an enclosed area. For example, these predatory insects control spider mites, aphids, slugs and thrips, reducing reliance on insecticides (McCartney and Lefsrud, 2018).

1.5. Fertigation systems

Fertigation is extensively practised in commercial horticulture by applying nutrients (fertilizer) in a liquid form to strawberries via an irrigation system (Smith, 2017). The fertigation system is generally used on high-value crops, including turf, vegetables, fruit trees and ornamentals (Nestb and Guery, 2017). It allows a timely water supply through drip irrigation, less wasteful than sprinklers with an accurate nutrient (fertilizer) application rate, improving crop nutrient uptake (Durner, 2018). Strawberries are grown in coir (figure 1.4), biodegradable, have an excellent capacity for aeration and water retention and have no fungus (Robinson Boyer et al., 2016).

Figure 1.4. Strawberry plants are grown in coir bags. Each bag is placed on raised beds and provided with drip irrigation systems for providing crops with water and nutrients.

Nitrogen (N_2) is the most commonly used plant nutrient (Hobbie, 2015). Naturally, occurring Nitrogen is a diatomic molecule that most plants cannot consume; therefore, the nitrogen used must be a component of other chemical substances that can be taken up by the plant (Singh et al.,2018). Generally, anhydrous ammonia, ammonium nitrate and urea are used as bioavailable forms of nitrogen (Kennedy, 2016). Other nutrients applied through the fertigation systems include monoammonium phosphate, diammonium phosphate in bioavailable forms and bioavailable sources of silicon (Artyszak, 2018; Gallegos-Cedillo et al., 2018). In fertigation, adjusting the potential of Hydrogen (pH) of irrigation water is essential as it allows optimal uptake of nutrients. Almanac (2020) mentioned that strawberries require a pH between 5.5 and 7.0 (figure 1.5) (slightly acidic to neutral) however, growers recommend a pH between 5.5 and 6.5, ideal for growing strawberries. The pH and EC levels alter the availability of essential nutrients in growing media (Hafiagroup, 2020). These pH levels (5.5 and 6.5) are required in the fertigation streams as very few UK strawberries are grown in soil. Moreover, pH levels above 8 can harm certain strawberry nutrients (such as iron levels in specific cultivars) (Hafiagroup, 2020). The availability of nutrients at varying pH in growing media is shown in figure 1.5.
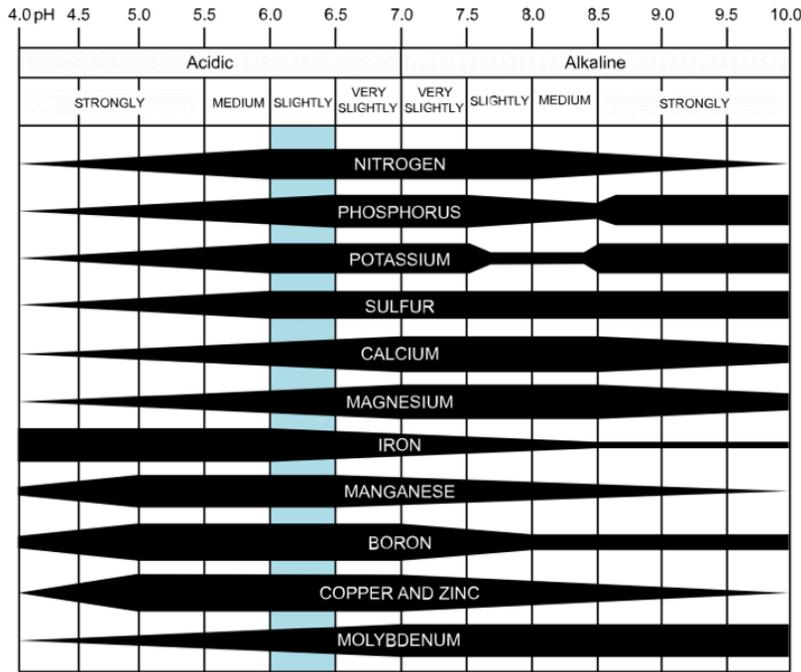
Figure 1.5. Availability of nutrients at varying pH values. Source: Almanac (2020). The image shows pH ranges of crop nutrients that are under the category of acidic to alkaline.

Electro-conductivity (EC) is of great importance in measuring the solution's ability to conduct electrical current, which can factor between 0.65ds/m and 0.7ds/m depending on the nutrient formulation (Lyaruu, 2010). The unit of measurement for the electroconductivity of soil is deciSiemens per metre (dS/m) or milliSiemens per centimetre (mS/cm) (Corwin and Yemoto, 2019). The EC measures the quantities of salts available in the soil. Higher levels can negatively impact crop yield, suitability, nutrient availability and activity of soil microorganisms and is an indicator of soil health (Corwin and Scudiero, 2020).

Additional problems associated with high sodium salts are toxicity, poor soil structure and poor infiltration and drainage. Electroconductivity of soil is affected by crop planting, irrigation, land use and application of manure, fertilizer and compost. Irrigation water salinity should also be measured as irrigating in amounts too low or too high in salts enables salts to accumulate in the root zone, thereby increasing EC (Almanac, 2020). An EC reading lesser than 1 dS/m in the soil is considered non-saline and does not affect most crops and soil microbial processes however, an EC reading more significant than 1 dS/m is considered saline and can affect critical microbial processes of soil. These include the production of nitrous and other N oxide gases, nitrogen cycling, respiration and decomposition, nitrogen losses and an increase in the populations of plant-parasitic nematodes (Corwin and Scudiero, 2020).

1.6.   Coir bags on raised soil beds under polythene tunnels

Raised soil beds are ideal for strawberries, which benefit from good drainage. Raised beds (figure 1.6) are made of coir bags or troughs on raised soil, which lifts the fruits off the soil and allows easy picking (Goodchild et al., 2018). Raised beds prevent grasses from invading the planting area. Using coir on raised beds eradicates the need for soil sterilization from soil-borne diseases and pests, an essential part of growing crops in soil beds (Nichols, 2013). Fleece is laid over crops in March after planting to boost the flowering, protect crops from harsh climate conditions such as heavy rainfall and frost and remove in April (Hall, Jin and Dodgson, 2016). Raised soil bed strawberries are in figure 1.6.
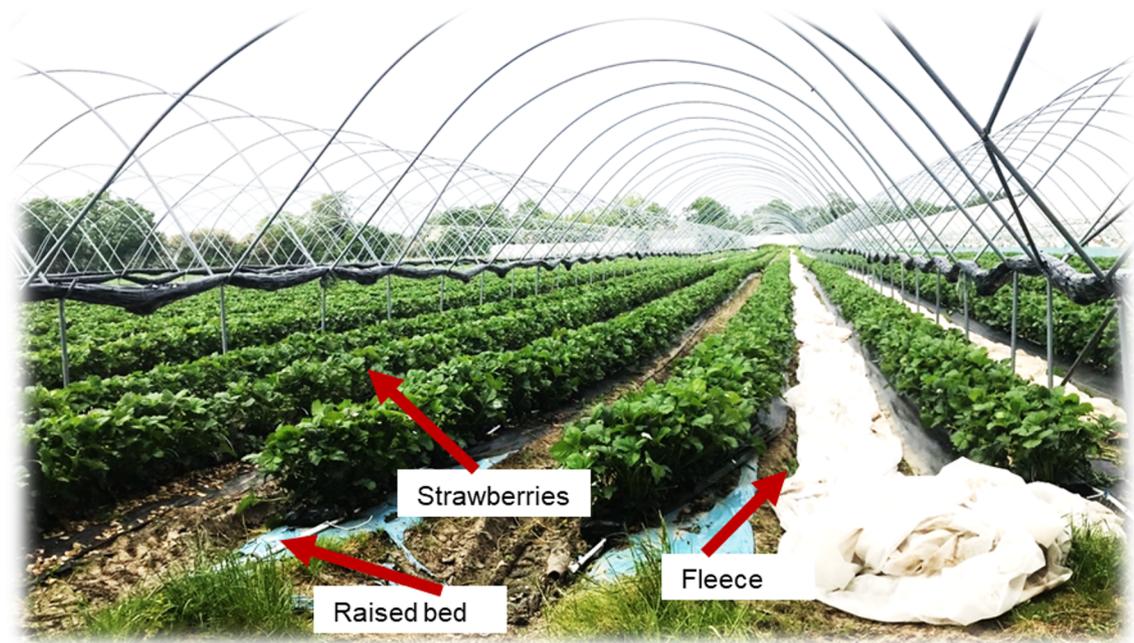
Figure 1.6. Raised soil bed strawberries

Fleece is usually spread over crops to encourage early flowering and keep crops away from frost. Refer to figure 1.7 to see fleece pulled over crops. (fleece is removed in figure 1.6).

1.7.   Fleece and mulch

1.7.1.   Fleece

Fleece and other floating films, known collectively as crop cover, are laid over plants hastening their growth and protecting against weather and pests (figure 1.7) (Marshall et al., 2018). Growers use these materials, which provide a small but significant amount of protection from cold or windy weather (Hall and Jin, 2016). Fleece-covered beds are shown in figure 1.7.
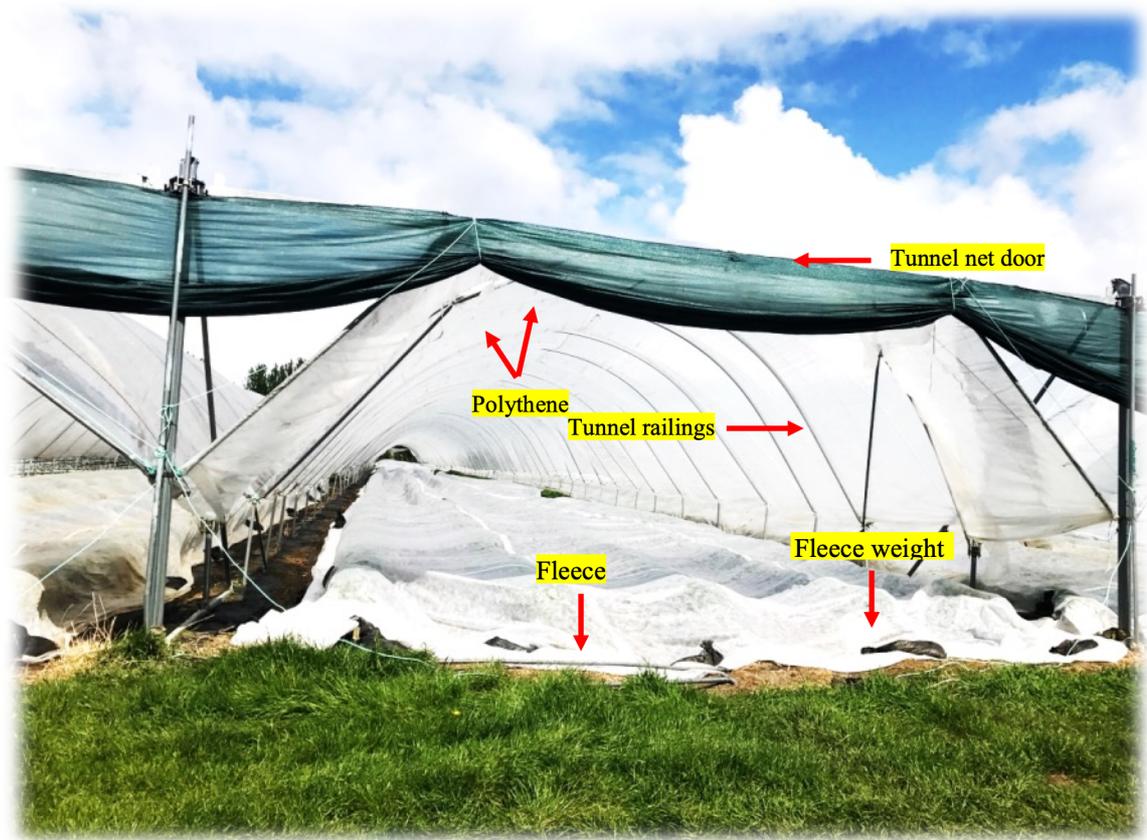
Figure 1.7. Fleece-covered strawberry beds under a polythene tunnel.

These fleeces are lightweight unwoven polypropylene fabric that allows filtered light to pass through them. The fleece also filters air movement, reducing wind chill, providing shade, and holding warm air. On fields, the fleece is pulled (like a blanket) over the crops from the beginning to the end of the field and held down with weight bags. Weights (as shown in figure 1.7 above) are placed on the edge of beds to secure the fleece from lifting with high winds. These weights are bags made from heavy-duty polyethene fabric, half filled with gravel, stone, pebbles or sand, with no specific standard measurement.

1.7.2. Mulch

In climates with cold winters, mulch is spread over strawberry plants to protect the plant from cold and extreme temperature fluctuation (Bano and Qureshi, 2016). Generally, mulch inhibits weeds from growing and protects plants from frost (Júnior et al., 2018). Plants show growth when the mulch is removed after the danger of frost is over (Singh et al., 2019). Figure 1.8 below are strawberry beds covered with fleece under a tunnel before the polythene is put over the tunnel hoops.
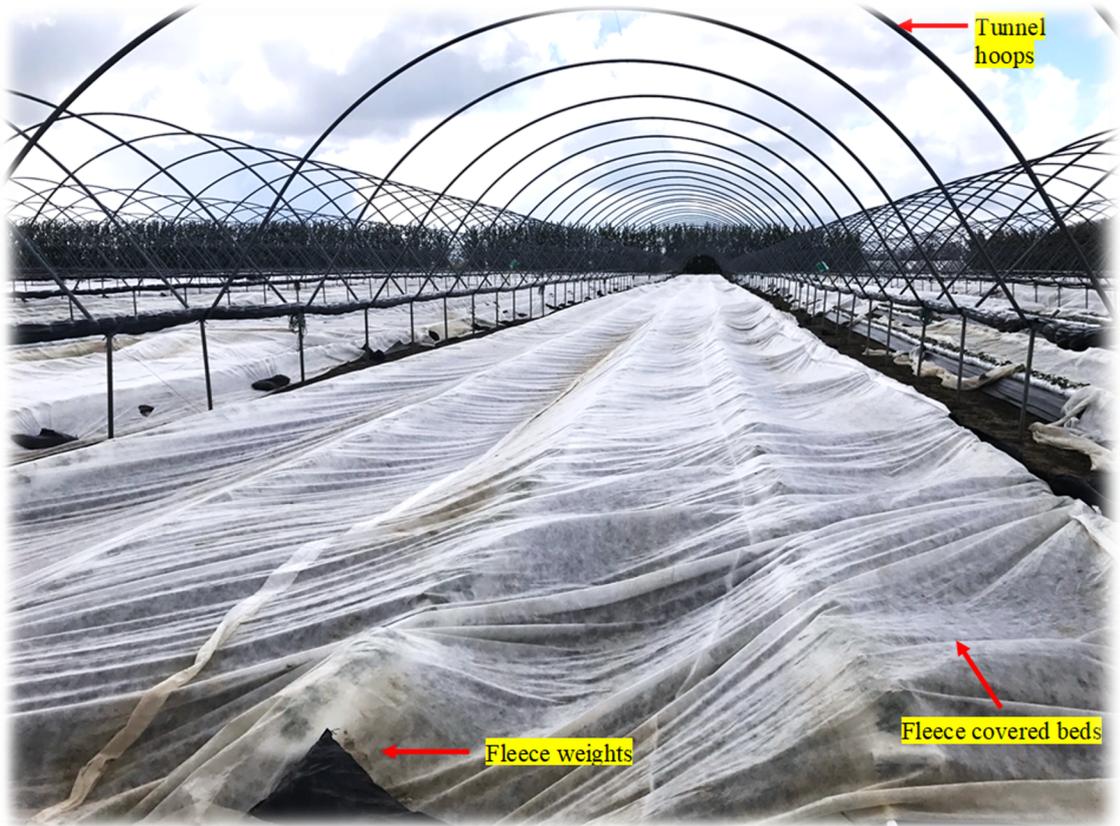
Figure 1. 8. Fleece-covered strawberries in spring (before the polythene is put on). Fleece is put over the crop for frost protection.

1.8. Tabletops

The use of tabletop growing systems (figure 1.9) adopted by commercial strawberry growers is rising (Daugaard, 2008). The plants are usually grown under polythene tunnels in plastic troughs approximately 1.5 metres above the ground containing compost and watered by trickle irrigation (Downing, 2016).

Figure 1 9. Tabletop strawberries. Source: (Palmers, 2018). The photo shows strawberries growing on tabletops on a field. During the fruiting season, fruits will hang down from the plants, as shown in figure 1.9, allowing easy picking compared to the crops growing on the ground.

Strawberry growers continue to invest in tabletop systems despite their high cost as they reduce picking costs by 30 to 40 per cent. Grimstad and From (2017) mentioned that the tabletop growing system not only creates ease of strawberry picking but makes strawberry picking a more attractive job as labour is increasingly difficult to obtain and manage. Tabletop strawberries decrease the amount of rot and poor harvest decay from *Botrytis* (De Preter, Anthonis and De Baerdemaeker, 2018).

1.9. Strawberry plant

Strawberries are low-growing herbaceous plants from the Rosaceae family with a fibrous root system and a crown, which arise compound leaves typically with three (saw-tooth edges) leaflets (figure 1.10 and table 1.5) (Stewart, 2016). The strawberry plant produces flowers on slender stalks known as the peduncle. As the strawberry plant ages, the root system becomes woody, sending out runners, thus enlarging the plant vegetatively (Visser and Konings, 2018). The strawberry fruit (figure 1.11 and table 1.5) is an accessory fruit consisting of a greatly enlarged flower receptacle with achenes commonly known as strawberry seeds (Nelson et al., 2018). An achene is a separate single-seeded fruit that does not open to release the seed (Ariza et al., 2016). The strawberries, *Fragaria L.*, are native to the temperate regions of the Northern Hemisphere, and cultivated varieties are grown worldwide (Kumar, 2018).

1.10. Strawberry plant breeding

Strawberry breeding was developed in England since the 1800s, and numerous varieties such as 'British Queen' (1840), 'Noble' (1884) and 'Jucunda' (1854) were introduced and were famous for their rich flavour or their resistance to cold and disease (Stewart, 2016). *Fragaria x ananassa* has developed into a large and tasty berry after years of hybridization and dominates modern strawberry cultivation (Jacobs, 1957; Darrow, 1966). The genus *Fragaria* has a primary chromosome number of (x=7) (Ichijima, 1926) and has four main groups, such as the diploids (2n=2x=14) chromosome number 14, which includes the model species for the genus, *F. vesca,* the tetraploids (2n=4x=28), including *Fragaria Orientalis*; the single hexaploid species *Fragaria moschata* (2n=6x=42); and four octoploid species (2n=8x=56): *F. chiloensis*, *F. iturupensis*, *F. virginiana* and the hybrid cultivated strawberry, *F. ananassa* (the *Fragaria ananassa* and *Fragaria vesca* are both diploids (2n=2x=14)) (Aristya et al., 2015).

Crossbreeding techniques have been established since the 20th century and are a traditional method of plant breeding (Folta, 2019). Crossbreeding involves allowing specifically chosen plants to sexually reproduce with other plants (Simpson, 2018). In other words, the crossbreeding techniques involve using plants with favourable characteristics (Schaart et al., 2016). Their offspring are raised, and a decision is made on a selection of plants with the best traits, and the process proceeds to the next generation (Nelson et al., 2018).

In crossbreeding, the pollen of the chosen plant is rubbed over the female receptacle of the other plant to achieve thorough pollination (Hancock, 2018). The strawberry plant is made up of five anatomical structures, which include the leaf (composed of 3 or 5 leaflets), root, crown, stolon and daughter plant (known as the runner) (Garcia, 2016). This structure is shown in figure 1.10 and explained in table 1.5.
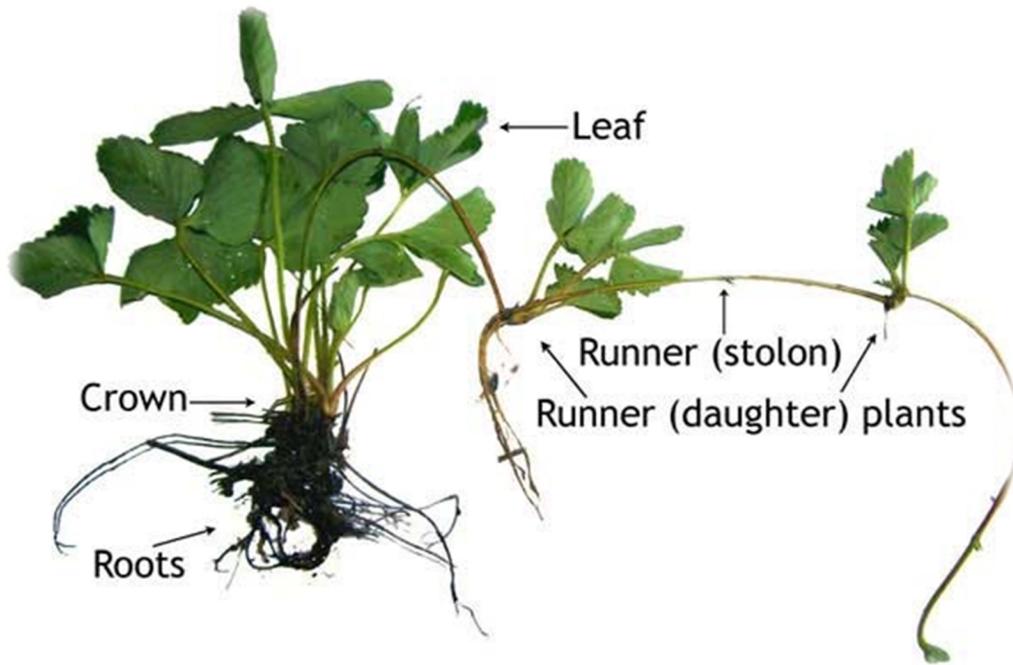
Figure 1.10. Anatomy of a strawberry plant. Source: Strawberryplants (2020). The fully established (mature) strawberry plant consists of woody/ fibrous roots (with secondary and primary roots), a crown from, which the leaves (with 3 leaflets) arise, flowers (not shown in the picture) and runners, also known as daughter plants.
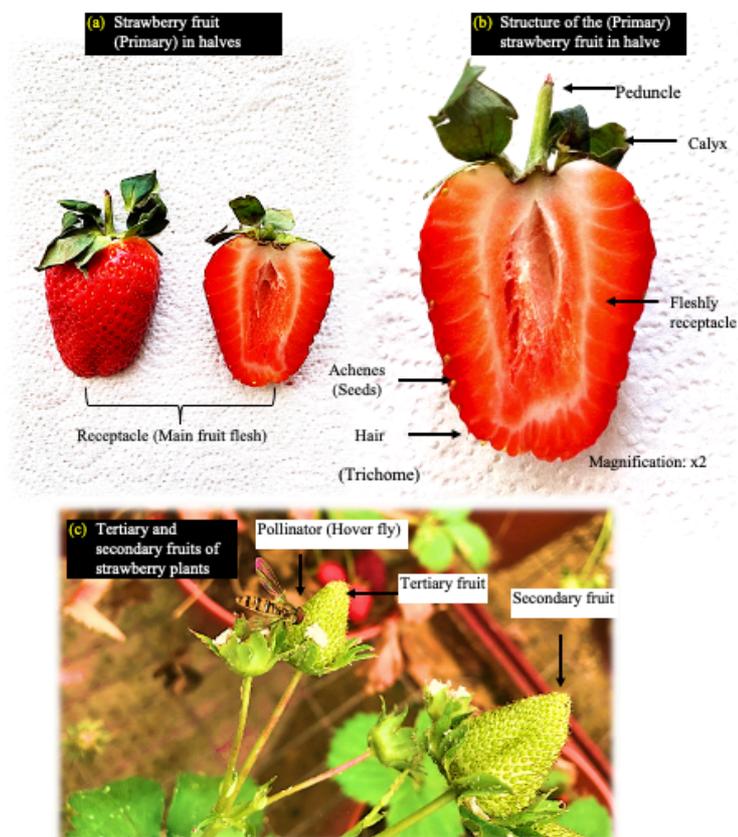
Figure 1.11. Anatomy of the strawberry fruit. (a) Strawberry plants in halves (b) Structure of the strawberry fruit and (c) Tertiary and secondary fruits of the strawberry plant.

16

Although strawberry plants can self-pollinate, the hoverfly (figure 1.11c) is one of several strawberry plant pollinators, including butterflies and wild bees such as bumblebees (Hodgkiss, Brown and Fountain, 2018; Kim et al., 2019). Driscoll's varieties of the strawberry plant became the most popular in Europe (Jublieestrawberries, 2020). Some of Driscoll's unique varieties include Driscolls Jubilee, Driscolls Elizabeth^{TM} and Driscolls Zara, which are preferred for their distinctive ruby colour and flavoursome taste (Jublieestrawberries, 2020). The anatomy of a strawberry plant is classified into 6 main parts: the root, crown, leaf, flower, fruit and runner, summarised in table 1.5.

Table 1.5. Strawberry plant anatomy and morphology

Plant structure	Characteristics
Crown	Consists of the central crown and branch (figures 1.10 and 1.14). The central crown is the plant's body, which holds the leaves, runners, branch crown and inflorescences that arise from it.
Leaf	In spring, new leaves grow from the crown and replace older leaves. Production of leaves stops when temperatures fall to 0°C in autumn. A well-established leaf canopy can supply the energy to initiate flower buds. (figures 1.10 and 1.14).
Root	Strawberries are sensitive to deficiency or excess water and high salts in the soil as they have shallow root systems. Most of its roots are produced in spring and autumn. Primary roots conduct water and nutrients to the crown, while feeder roots are generally for water and nutrient absorption. (figures 1.10 and 1.14).
Runner	Runners form during long days with warm temperatures, beginning in late spring and continuing until autumn. (figure 1.10).
Flower	The primary flower opens first and yields the largest fruit but is more susceptible to frost than flowers formed later. The secondary flowers normally open 1 to 2 days after the primary flower, followed by tertiary flowers. (figure 1.12).
Fruit	The strawberry 'fruit' is an enlarged receptacle with seeds (achenes) embedded in the surface. Each achene holds a seed. Primary berries are the largest and first to ripen, followed by secondary and tertiary fruits. (figures 1.11).

Images shown in figures 1. 12, and 1.13 below are from a glasshouse experiment showing flowers of strawberries and strawberry runners during their life cycle. Figures

1.14 and 1.15 show roots from a glasshouse experiment highlighting the parts of plants and the root system.

Figure 1.12. Strawberry plant flowers in a pot glasshouse experiment. (photo taken when automatic lights in the glasshouse were switched on).

Figure 1.13. Malling Centenary strawberry runners attached to mother plants (pot compost) in UH glasshouse experiment.

As shown in figure 1.14. and 1.15, the strawberry plant consists of both secondary (white) and primary (dark) roots. The secondary (young) roots, also known as feeder roots, are of great importance to the plants as they carry absorbed water and nutrients

from the soil or Hoagland's solution into the crown of the plant, as well as the primary (mature) roots, which act as a channel for the transportation of water and nutrients from the crown throughout the plant. The strawberry's primary roots are the main roots of the plant and can survive for multiple years; however, the secondary roots are short-lived and have a lifespan of days to weeks (Darrow, 1966).
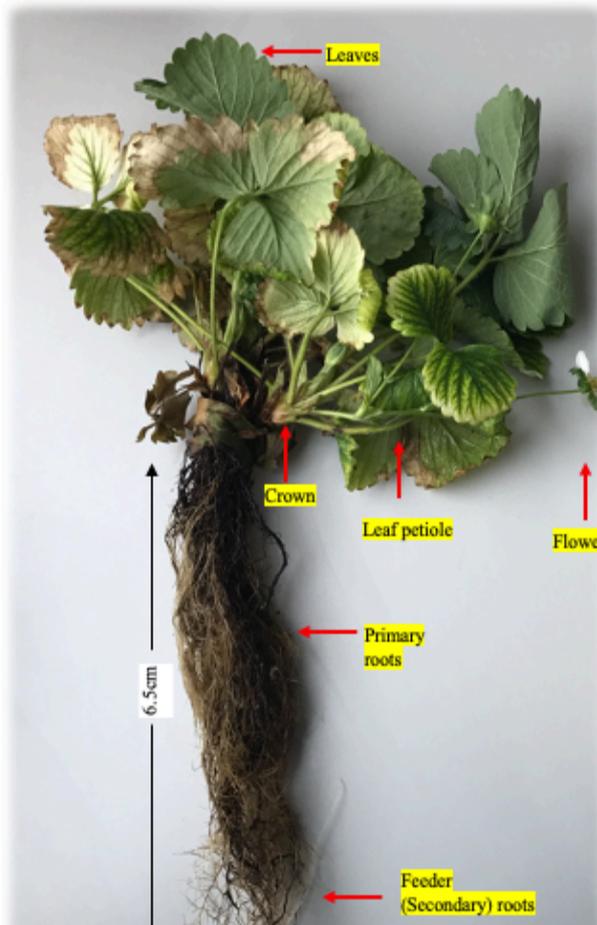
Figure 1.14. The appearance of a strawberry plant grown in hydroponics Hoagland's solution.
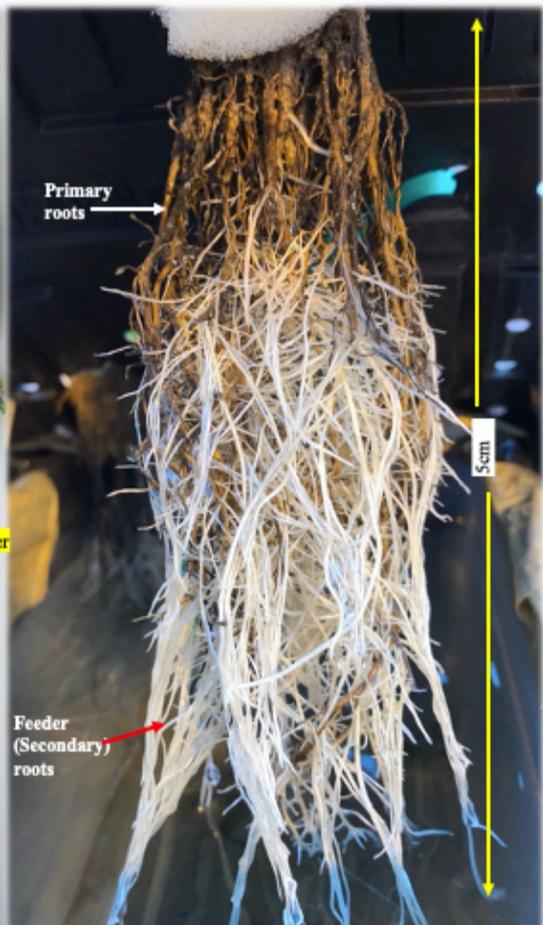
Figure 1.15. The appearance of strawberry plants 'roots' grown in a hydroponic Hoagland solution

1.11. Harvest and shelf life

Gibberellins are known to enhance the growth of plants and aid in the ripening of fruits (Ogas, 2000). Strawberries usually ripen 28-30 days after full bloom, and not all berries will ripen simultaneously (Poling, 2016). The ideal time of the day to pick (harvest) strawberries is in the early morning hours when the strawberries are still cool before the heat builds up in the fruits altering the quality (Anjom, Vovgioukas and Slaughter, 2018).

Strawberry pickers pick strawberries by grasping the peduncle between the forefinger and thumbnail while pulling and twisting simultaneously to allow the fruit to roll onto the palm of a hand with about one-quarter of the peduncle still attached as they are delicate and can be bruised easily. Unblemished strawberries will last longer and store better than bruised strawberries (Maurstad, 2018). Harvested strawberries are placed in punnets with a capacity of 250 to 450g away from sunlight (Sharma and Singh, 2019). Harvested strawberries are moved as quickly as possible to the cold room to preserve their freshness (Morris and Sistrunk, 2018). The significant fungi causing fruit decay include *Botrytis sp*, *Mucor sp* and *Rhizopus sp* (Siedliska et al., 2018). Strawberries maintain their freshness for 3 to 7 days in refrigeration at 4°C. After that, the quality of the fruit diminishes (Lamb and Chuah, 2018).

1.12. Pest and diseases of strawberries

In the UK, the major diseases are grey moulds such as *Botrytis cinerea* and powdery mildew (Hall, Jin and Dodgson 2016). The fungal infection caused by *Botrytis cinerea's* first symptoms appears as a grey mould at the end of the stalk of strawberries. In strawberries, the area of infection will enlarge, and a sunken brownish grey area will develop in the middle of the mould (Hill, Henshall and Beresford, 2017). *Botrytis cinerea* (figure 1.16a) affects fully ripe strawberries but can also infect green fruits and eventually cover the whole of each affected fruit (Llanos and Apaza, 2018).

Botrytis cinerea fruit rot is considered a significant challenge for strawberry growers, and when conditions favour disease development, losses from fruit rot can exceed 50% (Vorotnikova, VanSickle and Borisova, 2012). In the UK, *Botrytis* disease infection is the second most significant cause of crop losses to the horticulture sector by declining harvest yield and marketability with a cost estimated at £54 million to the industry (Abbey et al., 2019).

Other diseases of strawberries include red core disease, crown rot and *verticillium* wilt. The red core disease is caused by the soil-borne fungus *Phytophthora fragariae* (figure 1.16b). In an average year, it is estimated that the loss to the industry from red core *Phytophthora fragariae* is in the region of £3 to £4 million, and even though preventative measures are applied to most crops, these figures are doubled in bad years (Cross, Fitzgerald and Down, 2005). *Phytophthora fragariae* affects the root system of strawberry plants (Adams, 2019). The first symptoms of the red core disease are usually seen in the late spring during wet conditions when healthy crops resume growth and infected plants remain stunted (Parikka et al., 2016). Plants with the red core disease may die as the season progresses and become subject to increasing stress levels during high temperatures (Fry, 2012).

Crown rot (*Phytophthora cactorum)* (figure 1.16c) of strawberries is a soil-inhabiting pathogen that occurs in strawberries planted in poorly drained, over irrigated soil and during long periods of rain in warmer climates (Toljamo et al., 2016). Early symptoms

of the disease can be found in stunting growth and wilting of young leaves of strawberry plants. The potential loss of plants to crown rot can reach 20 to 30 %, and in 2016, 90,000 tonnes of strawberries were sold in the UK season, with a market value of £386 million. If 25% of plant loss occurs in the UK due to crown rot, the volume of fruit could be reduced by up to 22,500 tonnes, representing a value of £96 million (Xiangming, 2018).

Verticillium wilt in strawberries is caused by *Verticillium dahlia* (figure 1.16d), and symptoms include stunted growth, delayed development and yellowing of lower leaves (Washburn, 2018). As the disease progresses, the older leaves wilt and dry up, while the younger central leaves of the plant remain green until the plant dies and all foliage turns brown (Wu et al., 2019). Depending on the pathogen population in the soil and susceptibility of the cultivar, losses can vary between 5 to 90%. If 25% of plant losses occur in the UK due to *Verticillium* wilt, it will represent lost revenue of £29 million; however, techniques and measures implemented to tackle *Verticillium* wilt could save such potential losses (Xiangming, 2018).

Strawberry powdery mildew (*Podosphaera aphanis*) is a significant cause of crop yield losses (Hall, Jin and Dodgson, 2016). Strawberry powdery mildew can infect all parts of strawberry plants, including leaves, petioles, stolons, flowers and supporting stems (Maas, 1998). Pests may become problematic for strawberry plants during their development. The most common strawberry pest includes spider mites, *Tetranychus urticae*, aphids *Chaetosiphon fragaefoilii,* white fly *Aleyrodidae* and slugs *Gastropoda* (Nellist, 2018). Spider mites *Tetranychus* urticae attack strawberry plants by puncturing the plant cells to feed (Autunović, 2018). The aphids *Chaetosiphon fragaefoilii* are common sap-sucking insects which can cause a distortion in plant growth and transmit plant viruses to strawberries (HE and FU, 2018).

The white fly *Aleyrodidae* typically feeds on the under part of strawberry plant leaves and slugs *Gastropoda,* which attack tender strawberry fruits by forming holes that some insects may use to further damage (Castle, Grass and Westphal, 2019). An invasive pest of strawberries is the spotted wing drosophila SWD (*Drosophila suzukii*), a fruit fly that originated in Japan and has spread worldwide (Hennig and Mazzi, 2018). The spotted wing drosophila spread to the USA, then mainland Europe and then arrived in the United Kingdom in 2012 (Farnsworth et al.,2017). The invasive pest, similar to several species of fruit and vinegar flies, is now found in the UK (Rice et al., 2017). The adult males have a distinctive spot on each wing, while females have a saw-like appendage (ovipositor) used to pierce developing fruits (Hoskins, 2018). The female SWD lays eggs under the surface of the fruit's skin, which hatches into larvae that

contaminate the fruit and feed on the flesh resulting in fruit collapse (Graham and Brennan, 2018).
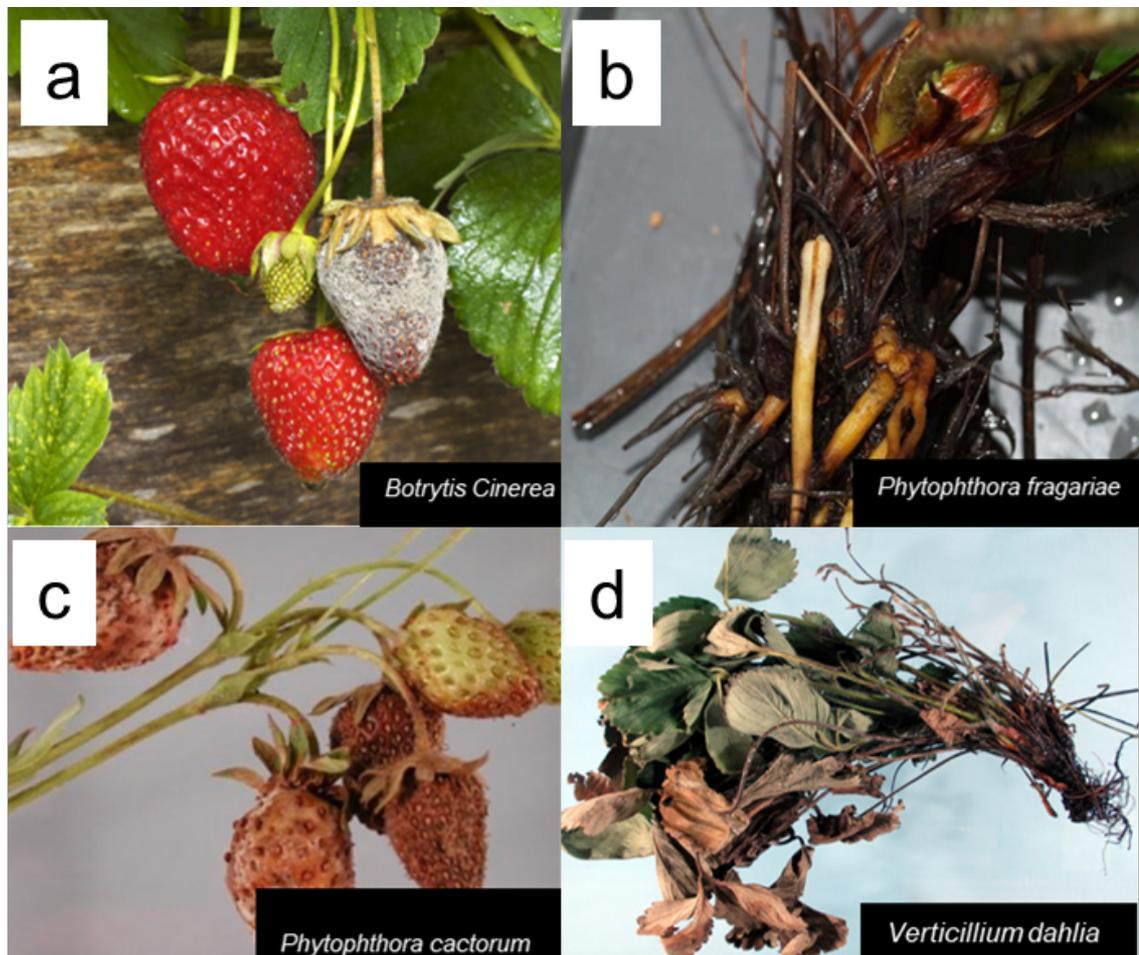
Figure 1.16. Diseases of strawberry plants. (a) *Botrytis cinerea* (fruit rot) (b) *Phytophthora fragariae* (red core) (c) *Phytophthora cactorum* (crown rot) (d) *Verticillium dahlia* (verticillium wilt). Source: Alchetron (2020); Pnwhandbooks.org (2020).

1.13. *Erysiphales* (Powdery mildews)

1.13.1. Introduction

Braun and Cook (2012) reported that there are over 650 species of *Erysiphales* (powdery mildew), and their order contains 1 family- *Ersiphaeae* and 16 genera. The *Erysiphales* are in the phylum Ascomycota (Takamatsu, 2013). They are obligate biotrophs (which obtain energy and nutrients from living cells) and infect almost every part of the plant (Spanu, 2012).

The term "powdery mildew" describes the appearance of whitish powder on infected plant parts as the conidiospores are produced in abundance on the surface of the host plants (Glawe, 2008). As this group of fungi cannot be grown on culture or artificial media, their taxonomy has been mostly based on morphological characteristics such as

ascocarps and ascospore morphology, conidial germination patterns, mycelial characteristics, appressoria morphology, and host range, among others (Meeboon and Takamatsu, 2015; Meeboon, Hidayat and Takamatsu, 2016). Nowadays, by using molecular techniques such as DNA sequencing and light and scanning electron microscopy, scientists can study the origin, distribution and migration of many species from this group in detail (Meeboon & Takamatsu, 2017). Figure 1.17 represents a phylogeny of tribes and genera of *Erysiphales*. The phylogeny is based on the 28S rDNA data set for 40 taxa of the *Erysiphales*, covering all known tribes and an outgroup taxon (Braun et al., 2006; Meeboon and Takamatsu, 2017).

1.13.2. General classification of *Erysiphales*

KINGDOM: Fungi
PHYLUM: Ascomycota (reproductive structure ascus)
CLASS: Leotiomycetes ascomycetes (primitive cup-fungi)
ORDER: *Erysiphales* (the powdery mildew fungi) Mycelium on the surface of the plant.
GENERA:
5 Tribes
FAMILY: *Erysiphaceae* (16 genera and up to 400 species)
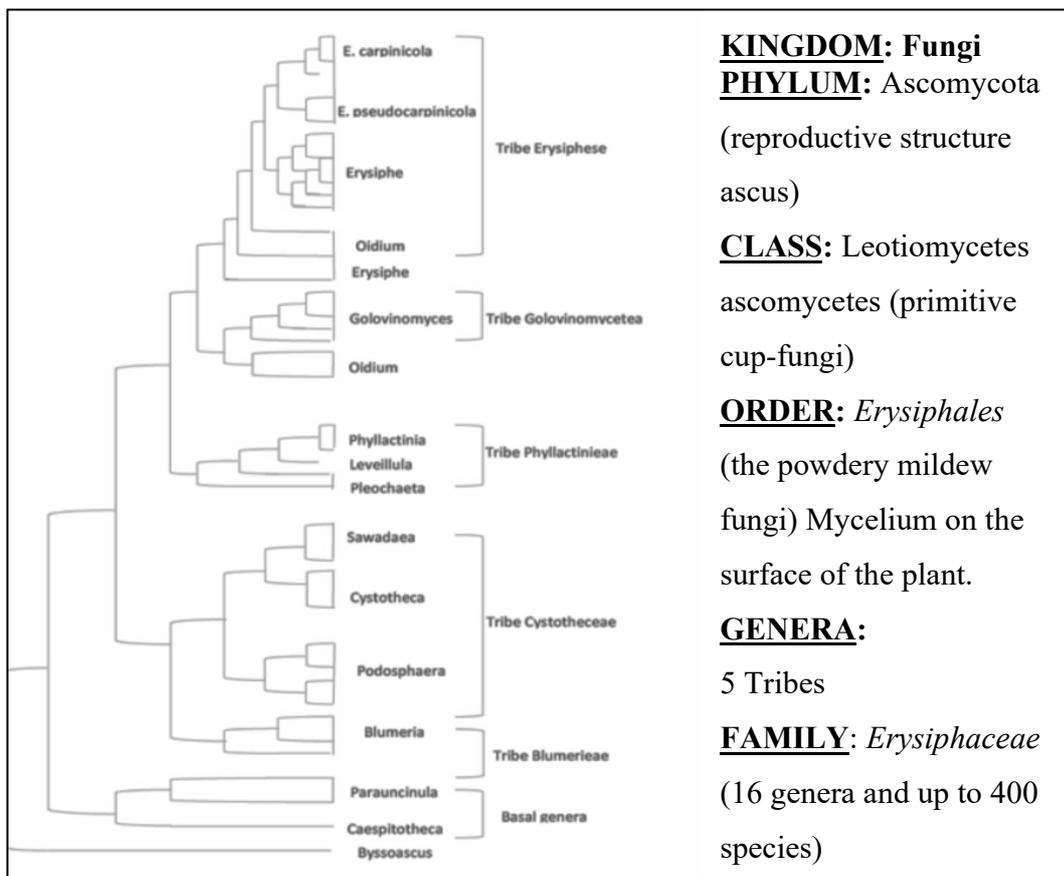
Figure 1.17. Phylogeny of tribes and genera of *Erysiphales.* Adapted from Braun, 2011 and Meeboon and Takamatsu, 2017

1.13.3. Infection

The process of infection of powdery mildew is initiated immediately after an ascospore or conidiospores lands on the surface of a host plant (Saharan et al., 2019; Glawe, 2008). The spore germinates and forms a germ tube that elongates at the tip to build an appressorium, from which hypha develops to produce the penetration peg (Glawe, 2006; Kabaktepe et al., 2017; Meeboon, Kokaew and Takamatsu, 2018). The penetration peg is used by the fungus to break the cuticle cell wall of the host and to

penetrate the epidermal cell through both enzymatic and mechanical pressure (Khodaparast, 2016). After penetration of the host plant, the fungus develops a haustorium, which is a feeding structure to supply the fungus with nutrients while the host cell is kept intact (Scholler et al., 2016).

1.13.4. The general life cycle of *Erysiphales*

The general life cycle of *Erysiphales* (powdery mildews) involves two stages asexual reproduction and sexual reproduction stage (Tulek and Canpolat, 2016). The asexual stage typically begins with the conidiospores landing on the host plant's surface, germinating, developing appressoria and penetrating the host cell wall (Braun, 1995). Conidiospores of powdery mildews are single-celled, colourless, and uninucleated and are usually produced in singles or chains (Tulek and Canpolat, 2016; Zhang et al., 2016).

The conidiospore germination can occur in a dry atmosphere as they are fully hydrated but may require relatively high humidity (Glawe, 2008). The conditions suitable are temperatures between 60 to 80°F (15 to 27°C) and moderate to high humidity, respectively (Hall, Jin and Dodgson, 2016). The dispersal of the conidium from the conidiospores (figure 1.18) depends on various factors such as electrostatic charges, wind, mechanical force and leaf shaking (Panstruga and Kuhn, 2019). The life cycle of strawberry powdery mildew is shown in figure 1.18.
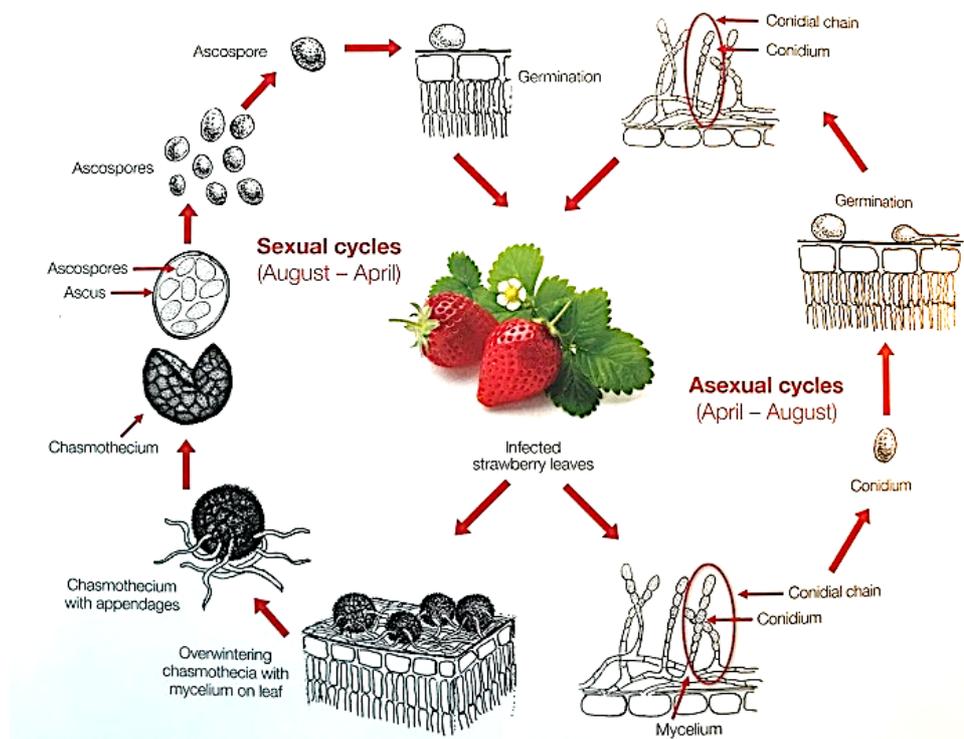
Figure 1.18. The life cycle of strawberry powdery mildew (*Podosphaera aphanis*).
Source: (Hall, Jin and Dodgson, 2016).

1.14. Strawberry powdery mildew *(Podosphaera aphanis)*

Podosphaera is a genus of the powdery mildew belonging to the tribe of *Cystotheceae* of *Erysiphaceae* (figure 1.17), and some other species of *Podosphaera* include; *Podosphaera macularis*, *Podosphaera Balsaminae*, *Podosphaera euphorbiae*, *Podosphaera clandestine*, *Podosphaera pannosa*, *Podosphaera spiraeae*, *Podosphaera fuliginea*, *Podosphaera clandestine var. cydoniae*, *Podosphaera leucotricha*, *Podosphaera mors-uvae*, *Podosphaera myrtillina*, *Podosphaera sp. Podosphaera* major and *Podosphaera fusca* (Takamatsu et al., 2010). Strawberry powdery mildew, *Podosphaera aphanis* (figure 1.18), is the most important fungal disease of protected strawberry crops, negatively impacting strawberry production worldwide (Hall & Jin, 2016). The disease accounts for up 70% yield loss, which amounts to a market value of £56.8 million (Hall, Jin and Dodgson, 2016).

1.14.1. The life cycle of *Podosphaera aphanis*

The fungus develops from spore germination, mycelium development and spore production, which is influenced by temperature and humidity (table 1.6). The optimum temperatures are between 15 – 30°C and relative humidity > 60% - 100% (Hall, Jin and Dodgson, 2016). Disease levels are mainly caused by the asexual conidiospores, which are similar genetically. At the same time, the sexual reproduction of *Podosphaera aphanis* leads to the formation of an ascus enclosed in a chasmothecium (figure 1.18) (Jin and Hall, 2012).

The mature chasmothecium contains one ascus and eight ascospores (figure 1.18). The chasmothecium forms multiple visible round black cases (ascocarps, which are genetically dissimilar in ascus and chasmothecia) on the lower surface of the leaf. The chasmothecia are the fungus's long-term survival structures involved in over-wintering the fungus on debris, green leaves and crop trash. The germination and spore production time for *Podosphaera aphanis* is 7-14 days under favourable conditions (Jin and Hall, 2012). The genetically similar asexual conidiospores are responsible for the disease level build-up, and reproduction begins from April to August. Jin and Hall (2012) explained that the chasmothecia (overwintering structures) are formed from August to April. Asalf et al. (2013) stated that the ascospores within the chasmothecium are formed by the sexual reproduction of the fungus.

Previous work has shown that the number of chasmothecia formed is determined by the level of infection during the summer season (Jin and Hall, 2012). They also mentioned that the percentage of mycelium on the leaf in the summer could significantly increase the number of chasmothecia formations during the autumn and winter season. An example is a percentage of mycelium (disease levels) found on leaves of strawberry plants in field experiments, which reached 30%, and levels of chasmothecia found were greater than 50% at the end of the strawberry growing season from September 2011 to 2012 (Jin and Hall, 2012).

1.14.2. Infection and symptoms

1.14.2.1. Infection

Hall, Jin and Dodgson (2016) pointed out that at the start of each season, it is important to consider the newly planted and over-wintered crops. In newly planted crops, the inoculum often develops during the process of propagation with the conidiospores and mycelium existing on plants at the time of delivery and planting (Oliveira, Braga and Rangel., 2018). In the case of over-wintered crops, the fungus survives within the crop through infected plants or as chasmothecia on debris, green leaves and dead leaves.

The fungus grows between the host cells, invading only a few cells to produce nutrient-absorbing structures known as Haustoria (Chowdhury, Coad and Little 2018). This enables the extraction of nutrients from the host plant through the hyphae penetrating the epidermal cells (Ellingham, 2017). After infection occurs, the leaves curl up, known as cupping (figure 1.19a). The leaf's surface is covered by white powdery patches known as the mycelium consisting of conidial chains, clusters of genetically similar asexual conidiospores (figure 1.19b), which spread from the lower to the upper surface of the leaf.

1.14.2.2. Symptoms

The disease displays a series of symptoms. Healthy strawberry leaves are naturally flat, but after infection, they begin to cup (figure 1.19a). However, other cupping causes could be a virus infection or pest infestation (Gaudery et al., 2010). Depending on the variety, blotches begin to form (figure 1.19b). Mycelium is formed (figure 1.19c), and the development of asexual conidiospores produces the powdery effect, which may be seen first on the lower than upper surfaces of the leaves and petiole. However, in some cases, the mycelium becomes visible first on the leaves, petioles and peduncles and spreads early to the fruit (Hall, Jin and Dodgson, 2016).
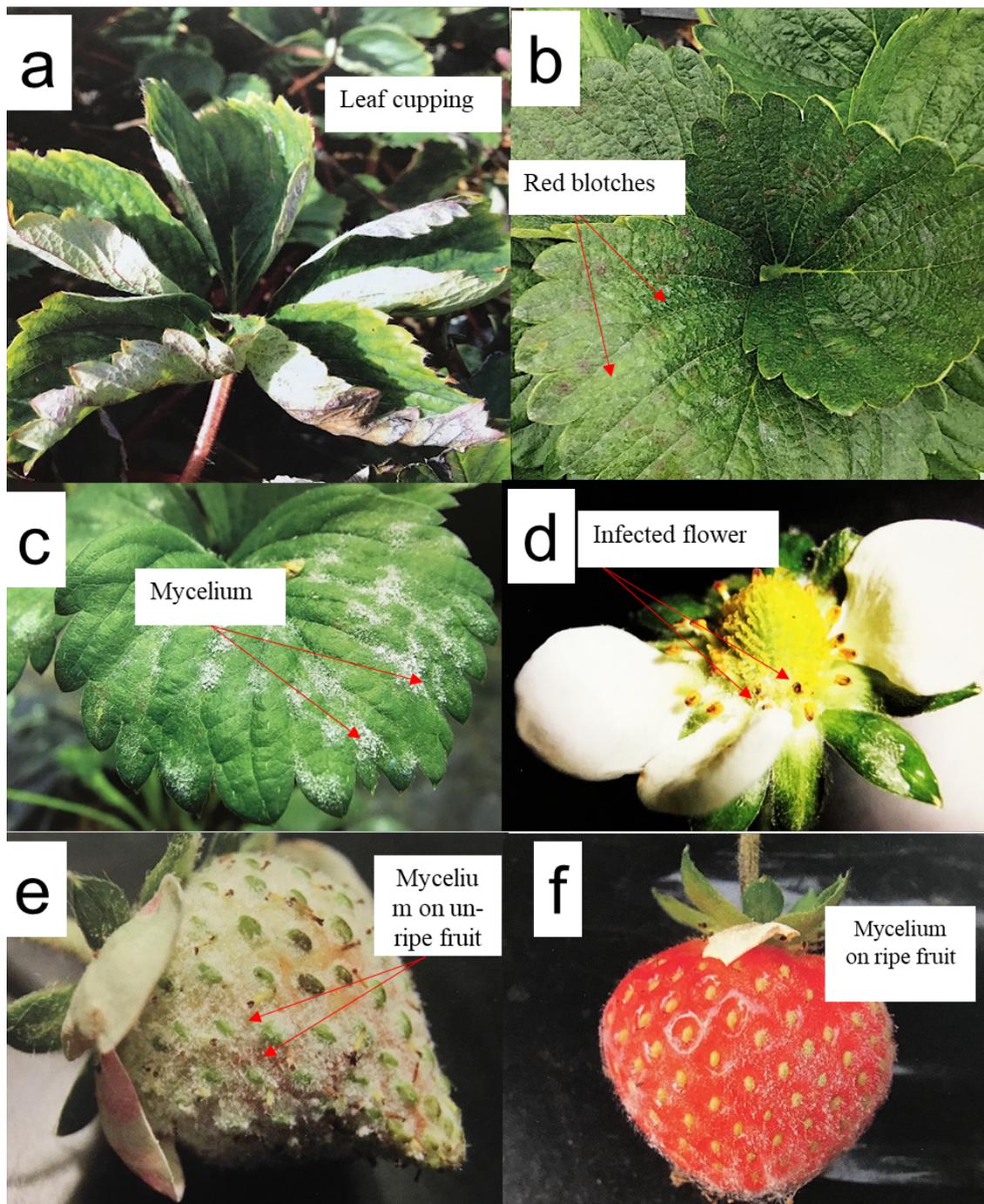
Figure 1.19. Symptoms of *Podosphaera aphanis* on strawberries. (a) Leaf cupping (b) Leaf blotching (c) Mycelium on leaves (d) Infected flower (e) Mycelium on unripe fruit, and (f) Mycelium on ripe fruit. Source: (Hall, Jin and Dodgson, 2016).

1.15. Epidemiology and the environment

1.15.1. Epidemiology and spread

The rate at which the disease spreads depend on the inoculum's presence and favourable environmental conditions, especially temperature and relative humidity. Table 1.6 summarises the conditions that affect the life cycle of *Podosphaera aphanis*. The

dispersal of the conidiospores depends on various factors such as electrostatic charges, wind, mechanical force, and leaf shaking (Stankevičiené, 2017). Conducive temperatures and relative humidity for powdery mildew development are shown in table 1.6.

Table 1.6. Conditions for strawberry powdery mildew conidia.

Variable		Germination	Infection	Sporulation
Temperature (°C)	Minimum	2 - 5	5	1 3
	Optimum	15.5 - 30	18 - 30	20
	Maximum	30 - 35	30	35
Relative humidity (%)	Minimum	8 -12	No effect	No effect
	Optimum	97 -100	No effect	No effect
	Maximum	100	No effect	No effect
Presence of free water (immersion time hours)		Up to 3	No effect	No effect
Time of day (hrs)	Minimum	No effect	No effect	20.00 – 8.00
	Maximum	No effect	No effect	12.00 – 16.00

Source: (Hall, Jin and Dodgson, 2016)

Powdery mildew disease infection and spread can be managed by the frequent use of fungicide (sprays) application using a crop sprayer (tractor) (figures 1.20a and b). The crop sprayer (tractor) sprays fungicides over each strawberry bed as it moves through the field tunnel. See how fungicides treatments are sprayed over crops in figure 1.20b below.
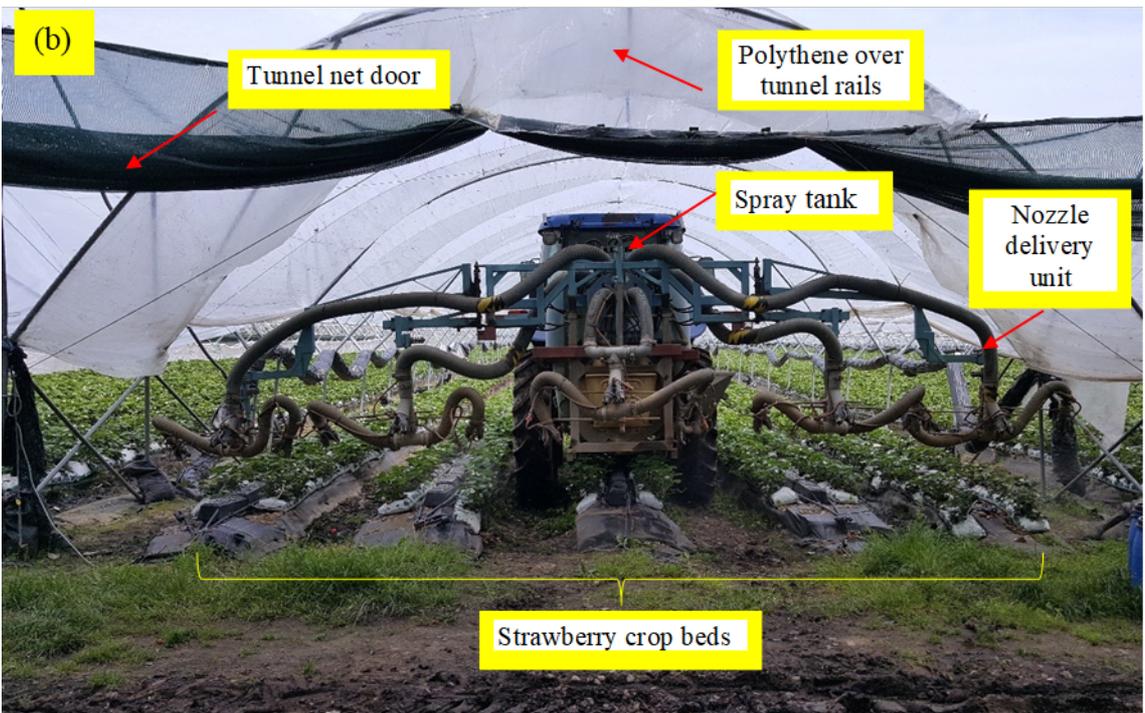
Figure 1.20. Fungicide crop sprayer on Ladybird field.

1.16. Plant defence mechanisms

1.16.1. Constitutive defence

Plant cell walls are formidable barriers to infection, and most organisms can only cause disease when the barrier (plant cell walls) is breached (Boots and Best, 2018). Most plants invest heavily in thick cell walls and cutin or suberin layers (Fuchs and Krauss, 2018). The suberin is a lipophilic macromolecule found in plants when insulation or protection is required, and suberized cells form epidermis. This tissue envelops

secondary stems as part of the bark and develops as the healing tissue (sealing) after wounding or leaf abscission (Graça, 2015). Figure 1.21 shows the cross-section of a strawberry leaf displaying the components of the plant's defence mechanism. In the case of an invasion of a pathogen, as explained by Debona et al. (2017), the silicon deposited (accumulated) is laid down mainly in the cuticle, epidermis and palisade layer, thereby hindering the appressorium of a fungus breaking through the cuticle wall to form a penetration peg.
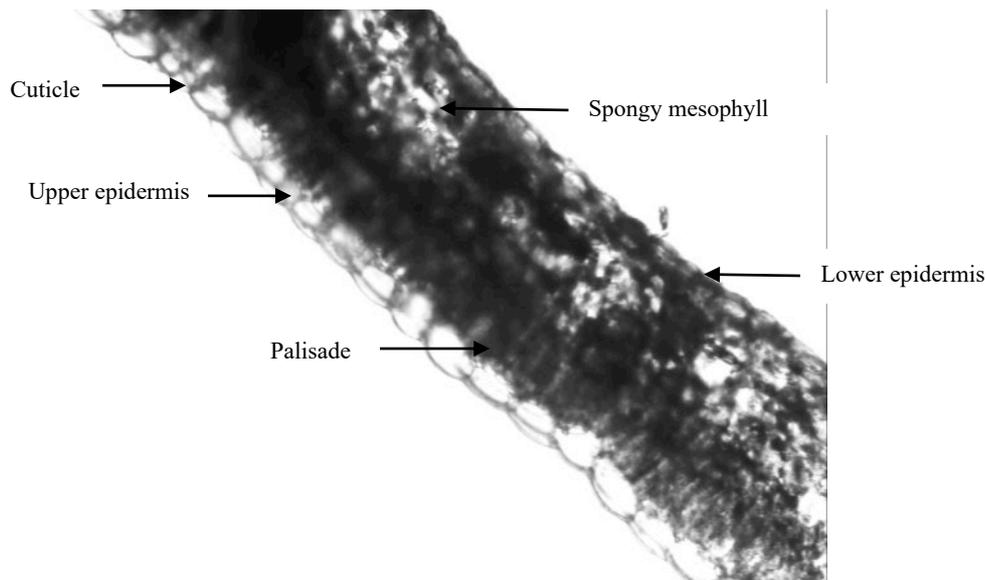
Figure 1.21. The cross-section of a strawberry leaf (UV light)

The constitutive defence mechanism is always present in the plant. It includes various preformed barriers such as cell walls, waxy epidermal cuticles and bark, which protect the plant from invasion and give it strength and rigidity (figure 1.21 and 1.22) (Pančić and Kiørboe, 2018). Nollet and Gutierrez-Uribe (2018) reported that compounds (phenols and quinones) were identified that explained the differential resistance of onion cultivars to the pathogen.

He explained that onion cultivars with outer scale leaves that were red or yellow were resistant to smudge caused by *Collectotrichum circinans,* while those with white scale leaves were susceptible. The usefulness of the constitutive defence mechanism is that if the infection is prevented through the constitutive defence pathway, there is no direct damage caused by the pathogen and little risk of damage due to this defence system (Boots and Best, 2018). The characteristics of plant defence mechanisms are described in figure 1.22.
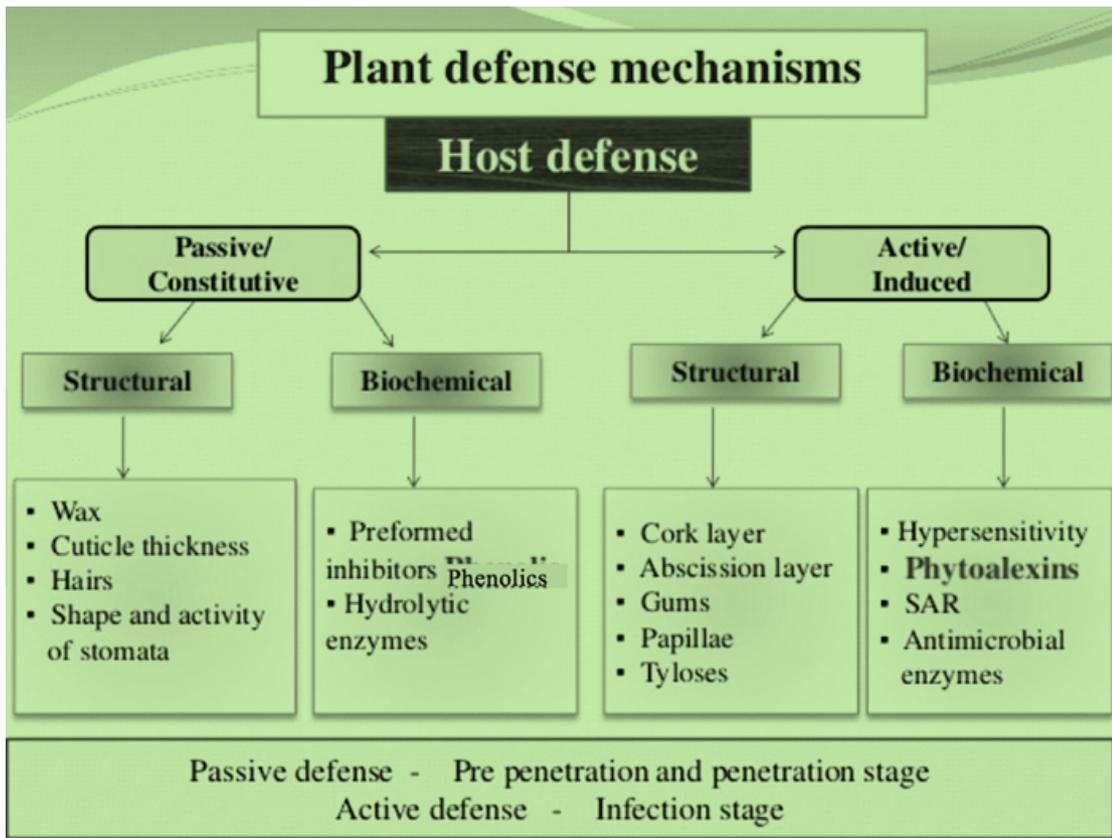
Figure 1.22. Plant defences. Source: Binder and Parniske (2018). The figure shows that plant defence mechanism are divided into two main pathways. The passive or the constitutive and the active or the induced defence mechanism.

Figure 1.23 below explains how a plant's defence mechanism is enhanced, which plays a role in deterring pathogen penetration and where the silicon is deposited in the epidermal cell of leaves of rice plants. This diagram is explained in figure 1.23 a, b, c below.

Debona et al. (2017) showed in figure 1.23 (a) that silicon formed a physical barrier beneath the cuticle and cell wall, which explains the cause of the reduction in rice blast severity. He explained (figure 1.23(b)) that the dis-uniformity in silicon deposition underneath the cuticle wall allowed the penetration peg of the fungus *P. oryzae* of the rice plant. He also showed that the sizes of the rice blast region were larger (figure 1.23 (c)) due to unlimited colonization of the epidermal and mesophyll cells by fungal hyphae.

31
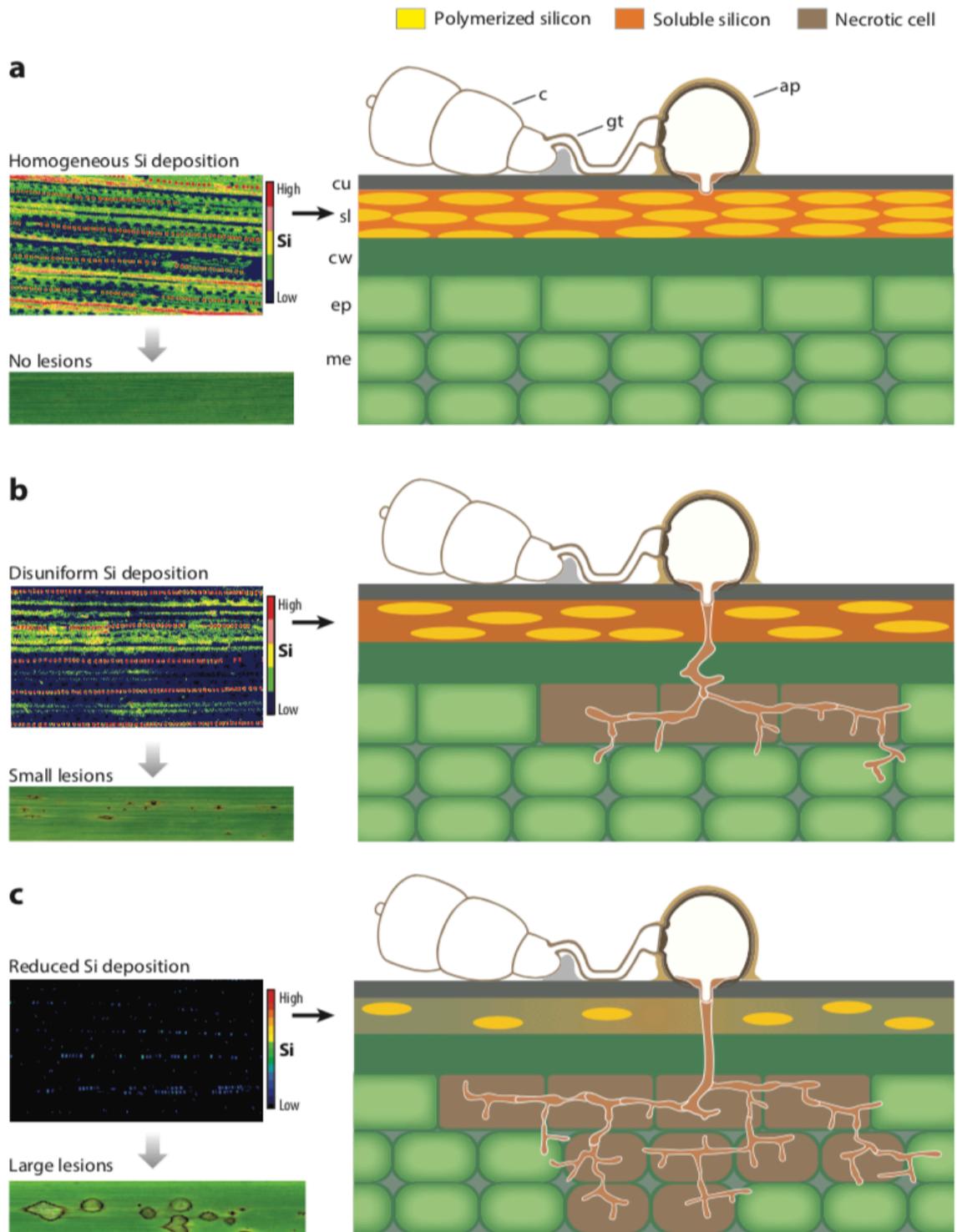
Figure 1.23. Silicon deposition in the epidermal cell of leaves of rice plants. Source: Debona et al. (2017).

Alhousari and Greger (2018) researched silicon and the mechanism of plant resistance to insect pests on rice plants. The work investigated found that bio-available silicon is deposited as a 2.5μm thick layer beneath the cuticle layer, forming a silicon-cuticle double layer in rice leaf blades. They mentioned that the abrasiveness of silicified wax,

cuticle, hairs and other tissues linked with plant protection, storage, support and strengthening leads to the irreversible wear of mouth parts while insects are feeding, therefore deterring chewing insects (Alhousari and Greger, 2018). Jin (2015) found that adding silicon to strawberries formed greater and thicker wax density on the adaxial leaf surface and reduced the disease levels of strawberry powdery mildew.

Silicon was shown to reduce disease levels of strawberry powdery mildew and increase the length and number of leaf hairs in strawberry plants (Fatema and Hall, 2012). Wang et al. (2017) explained that silicon accumulation in the epidermal tissue and thickening cell walls prevents pathogen penetration. He further explained that silicon-enhanced constitutive defence mechanism is associated with the density of silicified long and short epidermal cells, a thick layer of silica beneath the cuticle and thickness of the cell walls as physical barriers against pathogen penetration. Yang et al. (2017) showed that adding silicon to rice *Oryza sativa* improved the silicification of leaf sheaths that brown plant hoppers feed on, reducing the number of brown plant hoppers. Enhanced constitutive defence is believed to result from increased rigidity and reduced digestibility of plant tissue to pests through the addition and deposition of silicon in plants (Hernán et al., 2019).

1.16.2. Active defence

Defence against infectious disease arises from a complex set of interdependent mechanisms varying from mechanical barriers to the complex array of effectors in the immune system (Veloso and van Kan, 2018). The active (induced) plant defence is produced in reaction to infection, damage or stress caused by herbivores or diseases. It will only shorten the infectious period of the plant (Kupfer and Fessler, 2018). Nearly all living plants can detect invading pathogens and react with inducible defences such as pathogen-degrading enzymes, production of toxic chemicals and deliberate cell suicide (Pančić and Kiørboe, 2018). The active (induced) defence response often waits until a pathogen invasion before producing chemicals or defence-related proteins due to the high energy and nutrient requirements associated with their production and maintenance. Wang et al. (2017) found in a recent study that silicon, concerning the active defence pathway, acts by specifically building up in the area of infection to prevent it from further damage to the plant. Yang et al. (2017) published a study on silicon amendment is involved in the induction of plant defence response to a phloem feeder. It demonstrated that silicon amendment initiates plant resistance to herbivores through the plant's constitutive and active defence mechanism.

1.16.3. Silicon (Si)

In 1824, Silicon (Si) was first isolated and described as the seventh most abundant element in the universe and also known as the second most abundant element on the earth by Swedish chemist Jon Jacob Berzelius (Epstein, 1994). Silicon is in all plants (leaf hairs) and has various benefits. Silicon has an atomic number of 14 and is

generally in the form of ordinary sand; however, it can be found as rock crystals, quartz, amethyst, flint, opal, jasper and agate (Fauteux et al., 2005).

Silicon in a bioavailable form, is absorbed by most plants, conifers, sugar cane, ferns, moss, rice, wheat, potatoes, cassava, soybeans, sugar beet, barley, tomato and corn (Laing et al., 2015). The accumulated concentration can vary from 1% to above 10% (Datnoff and Rodrigues, 2007). Silicon accumulators accumulate high levels (>1.5%) of silicon in their tissue, "intermediate" accumulate moderate levels (0.5-1.5%), and non-accumulators have low levels (<0.5%) of silicon (Alhousari and Greger, 2018). Silicon accumulators are known to be able to take up and deposit silicon. Jin et al. (2012) maintains that although the strawberry plant is considered a non-accumulator of silicon, if the concentration of bioavailable silicon is greater than 1% of dry weight, then the plant is classified as a silicon accumulator. Plants absorb silicon through silicon transporter genes in the roots (Ouellette et al., 2017).

1.17. The effects of silicon on plant diseases

According to Ma and Yamaji (2006), silicon is a plant nutrient not considered essential for the plant. However, it has a positive impact on decreasing disease intensity. Today, bioavailable silicon reduces the powdery mildew of strawberries, apples, roses, cucumbers, wheat, peas, melon, soybean, banana, tomatoes etc. (Rodrigues et al., 2015; Wang et al., 2017). The work conducted in this thesis investigated silicon use in different root applications for three consecutive years from the start to the end of the growing season. Fatema and Hall (2012) identified silicon to have a positive impact in reducing the disease levels of strawberry powdery mildew and increasing the length and number of leaf hairs on the upper and lower surface of the strawberry leaf (Fatema and Hall, 2012).

Silicon acts on the plants by modifying the wax and cuticle and building up the plant's defence mechanism in response to infection (Jin and Hall, 2012). Some other positive effects of silicon include increased °Brix levels, where Jin (2015) showed that silicon nutrients significantly increased °Brix values in strawberry leaves and ripe fruits. Liu (2016) also showed a significant increase in °Brix of leaf petioles with silicon. °Brix is the total amount of sugar content in any aqueous solution determined by the refractometer (chapter 2, section 2.5.1). The average strawberry fruit °Brix reads 10, with 14 being good and 16 excellent (Harrill, 1998). Growers test strawberries for °Brix (sweetness) to ensure that it meets the demands of supermarkets such as Tesco. Silicon application has also been shown to encourage rapid growth and improve the ability of plants to withstand harsh environmental conditions (Pärtel, 2016). Research conducted at the University of Hertfordshire has shown that silicon nutrient applied once and twice a week in a fertigation field trial at Maltmas farm Wisbech significantly reduced the severity of the disease (Liu, 2016). Work at the University also investigated the

utilisation of silicon nutrients with and without the addition of Potassium Carbonate ($KHCO_3$) in a tank mixture to reduce disease levels (Fatema and Hall, 2012).

A recent study aimed to investigate the level of disease control using Potassium Bicarbonate (K50) and silicon-based wetter, both alone and in combination (Jin and Hall, 2012). The result described the control of the disease level when K50 alone was less significant than the addition of silicon. However, silicon applied alone significantly reduced the number of germinating ascospores and colonies in the trial (Fatema and Hall, 2012). This has shown that silicon can significantly reduce the susceptibility to strawberry plant disease. Jin (2016) reported that a concentration of silicon and silicon nutrient with K50 foliar spray treatment inhibited the disease level build-up of *Podosphaera aphanis* compared to the untreated plants. Liu (2016) found that using a silicon nutrient reduced susceptibility to *Podosphaera aphanis* and two-spotted spider mites. Previous work carried out also showed that silicon nutrients may play a positive role in raising °Brix (measurement of total sugar content in liquids or juices) of strawberry leaf, and petiole, improving pollen viability and influencing the length of flower receptacle and stamens (Liu, 2016).

Tibbits (2018) showed a significant increase in the seed yield of wheat when silicon nutrient was added to a hydroponic solution. The work showed a visual difference in awn straightness when silicon was added. High silicon-treated wheat plants had straight awns, and treatment with lower silicon concentration had twisted awns. Tibbits (2018) showed that the density of trichomes (leaf hairs) on awns increased significantly, and leaves of high silicon-treated wheat plants were stiffer and did not bend easily, suggesting that silicon provided structural support to the leaves of wheat plants. Korkmaz et al. (2018) showed a positive effect of silicon on yield and fruit quality in tomato crops grown in a closed hydroponic system. Diseases known to be effectively reduced (in severity) by silicon are shown in tables 1.7 and 1.8. Most research is mainly shown in silicon accumulators.

Tables 1.7 and 1.8 include the different crops: silicon accumulators, intermediate accumulators and non-accumulators. Tables 1.7 and 1.8 below show that silicon positively affects many diseases, and a fungus causes all diseases mentioned below. It is also shown in tables 1.7 and 1.8 that disease reduction is recorded in both silicon accumulators and non-accumulators plants. Silicon reduces several powdery mildews, and brown rust of wheat is the only disease silicon does not affect (table 1.7).

Table 1.7. Effects of silicon on disease severity

Crop Species	Disease	Pathogen	Effect of silicon on disease control	Uptake of silicon
Barley	Powdery mildew	*Erysiphe graminis f. sp. hordei*	Decreases	Silicon accumulators
Barley	Black point	*Alternaria spp.*	Decreases	Silicon accumulators
Corn	Stalk rot	*Pythium aphanidermatum.* *Fusarium moniliforme*	Decreases	Silicon accumulators
Rice	Brown spot	*Chochtiobolus miyabeanus*	Decreases	Silicon accumulators
Rice	Sheath blight	*Thanatephorus cucumeris*	Decreases	Silicon accumulators
Rice	Leaf scald	*Monographella abescens*	Decreases	Silicon accumulators
Rice	Stem rot	*Magnaporthe salvinti*	Decreases	Silicon accumulators
Rice	Grain discolouration	*Many fungal species*	Decreases	Silicon accumulators
Sorghum	Anthracnose	*Colletotrichum graminicola*	Decreases	Silicon accumulators
Sugarcane	Rust	*Puccinea melanocephala*	No effect	Silicon accumulators
Wheat	Powdery mildew	*Blumeria gramminis*	Decrease	Intermediate accumulators
Wheat	Brown rust	*Puccinia recondita*	No effect	Intermediate accumulators
Wheat	Foot rot	*Fusarium spp.*	Decreases	Intermediate accumulators
Wheat	Leaf spot	*Phaeosphaeria nodorum*	Decreases	Intermediate accumulators
Pea	Leaf spot	*Mycosphaeralla pinodes*	Decreases	Silicon accumulators
Soybean	Stem canker	*Diaporthe phaseolorum*	Decreases	Silicon accumulators

Table 1.8. Effects of silicon on disease severity continued

Crop Species	Disease	Pathogen	Effect on disease control	Uptake of silicon
Strawberry	Powdery mildew Pestalotia leaf spot Anthracnose fruit rot	*Podosphaera aphanis* *Pestalotia longisetula* *Colletotrichum acutatum*	Decreases	Non-accumulator
Cucumber	Powdery mildew Leaf spot Gray mould rot Crown and root rot Fusarium wilt	*Sphaerotheca xanthii* *Corynespora citrllina* *Botrytis cinerea* *Pythium ultimum* *p.aphanidermatum* *Fusarium oxysporum f.sp.* *Cucumerinum*	Decreases	Intermediate accumulators
Coffee	Coffee leaf rust	*Hemileia vastatrix*	Decreases	Silicon accumulators
Grape	Powdery mildew	*Uncinula necator*	Decreases	Intermediate accumulators
Melon	Powdery mildew Pink rot Fusarium	*Podosphaera xanthii* *Trichothecium roseum* *Fusarium semitectm*	Decreases	Intermediate accumulators
Lettuce	Pythium root rot	*Pythium spp.*	Decreases	Silicon accumulators
Yellow passion fruit	Bacterial spot	*Xanthomonas axnopodis pv. passiflorae*	Decreases	Silicon accumulators
Tomato	Powdery mildew	*Oidium neolicopersici* *Pseudomonas syringae pv. Tomato*	Decreases	Non-accumulators
Pumpkin	Powdery mildew	*Sphaerotheca xanthii*	Decreases	Intermediate accumulators
Rose	Powdery mildew	*Sphaerotheca pannosa*	Decreases	Intermediate accumulators
Zucchini squash	Powdery mildew	*Podosphaera xanthii*	Decreases	Silicon accumulators
Peach	Brown rot	*Monilinia fructicola*	Decreases	Intermediate accumulators
Rye	Powdery mildew	*Erysiphe graminis*	Decreases	Silicon accumulators

Chapter 2 - General material and methods for all chapters

This chapter covers i) A general introduction to the field experiment site used in this research project – Maltmas Farm, Wisbech; ii) A summary of general methods used for field, laboratory and glasshouse experiments.

2.1. Farm description

Experimental field experiments were established at a commercial strawberry-growing farm in Maltmas, Wisbech, Cambridgeshire, PE14 0HS (figure 2.1). The growers Harriet & Henry Duncalfe manage the County Council farm as a family business with 86 hectares. The farm site is commercially used for soft fruit production; 14 hectares are dedicated to strawberry production, 19 hectares for raspberry production and other arable crops, including wheat, 42 hectares, and oilseed rape, 24 hectares.

At Maltmas farm, strawberries are commercially grown in coir bags on raised beds under polythene tunnels (chapter 1, figure 1.7). The polythene tunnels are designed to protect crops from unfavourable climatic conditions. Fleeces and mulch (chapter 1, figure 1.6) are also used early in the season under the tunnels to stimulate early flowering and prevent plants from being damaged by frost. The farm uses a fertigation system to apply fertilizers, silicon nutrients and water to the crops. The Malling Centenary plants (June bearers), which are more susceptible to strawberry powdery mildew *Podosphaera aphanis,* were planted and established in the Ladybird field tunnel in the 2016 silicon fertigation field experiment (figure 2.3). Amesti plants (Ever bearers) more resistant to strawberry powdery mildew *Podosphaera aphanis* were planted and established in the Amelia field tunnel for the 2017 and 2018 silicon fertigation field experiments (figure 2.3). Figure 2.2 is the strawberry pack house located at Maltmas farm, and the legend below includes the process followed after strawberries are picked straight from the fields to the pack house.
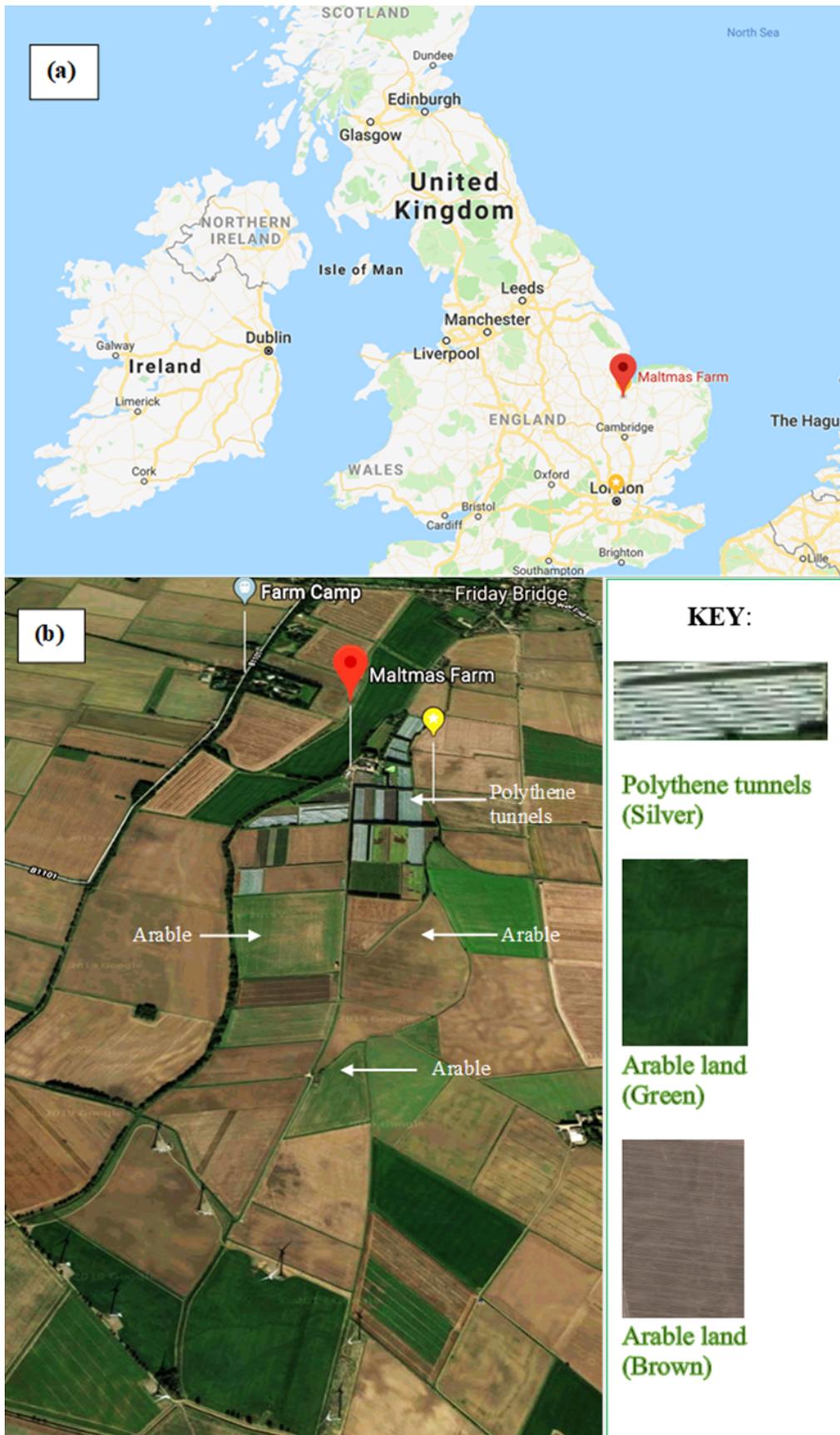
Figure 2.1. (a) Map location of Maltmas farm, Wisbech, Cambridgeshire. Source: Google Maps (2018). (b) Map location of Maltmas farm, Wisbech, Cambridgeshire. Source: Google Maps (2018).
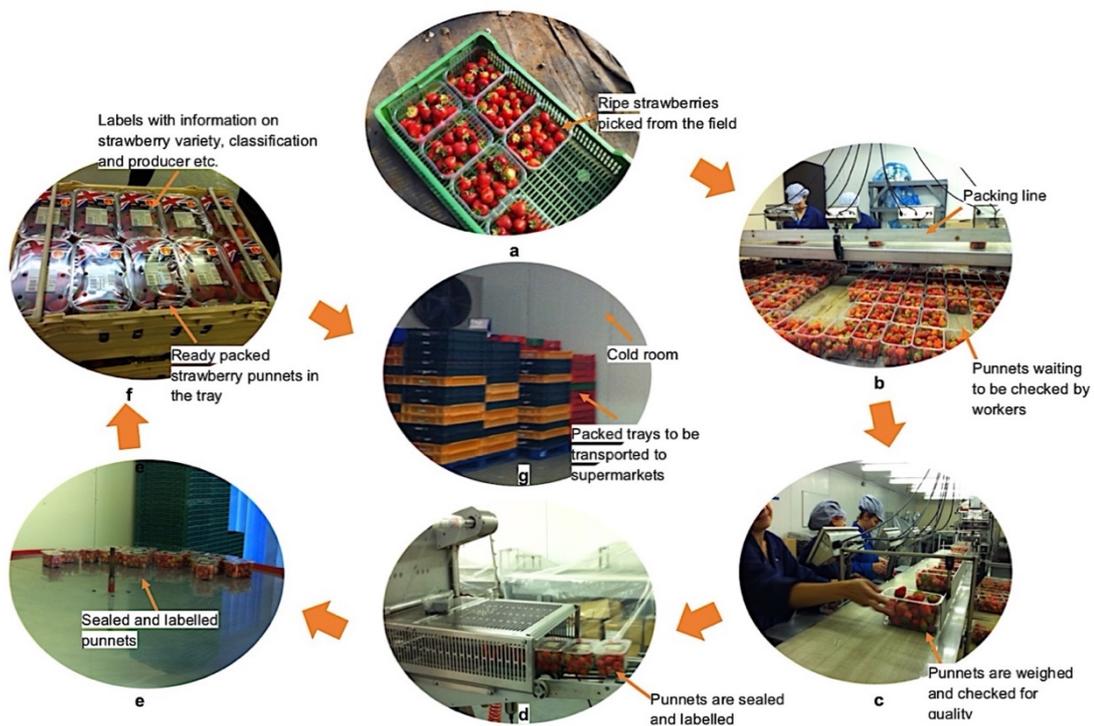
Figure 2. 2. heat sealed with the plastic film machine; e) Sealed strawberry punnets are separated; f) Labels with information on strawberry variety, classification and producer are requested by supermarkets; g) Strawberry punnets stored in cold room waiting to be transported to supermarkets. Source: Liu (2016).

2.1.1. Description of field experiments in 2016, 2017 and 2018

The silicon fertigation field experiments were carried out from 2016 to 2018 (table 2.1). Descriptions of all field experiments are listed below:

- **Silicon fertigation field experiments (chapter 3): 2016 Ladybird Field, 2017 and 2018 Amelia Field**

The silicon fertigation field experiments were established in the Ladybird tunnel in 2016 and the Amelia field in 2017 and 2018 (figure 2.3). The farm map with fields highlighted is in Appendices 7 and 8.

2.1.2. Effects of the use of silicon on disease levels of powdery mildew on strawberries: 2016 Ladybird field and 2017 to 2018 Amelia field

The silicon fertigation field experiment in the Ladybird field was carried out between 19 April and 9 August 2016, the silicon fertigation field experiment in 2017 was carried out between 9 April and 27 August 2017 and the 2018 silicon fertigation field experiment was carried out between 24 April and 21 September 2018. All silicon fertigation field experiments were set up in polythene tunnels. All polythene tunnels (Ladybird and Amelia field) were 180 metres long. A summary of all field experiments carried out at Maltmas Farm, Wisbech, between 2016 and 2018 is shown in table 2.1.

Table 2.1. A summary of all field experiments carried out at Maltmas Farm, Wisbech, between 2016 and 2018.

Year	Experiment field	Cultivation type	Area /ha	Strawberry variety	Summary of experiment
2016	Ladybird (figure 2.3)	Tunnel	1	Malling Centenary (June bearers) More susceptible to *Podosphaera aphanis*	▪ The effect of silicon nutrients on disease levels of strawberry powdery mildew (*Podosphaera aphanis*)- Disease assessments ▪ Silicon contents in leaves and fertigation water ▪ Silicon extraction from strawberry leaves
2017	Amelia (figure 2.3)	Tunnel	2	Amesti (Ever bearers) More resistant to *Podosphaera aphanis*	▪ The effect of silicon nutrients on disease levels of strawberry powdery mildew (*Podosphaera aphanis*)- Disease assessments ▪ Silicon contents in leaves and fertigation water ▪ Silicon deposition in the fertigation field experiment ▪ °Brix measurements
2018	Amelia (figure 2.3)	Tunnel	2	Amesti (Ever bearers) More resistant to *Podosphaera aphanis*	▪ The effect of silicon nutrients on disease levels of strawberry powdery mildew (*Podosphaera aphanis*) ▪ Disease assessments ▪ Silicon contents in leaves and fertigation water ▪ Silicon deposition in fertigation field experiments
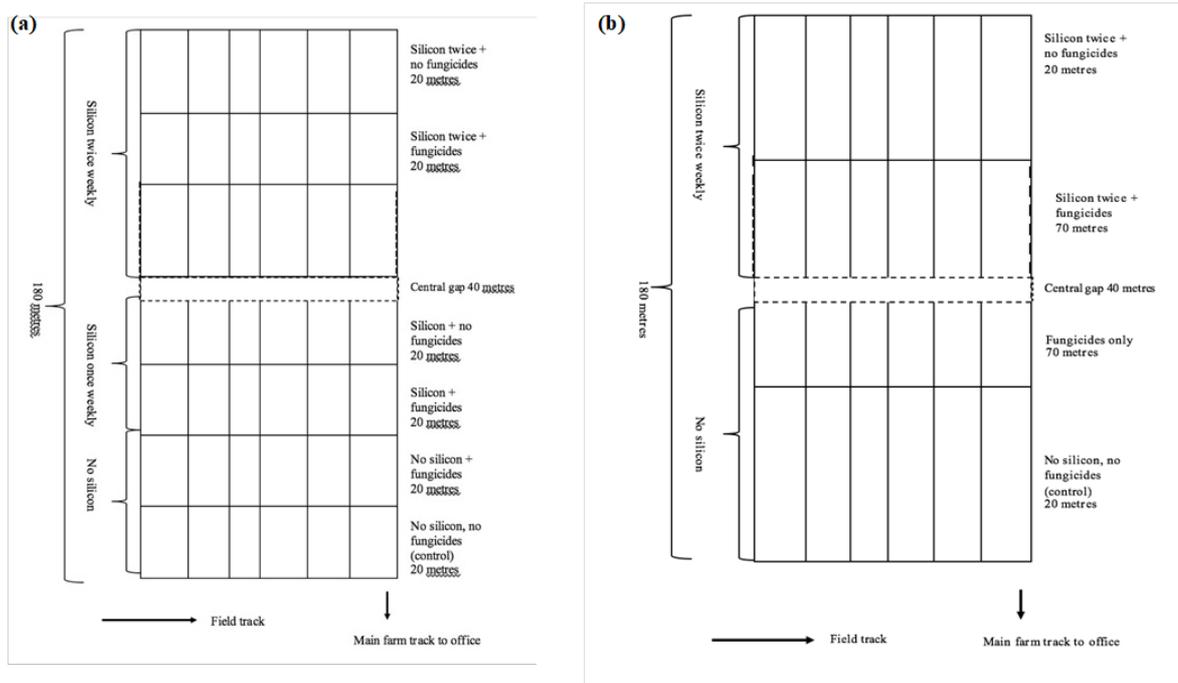
Figure 2.3. The Ladybird (a) and Amelia silicon field (b) experiment plan 2016 to 2018. Ladybird tunnel (180 metres) contains 6 treatments, while the Amelia tunnel (180 metres) contains 4 treatments. Silicon is applied through the fertigation tubes (drippers), and fungicides are applied through a crop sprayer (figure 1.20).

Treatments used in both field experiments (figure 2.3a and b) were set up in line with the fertigation system to enable commercial purposes such as strawberry picking during harvest season. The silicon nutrient used was 'Sirius,' a bioavailable silicon form containing 1-10% Polyether-modified polysiloxane, 0.5 – 1% Ethyl alcohol (ethanol), 70 – 80% Tetraethyl silicate and 10 – 20% Alkyloxypolyethyleneoxyethanol. For the Sirius composition, see Appendix 5. The fertigation system consists of tubes, which provide water and silicon to the crops. This design plan was established to enable the fertigation system work where some treatments on the field had silicon applied once and twice weekly (in 2016) while in the others, no silicon was applied in half the field and silicon applied in the other half of the field (2017 and 2018). However, limitations to this design containing adjacent treatments are that it can encourage the spread of disease across the block of treatments, thereby allowing contamination through the fields.

- **Field experiment methods**

This general material and methods chapter includes methods that apply to several chapters. In contrast, specific methods are provided in the appropriate chapters for reducing levels of disease in chapter 3, the silicon deposition experiment in chapter 4 and the strawberry growth parameters experiment in chapter 5. Field experiment methods mainly consist of; field sampling of strawberry leaves (fortnightly) for strawberry powdery mildew disease assessment, fruit and leaf petiole sampling (once a month) for ºBrix measurements, silicon extractions from strawberry leaves on the field

(once a month), silicon contents measurements in fertigation field water, silicon deposition assessments in strawberry plants in a glasshouse experiment in 2017 and field experiments in 2017 and 2018, measurements of growth parameters in untreated and silicon-treated strawberry plants in a hydroponic glasshouse experiment.

2.2. Leaf sample collection, storage and assessments

2.2.1. Leaf sampling for disease assessments

Cupping is the first sign of strawberry leaves infected with *Podosphaera aphanis* (chapter 1, figure 1.19a). Fully infected leaves can be observed with white mycelium on and below the surface of the leaves and petioles (chapter 1, figure 1.19c). Fully expanded leaves showing disease symptoms (cupping or mycelium development on the surface) were sampled by clipping the petioles of the plants with scissors and placing them in zipped polythene sample bags. Leaves were sampled randomly following a zig-zag motion from different plants in beds from each treatment in the silicon fertigation field experiments.

Strawberry leaves were sampled fortnightly from April to August from the 2016 silicon fertigation field experiment. On each sample day, 15 leaves were sampled (per bed) in replicates of 5 beds, such as beds a, b, c, d and e. Therefore, 75 leaves were sampled per treatment (such as 15 untreated leaves from 5 strawberry beds, for example), and 450 leaves were sampled per sample day. In the 2017 silicon fertigation field experiment (April to August), 15 leaves were sampled (per bed) in replicates of 4 beds. Therefore, 60 leaves were sampled per treatment and 240 per sample day. In the 2018 silicon fertigation field experiment (April to September), 15 leaves were sampled (per bed) in replicates of 4 beds. There were 60 leaves sampled per treatment and 240 on each sample day. The total number of strawberry leaves sampled varied between the 2016 to 2018 fertigation field as 5 beds were used (as replicates) in the 2016 field experiment, and only 4 beds (as replicates) were sampled from and used in the 2017 and 2018 field experiments.

After sampling, leaves were put in polythene sample bags labelled with the sample date, treatment and bed, respectively. Sample bags were then transferred into labelled plastic storage boxes and transported back to the cold room in the Science Building (at a constant temperature of 4°C) located in the College Lane Campus, University of Hertfordshire, Hatfield, Hertfordshire, AL10 9AB. The samples stored in the cold room were then assessed the following day for percentage (%) of mycelium cover using the strawberry powdery mildew assessment key (appendix 4) in preparation for further analysis. All 15 leaves per treatment bed (single leaf with 3 leaflets attached) were assessed using a dissection microscope (GX microscopes, 1x & 3x objectives and 10x eyepieces magnification, GT Vision Ltd, Suffolk, CO108LY). Fortnightly leaf samples were taken from the 2016 Ladybird field experiment (figure 2.3), 2017 Amelia field experiment and 2018 Amelia field experiment silicon fertigation field

experiments (figure 2.3). The disease levels assessment key was based on a revised strawberry powdery mildew assessment key developed by Xiaolei Jin (Jin, 2015) (appendix 4).

- **Leaf sampling for silicon extraction**

In the 2016 to 2018 silicon fertigation field experiments (chapter 3), strawberry plants from the fertigation fields used for disease assessment (section 2.2.1) were also used for silicon extraction to measure silicon contents in strawberry leaves after silicon nutrient treatment. Twenty leaves (sampled separately) were placed in foil boats selected from each strawberry bed. For example, in the untreated, 20 leaves from bed a, 20 leaves from bed b, 20 leaves from bed c, 20 leaves from bed d and 20 leaves from bed e etc. Strawberry leaves for silicon extraction were sampled only once a month from April to September (2016 to 2018).

2.3.   Silicon extraction

The Autoclave Induced Digestion (AID) method was used for silicon extractions (Epstein, 1994). The extraction of silicon was carried out once on each sample month. The extraction process followed these steps: Twenty leaves were sampled once a month for silicon extraction and replicated by five strawberry beds per treatment (for example, 20 leaves x 5 beds). The leaves were placed in each foil boat labelled (with treatment name and bed) and oven dried at 60°C for 48 hours. Dried samples of leaves were crushed to powder form using a porcelain pestle and mortar.

100mg (0.1g) of ground leaves samples (from each treatment) were weighed and placed into autoclave-resistant polyethene bottles and labelled according to treatments. Five replications per sample were carried out for consistency. The leaf samples were moistened with 3mls of 50% H_2O_2, followed by the addition of 3mls of 50% N_aOH. The bottles were covered individually with loose-fitting caps and gently mixed using a vortex machine. Mixed samples were placed in large autoclave beakers, wrapped with foil and labelled before being sent into the autoclave at 138 Pka at 120°C for one hour (Taber, Shogren and Lu, 2002). After one hour, samples were allowed to cool down and for sediments to settle before being transferred into labelled 15mL polycarbonate tubes (flacons).

One millilitre of clear dissolved content was drawn from each polyethene bottle using a pipette (P1000) and brought to a final volume of 5ml with deionized (DI) water. Silicon extractions were carried out monthly from April to September in the 2016 to 2018 field experiment. In 2016, each treatment used for silicon extraction had 5 samples (beds) replicates. For example, there were 5 Untreated beds, 5 No silicon with fungicides beds, 5 silicon once a week with fungicides beds, 5 silicon once a week without fungicides beds, and silicon twice a week with fungicides beds and 5 silicon twice without fungicides beds. In 2017 and 2018, each treatment used for silicon

extractions had 4 samples (beds) replicates. For example, there were 4 untreated beds, 4 fungicides-only beds, 4 silicon twice a week with fungicides beds and 4 silicon twice without fungicides. Replicates differed between years (2016 to 2018) as the number of beds and treatments in each field differed.

The following procedures were carried out to measure the silicon in the above-diluted samples.

i) To each sample, 0.75mL of 2.5% boric acid was added. The tubes were then gently mixed with a vortexer. (pipette tips were replaced, and pipette swabbed with 70% industrial methylated spirit (IMS) to prevent contamination).

ii) Ammonium molybdate (0.25 mL; 54 g/l, pH 7.0) was added to the sample, vortexed and left to stand for 5 minutes.

iii) After five minutes, 0.125mls of tartaric acid was added to the solution and vortexed.

iv) Lastly, 0.125mL of reducing solution, which is a combination of two parts; A) 2g of N_aSO_3 $_{and}$ 0.4g of 1-amino-2-naphthol-4-sulfonic acid in 25ml DI water) and B (25g of $NaHSO_3$ in 200ml of DI water). Solutions A and B were combined and brought to 250ml final volume with de-ionized (DI) water.

v) The prepared solution was vortexed and left to stand for 30 minutes before measuring using a spectrophotometer.

vi) The spectrophotometer (CECIL 1021, 1000 series, Cambridge, CB24 6AZ) reading optical density (OD) was set at 650nm. According to their treatment, one millilitre of the prepared solution was transferred from the polycarbonate tube (Falcons) to the semi-micro non-UV cuvettes.

vii) Semi-micro non-UV cuvettes containing 1mL of each solution were placed vertically into the spectrophotometer to read the absorbance one at a time. (spectra was at zero (blank) before absorbance readings were taken).

- **Silicon standard curve**

A silicon calibration curve indicated each sample's true amount of silicon. For the standard measure, 15 of 15mL Falcon tubes were used, and the concentration used for the standard curve was 250mg of 99% silicate powder (25mg/mL). The silicate powder was diluted into 10 mL of deionized water, which was used as a known silicon concentration. The solution was then subsequently diluted into series of concentration of 0.000078, 0.00015, 0.00031, 0.00062, 0.00125, 0.0025, 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5 mg/mL. Find an example (presented below) of a silicon standard curve graph in figure 2.4 below. The points on the linear graph below were produced using the absorbance of the above-known concentrations above (mg/mL) (figure 2.4). The silicon concentration within a sample can be evaluated using the equation provided by the graph Y= 9.9868x.
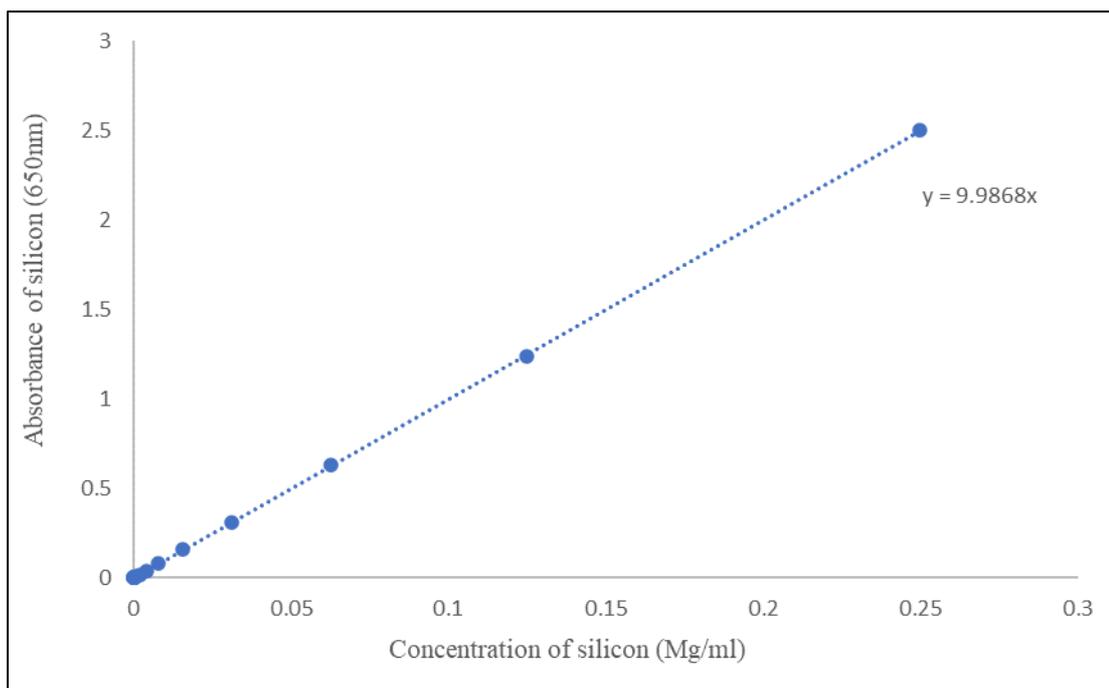
Figure 2.4. Silicon concentration standard curve

Figure 2.4 is an example of a silicon standard curve. To calculate each sample concentration based on the standard curve, first find the concentration for each sample absorbance on the standard curve. Multiply the concentration by the dilution factor for each sample. The points on the silicon standard curve above are the absorbance of silicon on the Y axis (vertical) versus silicon concentrations on the X axis (horizontal).

2.4. Silicon content in fertigation water 2016, 2017 and 2018

2.4.1. Sampling

The fertigation water experiment was designed to determine the contents (amounts) of silicon in water flowing through the tubes at different set times when silicon treatments were applied and to understand if the water travelled the whole lengths of each field tunnel used for the experiment. The step-up of the fertigation experiment (2016 to 2018) was in the same experiment fields used for disease assessments. Water samples were collected from the same fields monthly and assessed in the laboratory. The fertigation system was used to allow the smooth transmission of water and silicon nutrients to the strawberry plants as there are insufficient nutrients in coir (which was used on the farm). The fertigation water flows through the drippers (figure 2.5) at 1.8 L per hour. Each burst of watering is 6 minutes. Normal irrigation (fertigation) session is up to 8 times a day depending on the weather example, 8 times in hot weather conditions and up to 6 times in cooler weather temperatures. In the 2016 field experiment, silicon was applied once and twice a week for two and four minutes, while in the 2017 and 2018 field, silicon was applied twice a week only for three and five minutes.

Sample points were from the near to far-end point of the silicon delivery system (figure 2.6 and 2.7). To replicate samples, four sample bottles were placed on each sample point for set times. For example, for 2 and 4 minutes, 2 bottles are placed on each bed side. Sampling was carried out once a month.

Water samples were collected at various set times and sample points (figure 2.7) to assess the differences in silicon accumulation between times and sample points. Before the sample day, plastic storage bottles were labelled according to sample times and points on the field. The collection was achieved by inserting drippers uprooted from coir bags into sample bottles, as shown in figure 2.5. After water sample collection, the bottles were placed in a sample basket and transported to the cold room at 4°C in the Science Building at the University of Hertfordshire for silicon contents in the fertigation water measurements. Figure 2.5 a to d shows the process of water sampling from the fertigation field.

Figure 2. 5. Fertigation water sampling from strawberry beds. (a) Sample bottle (b) Fertigation dripper in a coir bag (c) Fertigation dripper out of a coir bag (d) Fertigation dripper inserted in a sample bottle.

2.4.2. Measurement

The amount of silicon in each water sample bottle was evaluated using the same silicon titration method as in section 2.3, without using an autoclave. A silicon standard curve was used to determine silicon's (unknown) concentration in the water samples. The spectrophotometer reading optical density (OD) was set at 650nm to obtain each absorbance value. Samples were then transferred from polycarbonate tubes (Falcon) to the semi-micro non- u/v cuvettes according to their treatments. Before treatments were read, the spectra reading was brought to zero (0) using a blank solution (without silicon). Spectra was at zero before actual readings were taken. Cuvettes containing 1ml of each (titrated) water sample were placed vertically into the spectrophotometer one at a time, and readings were recorded respectively. Water and silicon were applied using a fertigation system (figure 2.6). The delivery system is monitored and timed for 5 minutes. See this system below.
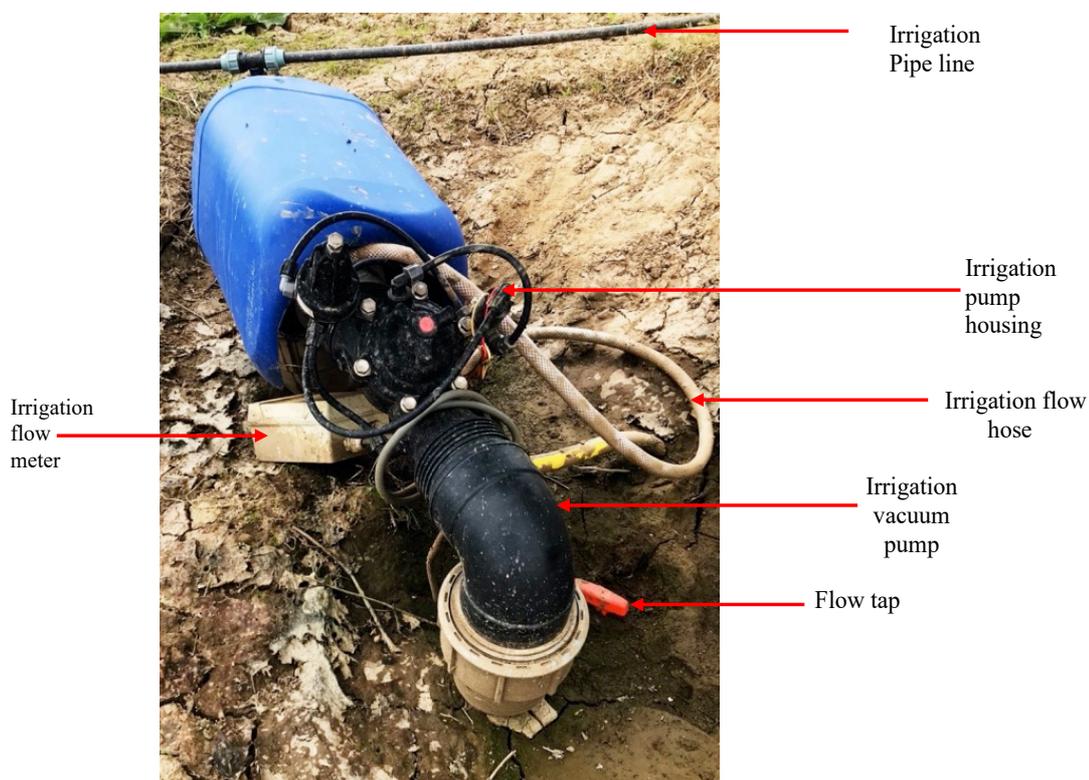
Figure 2.6. Silicon delivery through the fertigation system. Silicon is added into the delivery system, which flows with the irrigation water through the irrigation pipes

Figure 2.7 below is the fertigation plan showing the sample points used in sampling water from the fertigation field.

Tunnel exit (End of field tunnel)
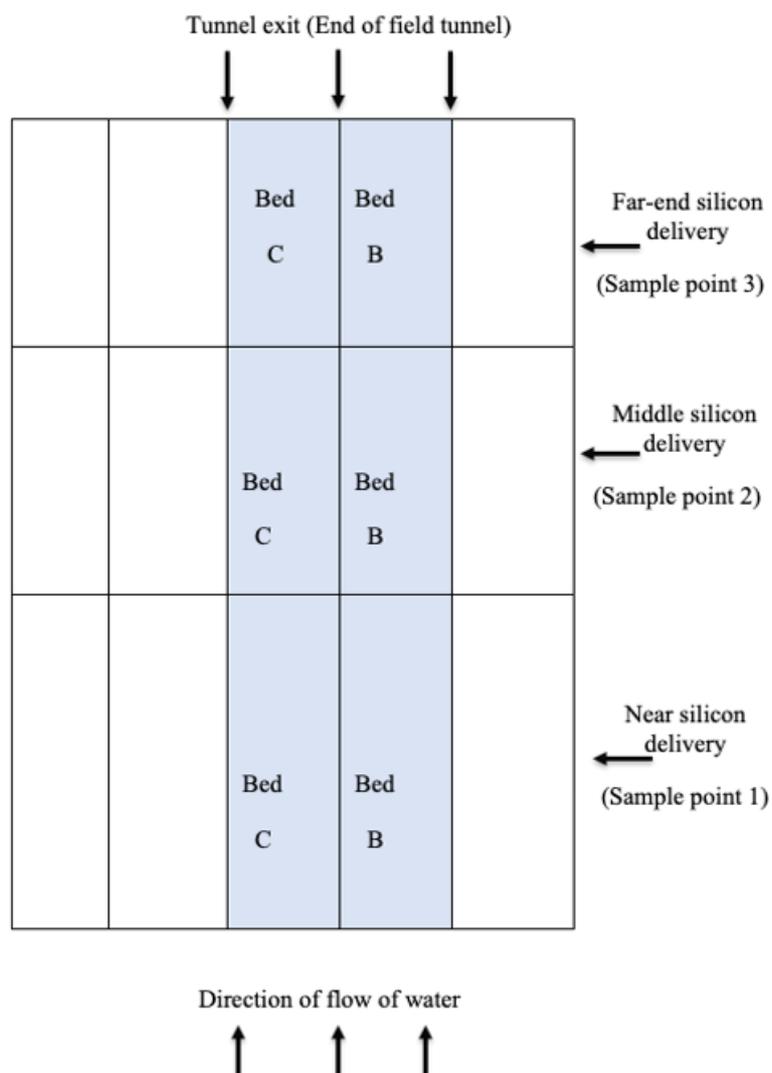
Figure 2.7. Silicon in fertigation field water sample points on the fertigation field. The plans consist of the three sample points used in the fertigation field water experiment. The blue highlighted column are beds b and c out of 5 beds, which were the areas sampled from.

2.5.   Laboratory experiment methods

Overall laboratory experiments covered in this thesis include leaf disease assessments for strawberry powdery mildew *Podosphaera aphanis,* silicon extraction from strawberry leaves, silicon contents in fertigation field water measurements and Degree Brix (°Brix) measurements on strawberry fruits and leaf petioles.

- **Strawberry fruit and leaf petiole sampling for °Brix measurement**

Strawberry fruits and leaf petioles were sampled for °Brix measurements in the 2017 silicon fertigation field experiment. See section 2.5.1 below. Strawberry leaves with long petioles attached were collected from the fertigation field for disease assessments, and petioles were separated in preparation for °Brix measurements.°Brix measurements were for 5 leaf petioles (replicated by 4 treatments), and 10 strawberries (replicated by 4 treatments) were taken monthly from the silicon field experiment in 2017.

2.5.1. °Brix Measurements

All °Brix measurements were carried out according to the standardized instructions provided in the user manual of the hand-held refractometer Eclipse (ThermoFisher Scientific. the UK). The device (figure 2.8b) is specially designed to measure plant juices' refractive index to estimate the sugar contents in plants. Crop juices' refractive index is calibrated in degree °Brix or per cent sucrose.

A °Brix refractometer (figure 2.8b) (Eclipse, Sugar % (°Brix): 0 to32) was used to measure the degree °Brix strawberry fruits and leaf petioles. Petioles were cut into small sections and then placed into a garlic crusher. Liquid from petioles was obtained using the garlic crusher (figure 2.8 a and b). The juices of the strawberry fruits were retrieved by cutting off the tips of the strawberries and gently squeezing the strawberries in a rotating motion. Juices collected from each fruit were transferred directly onto the reactive plastic screen of the refractometer (figure 2.8b). The lid is shut and held up to the eye to view through the optical lens. °Brix readings were recorded for each sample after viewing. The reactive plastic screen was wiped clean with deionised water each time a sample was read. Figure 2.8 below is a photograph of a garlic crusher (a) used in crushing leaf petioles and a refractometer (b) used in measuring sample juices.
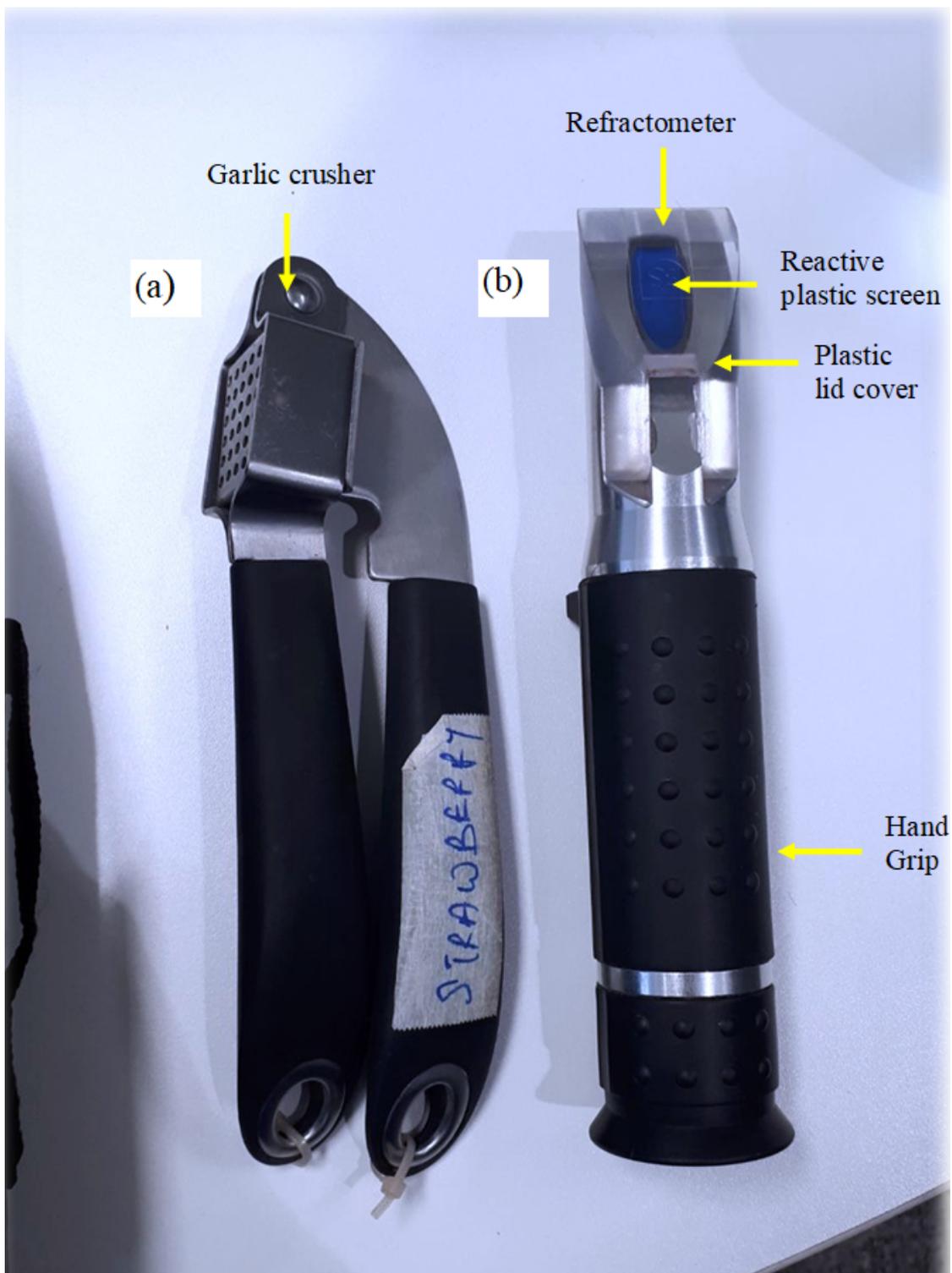
Figure 2.8. (a) Garlic crusher (Left) and (b) refractometer (Right)
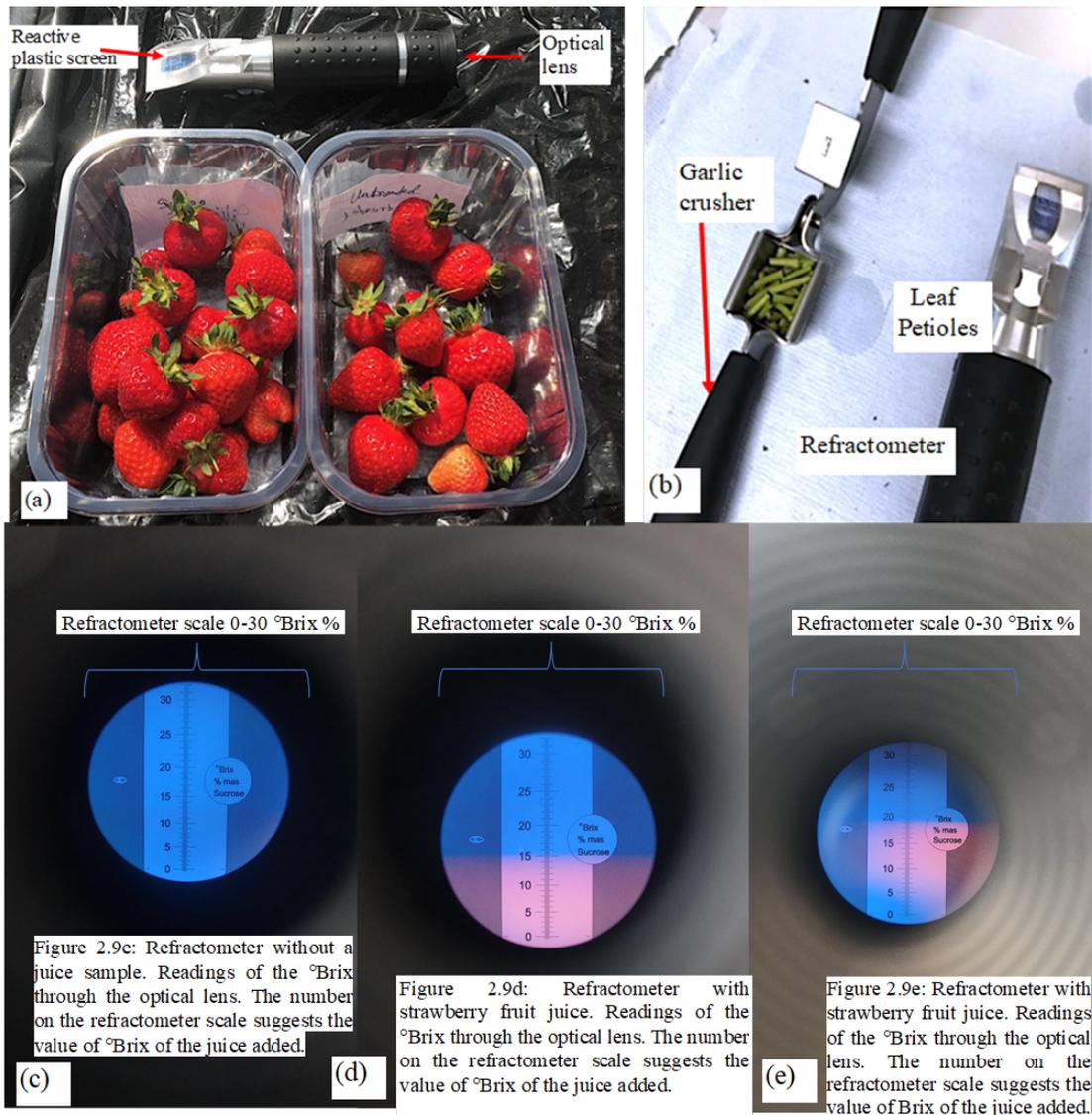
Figure 2.9. Strawberry °Brix measurements. (a) The refractometer and strawberry fruits; (b) The garlic crusher and a refractometer on a lab bench during sample measurements; (c) Blank reading of °Brix through the optical lens; (d) Readings of °Brix with strawberry juice reading 15 through the optical lens; (e) Readings of °Brix with strawberry juice reading 19 through the optical lens.

The number on the refractometer scale indicates the °Brix value (% mass sucrose) of the solution. The sweetness of plant fruit juices can vary, meaning their °Brix levels will read differently. As mentioned earlier, the average strawberry reads 10, with 14 being good and 16 excellent (Harrill, 1998). The refractometer displays a scale (figure 2.9 c to e) of 0 – 30 (%). This means a strawberry °Brix of 6 is poor (Harrill, 1998). Plant juices are measured on the percentage (%) at which liquids (fruit juices) bend through refraction. Figures 2.9 c to e above are visual presentations to show the different °Brix readings per plant juice.

2.6. Glasshouse experiment methods (pot and compost)

Glasshouse experiments were carried out in 2017 (compost) and 2018 (Hydroponics). Malling Centenary strawberry plants were potted in compost at the University of Hertfordshire, Hatfield College Lane campus glasshouse near the CP Snow building, AL10 9AB (figure 2.10 and figure 2.12) and the UH Bayfordbury Research Station, Lower Hatfield Road, Hertford, SG13 8LD (figure 2.11 and 2.12) for a silicon deposition experiment.

Figure 2.10. a and b: Location map of Bayfordbury Source: Google maps (2020)

The New Horizon multi-purpose compost and the Westland Garden Health Organic Chicken Manure Pellets were used for planting (potting) strawberry plants in both Hatfield and Bayfordbury glasshouses. Strawberry plants were watered manually using a plastic watering can before weekly treatments were added. The sensor watering system automatically detected that the mat and polythene felt were dry at the Hatfield campus glasshouse. Lights in the Hatfield glasshouse were 16 hours daily, and temperatures were kept at 25 °C daily and 8°C at night.
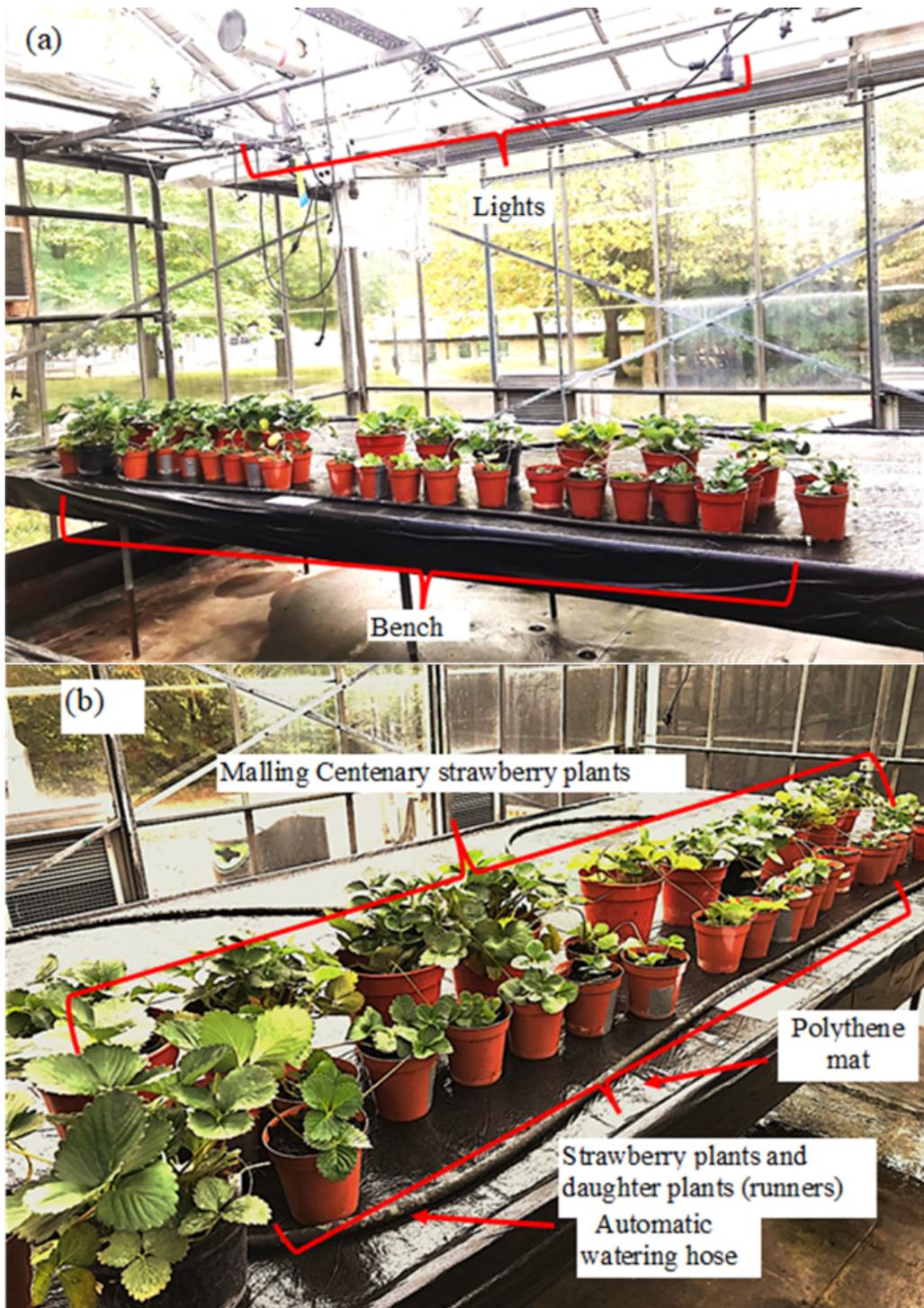
Figure 2.11. a and b Glasshouse experiment at the University of Hertfordshire, Hatfield. Automatic window vents in the glasshouse were opened when temperatures were above 25°C. At the Bayfordbury glasshouse, automatic watering was set for 30 minutes twice daily. The lights (ValuTekTM 400Wn Metal Halide Low Bay Light) and heating in the glasshouses were off during summertime, and the vents in the roof opened automatically when the temperatures were greater than 25°C. During the winter, the lights were on 12 hours per day and temperatures were kept at 25°C on days and 8°C at night to keep temperatures above freezing.
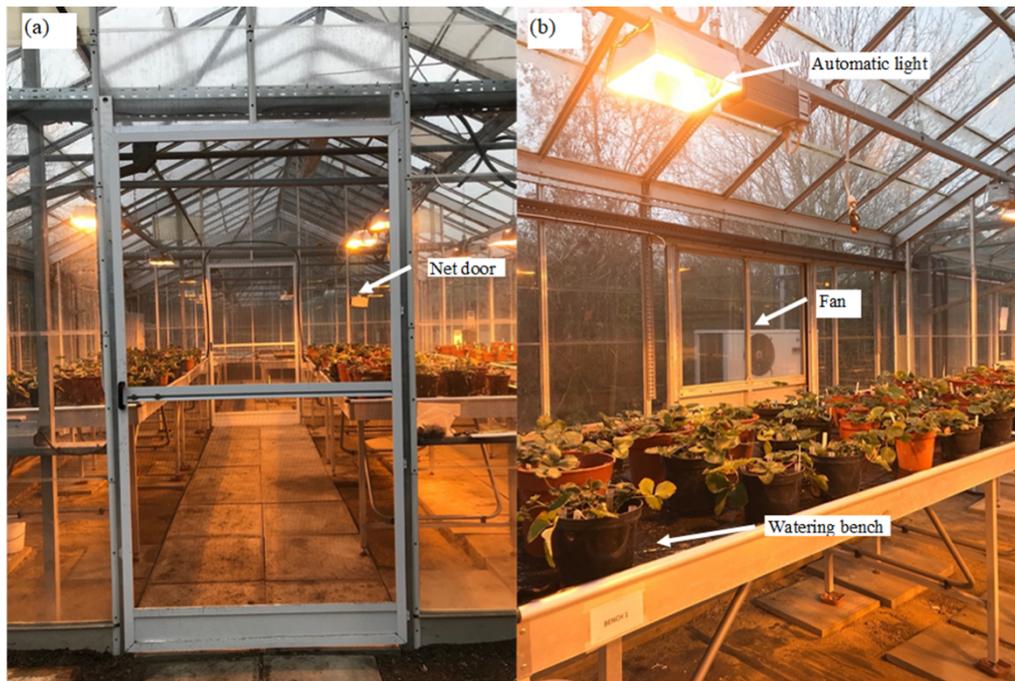
Figure 2.12. a and b Experiments at UH Bayfordbury glasshouse in 2017 (colour of glasshouse photos is caused by an automatic light in the glasshouse, which is programmed to switch on when the sun goes down).

In the 2017 UH Bayfordbury glasshouse experiment (figure 2.12 and 2.13) (pot and compost), 16 Malling Centenary strawberry plants were planted in plastic pots that were 12cm in diameter and 10cm deep (figure 2.13) and moved to the University of Hertfordshire, Hatfield glasshouse (figure 2.11). The plants were divided into two treatments, untreated (control) and treated (with silicon nutrient). All plants were randomly placed on the bench and were manually watered (along with the automatic watering) and then treated once weekly with silicon nutrient (treated) and deionised water (untreated). The duration of the 2017 glasshouse experiment was for eight weeks. Runners (daughter plants) from the Malling Centenary plants were planted in separate pots attached to their mother plants (figure 2.11) and detached when they were mature enough. Figure 2.13 below is a strawberry plant pot with compost from the glasshouse experiment showing how silicon treatments and de-ionized water were applied through the roots of strawberry plants via Gilson pipette tips (once weekly). See table 2.2 for treatments used.

55

Figure 2.13. Strawberry plant in the Bayfordbury glasshouse pot (compost) experiment. Silicon treatment and de-ionized water were added via a 50 ml syringe of 50 ml per plant pot via Gilson pipette tips, as shown in the figure.

Thirty bare root Malling Centenary strawberry plants were provided by Maltmas farm from a propagator, and 20 of those plants were then planted in a Hoagland's solution for a hydroponic experiment (to test for strawberry plants growth parameters). The remaining spare plants were potted with compost and chicken manure pellets on 24 January 2018. Bare roots strawberry plants are not planted in soil. They need 1500 hours of cold to recover from their dormant phase (Pestana et al., 2012). The strawberry plants were divided into two treatments. Ten treated (with silicon nutrient) and 10 untreated (control) were treated through a root application via Gilson pipette tips (figure 2.13) and monitored once weekly from January to June 2018. Table 2.2 shows experiments conducted in the glasshouse in 2017 and 2018.

Table 2.2. Glasshouse experiments in 2017 and 2018

Year	Location	Cultivar	Number of plants used	Treatments	Assessments
2017	Hatfield Silicon deposition experiment (in pot and compost)	Malling Centenary (June bearers)	16	0.017% (v/v) silicon root application, 50 ml per pot. De-ionized water in untreated (control) root application, 50 ml per pot.	Silicon deposition in leaves, leaf petioles and roots. (see chapter 2, section 2.3.3).
2018	Bayfordbury Hydroponic experiment (Hoagland's solution)	Malling Centenary (Ever bearers)	20	0.017% (v/v) silicon root application, 50 ml per hydroponic tub De-ionized water in untreated (ccontrol) root application, 50 ml per pot.	Strawberry plants' growth parameters and quality concerning fruit quality between silicon-treated and untreated plants

2.7. Data analysis

Statistical analysis was applied to data collected in the experimental chapters of this thesis. Firstly, data collected were tested for normality, and statistical tests were applied to understand the mean differences between silicon treatments in the silicon fertigation field experiment (effects of silicon on disease severity reduction) in 2016 and the relationship (correlation) between silicon extraction and disease levels in 2016 in chapter 3. Chapter 4 shows the quantification assessments and analysis of mean differences in cross-section fluorescence intensities of silicon-treated and untreated plants in 2 silicon deposition field experiments in 2017 and 2018. In chapter 5, statistical assessments were conducted to distinguish and understand the mean differences between growth parameters of silicon-treated plants and untreated plants from a glasshouse hydroponic experiment in 2018.

Raw data collected in all experiments were analysed using the tests mentioned below. Firstly, raw data were entered and stored in spreadsheets in Microsoft Excel (2016). Graphs provided in chapter 3 (disease assessments) for the silicon fertigation field experiment from 2016 to 2018 were also created using Microsoft Excel (2016). Statistical analysis throughout this thesis was done using both SPSS, version 26.0 and Microsoft Excel Data Analysis (2021). Before conducting any statistical tests, each data was tested for normality; to determine whether the data was standard or not normally distributed. Checking for normality of data used in chapters 3 to 5 was done using the Shapiro-Wilk test. Statistical tests used in the experimental chapters (3 to 5) were the

parametric test: One and Two-way analysis of Variance (ANOVA), Paired Samples test, the nonparametric tests: Spearman's Rank Correlation, Wilcoxon Signed-Rank test and Kruskal-Wallis test. Mean comparisons were evaluated to calculate the differences between silicon-treated and untreated samples and to understand the significance P-value. The probability value (P-value) measures the probability that the difference in observation could have occurred just by random chance. Therefore, the greater the P value, the lower the statistical significance of the observed difference. If the P-value is below the 0.05 level, reject the null hypothesis.

The correlation coefficient or (r) examines the relationship between two quantitative variables. The Spearman's rank correlation was used to analyse the relationship between levels of disease in strawberry plants and amounts of silicon extracted in the 2016 silicon fertgation field experiment, chapter 3. Spearman's rank correlation is a statistical test investigating the degree to which two data sets are correlated and if there is any correlation. To apply Spearman's rank correlation, the researcher must have paired sets of data that are related (Akoglu, 2018).

The One-way ANOVA and Two-way ANOVA are statistical tests that explore the means between data sets to determine their differences. The One-way ANOVA and Two-way ANOVA were used to analyse fluorescence intensity data of the cross-section of leaves, petioles and roots of plants in a glasshouse and field experiments (chapter 4). The ImageJ multidimensional processing software was used to quantify fluorescence intensities between sample cross-sections examined in chapter 4 (Collins, 2007; Jensen, 2013; Bankhead, 2014). The Paired Sample test, nonparametric tests, Wilcoxon Signed-Rank test and Kruskal-Wallis test, were used to determine and calculate the mean differences between the silicon-treated and untreated plant growth parameters in the glasshouse hydroponic experiment. (chapter 5).

2.7.1. Comparisons of disease levels between treatment

The Area Under the Disease Progress Curve (AUDPC) measures disease intensity and resistance. This method works by estimating percentages of affected leaf areas recorded at different times during disease levels and indicating the level of suppression at the start of disease levels (Wu, 2016). (chapter 3, section 3.4). Results were obtained using the formula (Schandry, 2017).

$$AUDPC = \sum_{i=1}^{n-1} \frac{(x_{i+1} + x_i)}{2} (t_{i+1} - t_i)$$

Therefore,

X_i is a measure of disease severity at initial observation,

t is a measure of time,

n is the total number of observations.

Chapter 3 - The use of silicon nutrients in reducing disease levels of strawberry powdery mildew

This chapter covers i) An introduction to the use of a silicon nutrient in reducing susceptibility to strawberry powdery mildew *Podosphaera aphanis*; ii) Rationale, aim, hypothesis and objectives for the 2016 to 2018 silicon fertigation field experiment at Maltmas farm; iii) Material and methods for the 2016 to 2018 silicon fertigation field experiments; iv) Experiment results; v) Discussion and vi) Conclusion.

3.1.   Introduction

The work conducted in this research was a continuation of previous work regarding the use of a bioavailable form of silicon in reducing powdery mildew of strawberries caused by *Podosphaera aphanis* by Jin (2015) and Liu (2016). However, this experiment investigated further using different cultivars, Malling Centenary and Amesti, (refer to table 2.1 for a description). A resistant cultivar (Amesti) was used to determine if silicon affected disease reduction as in a susceptible cultivar. Work by Jin (2015) and Liu (2016) showed that the addition of a silicon nutrient through a fertigation system in field experiments reduced strawberry powdery mildew *Podosphaera aphanis.* All strawberry plants used on the farm for the field experiments were chosen by the growers each year for commercial purposes, mainly for fruit production. The Malling Centenary cultivar was used in 2016 and Amesti in the 2017 and 2018 silicon fertigation field experiments. Growers are moving cultivation techniques and are now using all Ever bearer cultivars. Since moving from June bearers to Ever bearers at Maltmas farm, these Ever bearers crops were the crops available and provided for all experiments by the grower.

The cultivars Malling Centenary (June bearers and susceptible) and Amesti (Ever bearers and disease tolerant) were used to understand if silicon is effective in reducing disease levels, particularly in different cultivars, and also to determine if silicon was efficiently taken up by all cultivars, the differences in silicon uptake in these different cultivars, to determine whether applying silicon to strawberry plants twice a week is more beneficial than a once-a-week treatment.

3.2.   Bioavailable silicon in plants

The general introduction to silicon in plants is provided in chapter 1, section 1.17; however, there is increasing evidence from research at the University of Hertfordshire that shows benefits from the application of silicon in reducing biotic and abiotic stresses (Fatema, 2014; Jin, 2015; Liu, 2016). Silicon in soil (pH 5.5 to 6) can only be absorbed by plants in the bioavailable form of silicic acid $Si(OH)_4$ (Scholey et al., 2018). The amounts of silicon in plants may vary from 0.1% to 10% of the plant's dry weight (Frew

et al., 2017). The silicon used is 'Sirius' (the main active ingredient is 70 – 80 Tetraethyl silicate: other compounds include silicon-based adjuvant and two other non-silicon compounds. Silicon concentration applied to plants in the silicon field experiment was at 0.017%. Fatema (2014) showed that bioavailable silicon increased the density of leaf hairs of strawberry plants in a glasshouse experiment. Work carried out by Jin (2015) showed that silicon increased cuticle thickness and wax formation in the adaxial surface of the leaves of the cultivar Shelley of strawberry plants and reduced powdery mildew disease levels. Liu (2016) also showed in the cultivar Sonata a reduction in strawberry powdery mildew disease levels and the number of two-spotted spider mites of strawberries with silicon in field experiments. The use of the silicon nutrient also showed an increase in the °Brix levels of strawberries (Liu, 2016).

3.2.1. Rationale

Previous work has shown that the application of silicon nutrient can cause a reduction in the levels of powdery mildew disease in strawberry plants cultivars such as Vibrant, Sonata, Shelley, Alexandra and Elegance when applied through the roots and sprays once a week in field experiments. The work reported here in chapter 3 explored the use of silicon on cultivars such as Malling centenary and Amesti applied through the roots of strawberry plants once and twice- a-week on field experiments to determine the effects and differences between the two treatments using different cultivars.

3.2.2. Aim

To investigate the effects of the silicon nutrient delivered through the fertigation system on the development of strawberry powdery mildew (*Podosphaera aphanis*) disease levels in different strawberry plant cultivars.

3.2.3. Hypothesis

Applying silicon nutrient applied once and twice a week through the fertigation system of strawberries can reduce the severity of *Podosphaera aphanis.*

3.2.4. Objectives

▪ Measure strawberry powdery mildew mycelium percentage cover on strawberry leaves treated with and without silicon nutrient. Disease assessments.

▪ Determine the relationship between the level of disease infection and the amount of silicon in strawberry plants from the silicon fertigation field experiment 2016.

▪ Compare the distribution of silicon in strawberry plants through silicon fertigation field experiments (2016 to 2018). Silicon extraction from strawberry leaves.

- Quantify the amount of silicon in the fertigation water from the silicon fertigation field experiments from 2016 to 2018. Silicon contents in water measurements.

3.3. Material and methods

Methods used in this chapter investigating the use of a silicon nutrient in reducing susceptibility to *Podosphaera aphanis* in the 2016 to 2018 fertigation field experiments include.

i) Strawberry leaf sample collection and storage (chapter 2, section 2.2)

ii) Disease assessments (chapter 2, section 2.2.1)

iii) Silicon extraction from strawberry leaves from the silicon fertigation field experiment from 2016 to 2018. (chapter 2, section 2.3).

iv) Silicon content measurements in fertigation water 2016 to 2018 (chapter 2, section 2.4 (i)).

3.3.1. Cultivars

Strawberry plants used in field experiments were planted in coir bags on raised soil beds and conducted under commercial conditions. The cultivars used were chosen by the growers each year. Malling Centenary (June bearer) cultivars were planted in March 2016 in the Ladybird field tunnel for the silicon fertigation field experiment. Amesti strawberry plants (Ever bearer) cultivars were planted in March 2017 in the Amelia field tunnel for the silicon fertigation field experiment 2017. Amesti strawberry plants were also planted in March 2018 in Amelia field tunnel for the silicon fertigation field experiment 2018. Description of Malling Centenary (June bearer) and Amesti (Ever bearer) cultivar strawberry plants are in chapter 1, table 1.2. As mentioned earlier, growers are moving cultivation techniques from growing Junebearing strawberries to growing all Everbearing strawberries. The grower chose these cultivars for the experiments in this chapter to determine if both cultivars acted similarly to silicon (disease reduction and silicon uptake).

3.3.2. Treatments

Six treatments (table 3.1) were applied to Malling Centenary strawberry plants in the silicon fertigation field experiment in 2016. All three fertigation field experiments were 180 metres long, including a central gap (un-sampled and untreated area) of 40 metres. All five strawberry beds from the silicon fertigation field experiment in 2016 were approximately 1 metre apart. In 2017 and 2018 (chapter 2, figure 2.3), four treatments were applied to four strawberry beds, also approximately 1 metre apart. The silicon twice weekly plus fungicides and fungicides only were 70 metres longer than other treatments because of the unusual commercial design. Sample replication is fully presented in chapter 2, section 2.2.1. Refer to appendix 9 to 12 for the list of fungicide

sprays used in the silicon fertigation field experiments from 2016 to 2018. In 2016 and 2017, the experiment ended in August, and in 2018, the experiment ended in September.

Table 3.1. Treatments used in the silicon fertigation field experiments from 2016 to 2018

Year	Treatments
2016 (Ladybird field) Malling Centenary Crops were planted in coir bags in March. Silicon and fungicides treatment started on 19 April 2016, and the experiment ended on 9 August 2016 (the end of the growing season).	• Untreated (no silicon nutrient and no fungicides • No silicon nutrients + fungicides • silicon once weekly + fungicides • silicon once weekly without fungicides • silicon twice weekly + fungicides • silicon twice weekly + no fungicides
2017 (Amelia field) Amesti Crops were planted in coir bags in March 2017. Silicon and fungicides treatment started on 9 April 2017, and the experiment ended on 27 August 2017 (the end of the growing season).	• Untreated (no silicon nutrient and no fungicides) • fungicides-only • silicon twice weekly + fungicides • silicon twice weekly + no fungicides
2018 (Amelia field) Amesti Crops were planted in coir bags in March 2018. Silicon and fungicides treatment started on 24 April 2018, and the experiment ended on 21 September 2018 (the end of the growing season).	• Untreated (no silicon nutrient and no fungicides) • fungicides-only • silicon twice weekly + fungicides • silicon twice weekly + no fungicides

All field and glasshouse experiments conducted ended in 2018. There were no experiments carried out in 2019.

3.3.3. Leaf sampling

Strawberry leaves were sampled randomly (following a zig-zag pattern) every two weeks from each strawberry bed in all treatments from the silicon fertigation field experiment from 2016 to 2018. See chapter 2, section 2.2.1, for a general description of leaf sampling methods used in the silicon fertigation field experiment from 2016 to 2018.

3.3.4. Disease assessments: Strawberry powdery mildew (*Podosphaera aphanis*) on the strawberry leaf surface from 2016 to 2018

Fifteen strawberry leaves from 5 strawberry beds (75 leaves per treatment and 450 leaves in total per sample date) were sampled in 2016. Fifteen leaves per 4 strawberry beds (60 leaves per treatment and 240 leaves in total per sample date) in 2017 and 2018 were assessed for strawberry powdery mildew using the disease assessment key (appendix 4). The Area Under the Disease Progress Curve (AUDPC) was calculated to measure disease severity based on the mean percentage of powdery mildew (*Podosphaera aphanis*) coverage per strawberry leaf and sampling date (chapter 2, section 2.2.1).

The AUDPC measures the area under the disease progress curve. The formula for the AUDPC is in chapter 2, section 2.7.1. This method quantifies the size of the disease level based on the samples assessed throughout the season, such as the percentage cover of strawberry leaves (chapter 2, section 2.2.1(i)) and the severity of the disease under different treatments. Refer to chapter 2, section 2.7.1, for the formula of AUDPC. Data were first tested for normality using the Shapiro-Wilk test. The Parametric test, Paired samples test and Nonparametric test; Wilcoxon Signed-Rank test and Kruskal-Wallis test were used to evaluate the mean differences in the level of disease in the field experiment in 2016 (chapter 2, section 2.7).

3.3.5. Water sampling

Silicon content in the fertigation system water was measured to determine whether the silicon applied via the fertigation system was found throughout the fertigation water in the 2016 to 2018 field experiment. Water samples were collected and assessed every month from 2016 to 2018; all experiments ended when the crop season ended. Refer to chapter 2, section 2.4, for the method description used in this experiment.

3.3.6. Silicon extraction

Strawberry plants used for strawberry disease assessments in the silicon fertigation field experiments from 2016 to 2018 were also sampled for leaves used in silicon extractions. Refer to chapter 2, section 2.2.1, for the method description used.

3.4. Results

Figure 3.1 shows disease progress levels of Malling Centenary plants in the silicon fertigation field 2016 experiment from the start of silicon treatments in April to the end of the experiment in August 2016.

3.4.1. Disease levels of strawberry powdery mildew in the silicon fertigation field experiment 2016 (Malling Centenary)
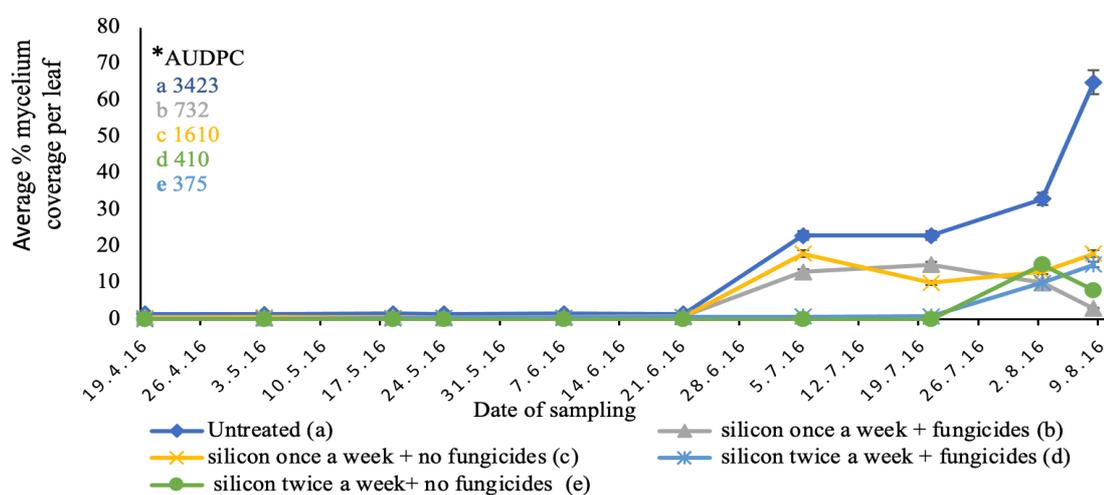
Figure 3.1. Disease levels in the 2016 silicon fertigation field experiment.

The lines in the graph distinguish the different treatments. The vertical axis indicates the average percentage (%) of mycelium covered per leaf from each treatment. The horizontal axis indicates the date of sampling from each treatment. Silicon nutrient (Sirius 0.017v/v) was applied through the fertigation system once and twice weekly in this experiment. The AUDPC values of five treatments are displayed on the top left side of the graph. Silicon applied twice a week with fungicides (d) AUDPC, 375, and silicon applied twice a week without fungicides (e) AUDPC, 410 had the lowest disease levels. The highest disease levels (AUDPC, 3,423) were found in the untreated plot (a).

The effect of fungicides-only application on crops in the plot without silicon is not seen on this graph as there was an alteration of results due to flooded areas of field beds, which appears as if the fungicides treatment had a minimal reduction in disease levels. The flooding in the tunnel was an unexpected result of the field shown on the graph above (figure 3.1). This means the no silicon + fungicides treatment was flooded and excluded from the graph.

Typically, a disease level graph does not go down however, in figure 3.1, results show the silicon once-a-week + fungicides and silicon twice-a-week + fungicides treatments which may have been due to a sampling error. An example of sampling error is sampling leaves without disease towards the end of the experiment or season. The Shapiro Wilk test was used for normality and the parametric tests; Paired Samples test in SPSS version 26.0 were used to process and calculate the differences between treatment means from the 2016 silicon field experiment. The Paired Samples test, a parametric test, was used as the data collected from the 2016 field experiment disease

assessments followed a normal distribution. The Paired Samples t-test compared the means of treatment variables. For example, untreated strawberry plants against silicon once-a-week treated crops or the untreated against fungicides-only treated crops to determine the differences in levels of disease between them.
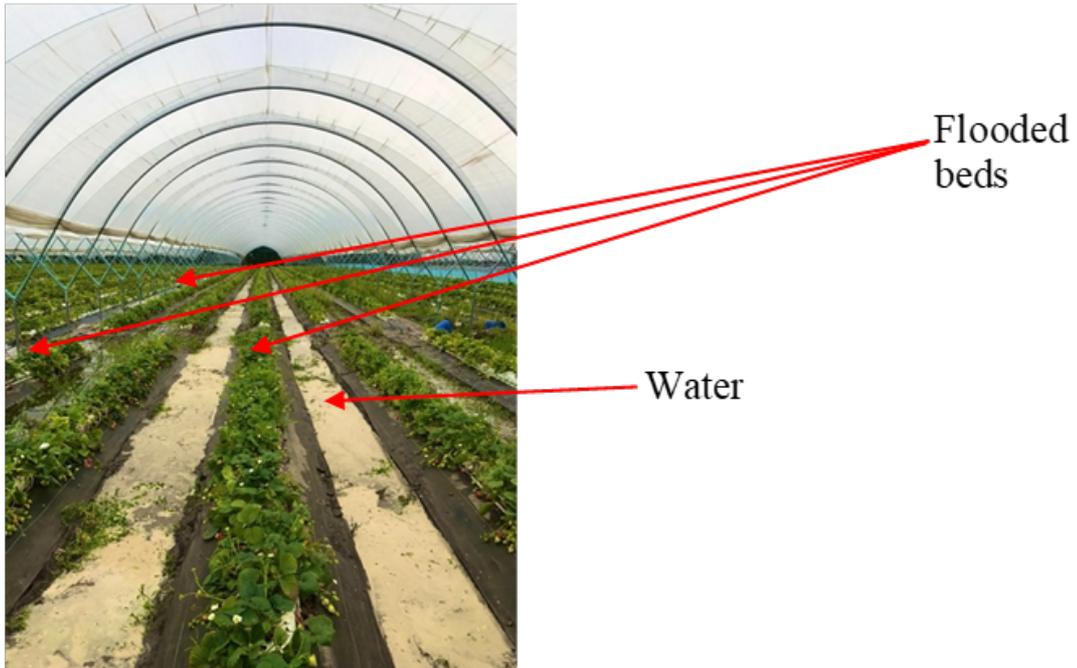
Figure 3.2. Flooded Ladybird in 2016

Similarly, the AUDPC shows that the lowest disease level occurred in treatments with twice weekly silicon + fungicides (AUDPC = 410) and twice weekly silicon + no fungicides (AUDPC = 375). In contrast, the largest disease level occurred in untreated strawberry plants (AUDPC =3,423). Statistical analysis is provided (appendix 14) using the paired samples t-test to find the differences between disease levels in the treatments from the silicon fertigation field experiment in 2016. The Shapiro-Wilk test was used to test for normality before applying statistical tests. Table 3.2 below is a summary table of findings from the disease levels graph 2016, shown in figure 3.1.

Table 3.2. Summary of findings for disease levels of strawberry powdery mildew in the silicon fertigation field experiment 2016 (Malling Centenary)

Year	Treatments	Statistical test applied	Statistical findings (see appendix 14 for statistical workings)
2016	Untreated control (no fungicides, no silicon) and silicon once + fungicides	Test for normality using Shapiro-Wilk test and average values of statistical differences were tested using parametric test; t-test Paired samples test	There were reduced levels of disease in the silicon once-a-week + fungicides compared to untreated control (no fungicides, no silicon) treatment. There was a statistical difference between both treatments (P<0.05). The null hypothesis (H0) is rejected.
2016	Untreated control (no fungicides, no silicon) and silicon once + no fungicides	Test for normality using Shapiro-Wilk test and average values of statistical differences were tested using parametric test; t-test Paired samples to test.	There was no statistical difference between disease levels of untreated control (no fungicides, no silicon) and silicon once-a-week + no fungicides (P>0.05). The null hypothesis (H0) is accepted.
2016	Untreated control (no fungicides, no silicon) and silicon twice + fungicides	Test for normality using Shapiro-Wilk test and average values of statistical differences were tested using parametric test; t-test Paired samples to test.	There were reduced levels of disease in the silicon twice-a-week + fungicides compared to untreated control (no fungicides, no silicon) treatment (P<0.05). The null hypothesis (H0) is rejected.
2016	Untreated control (no fungicides, no silicon) and silicon twice + no fungicides	Test for normality using Shapiro-Wilk test and average values of statistical differences were tested using parametric test; t-test Paired samples to test.	There were reduced levels of disease in the silicon twice-a-week + no fungicides compared to untreated control (no fungicides, no silicon) treatment (P<0.05). The null hypothesis (H0) is rejected.

In the 2016 silicon fertigation field experiment above (figure 3.1) and appendix 14, results showed a twice weekly application of silicon nutrient (Sirius 0.017% (v/v)) with fungicides and without fungicides had significantly ($P<0.05$) reduced susceptibility to *Podosphaera aphanis* throughout the experiment period. A parametric Paired Samples t-test showed that disease levels in the untreated were not statistically different ($P>0.05$) from the silicon once weekly without fungicides however, a reduction in disease levels and statistical difference (P<0.05) is seen in the silicon twice weekly + fungicides and silicon twice weekly + no fungicides compared to the untreated (no fungicides, no silicon) treatments. It is important to understand temperature and humidity can influence the growth and development of *Podosphaera aphanis* (Hall and Jin, 2016).

The paired t-test analysis conducted on means of disease levels between treatments in the 2016 silicon fertigation field experiment showed that there was a significant difference ($P < 0.05$) found between the untreated (control), the silicon once weekly + fungicides, silicon twice weekly + fungicides and silicon twice weekly + no fungicides treatment in the silicon fertigation field experiment in 2016. (figure 3.1 and table 3.2).

As mentioned previously, the flooded treatment was removed from the graph. The results from disease assessments suggested that disease intensities increased as temperatures increased due to flooding (figure 3.2). Flooding in the tunnel did not impact silicon levels in treatments containing silicon. However, the AUDPC results have confirmed that all four treatments with silicon nutrient showed lower disease levels of *Podosphaera aphanis*. In this case, figure 3.1 and table 3.2 above suggest that the aim that silicon can cause a reduction in strawberry powdery mildew disease levels was met in the 2016 silicon field experiment where treatments such as silicon once- a-week + fungicides, silicon twice a week and fungicides, and silicon twice a week without fungicides were applied, however not in treatments where the silicon once- a-week + no fungicides were applied. The bar chart in figure 3.3 below shows silicon extractions from the leaves of strawberry plants from the 2016 silicon fertigation field experiment (April to August).

3.5. Silicon content in strawberry leaves from the silicon fertigation field experiment 2016 (Malling Centenary)
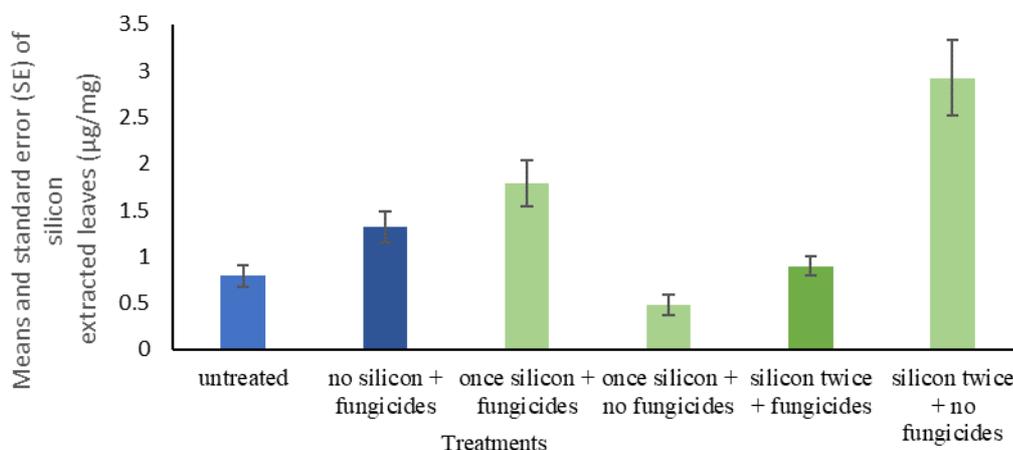
Figure 3.3. Silicon extraction from strawberry leaves (µg/mg) in the fertigation field in 2016 (at the end of the growing season in August)

Results from figure 3.3 show mean silicon extraction results at the end of the silicon fertigation field experiment season in 2016. The different colour bars indicate the different treatments used in this experiment. The error bars are standard error bars extracted from Excel (2022). The bar chart was produced from the mean silicon contents available (silicon accumulated) in strawberry plants at the end of the season. Each bar (figure 3.3) shown are replicated leaves. Silicon extraction was done from 20 leaves from each bed and each different treatment.

All leaves were sampled randomly through the length of each treatment bed. The Paired Two Samples for Means in Excel (version 16.43) was used to analyse the means of silicon extraction between treatments in the silicon fertigation field experiment in 2016. See table 3.3 below for a summary of the findings of silicon extracted from strawberry leaves from the silicon fertigation field experiment in 2016. Refer to appendix 17 for the statistical workings of silicon extraction between all treatments.

Table 3.3. Summary of silicon extraction from strawberry leaves in the 2016 field experiment

Year	Treatment	Statistical test applied	Statistical findings (see appendix 17 for statistical workings)
2016	Untreated control (no fungicides and no silicon) and silicon once + no fungicides	t- Test Paired sample for average values	$P > 0.05$; there was no statistical difference between silicon levels in the untreated control (no fungicides and no silicon) compared with the once silicon once-a-week + no fungicides. The null hypothesis (H0) is accepted.
2016	Untreated control (no fungicides and no silicon) and silicon once + fungicides	t- Test Paired sample for average values	$P > 0.05$; there was no statistical difference found between levels of silicon in untreated control (no fungicides and no silicon) compared with the silicon once-a-week + fungicides. The null hypothesis (H0) is accepted.
2016	Untreated control (no fungicides and no silicon) and silicon twice-a-week + fungicides	t- Test Paired sample for average values	$P > 0.05$; there was no statistical differences found between levels of silicon in untreated control (no fungicides and no silicon) compared with the silicon twice-a-week + fungicides. The null hypothesis (H0) is accepted.
2016	Untreated control (no fungicides and no silicon) and silicon twice + no fungicides	t- Test Paired sample for average values	$P < 0.05$; there was a statistical difference found between levels of silicon in untreated control (no fungicides and no silicon) compared with the silicon twice + no fungicides. The null hypothesis (H0) is rejected.

Results from table 3.3 above exhibit a statistical increase ($P<0.05$) in the levels of silicon extracted from the leaves of plants treated with silicon twice-a-week without fungicides compared with the untreated control. Results showed no statistical increase

(P>0.05) between the levels of silicon extracted from the leaves of the silicon + fungicides, once silicon + no fungicides, silicon twice + fungicides compared to the untreated ones. The bar chart presented in figure 3.3 above shows the levels of silicon remaining in strawberry plants at the end of the season, and no account was taken of the age of strawberry leaves (young and old leaves); however, the experiment was revised and conducted in the following year 2017 where only same age leaves were sampled.

A Spearman's Rank correlation analysis between disease levels and amounts of silicon extracted from the 2016 field experiment revealed a moderate relationship between both variables with no significant difference (P>0.05). Refer to appendix 18 for the statistical workings.

Silicon contents in the fertigation water were carried out once a month and were sampled at 3 delivery points, and the results are shown in table 3.4.

3.5.1.1. Silicon content in fertigation water from the silicon fertigation field experiment 2016

The experiment (table 3.4) was set up in May 2016, and a pre-assessment (initial) was not conducted and examined; however, the experiment was revised to include a pre-assessment sample in the following month, June (table 3.4) and for the 2017 and 2018 fertigation field water experiment. The sampling time was also extended to the end of each season rather than only 2 months in 2016.

The experiment aimed to determine the amounts of silicon that travelled (at different lengths of time) throughout the fertigation field. These measurements were also conducted to show evidence that the silicon applied through the fertigation system is distributed throughout the tunnel. No silicon is present in the water used in fertigation (Liu, 2016). From table 3.4, the near silicon delivery indicates a sample point near the silicon irrigation system. Middle silicon delivery indicates the sample point in the centre of the 3 samples. The far end is the end point of silicon delivery (chapter 2, figure 2.8).

The pre-assessment in June (table 3.4) showed no silicon contents in the samples. This was pure irrigation water flowing through the fertigation tubes sampled first before silicon was added for measurements. Table 3.4 shows a range of results of silicon travelling the length of the 2016 tunnel; however, the expected readings of silicon at the concentration of 0.017% (0.025ml to 0.027ml of bioavailable silicon) added in the fertigation system should be around the same amount of silicon extracted $0.003mg/cm^3$. No measurements were taken in a steady state example, 1 hour or 24 hours after the application of silicon as the silicon remaining in the water would have been washed out

by the next fertigation event. The farm water contains no silicon, and the farm's reservoir combines drinking and rainwater as the groundwater is saline.

Table 3.4. Silicon concentration (mg/ml) in fertigation field water 2016

Sample point May 2016	Time of sampling after delivery of silicon/min	Silicon content (mg/ml) (averages of two samples) Mg/ml
Near silicon delivery	2	0.011 mg/ml
Near silicon delivery	4	0.016 mg/ml
Middle silicon delivery	2	0.015 mg/ml
Middle silicon delivery	4	0.022 mg/ml
Far-end silicon delivery	2	0.007 mg/ml
Far-end silicon delivery	4	0.019 mg/ml
Sample point June 2016	**Time of sampling after delivery of silicon/min**	**Silicon content (averages of two samples) Mg/ml**
Near silicon delivery	0 (i.e., before addition)	0.000
Near silicon delivery	2	0.010 mg/ml
Near silicon delivery	4	0.018 mg/ml
Middle silicon delivery	2	0.010 mg/ml
Middle silicon delivery	4	0.023 mg/ml
Far-end silicon delivery	2	0.015 mg/ml
Far-end silicon delivery	4	0.028 mg/ml

Moreover, the suggested rate for silicon nutrient used in agriculture is 50 – 100 mL in 100 mL in 100 – 600 L of water (Jin, 2015; Liu, 2016). The grower at Maltmas farm use 100 mL of silicon 'Sirius' in 600 L per hectare, which is calculated as:

$$100 \ mL \ in \ 600 \ L \ = \ 0.017\% \ Sirius \ (v/v)$$

In 6 minutes, each irrigation tunnel received.

$$6 \ minutes \ / \ 60 \ x \ 2.2 \ L \ / \ hour \ x \ 4 \ drippers \ x \ 180 \ m \ x \ 15 \ beds \ = \ 1584 \ L \ water$$

Therefore, the amount of silicon (0.017% Sirius v/v) for one irrigation tunnel is:

$$0.00017 \ x \ 1584 \ L \ = \ 0.269 \ L \ \cong \ 270 \ mL$$

Each strawberry plant received approximately 270 mL:

$$270 \ mL \ / \ 5000 - 5300 \ plants \ x \ 2 \ tunnels$$
$$= \ 0.026 - 0.027 \ mL \ Sirius \ per \ plant$$

Sirius contains four different compounds (main active ingredient; 70 – 80 Tetraethyl silicate, silicon-based adjuvant and two other non-silicon compounds); therefore, the actual amount of soluble silicon that is taken up and remaining in the plants and water may be lower than the amounts calculated above (Jin, 2015; Liu, 2016).

3.6. Levels of disease in untreated (uncontrolled) strawberry plants in the silicon fertigation field experiment 2017 (Amesti)

Figure 3.4 is a disease assessment in the 'Amesti cultivar' in the silicon field experiment in 2017 (Amelia field). Disease assessments were carried out in the Amelia field from 9 April to 27 August 2017. Results in figure 3.4 show minimal disease levels in the untreated control plot only, and no disease levels occurred.
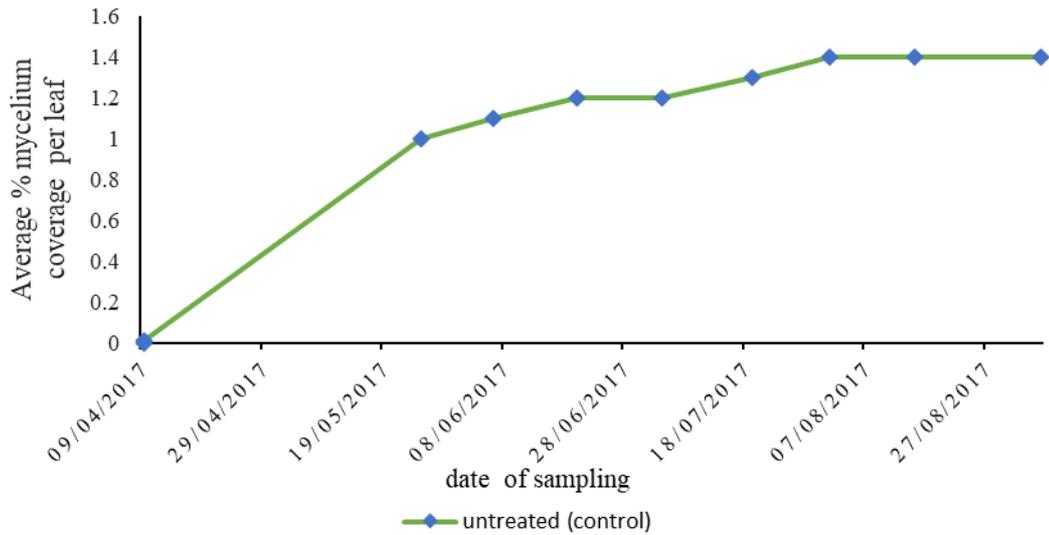
Figure 3.4. Disease levels in the 2017 silicon fertigation field experiment.

As there were deficient disease levels in the 2017 field experiment, figure 3.4 only shows the untreated control plot. No disease was found in all other treatments where silicon nutrient or fungicides were applied. No disease was detected in any other treatment (fungicides only, silicon twice + fungicides and silicon twice + no fungicides) in the silicon fertigation field experiment in 2017 as the cultivar Amesti, which is a more resistant cultivar and less susceptible to *Podosphaera aphanis* than Malling Centenary strawberry plants used in the 2016 fertigation field experiment. The growers specifically chose these cultivars. The experiment aimed to understand the effect of silicon in various cultivars besides moving to Ever bearer cultivars, as mentioned previously. A range of other cultivars grew on the farm; however, no Malling Centenary was grown in 2017 and 2018.

Also, the farm was experiencing lower disease pressures in 2017 than in 2016. The lower disease pressures experienced in the 2017 experiment were shown by the minimal amounts of disease only found in the untreated control of the field experiment, suggesting minimal pressures in disease intensities. In the 2016 field experiment, disease levels were higher, suggesting higher intensities of disease (pressures). Temperatures appeared to be less conducive to the development of fungus (*Podosphaera aphanis*), as shown by disease levels in 2017 compared to 2016. Conducive temperatures for powdery mildew fungus development are between 18 to 30°C (Hall and Jin, 2016). The measurement of temperatures under each field experiment was not stored for this experiment as the minimal amounts of disease seen throughout the experiment were not expected (2017 and 2018). Nonetheless, fungicide sprays continued in both experiments. For a list of fungicides used, see appendices 9 to 12.

3.6.1. Silicon content in the strawberry leaves from the silicon fertigation experiment 2017 (Amesti)

Silicon extractions from the leaves of strawberry plants from the 2017 silicon fertigation field experiment (April to August) are presented in the bar chart.
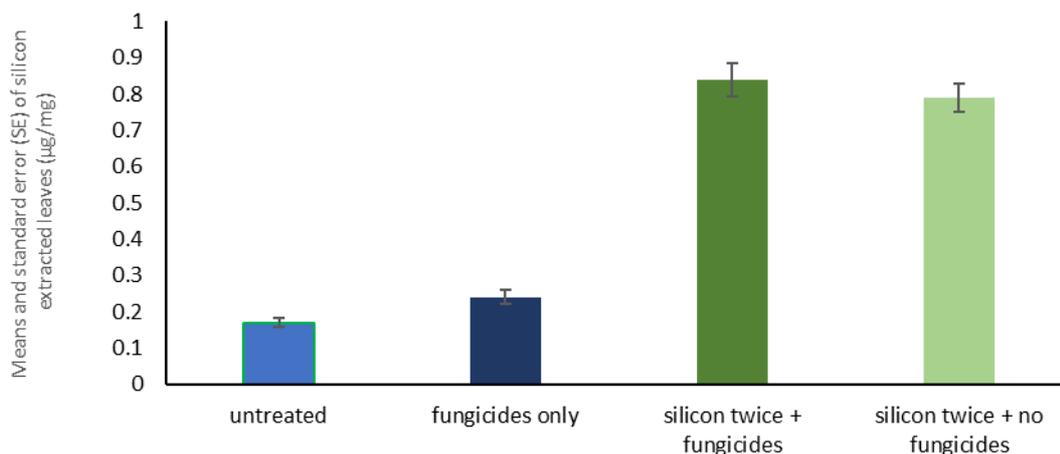
Figure 3.5. Silicon extraction from strawberry leaves (μg/mg) in the fertigation field in 2017 (at the end of the growing season in August)

Results from Figure 3.5 is a bar chart showing mean silicon extractions results at the end of the silicon fertigation field experiment season in 2017. The colours on the bars indicate the different treatments used in this experiment. The error bars are standard error bars. The bar chart was produced from silicon extraction results of untreated strawberry leaves and silicon-treated strawberry leaves.

The bar chart in figure 3.5 above shows means of 20 leaves per bed (the replicates are the 4 beds), where 20 leaves x 4 beds are 80 leaves per treatment. Refer to appendix 20 for statistical t-Tests Paired Sample for Means for silicon extraction comparisons (differences) between the untreated and silicon treatments in 2017. Table 3.5 summarises the findings of silicon extracted from strawberry leaves at the end of the silicon fertigation field experiment in 2017.

Table 3.5. Summary table of silicon extraction from strawberry leaves in the silicon fertigation field experiment 2017

Year	Treatment	Statistical test applied	Statistical findings (see appendix 20 for statistical workings)
2017	Untreated control (no fungicides and no silicon) and fungicides-only	t- Test Paired sample for average values	$P > 0.05$; no statistical differences were found between levels of silicon in untreated compared with the fungicides-only treatment. The null hypothesis (H0) is accepted
2017	Untreated control (no fungicides and no silicon) and silicon twice-a-week + fungicides	t- Test Paired sample for average values	$P < 0.05$; there was a statistical difference found between levels of silicon in untreated compared with the silicon twice + fungicides. The null hypothesis (H0) is rejected.
2017	Untreated (no fungicides and no silicon) and silicon twice-a-week + no fungicides	t- Test Paired sample for average values	$P < 0.05$; there was a statistical difference found between levels of silicon in untreated compared with the silicon twice-a-week + no fungicides. The null hypothesis (H0) is rejected.

Results from the 2017 field experiment have shown that there was an increase ($P < 0.05$) in silicon contents extracted from the leaves in the silicon twice a week + fungicides and no fungicides compared to the untreated, but no differences ($P > 0.05$) were found between the fungicides only treatment and the untreated ones. Silicon extraction measurements from the silicon fertigation water in the 2017 field experiment are shown in table 3.6 below. There were also three sample points, and the water sampled each month included a pre-assessment sample.

3.6.1.1. Silicon content in fertigation water from the silicon fertigation field experiment 2017

Table 3.6. Silicon content (mg/ml) in fertigation field water 2017

Sample point (May 2017)	Time of sampling after delivery of silicon /min	Silicon content (mg/ml) (averages of two samples)
Near silicon delivery	--	--
Near silicon delivery	3	0.024 mg/ml
Near silicon delivery	5	0.029 mg/ml
Middle silicon delivery	3	0.054 mg/ml
Middle silicon delivery	5	0.032 mg/ml
Far-end silicon delivery	3	0.001 mg/ml
Far-end silicon delivery	5	0.009 (mg/ml
Sample point (June 2017)	**Time of sampling after delivery of silicon**	**Silicon content (mg/ml) (averages of two samples)**
Near silicon delivery	Pre-addition of silicon	0.000
Near silicon delivery	3	0.010 mg/ml
Near silicon delivery	5	0.002 mg/ml
Middle silicon delivery	3	0.006 mg/ml
Middle silicon delivery	5	0.011 mg/ml
Far-end silicon delivery	3	0.005 mg/ml
Far-end silicon delivery	5	0.022 mg/ml
Sample point (of July 2017)	**Time of sampling after delivery of silicon**	**Silicon content (mg/ml) (averages of two samples)**
Near silicon delivery	--	--
Near silicon delivery	3	0.011 mg/ml
Near silicon delivery	5	0.023 mg/ml
Middle silicon delivery	3	0.009 mg/ml
Middle silicon delivery	5	0.018 mg/ml
Far-end silicon delivery	3	0.014 mg/ml
Far-end silicon delivery	5	0.030 mg/ml

Results from table 3.6 show that silicon was found throughout the length of the fertigation experiment in 2017 during silicon application. Although silicon contents vary between samples months, there were no abnormalities in the results presented in table 3.6. The water flowing through the fertigation drippers at 1.8 L per hour allows a burst of watering for 6 minutes; however, one of the aims of measuring the fertigation

water for silicon levels at these separate times was to determine whether the silicon added travelled the lengths of the tunnel during different set times.

3.7. Levels of disease in untreated (uncontrolled) strawberry plants in the silicon fertigation field experiment 2018 (Amesti)

Figure 3.6 is disease assessment also in the 'Amesti cultivar' in the silicon field experiment in 2018 (Amelia field). Disease assessments were carried out in the Amelia field from 24th April to 21st September 2018. Results in figure 3.6 show low disease levels in the untreated control plot only, and no disease levels occurred.
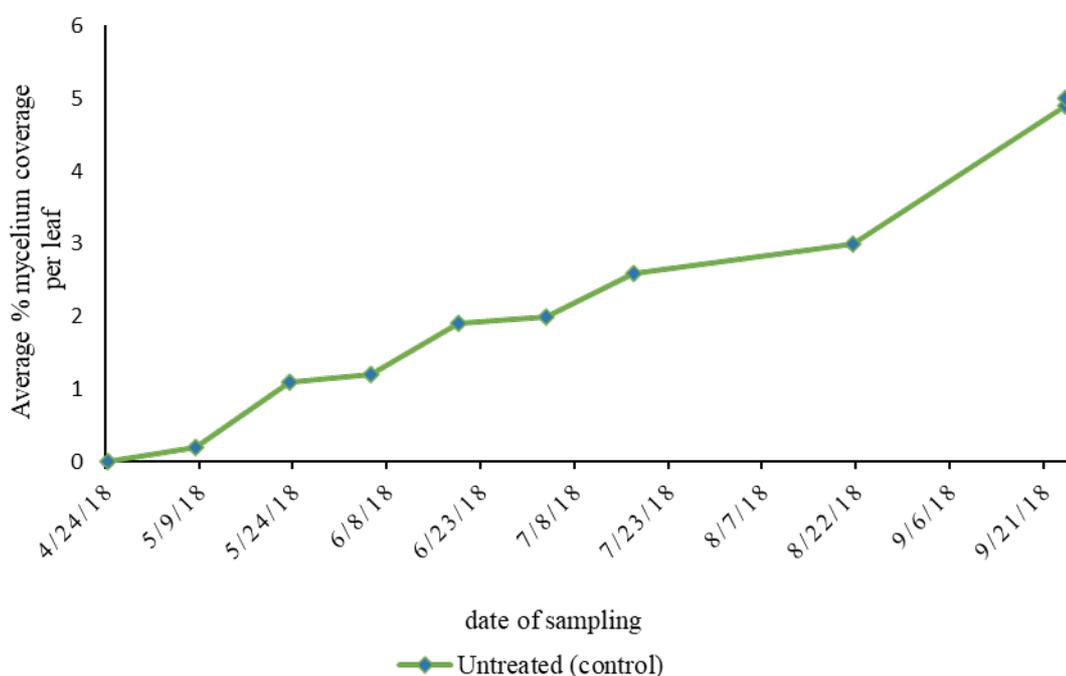
Figure 3.6. Disease levels in 2018 silicon fertigation field experiment.

Figure 3.6 also showed minimal levels of disease in the untreated control. However, no disease levels occurred in the 2018 silicon fertigation field experiment as the cultivar Amesti was also used (refer to section 3.6). No disease was found in the treatment plots where silicon nutrient was applied. This may be a result of using the same cultivar Amesti. The grower still applied fungicide sprays throughout the experiment, supported by a complete list of fungicide sprays used for the silicon fertigation field experiment in 2018 in appendix 9 to 12. Although no disease levels occurred in this experiment (figure 3.6). Silicon extractions from the leaves of strawberry plants from the 2018 silicon fertigation field experiment (April to September) are presented in the bar chart below.

3.7.1. Silicon content in the strawberry leaves from the silicon fertigation field 2018 (Amesti)
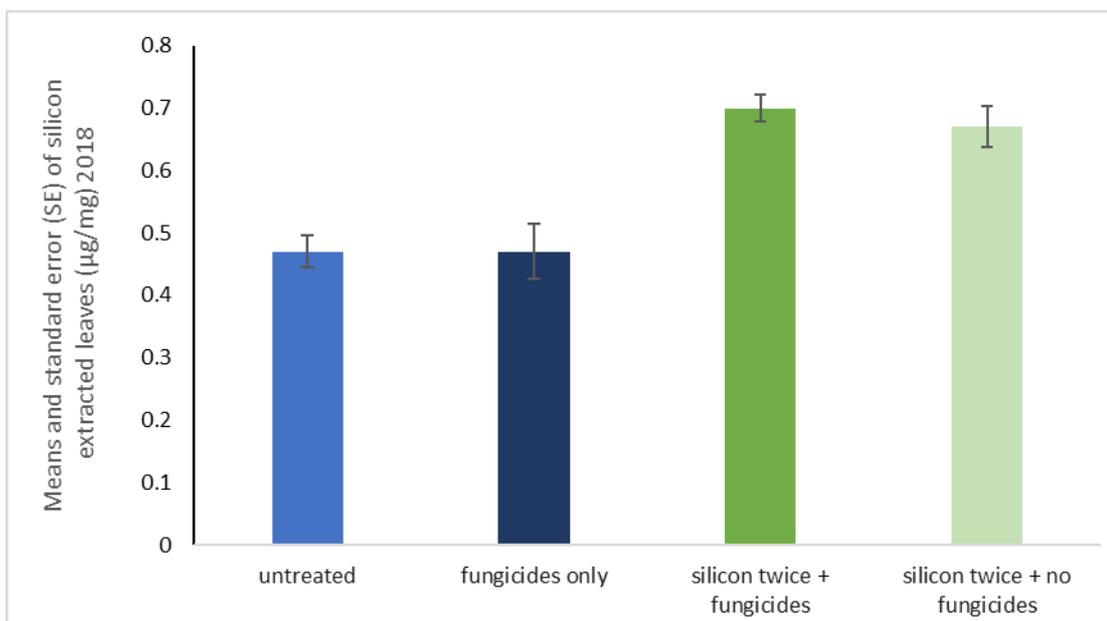
Figure 3.7. Silicon extraction from strawberry leaves (µg/mg) in the silicon fertigation field experiment 2018. (at the end of the growing season in September)

Figure 3.7 silicon extraction results proved that the plants take up the silicon nutrient delivered via the fertigation system. The different bars represent silicon extraction levels. They show that there is a larger quantity of silicon in the silicon twice weekly with and without fungicides compared to the untreated and fungicides-only treatment at the end of the experiment and season. Refer to appendix 22 for the statistical t-Test Paired Two Sample for means results of silicon extraction between the untreated and silicon-treated plants (untreated, fungicides only, silicon twice + fungicides and the silicon twice + no fungicides treatments) in the 2018 field experiment. A summary of findings of silicon extracted from strawberry leaves at the end of the silicon fertigation field experiment in 2018 is in table 3.7.

Table 3.7. Summary table of silicon extraction from strawberry leaves in the silicon fertigation field experiment 2018

Year	Treatment	Statistical test applied	Statistical findings (see appendix 22 for statistical workings)
2018	Untreated control (no fungicides and no silicon) and fungicides-only	t- Test Paired sample for average values	P>0.05; there was no statistical differences or increase found between levels of silicon in untreated control (no fungicides and no silicon) compared with the fungicides-only treatment. The null hypothesis (H0) is accepted.
2018	Untreated control (no fungicides and no silicon) and silicon twice-a-week + fungicides	t- Test Paired sample for average values	P<0.05; there was a statistical difference found between levels of silicon in untreated control (no fungicides and no silicon) compared with the silicon twice-a-week + fungicides. The null hypothesis (H0) is rejected.
2018	Untreated and silicon twice-a-week + no fungicides	t- Test Paired sample for average values	P>0.05; there was no statistical difference found between levels of silicon in untreated control (no fungicides and no silicon) compared with the silicon twice-a-week + no fungicides. The null hypothesis (H0) is accepted.

Results from the 2018 field experiment has shown that there was an increase (P<0.05) in the silicon contents extracted from strawberry leaves from the silicon twice + fungicides compared with the untreated. The results also showed that there were no statistical differences (P>0.05) found between the levels of silicon extracted from the leaves of the silicon twice + no fungicides and the untreated control. Silicon contents in the fertigation water for 2018 is presented in table 3.8 a and b below.

3.7.1.1. Silicon content (mg/ml) in water from the fertigation field experiment 2018

Silicon contents in the silicon fertigation water from the 2018 field experiment are presented in tables 3.8 a and b.

Table 3.8a. Silicon content (mg/mL) in fertigation field water

Sample point (as in figure 3.4) in May 2018	Time of sampling after delivery of silicon/ min	Silicon content (mg/ml) (averages of two samples)
Near delivery	Pre-addition of silicon	--
Near delivery	3	0.010 mg/ml
Near delivery	5	0.002 mg/ml
Middle delivery	3	0.006 mg/ml
Middle delivery	5	0.011 mg/ml
Far-end delivery	3	0.005 mg/ml
Far-end delivery	5	0.022 mg/ml
Sample point (as in figure 3.4) in June 2018	**Time of sampling after delivery of silicon/ min**	**Silicon content (mg/ml) (averages of two samples)**
Near delivery	Pre-addition of silicon	--
Near delivery	3	0.010 mg/ml
Near delivery	5	0.014 mg/ml
Middle delivery	3	0.009 mg/ml
Middle delivery	5	0.020 mg/ml
Far-end delivery	3	0.012 mg/ml
Far-end delivery	5	0.028 mg/ml
Sample point (as in figure 3.4) in July 2018	**Time of sampling after delivery of silicon/ min**	**Silicon content (mg/ml) (averages of two samples)**
Near delivery	Pre-addition of silicon	--
Near delivery	3	0.014 mg/ml
Near delivery	5	0.013 mg/ml
Middle delivery	3	0.004 mg/ml
Middle delivery	5	0.017 mg/ml
Far-end delivery	3	0.005 mg/ml
Far-end delivery	5	0.008 mg/ml

Table 3.8b Silicon content (mg/mL) in fertigation field water

Sample point (as in figure 3.4) of August	Time of sampling after delivery of silicon	Silicon content (mg/ml) (averages of two samples)
Near delivery	Pre-addition of silicon	--
Near delivery	3	0.018 mg/ml
Near delivery	5	0.026 mg/ml
Middle delivery	3	0.012 mg/ml
Middle delivery	5	0.014 mg/ml
Far-end delivery	3	0.017 mg/ml
Far-end delivery	5	0.021 mg/ml
Sample point (as in figure 3.4) in June 2018	**Time of sampling after delivery of silicon**	**Silicon content (mg/ml) (averages of two samples)**
Near delivery	Pre-addition of silicon	--
Near delivery	3	0.016 mg/ml
Near delivery	5	0.021 mg/ml
Middle delivery	3	0.013 mg/ml
Middle delivery	5	0.032 mg/ml
Far-end delivery	3	0.029 mg/ml
Far-end delivery	5	0.031 mg/ml

Results above in table 3.8 a and b show that the silicon nutrient delivered (via the fertigation system) is distributed throughout the field experiment. No samples were collected 24 hours after silicon was added to the fertigation system as the fertigation system did not run for 24 hours. Refer to chapter 2, section 2.4, for a full description and explanation of the fertigation experiment. The fertigation experiment aimed to determine the amounts of silicon (at different set times) that travelled the lengths of the tunnels and to show that the silicon delivered is distributed throughout the tunnel. The aim of this experiment was met as silicon was found throughout the fertigation fields in 2016 and 2018.

3.8. Discussion

3.8.1. The use of silicon nutrients reduced levels of strawberry powdery mildew *Podosphaera aphanis* in the silicon fertigation field experiment from 2016 to 2018

The experiment conducted in this chapter aimed to investigate further the work reported by Jin (2015), which investigated the use of silicon applied via sprays and roots through a fertigation system once a week only in a 2012 and 2013 (two years) field experiment on cultivars Elegance, Alexandra and Sonata. Liu (2016) showed silicon via root application in reducing the disease levels of strawberries on the cultivar Sonata.

The experiment reported in this chapter investigated in more detail, comparing silicon applied once- a-week with silicon applied twice- a-week treatment through a fertigation system, in reducing levels of strawberry powdery mildew using different cultivars Malling Centenary and Amesti cultivars for three complete growing seasons and measuring silicon contents in the leaves of strawberries and fertigation water for 3 consecutive years. Results from the silicon fertigation field experiment in 2016 showed that the strawberry crop from the silicon twice weekly plus fungicide, silicon twice weekly without fungicides and silicon nutrient once weekly plus fungicides had significantly ($P<0.05$) lower disease levels compared with the silicon nutrient once weekly without fungicides and the untreated (control) (figure 3.1). The disease levels graph (figure 3.1) revealed that the lowest disease levels occurred in the silicon twice weekly plus fungicides AUDPC (410) and the twice weekly plus no fungicides AUDPC (375) treatment. At the same time, the graph also showed that the most significant levels of disease occurred in the untreated (control) with the highest AUDPC (3,423).

A spearman's correlation analysis (appendix 18) was conducted in the silicon fertigation field experiment in 2016 to determine if the amount of silicon extracted had a relationship with disease infection levels and showed a moderate relationship between them. Using a silicon nutrient in the fertigation field experiment in 2016 showed similar results to the work done by Jin (2015). The work reported that the use of a silicon nutrient in a silicon fertigation field experiment in 2012 and 2013 caused a delay (more than 2 weeks) in the start of disease levels of strawberry powdery mildew on a strawberry field. Work done by Liu (2016) also found that silicon nutrient had significantly reduced the disease severity in treatments where silicon nutrient was applied, and the disease was significantly lower ($P<0.05$) in treatments where silicon nutrient had been applied.

Results from the 2017 and 2018 silicon fertigation field experiments have shown that no disease levels occurred; however, a small amount of disease was found only in the untreated control of strawberry plants in the silicon fertigation field experiments in 2017 and 2018. The farm experienced low disease pressures with high temperatures up

to and above 30°C, which is too high for disease development (Ouellette et al., 2017). Although no disease levels occurred in 2017 and 2018 field experiments, results from this thesis have shown that the addition of a silicon nutrient significantly ($P<0.05$) reduced the disease severity of *Podosphaera aphanis* in 2016, and similar results have been achieved in previous work done by Jin (2015) and Liu (2016). Jin (2015) showed in her thesis that the application of silicon via foliar and root 'once a week' can reduce the effects of powdery mildew. This chapter shows that silicon reduces powdery mildew in Malling Centenary plants treated once and twice a week via a fertigation system.

3.8.2. Silicon extraction from leaves of strawberry plants from the silicon fertigation field experiment 2016 to 2018

Previous preliminary work at the University of Hertfordshire by Jin (2015) and Liu (2016) has shown that the silicon delivered through (foliar and root) of strawberry plants 'once weekly' in field experiments contained more silicon compared with the untreated plants. Results from this experiment showed that in 2016, there was a statistical increase ($P<0.05$) found in the levels of silicon extracted from leaves silicon twice a week + no fungicides treatments compared to the untreated control and no statistical differences ($P>0.05$) in the silicon extracted from the leaves in the silicon once a week + fungicides, silicon once-a-week + no fungicides, silicon twice + fungicides compared to the untreated at the end of the experiment.

Results showed that in the 2017 field experiment, there was a statistical increase ($P<0.05$) between the levels of silicon contents of the leaves of silicon-treated plants compared to the untreated control. In the 2018 field experiment, there was a statistical increase ($P<0.05$) in the levels of silicon extracted from the leaves in the silicon twice + fungicides and the untreated ones. No differences ($P>0.05$) were found in the silicon twice + no fungicides treatment and the untreated ones. Silicon extraction results did not show a trend in the amounts of silicon extracted between the 2016 to 2018 silicon fertigation field experiments. However, other factors could have played a role in the differences in the quantity of silicon remaining in plants after treatments.

3.8.3. Silicon contents in water from the silicon fertigation field experiment from 2016 to 2018

Silicon contents in fertigation water in the silicon fertigation field experiments were measured to show that the silicon delivered through the fertigation system is transported throughout the field. Although previous work by Liu (2015) and Jin (2016) has found that the silicon delivered is distributed in the fertigation water in a one-year experiment, the work reported in this chapter extended the experiment for 3 years consecutively. Conducting this experiment for three years allowed the observation for consistency in

the presence of silicon in the fertigation system after application. This experiment showed that the fertigation system always worked each year and can be a reliable means for transporting silicon, water and all other nutrients to crops. This work suggests that the fertility system's silicon is always transported throughout the fertigation field. Using a fertigation system, silicon nutrient are delivered through the roots of strawberry plants.

3.8.4. Agronomic differences between June bearers and Ever bearer strawberry plants

Malling Centenary strawberry plants were more susceptible to strawberry powdery mildew with high disease levels, as shown in the 2016 silicon fertigation field experiment graph (figure 3.1), while the Amesti strawberry plants used in the silicon fertigation field experiment in 2017 and 2018 were tolerant to the disease with minimal disease levels found only in the untreated plot of both fields. Temperatures (section 3.6) could have also contributed to less disease development in both fields; however, this thesis does not confirm the impact of these temperatures. This chapter suggests, in summary, that silicon can reduce disease levels of strawberry powdery mildew *Podosphaera aphanis* in 2016 (figure 3.1). Silicon was effectively absorbed by strawberry plants (silicon extractions from leaves), and silicon was always found throughout the length of the tunnel and in the fertigation water (silicon contents in fertigation water) in field experiments from 2016 to 2018.

3.9. Conclusion

This chapter's main findings showed that silicon effectively reduces strawberry powdery mildew disease in different strawberry plant cultivars, such as the Malling Centenary cultivars 2016. It showed that silicon as a treatment could delay the start of disease level build-up by up to 29 days (2016). Therefore, the aim to investigate the effects of silicon in reducing strawberry powdery mildew was met in 2016. Although this chapter has shown that silicon can reduce the disease severity, the following chapter (chapter 4) explores how silicon can be involved in enhancing the passive defence pathway of strawberries, thereby reducing susceptibility to strawberry powdery mildew disease.

Chapter 4 – Silicon deposition in strawberry plants

This chapter covers i) An introduction to the deposition of silicon in plants; ii) Rationale, aim, objectives and hypothesis; iii) Material and methods of the chapter; iv) Results; v) Discussion and vi) Conclusion.

4.1. Introduction

In chapter 3, results showed that a silicon nutrient application in a fertigation field experiment in 2016 reduced susceptibility to strawberry powdery mildew (*Podosphaera aphanis*) and also in previous years' field experiments showed by Jin (2015) and Liu (2016). This chapter investigates the deposition, amount and pattern of distribution of silicon and its role in enhancing the passive defence pathway of strawberry plants about strawberry powdery mildew (*Podosphaera aphanis*) disease reduction (Debona et al., 2017).

4.1.1. Silicon deposition in plants

This chapter explores how strawberry powdery mildew disease reduction can be attained with the use and deposition of silicon in strawberry plants. This chapter also explains the location of silicon deposited in plants. Firstly, silicon is present in all plants (Greger, Landberg and Vaculík, 2018). Silicon accumulation differs significantly between plant species due to differences in silicon uptake by the roots (Rodrigues et al., 2015). Work done by Jin (2015) established in a glasshouse experiment on strawberry plants revealed that the silicon nutrient administered through the roots of strawberries was deposited in the epidermal layer of the strawberry leaves and the xylem and epidermis of the petioles of treated strawberry plants. SEM images showed that the wax formation of strawberry leaves was greater and thicker in silicon-treated plants compared with the untreated control (Jin, 2015). Debona et al. (2017) found that silicon deposition and polymerization beneath the cuticle, cell walls and bulliform cells formed a physical barrier causing a reduction in the severity of rice blast disease.

4.2. Rationale, aim, objectives and hypothesis of silicon deposition in strawberry plants

4.2.1. Rationale

Silicon nutrient can reduce levels of strawberry powdery mildew. However, this can be linked to the accumulation (deposition) of silicon in strawberry plants' tissues (leaves and petioles). This chapter sought to examine the amount and pattern of deposition of silicon in strawberry plants. This experiment examined plants growing in a glasshouse-controlled environment and field tunnels.

4.2.2. Aim

This chapter aimed to examine the amounts and pattern of distribution of silicon in leaves, petioles and roots of strawberry plants in glasshouse and field experiments.

4.2.3. Objectives

- Assess the cross-sections of strawberry leaves, leaf petioles and roots of silicon-treated and untreated strawberry plants from a glasshouse and field experiments with a LysoTracker Yellow HCK-123 dye.

- Quantify the amounts of silicon deposits in cross-sections (leaves, leaf petioles and roots) of strawberry plants from a glasshouse and field experiments from stained samples viewed through a fluorescence microscope.

- Determine the pattern of silicon deposition in leaves, leaf petioles and roots in both glasshouse and field experiments.

4.2.4. Hypothesis

- There can be higher silicon levels in treated plants compared to untreated plants.

- The silicon can be deposited in a regular manner (form) in the leaves.

4.3. Material and methods

4.3.1. Silicon deposition

The deposition of silicon in strawberry plants experiment was established in the University of Hertfordshire, Hatfield glasshouse in 2017 and the silicon fertigation field experiment in 2017 and 2018. See chapter 2, table 2.2, for treatments used. Strawberry plants in the 2017 and 2018 silicon fertigation field experiments, established for disease assessments, silicon extractions and measurements of silicon content in fertigation water, were harvested for a silicon deposition experiment at the end of the season.

Harvested strawberry plants were placed in large polythene bags and labelled. Eight whole plants per treatment and 32 were harvested from the silicon fertigation field experiments for the deposition examination. Plants were stored in a cold room at 4°C in the Science Building, College Lane Campus at the University of Hertfordshire. Before the examination, the leaves, leaf petioles and roots were carefully detached from the plants using a sharp single-edged surgical carbon-steel blade to prevent any damage.

Ten cross-sections of the leaves, leaf petioles and roots per treatment and 60 cross-sections in total were sectioned (from glasshouse and field experiments) each time using new sets of the single-edged surgical carbon-steel blades, following the old traditional method by placing each plant part (leaf, leaf petioles and root) in a carrot and slicing of the tips severally to achieve a one-cell thick section each time. Surgical blades were replaced after four uses. Each cross-section was picked up using a paintbrush and transferred onto labelled 8 x 6 x 2.5 cm microscope glass slides for fluorescence dye staining.

4.3.2. Plant sectioning

At the end of each experiment, ten leaves, ten petioles and ten roots per treatment were sampled from each treatment in the glasshouse and field experiments. All cross-sections were stained using the LysoTracker dye. See section 4.3.3 below for details on the fluorescence dye staining.

4.3.3. LysoTracker Yellow HCK-123 fluorescence dye staining

The LysoTracker Yellow HCK-123, a fluorescence dye, was used in staining cross-sections of plants (Desclés et al., 2008; Gröger, Sumper and Brunner, 2008; Shetty et al.,2012; Oh et al., 2018). The LysoTracker dye was obtained from the ThermoFisher Scientific manufacturer. Each plant section was stained separately on slides for two hours in the dark. The Vectashield mounting media from the ThermoFisher Scientific manufacturer was used in drops to preserve fluorescence in samples while staining. After two hours of staining, samples were moved from the microbiology laboratory to the microbiology preparation room for observation. Cross-sections were viewed using the GXML3201 LED fluorescence microscope. Images viewed were saved onto Secure Digital Cards (SD) for further observations and a silicon quantification analysis. Silicon fluorescence intensities (density) were assessed and quantified using the software program ImageJ, which measures the total amounts of fluorescence within each sample. See appendix 23.

4.3.4. GXML3201 LED fluorescence microscope

Each sample slide containing a leaf, leaf petiole and root cross-section was viewed using the GXML3201 LED fluorescence microscope (figure 4.1) to determine the location of silicon deposits through the accumulation of fluorescence intensity. Each

sample slide was viewed at x40 and x400 magnification using a green filter and a wavelength of 450nm. Photos were saved each time by selecting the "capture icon' on the microscope screen onto a removable Secure Digital Card (SD) card for fluorescence intensity measurements. Measurements of fluorescence intensities were conducted using the ImageJ processing program designed for multidimensional scientific images for quantification. Refer to appendix 24 for the protocol for using ImageJ.

4.3.5. Silicon accumulation (fluorescence intensity) measurement

Fluorescence intensities were measured using ImageJ, a multidimensional imaging processing software involving quantifying the fluorescence intensities by downloading direct images from a microscope (Collins, 2007; Jensen, 2013; Bankhead, 2014). The unknown area (blank) of the image (cell) is subtracted from the integrated density (total fluorescence) of the cell, which equals the total quantity of fluorescence (without background fluorescence). The total fluorescence (integrated density) given is divided by the area of the cell, which then equals the actual quantity of fluorescence per given sample.

Therefore:

$$Actual\ Fluorescence = \frac{Fluorescence\ of\ the\ cell}{Area\ of\ the\ cell}$$
$$= Integrated\ Intensity - Blank\ (unknown\ area\ of\ the\ cell)$$

Fluorescence intensities are recorded in Relative Fluorescence Units (RFU) or counts per second (cps) arbitrary units (Peruski, Johnson and Peruski Jr, 2002; Neumeier, Heck and Feldmann, 2019). Refer to appendix 19 for a protocol for using ImageJ.
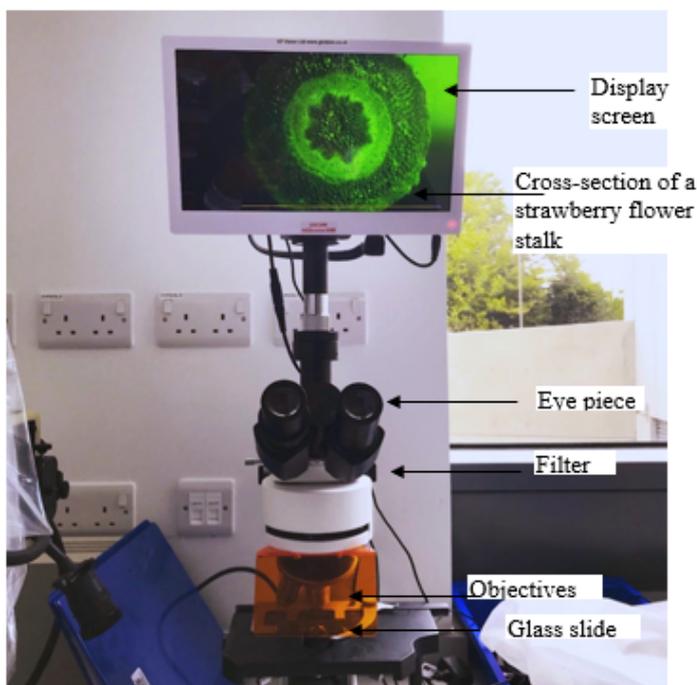
Figure 4.1. GXML3201 LED fluorescence microscope with a cross-section sample at x 40 magnification.

4.3.6. Treatments

The glasshouse pot compost experiment had only two treatments; the silicon-treated (0.017% v/v at 50mls per plant pot) and the untreated control (50mls of de-ionized water). Field experiments only used silicon treatments (0.017% v/v via fertigation system) and the untreated for deposition comparison and statistical analysis. Silicon was applied through the roots in both experiments.

4.3.7. Cultivars

The cultivar Amesti was used in the silicon fertigation field experiment in 2017 and 2018. The cultivar Malling Centenary was used in the glasshouse pot compost experiment. To describe the Amesti and Malling Centenary cultivars used in this experiment, refer to chapter 1, table 1.4.

4.4. Results

4.4.1. Pattern and deposition of silicon in the leaves, leaf petioles and roots from a glasshouse experiment 2017 (quantification of fluorescence intensity)

See results from the silicon deposition in a glasshouse experiment (pot compost) 2017 in figure 4.2, table 4.1 to 4.2 (appendix 25 to 33). Table 4.1 includes mean fluorescence intensities (untreated and silicon-treated cross-sections) from ImageJ recorded in relative fluorescence units (RFU) and counts per second (cps).

At the end of the glasshouse experiment, cross-sections of ten leaves, ten petioles and ten roots were sampled from each treatment (untreated and silicon-treated). All cross-sections were stained using the LysoTracker Yellow HCK-123 dye. The data collected for the 2017 glasshouse experiment was first tested for normality before using the one-way ANOVA to determine the differences in fluorescence intensities (levels of silicon deposits) among treatments such as untreated and silicon-treated. Refer to appendix 25 to 33 for all (total) replicates of fluorescence (cross-sections) images from the glasshouse experiment.
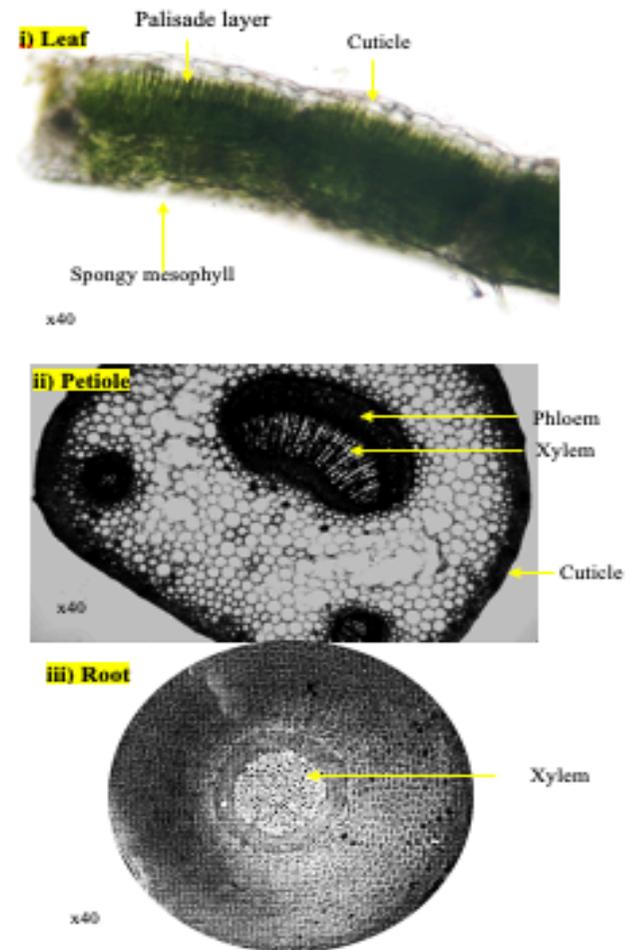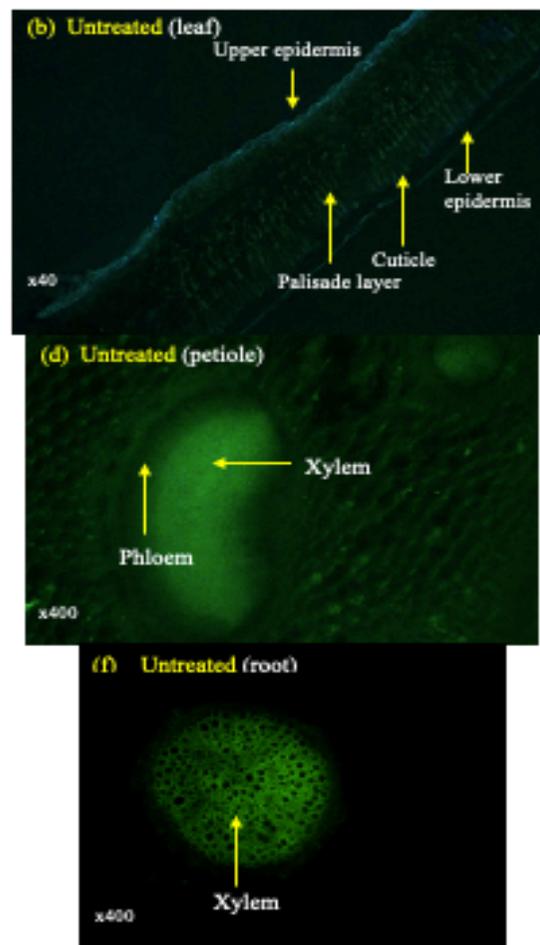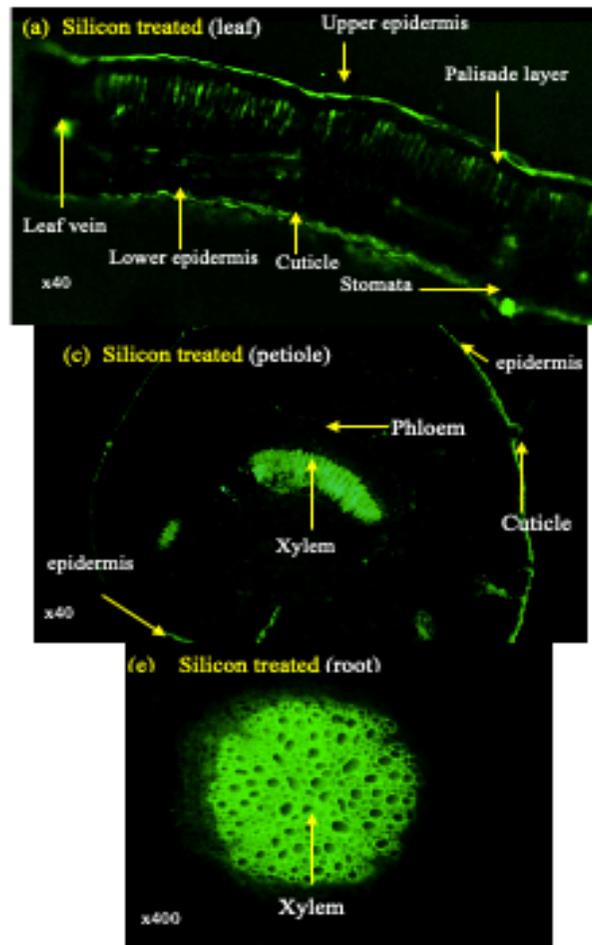
Figure 4.2. Silicon deposition in glasshouse experiment 2017.

Silicon deposits in green fluorescence. Viewed at x 40 and x 400 magnification. (a) Silicon-treated (leaf) More silicon was found in the upper and lower cuticle, epidermis, palisade layer and leaf vein compared with untreated (b). (c) Silicon-treated (petiole) More silicon was found in the cuticle and xylem than untreated (d). e) Silicon-treated (root) More silicon was found in the treated compared with untreated (f). Fluorescence Intensity quantification - Counts per second (cps) is (a) 7.9cps for the silicon-treated (leaf) (b) 2.2cps for untreated (leaf) (c) 7.7cps for the silicon-treated (petiole) (d) 1.9cps for untreated (petiole) (e) 11.6cps for the silicon-treated (root) and (f) 1.2cps from untreated (root).

Figure 4.2 above show some images of the different intensity levels and deposition pattern in the 2017 glasshouse experiment. Figure 4.2(a) shows the amounts of fluorescence intensity and pattern of silicon deposition in the leaf (7.9cps) that the silicon was laid down regularly in the upper and lower epidermis, palisade layer, xylem and vein of the leaf in silicon-treated plants (b) in the untreated, there was only a background level of silicon (2.2cps), found in the leaf, (c) shows the amounts of fluorescence intensity and pattern of silicon deposition in (7.7cps) in the silicon-treated leaf petioles and is laid down in the epidermis and xylem (d) in the untreated, there is only a background level of silicon (1.9cps), found in the xylem (e) shows the amounts of fluorescence intensity and pattern of silicon deposition in a silicon-treated root (11.6cps), there was only a background level of silicon found in the xylem (f) In the untreated root, (1.2cps). Refer to appendix 25 to 33 for fluorescence images from the glasshouse experiment replicates. Mean readings from fluorescence intensities (integrated density) in the leaf, leaf petiole, and root from the glasshouse experiment in 2017 is in table 4.1

Ten leaves, ten petioles and ten roots per treatment were sampled for ten cross-sections each from the glasshouse experiment in 2017. Table 4.1 below is a summary table of findings from the glasshouse experiment. Treatments below include the leaf, leaf petiole and root from the untreated and silicon-treated plants. A mean reading was achieved each time from 3 readings per cross-section by ImageJ.

Fluorescence intensities from ImageJ presented in table 4.1 below show the leaves, leaf petioles and roots of silicon-treated plants compared to the untreated ones. Silicon was found throughout (leaves, leaf petioles and roots) of the strawberry plants in the glasshouse (pot compost) experiment in 2017. The images in appendix 25 to 33 were used to produce table 4.1below. Refer to appendix 60 for statistical workings to calculate the differences between the means sample of untreated and silicon-treated plant cross-sections presented in table 4.1 below. A summary table for the means of 10 cross-sections of strawberry plant leaves from the glasshouse experiment in 2017 is in table 4.2.

Table 4.1. Readings from fluorescence intensities (integrated density) in the leaf, leaf petiole and root from a glasshouse experiment 2017. Values are the mean of 3 readings per leaf, leaf petioles and roots.

Strawberry cross-section	Untreated	Silicon-treated
Leaves	2.2cps	4.7cps
	1.2cps	2.0cps
	1.1cps	1.9cps
	1.6cps	4.0cps
	3.8cps	5.7cps
	1.9cps	12.1cps
	4.5cps	6.3cps
	1.9cps	7.7cps
	3.7cps	3.5cps
	2.2cps	7.9cps
Leaf petiole	4.1cps	5.0cps
	3.6cps	5.5cps
	2.3cps	10.7cps
	3.4cps	8.3cps
	2.2cps	7.7cps
	1.8cps	9.1cps
	2.0cps	9.0cps
	1.1cps	8.5cps
	3.5cps	5.9cps
	4.0cps	9.4cps
Root	3.2cps	9.2cps
	6.4cps	13.3cps
	4.8cps	8.6cps
	8.1cps	1.7cps
	7.4cps	9.9cps
	5.9cps	10.1cps
	4.3cps	7.9cps
	5.3cps	2.6cps
	7.3cps	8.7cps
	1.2cps	11.6cps

Table 4.2. Summary of 10 fluorescence intensities (integrated density) in the leaf, leaf petiole and root from a glasshouse experiment 2017

Cross-section	Treatments	Statistical test applied	Statistical findings (see appendix 60 for statistical workings)
Leaves	Untreated control (no fungicides, no silicon) and silicon twice-a-week treated	Shapiro-Wilk test for normality and One Way ANOVA to find the average values of differences between untreated and silicon-treated plants.	There were statistically significant differences between group means as determined by One Way ANOVA (F (1,18) = [7.142], P = 0.016.
Leaf petioles	Untreated control (no fungicides, no silicon) and silicon twice-a-week treated	Shapiro-Wilk test for normality and One Way ANOVA to find the average values of differences between untreated and silicon-treated plants.	There were statistically significant differences between group means as determined by One Way ANOVA (F (1, 18) = [22.604], P = 0.000.
Roots	Untreated (no fungicides, no silicon) and silicon twice-a-week treated	Shapiro-Wilk test for normality and One Way ANOVA to find the average values of differences between untreated and silicon-treated plants.	There were statistically significant differences between group means as determined by One Way ANOVA (F (1, 18) = [31.283], P = 0.000.

The work in this chapter suggests that there are more silicon deposits ($P<0.05$) in the leaves, leaf petioles and roots of silicon-treated plants compared to the untreated from the 2017 glasshouse experiment. All samples were replicated as shown by their means in table 4.1 and appendix 25 to 33.

Previous work done by Jin (2015) on silicon deposition in the leaf and leaf petiole of strawberry plants in a glasshouse experiment only showed that more silicon was found in the silicon-treated plant's leaf and leaf petiole compared to the untreated ones. However, the level of deposition of silicon nutrients via fluorescence intensities was not quantified or shown in the roots. This chapter also found that silicon nutrients applied through the roots of strawberries in a glasshouse experiment accumulated silicon throughout the plant, such as the leaves, leaf petioles and roots. The work showed the regularity of silicon deposited in the leaves, leaf petioles and roots.

4.4.2. Pattern and deposition of silicon in the leaves, leaf petioles and roots from the silicon fertigation field experiment 2017 (quantification of fluorescence intensity)

Figure 4.3 and appendix 34 to 42 show where silicon is deposited in the plants harvested from the 2017 field experiment. Table 4.3 shows the mean quantification of fluorescence intensities in the leaves, leaf petioles and roots from the 2017 field experiment. The silicon deposition and quantification experiment give a precise idea of the location and amount of silicon within the plant before silicon extraction. At the end of the 2017 field experiment, eight whole strawberry plants per treatment were sampled for ten leaves, ten leaf petioles and ten roots from each treatment (untreated and silicon twice only treatment). All cross-sections were stained using the LysoTracker Yellow HCK-123 dye. See appendix 34 to 42 for more fluorescence images from this experiment in 2017. Refer to appendix 61 for statistical workings. The data collected from the 2017 field experiment was first tested for normality before using the two-way ANOVA to determine the differences in fluorescence intensities among mean treatments. The images presented in figure 4.3 are samples taken from the untreated and silicon twice-a-week only treatment. Means of three readings were done in every ten cross-sections, as shown below. Figure 4.3 shows examples of results images from the fluorescence microscope. Refer to results in table 4.3 for fluorescence intensities measurements, appendix 34 to 42 and appendix 61 for statistical workings.
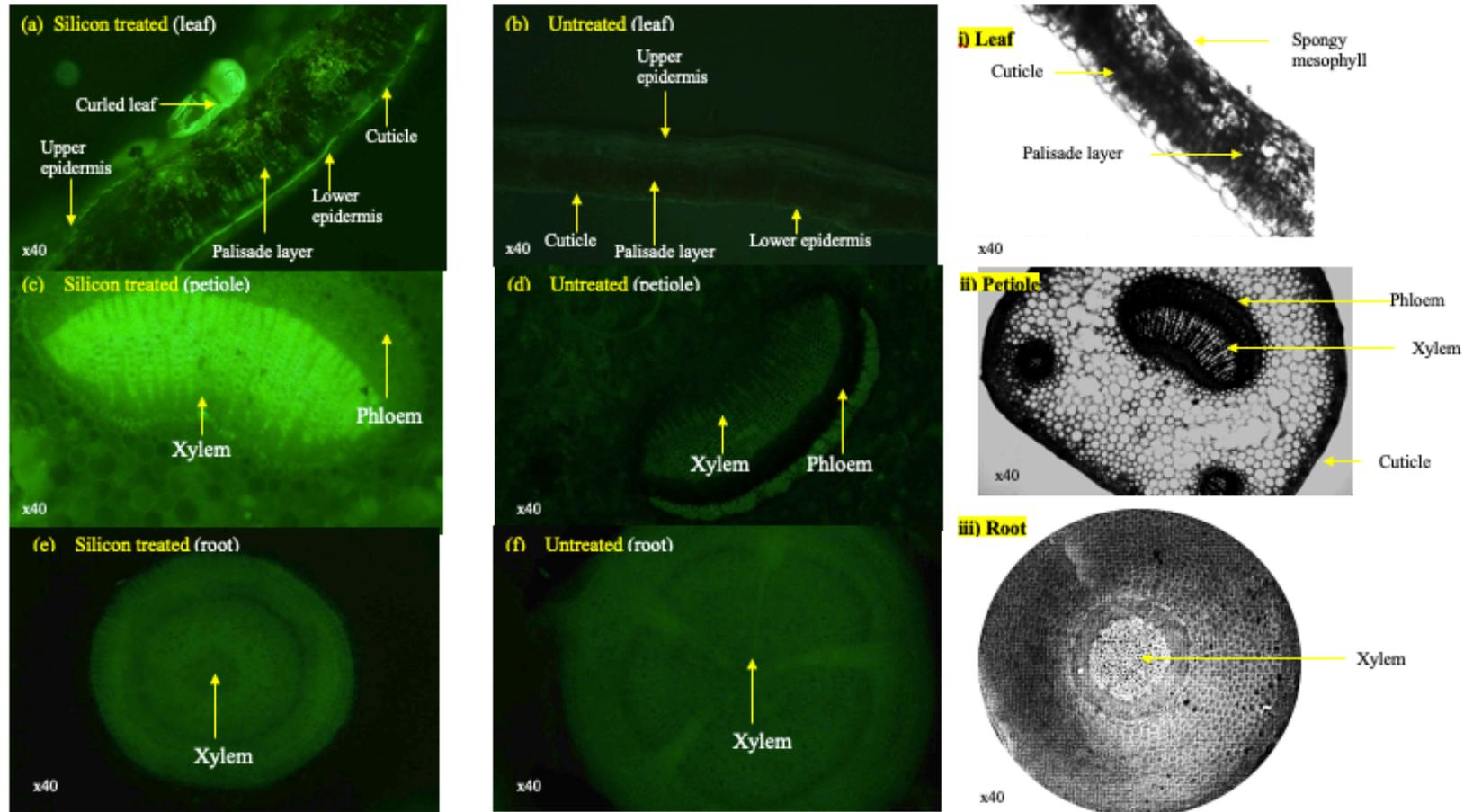
Figure 4.3. Silicon deposition in silicon field experiment 2017. Silicon deposits in green fluorescence.

Viewed at x 40 and x 400 magnification(a), Silicon-treated (leaf) showed that more silicon was found in the upper and lower cuticle, epidermis and palisade layer compared with untreated (b). (c) Silicon-treated (petiole) More silicon was found in the xylem compared with untreated (d) (e) Silicon-treated (roots) show no silicon was found in both the treated and untreated (f). The fluorescence intensity quantification for each figure is 19.4 cps for (a) silicon twice only (leaf), (b) 7.9 cps for untreated (leaf), (c) 16.7cps for the silicon twice only (petiole), (d) 10.2cps for untreated (petiole) (e) 6.9cps for the silicon twice only (root) and (f) 6.0 cps for untreated (root). Note: the best images captured from the GXML3201 LED fluorescence microscope are provided in the whole of the chapter; refer to appendix 15 for replicates of cross-sections.

The images in figure 4.3 above show some examples of the different intensities and deposition patterns in the 2017 field experiment. Figure 4.3(a) shows the amounts of fluorescence intensity and pattern of silicon deposition in the leaf (19.4cps) that the silicon was laid down regularly in the upper and lower epidermis, cuticle, and palisade layer in silicon-treated plants (b) in the untreated, there was only a background level of silicon (7.9cps), found in the leaf, (c) shows the amounts of fluorescence intensity and pattern of silicon deposition (16.7cps) in the silicon-treated leaf petioles and is laid down in the epidermis and xylem (d) in the untreated, there is some level of silicon (10.2cps), found in the xylem (e) shows the amounts of fluorescence intensity and pattern of silicon deposition in the silicon-treated root (6.9cps), there was only a background level of silicon found in the xylem (f), and in the untreated root, there was also only background level of silicon (6.0cps), found in the xylem. Refer to appendix 34 to 42 for replicates of fluorescence images from the 2017 field experiment. The amounts of silicon in the different treatments were statistically analysed using the cross-section means presented in table 4.3 below.

Ten leaves, 10 petioles and 10 roots per treatment were sampled for 10 cross-sections each from the field experiment in 2017. Treatments below include the leaf, leaf petiole and root from the untreated and silicon twice only treatment (for statistical analysis). Mean readings were achieved each time from 3 readings per cross-section by ImageJ.

From table 4.3 below (2017 field experiment), it appears that more silicon is found in cross-sections of the leaves, leaf petioles and roots of plants treated with silicon compared to the untreated ones. Refer to appendix 61 for statistical tests used to evaluate the means of both treatment means. A summary table for the means of ten cross-sections of strawberry plant leaves, leaf petioles and roots from the field experiment in 2017 is presented in table 4.4

Table 4.3. Fluorescence intensities (integrated density) in the leaf, leaf petiole and root from a field experiment 2017

Strawberry cross-section	Untreated	Silicon-treated
Leaves	7.99cps	19.4cps
	3.82cps	12.13cps
	4.34cps	8.24cps
	6.91cps	8.34cps
	7.71cps	6.61cps
	8.64cps	10.33cps
	6.53cps	12.63cps
	2.84cps	7.46cps
	6.84cps	10.16cps
	5.32cps	8.32cps
Leaf petiole	6.24cps	16.7cps
	6.53cps	8.96cps
	7.11cps	12.2cps
	3.61cps	7.95cps
	4.73cps	4.66cps
	6.71cps	13.8cps
	8.41cps	10.56cps
	7.84cps	9.65cps
	5.32cps	3.33cps
	4.43cps	5.22cps
Root	6.34cps	6.94cps
	2.27cps	7.97cps
	4.45cps	8.44cps
	3.99cps	5.41cps
	6.01cps	6.94cps
	7.73cps	8.42cps
	3.24cps	8.14cps
	3.37cps	8.15cps
	6.45cps	10.35cps
	5.45cps	7.85cps

Table 4.4. Summary of 10 fluorescence intensities (integrated density) in the leaf, leaf petiole and root from a field experiment in 2017

Cross-section	Treatments	Statistical test applied	Statistical findings (see appendix 61 for workings)
Leaves, leaf petioles and roots	Untreated and silicon twice-a-week only treatment	Shapiro-Wilk test for normality and Two Way ANOVA to find the average values of differences between untreated and silicon twice-treated plants.	There was a statistically significant difference between treatment means as interpreted by the Two Way ANOVA (F (3) = 10.047, P = 0.000. However, the interaction between the block and treatment terms was not significant, (F (3) = 1.465, P = 0.197 as shown by the output model.

Examples of the 2017 silicon fertigation field experiment results are shown in figure 4.3. Results have shown that all plants contain little silicon; however, silicon treatments enhanced the amount of silicon naturally present within the plant. Results (figure 4.3 and Appendix 61) showed that more silicon had accumulated in silicon-treated plants than in untreated ones. In strawberry leaves silicon-treated, more silicon deposits were laid down regularly in the upper and lower cuticle, epidermis and palisade layers compared to the untreated ones.

In strawberry leaf petioles, silicon was found mainly in the xylem of silicon-treated plants compared with the untreated ones. The cross-section of the roots of treated and untreated plants shows that no additional silicon was deposited in the roots (figure 4.3). However, fluorescence quantification has shown that there was little silicon found in the roots of silicon-treated and untreated ones. Images in Figures 4.3i, ii and iii are unstained cut cross-sections (as in figure 4.2) of a strawberry leaf, leaf petiole and root viewed under UV light at x40 magnification through the GXML3201 fluorescence microscope. This chapter presents these images to show the plant's anatomy without the Lysotracker fluorescence dye.

4.4.3. Pattern and deposition of silicon in leaves, petioles and roots from the silicon fertigation field experiment 2018 (quantification of fluorescence intensity)

Figure 4.4 shows some examples of fluorescence images of the cross-section of the leaves, leaf petioles and roots of plants from the 2018 silicon fertigation field experiment. The means used in analysing the cross-sections from this experiment are provided in table 4.5 below.
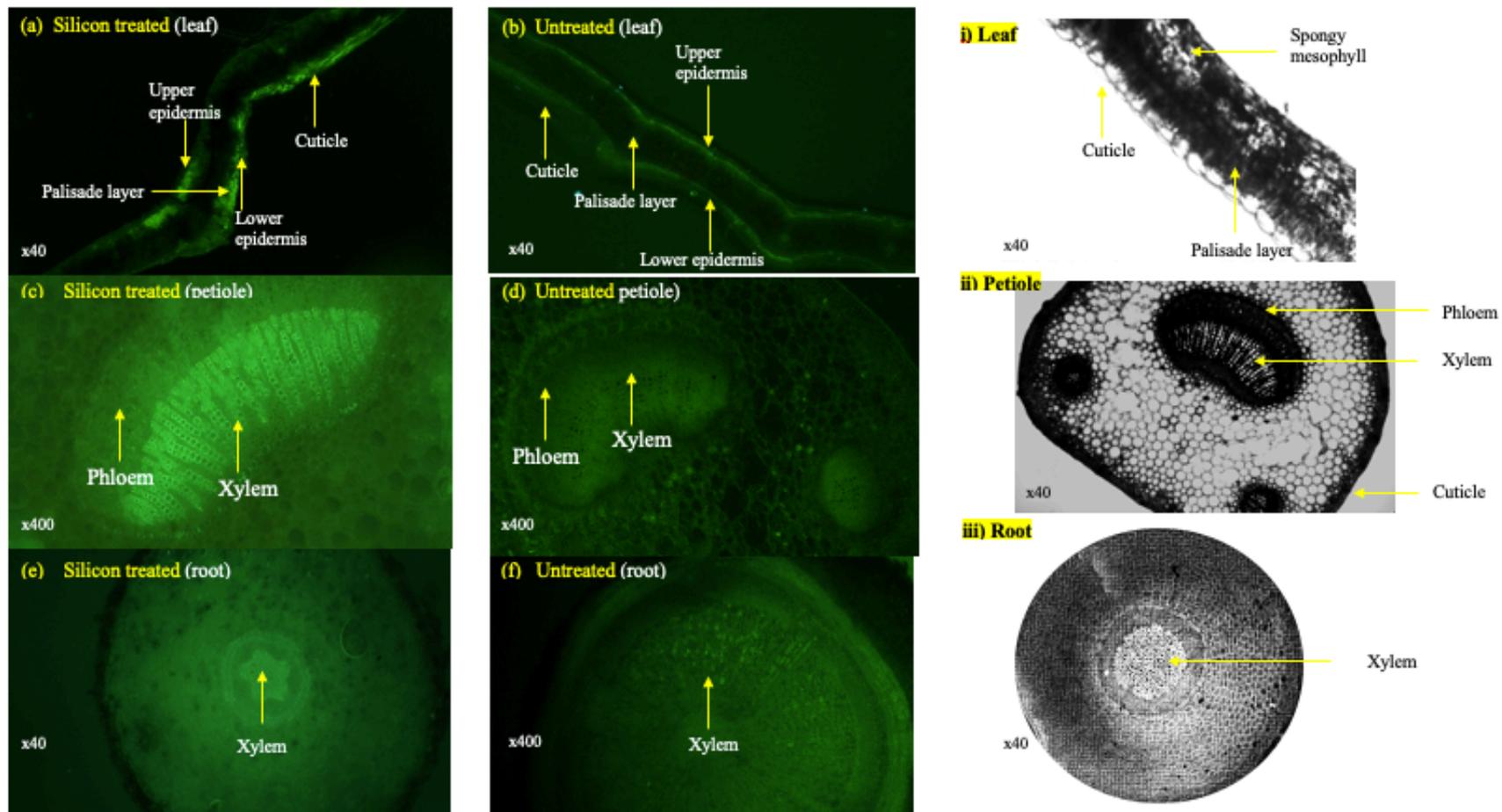
Figure 4.4. Figure Silicon deposition in silicon field experiment 2018.

Silicon deposits in green fluorescence. Viewed at x 40 and x 400 magnification. (a) Silicon-treated (leaf) showed that more silicon was found in the upper and lower cuticle, epidermis and palisade layer compared with untreated (b). (c) Silicon-treated (petiole) showed that more silicon was found in the xylem than untreated (d). e) Silicon-treated (root) showed that no silicon was found in both the treated and untreated (f). The fluorescence intensity quantification is as follows: (a) 4.98cps silicon twice only (leaf) (b) 2.2 cps for untreated (leaf) (c) 3.72 cps for the silicon twice (petiole) (d) 1.98cps for untreated (petiole) (e) 4.97 cps for the silicon twice (root) and (f) 1.76 cps for untreated (root).

The images in figure 4.4 above show some of the different intensities and deposition patterns in the 2018 field experiment. Figure 4.4(a) shows the amounts of fluorescence intensity and pattern of silicon deposition in the leaf (4.98cps), that the silicon was laid down systematically in the upper and lower epidermis, cuticle and barrier layer of the leaf in silicon-treated plants (b) in the untreated, there was only a background level of silicon (2.2cps), found in the leaf, (c) shows the amounts of fluorescence intensity and pattern of silicon deposition in (3.72cps) in the silicon-treated leaf petioles and is laid down mainly in the xylem (d) in the untreated, there is only a background level of silicon (1.98cps), found in the xylem (e) shows the amounts of fluorescence intensity and pattern of silicon deposition in the silicon-treated root (4.97cps). The untreated shows a similar background level of silicon found in the root's xylem (f) (1.76cps). Refer appendix 43 to 51 for the remaining replicates of fluorescence images from this field experiment. The amounts of silicon in the different treatments were statistically analysed using means of replicates from table 4.5, shown below.

Table 4.5. Readings from fluorescence intensities (integrated density) in the leaves, leaf petioles, and roots from the silicon fertigation field experiment 2018.

Strawberry cross-section	Untreated	Silicon-treated
Leaves	2.54cps	2.90cps
	3.63cps	3.85cps
	3.71cps	6.54cps
	3.32cps	5.25cps
	3.15cps	3.88cps
	2.22cps	4.96cps
	5.14cps	7.47cps
	4.33cps	5.56cps
	5.79ps	3.36cps
	3.99cps	5.94cps
Leaf petiole	2.09cps	3.72cps
	4.33cps	4.76cps
	4.44cps	6.56cps
	6.31cps	7.74cps
	6.47cps	4.33cps
	1.98cps	2.97cps
	4.56cps	7.43cps
	2.54cps	1.38cps
	3.85cps	3.66cps
	6.50cps	5.35cps
Root	1.76cps	2.03cps
	5.35cps	3.24cps
	4.68cps	2.43cps
	5.63cps	4.04cps
	3.33cps	3.71cps
	1.90cps	4.9cps
	5.53cps	4.34cps
	6.21cps	4.39cps
	1.90cps	3.85cps
	2.44cps	4.90cps

Fluorescence intensity images from table 4.5 above show that silicon was mainly found in the leaves and petioles. Still, some amounts were found in the roots of strawberry plants (no additional silicon) in the silicon fertigation field experiment in 2018. Refer to appendix 62 for a statistical test to evaluate the mean differences between both (cross-section) treatments. A summary table for the means of ten cross-sections of strawberry plant leaves, leaf petioles and roots from the field experiment in 2018 is presented in table 4.6 below.

Table 4.6. Summary of 10 fluorescence intensities (integrated density) in the leaf, leaf petiole and root from a field experiment in 2018

Cross-section	Treatments	Statistical test applied	Statistical findings (see appendix 62 for workings)
Leaves, leaf petioles and roots	Untreated and silicon twice only treatment	Shapiro-Wilk test for normality and Two Way ANOVA to find the average values of differences between untreated and silicon twice-treated plants.	There was a statistically significant difference between block means as interpreted by the Two Way ANOVA (F (2) = 7.967, P = 0.001. However, the interaction between the block and treatment terms was not significant, (F (6) = 1.042, P = 0.403.

Table 4.6 above showed the interpretation of the Two Way ANOVA conducted on treatments means from the 2018 field experiment. It revealed that there was a significant difference between block as sig. value P = 0.001 but the interaction between the treatment and block terms is not significant as sig. value is 0.403.

4.5. Discussion

4.5.1. Silicon deposition in strawberry plants from a silicon fertigation field experiment in 2017 and 2018 and a glasshouse experiment (pot compost) in 2017

This chapter aimed to examine the amounts and pattern of distribution of silicon in leaves, petioles and roots of strawberry plants in glasshouse and field experiments. The hypothesis was that there would be higher levels of silicon in treated plants compared to untreated plants, and the silicon would be deposited regularly (form) in the leaves. Although all plants, including the untreated ones, have a background level of silicon, there will be higher levels of silicon in silicon-treated plants than in untreated plants.

Figure 4.2 and table 4.1 show the results from silicon deposition in a glasshouse experiment in 2017 of means of ten cross-sections per leaf, leaf petioles and root results

showed that the strawberry leaves of silicon-treated strawberry plants had more (P<0.05) deposits of silicon laid down regularly in the upper and lower cuticle, epidermis, palisade layer and leaf vein. In the leaf petioles, more (P<0.05) silicon was found deposited in the cuticle and xylem of silicon-treated plants compared to the untreated ones and the roots, more silicon (P<0.05) was found deposited mainly in the xylem of silicon-treated plants compared to the untreated ones. Means of three readings of each of the ten replicates (leaves, leaf petioles and roots) were used to produce the statistical tests. Strawberry leaves from the silicon fertigation field experiments in 2017 and 2018 showed that more (P<0.05) silicon was found in the cuticle and palisade layer of the silicon-treated plants compared with the untreated ones.

Domiciano et al. (2013) showed silicon accumulation was found in foliar tissues, which delayed pathogen ingress into epidermal cells and reduced fungal colonization. Sousa et al. (2013) reported how the amount of silicon deposited in wheat leaves restricted hyphal entry to the epidermal cells, while hyphae successfully invaded several neighbouring leaf cells where silicon was not found. In a glasshouse experiment, Jin (2015) recently experimented with a glasshouse at the University of Hertfordshire to locate silicon accumulation in strawberry plants. The work Jin (2015) conducted revealed through confocal microscopic images that silicon-treated strawberry plants contained more silicon than untreated strawberry plants; moreover, some silicon was found in untreated plants. Compared with the untreated, Silicon was found mainly in the epidermis of a silicon-treated strawberry leaf. Silicon was found in the epidermis and the vascular tissue in the leaf petiole compared with the untreated ones.

More recently, work conducted by Mvondo-she and Marais (2019) investigated silicon localization and accumulation in Citrus plants and the Cultivars used for the study were Valencia 'Delta' and Clementine 'Nule'. The experiment was done in a glasshouse at the Experimental Farm of the University of Pretoria in pots containing an artificial growing medium. Coir-Perlite and silicon were applied via the roots (Mvondo-she and Marais, 2019). Their work found through statistical findings that there was a higher amount of silicon deposits in silicon-treated leaves compared with the untreated control. Lastly, this experiment also identified the presence of silicon granules on the surface and around the outer cell surface, forming a double cuticle layer of the lower epidermis in the leaves of silicon-treated Citrus plants compared with the untreated control (Mvondo-she and Marais, 2019).

Figures 4.2 to 4.4 and tables 4.1 to 4.6 showed that in the leaf petioles of silicon-treated plants, more (P<0.05) silicon deposits were laid down regularly in cuticle and xylem compared with the untreated in the glasshouse and field experiment in 2017. However, statistics also revealed that silicon deposits of untreated leaf petioles were not higher (P>0.05) than the silicon-treated leaf petioles in the silicon field experiment in 2018 (figure 4.4). Jin (2015) showed in her thesis that in the leaf and leaf petiole of silicon-treated plants, there was more silicon found in the cuticle and vascular tissue of silicon-treated plants compared with the untreated ones. However, the roots were not tested,

nor were the results quantified or tested for significance. Rao and Susmitha (2017) also found that silicon accumulation in silicon-treated plants formed a cuticle-silicon double layer, which maintained the erectness of rice leaf blades. Figure 4.2 to 4.3 showed that there was more silicon ($P<0.05$) deposited in the roots of silicon-treated plants in the 2017 glasshouse and field experiment, and no differences ($P>0.05$) were found in silicon deposits between the leaf petioles and untreated roots from the 2018 field experiment.

Results from this chapter indicated that fluorescence intensity quantification (table 4.1, 4.3 and 4.5) found more silicon in strawberry leaves and petioles, but little amounts found in the roots of strawberry plants in the 2017 and 2018 field experiments. Results from the field experiments in 2017 and 2018 (figure 4.3 and 4.4 and table 4.3 to 4.6) showed that silicon accumulated mainly in the leaves and petioles of strawberry plants compared with the glasshouse pot experiment (figure 4.2 and table 4.1) where large quantities of silicon were found throughout the leaves, petioles and roots of strawberry plants than results from field experiments (figure 4.3 and 4.4 and table 4.1 to 4.6). However, the results from the roots in the field experiments were different and unexpected, in that there were no significant differences seen in quantities of silicon between treatments (untreated and twice only treatment) in the field experiments.

The strawberry plants in the glasshouse were permanently exposed to silicon because they were in pots. In the fields, there were only exposed to the silicon twice a week (two out of 42 fertigation events). Thus, the exposure to silicon was far less in the fields. The hypothesis is that the silicon is taken up by the roots (via a silicon transporter gene) (Ma et al., 2004). The bio-available silicon is transported in the water in the xylem, distributed to the leaves via the petiole, and distributed throughout the leaves in the leaf vein. At the leaf surface, water leaves the plant by guttation, and water vapour leaves the plant by evaporation. The hypothesis is that the silicon in the water in the leaf becomes more concentrated in the leaves and is deposited in the cuticle and on the cell walls.

There was a difference in the amount of silicon deposited in the roots between the glasshouse and the field experiment (table 4.1 to 4.6), and the explanation for this can be the differences in the exposure time of silicon in the glasshouse (pot compost) and field experiment (fertigation). This chapter shows that silicon appears to be deposited within the plant using 10 leaves, 10 leaf petioles, and 10 roots from the glasshouse and fertigation field experiment. The aim, objectives and hypothesis that silicon delivered through a glasshouse experiment and in field experiments is distributed around the plants were met.

4.6. Conclusion

This chapter revealed that the bioavailable silicon nutrient delivered via the fertigation through the roots of strawberry plants was deposited and laid down regularly (distinct crystalline form) (Debona et al., 2017). This chapter showed that silicon could enhance the features of the passive defence pathway (through silicon deposits in the upper and lower epidermis, palisade layer, and leaf vein). It quantified the amounts of silicon deposited (fluorescence intensities) in strawberry plants, which appear not to be shown particularly in strawberries. The deposition and quantification of amounts of silicon in strawberry plants shown in this thesis provided actual measurements of silicon accumulation over time. The aim and objectives of this chapter were to examine the amounts and pattern of distribution of silicon in the leaves and petioles of strawberry plants in a glasshouse and field experiments. These aims and objectives were met. The measurements of the benefits of silicon, such as strawberry plant growth parameters and fruit quality in field and glasshouse experiments, are presented in the following chapter (chapter 5).

Chapter 5 - Measurements of strawberry plant growth parameters and quality about silicon

This chapter covers i) An introduction to the measurements of strawberry plant growth parameters and quality concerning silicon; ii) Rationale, hypothesis, aim and objectives; iii) Material and methods; iv) Results; v) Discussion and vi) Conclusion.

5.1. Measurements of strawberry plant growth parameters and quality concerning silicon

The literature suggests that silicon as a nutrient has significant benefits in growing crops such as rice, wheat, melon, cotton and citrus (Rodrigues et al., 2015). Silicon has been shown to have benefits on non-accumulators; see table 5.1. Table 5.1 suggests that there are benefits in non-silicon accumulators such as an increase in flowering, fruit number, fresh weights, fruit shelf life etc. Although some strawberry growers are concerned about toxicity, the literature suggests that silicon is shown to reduce the toxicity of metals (Liang et al., 2005).

As explained by Liang et al. (2005), one of the main effects of silicon toxicity is reducing the uptake and translocation of metals in plants. Growers fear excess silicon may cause white strawberry fruits (albinism) and reduced yield (AHDB, 2011). AHDB advises using no more than 22mg/L of silicon (optimal ranges) (AHDB, 2011). Work reported in the chapter has focused on the benefits of silicon on strawberry plants. The experiment reported here found some benefits linked with using silicon in growing strawberry plants. Silicon nutrients applied through the roots of strawberry plants growing in Hoagland's solution in a hydroponic experiment increased the numbers of leaves, runners, fruits, chlorophyll contents in the leaves and fresh weights biomass in silicon-treated plants compared with the untreated ones. Refer to chapter 1, section 1.22, and tables 1.7 and 1.8 to review some of the benefits of silicon use.

An example is an experiment conducted by Li et al. (2018), who reported that silicon increased the biomass of cotton plants and bast fibre in plants treated with silicon compared with the control plants. Johnson et al. (2018) also mentioned that silicon promoted yield and upright growth, including stronger and thicker stems, which prevented lodging, promoting conducive exposure of light to the leaves of wheat plants. It is reported that silicon deficiency in plants can affect the normal development of healthy leaves, petioles and roots, causing the plant to be more susceptible to disease infections (Hajiboland et al., 2018). Deus et al. (2019) showed that silicon deficiency symptoms in rice leaf blades included reduced photosynthetic activity and increased susceptibility to diseases such as blast caused by *Pyricularia oryzae* or brown spot caused by *Helminthosporium oryzae*, reduction in grain yield and prone to lodging. The

work reported in this chapter compares non-silicon with silicon-treated plants for silicon deficiency symptoms and the measurements of strawberry plant growth parameters in a glasshouse hydroponic and field experiment (°Brix). Part of the work reported here followed previous work at the University of Hertfordshire. Jin (2015) and Liu (2016) found that silicon increased °Brix levels of strawberries growing on a commercial strawberry farm. Table 5.1 below shows some of the benefits of the application of silicon to plants that are non-accumulators of silicon.

Table 5. 1 Benefits of using silicon on non-accumulators of silicon

Crop species	Uptake of silicon	Benefits	References
Strawberry	Non-accumulators	Enhanced fruit diameter, weight, glucose, fructose content and shelf life.	(Peri-Felipo,2020)
		Enhanced size, weight, firmness, ºBrix of fruit.	(Zahedi et al., 2020)
		Increase in chlorophyll contents, area and numbers of strawberry leaves.	
Tomato	Non-accumulators	Increased yield of fruit and vegetative growth.	(Hoffmann et al., 2020)
		Increased commercial productivity and reduced occurrence of cracked fruits.	
		Enhanced vitamin c content and fruit firmness.	(Marodin et al., 2014)
Sunflower	Non-accumulators	Early flowering, increased stem diameter.	(Kamenidou, Cavins and Marek; 2008)
		Improved biomass and increased tolerance to salinity.	(Saqib et al., 2011)
Gerbera	Non-accumulators	Increased diameter of flower and thickness of flower stems.	(Savvas et al., 2002)
Petunia	Non-accumulators	Thicker leaves.	(Jana and Jeong, 2014)
Begonia	Non-accumulators	Increased biomass parameters.	(Mills-Ibibofori et al., 2019)

Table 5.1 includes only the benefits of using silicon on non-accumulators of silicon, as the strawberry plant is a non-accumulator of silicon. The benefits of silicon to silicon accumulators are well known.

5.2. Rationale

Silicon is not considered an essential element for plant development. However, previous work has shown that silicon can improve strawberry plants' overall quality, ranging from enhanced pollen fertility to the °Brix and firmness of strawberry fruits. The work reported in chapter 5 aims to quantify the growth parameters of strawberry plants in silicon's presence (with) and absence (without).

5.3. Aim

- Evaluate °Brix levels of silicon-treated fruits and leaf petioles with untreated fruits and leaf petioles.

- In a glasshouse hydroponic experiment, measure growth parameters of strawberry plants in the absence and presence of silicon.

5.4. Objectives

- Measure °Brix levels of fruits and leaf petioles in a field experiment

- Conduct weekly assessments on growth parameters; leaf number, runner number, flowering, fruit number, °Brix levels, chlorophyll content and fresh weight biomass at the end of the experiment

- Monitor strawberry plants treated with and without silicon for physiological symptoms in a glasshouse hydroponic experiment.

5.5. Hypothesis

- Plants treated with silicon can show improved growth parameters compared with untreated plants growing without silicon in a glasshouse hydroponic experiment.

- Plants treated with silicon in a silicon field experiment can have elevated °Brix levels compared with untreated plants.

5.6. Material and methods

Material and methods used include strawberry fruits and leaf petioles °Brix measurements (chapter 2, section 2.5.1 and figure 2.8 and 2.9), measurements of strawberry plant growth parameters; numbers of leaves, runners and fruit counting once a week, chlorophyll content measurements (in the leaves) once-a-week, fresh weight

biomass at the end of the experiment in the hydroponic glasshouse are in sections 5.7.1 to 5.7.7 of this chapter. The data collected from field °Brix measurements and the hydroponic glasshouse weekly assessments of strawberry plant growth parameters were first tested for normality, and the Paired Two Samples for means t-test, Paired Sample test and Wilcoxon-Sign Ranked test was then used to analyse the sample means (compare treatment means) collected from both the field and glasshouse hydroponic glasshouse experiment.

5.7.   Hydroponics (methods)

Malling Centenary (June bearer) plants were used for the glasshouse hydroponic experiment. This cultivar was chosen to enable the completion of the experiment throughout the growing season. The experiment was set up at the University of Hertfordshire, Bayfordbury glasshouse in 2018. Twenty bare-root Malling Centenary strawberry plants were planted in 5 L tubs (not glass, which contains silicon) containing Hoagland's solution (table 5.2 and figure 5.1). Hoagland's solution is a complete plant nutrient developed by Hoagland and Snyder, which contains the necessary nutrient needed for plants' normal function and growth (Hershey, 1994). The tubs were wrapped with black polythene to reduce light penetration, root growth and encourage algae to grow (figure 5.1). Circular holes were drilled into black plastic lids, and each plant was wrapped below its crown in foam stopper bungs (before inserting through the lid) to keep the crown above the liquid to prevent it from getting wet while its roots remained in the solution (figure 5.2). Only the roots of strawberry plants were inserted into Hoagland's solution. Crowns of strawberry plants were elevated from the solution.

Two strawberry plants were planted per tub and spaced apart to minimize tub crowding (table 5.2 and figure 5.1). Sufficient air supply to the roots was provided by two Oase Pontec PondAir aeration pumps from Aqautix-2U. Eheim T junctions were attached to the aeration tubes to enable the spread and circulation of oxygen to the roots of the strawberry plants in all tubs. (figure 5.1 and 5.2 and table 5.2). Treatments consisted of weekly 50ml applications of silicon nutrient per hydroponic tub. The concentration of silicon nutrient used was 0.017% of Sirius, estimated as 0.025ml to 0.027ml ($0.003mg/cm^3$) (Liu, 2016) for the treated strawberry plants. The untreated set (no silicon nutrient) used de-ionized water only. Refer to figures 5.1, 5.2 and 5.3 and table 5.2 for the materials used and the setup of the hydroponic system.

The hydroponic tubs were topped up once weekly with Hoagland's solution as levels ran low as strawberry plants grew throughout the experiment. Refer to table 5.2 for nutrient components used to prepare Hoagland's solution (Hoagland and Arnon, 1950). Photos of the hydroponic experiment set-up are in figures 5.2 a and b, while the recipe for Hoagland's solution is in appendix 63. Figure 5.1 below is the design and treatment plan used in the glasshouse hydroponic experiment.

Tubs	Treatments
U T U U T U T U T U	Untreated = 5 tubs Number of plants = 10 Silicon-treated = 5 tubs Number of plants = 10
T U T T U T T U T U	Untreated = 5 tubs Number of plants = 10 Silicon-treated = 5 tubs Number of plants = 10 Total plants used = 20

Figure 5. 1. A design plan showing the treatment and plants used for the hydroponic experiment in 2018. T = Treated - with silicon. U = Untreated - without silicon, Total number of plants used in experiment = 10 silicon-treated and 10 untreated = 20.

Figure 5.2. Hydroponic experiment in at UH Bayfordbury glasshouse 2018. Strawberry plants were arranged in plastic tubs wrapped in black polythene and treated once-a-week.

Table 5. 2 Nutrient components for Hoagland's solution

Component	Stock solution	mL Stock Solution/1L
Macronutrients		
2M KNO_3 (Potassium nitrate)	202 g/L	2.5
1M Ca $(NO_3)_2$ .4H_2O (Calcium nitrate)	236 g/0.5L	2.5
Iron (Sprint 138 Iron chelate) Ferric EDTA	15 g/L	1.5
2M $MgSO_4$.7H_2O Magnesium sulfate	493 g/L	1
1M NH_4NO_3 Ammonium Nitrate	80 g/L	1
Micronutrients		
H_3BO_3 Boric Acid	2.86 g/L	1
$MnCl_2$ .4H_2O Manganese chloride	1.81 g/L	1
$ZnSO_4$ .7H_2O Zinc sulfate	0.22 g/L	1
$CuSO_4$ .5H_2O Copper 11 sulfate	0.051 g/L	1
H_3MoO_4 .2H_2O Molybdic Acid	0.09 g/L	1
1M KH_2PO_4 (pH to 6.0)	136 g/L	0.5

Source: Hoagland and Arnon, (1950); Hothem, Marley and Larson, (2003). Protocol for preparation of Hoagland's solution is in Appendix 63.

Figure 5.3a: Roots of silicon treated plants at second week of planting

Figure 5.3b: Roots of silicon treated plants at four weeks of planting

Figure 5.3c: Roots of silicon treated plants at 20 weeks of planting (Near end of experiment)

Figure 5.3d: Roots of silicon treated plants at end (22 weeks) of experiment handheld by author with plastic lid

Figure 5.3. Stages of silicon-treated strawberry plant root development in Hoagland's solution in the UH Bayfordbury glasshouse (a) Roots of silicon-treated plants at the second week of planting. (b) Roots of silicon-treated plants at four weeks of planting. (c) Roots of silicon-treated plants at 20 weeks of planting (near the end of the experiment). (d) Roots of silicon-treated plants at the end (22 weeks) of the experiment were hand-held by the author with a plastic lid.

5.7.1. Number of Leaves

The total number of leaves per plant and treatment was counted once-a-week from the experiment's start (24 January 2018) to end (27 June 2018). When plants stopped producing newer leaves, their numbers were recorded as the same and unchanged.

5.7.2. Number of runners

Strawberry plant runners (daughter plants) produced in this experiment were counted once a week until the experiment ended. Each runner was potted in compost (still attached) growing beside the mother plant established in Hoagland's solution. All runners (figure 5.4) were cut off from the mother plant as soon as they were mature enough on their own.
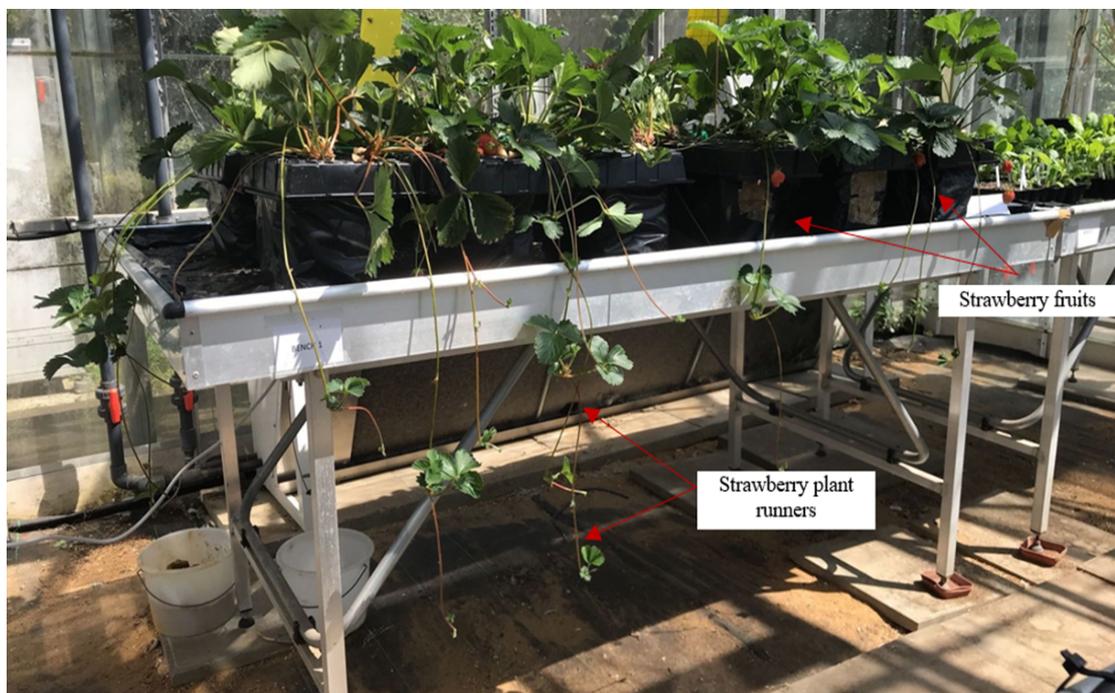
Figure 5. 4. Strings of strawberry daughter plants (runners) in the hydroponic experiment.

5.7.3. Flowering

Flowering dates on strawberry plants from silicon-treated and untreated plants were recorded as soon as plants began to produce their flowers.

5.7.4. Fruit number

The numbers of fruits produced per plant and per week from the silicon-treated and untreated plants were recorded and picked in preparation for fruit °Brix measurements.

Figure 5. 5. Fruiting in strawberry plants in the hydroponic experiment 2018

5.7.5. Brix measurements

- **Field experiment °Brix (Tesco's method) strawberry fruit**

The grower used Tesco's method (the grower supplied Tesco). The method used in 2018 was cutting ten individual ripe strawberries around the circumference (middle). Then the juice was squeezed and placed directly on a digital refractometer, and the readings were recorded. Several years ago, Tesco's method was ten mashed strawberries and using a sample of juice to gain the reading with a refractometer (chapter 2, figure 2.8 and 2.9) (Tesco operating procedures). Growers measure petiole °Brix as an indication of how the fruit will develop. Strawberry fruits were sampled three times in July when fruiting was consistent and once in August 2017, when the fruiting season ended in the silicon field experiment 2017. Refer to chapter 2, section 2.5.1 for °Brix sampling methods.

- **Field experiment °Brix (leaf petioles)**

°Brix of strawberry leaf petioles of silicon-treated and untreated plants was also evaluated in the 2017 silicon fertigation field experiment. Strawberry leaf petioles were sampled for ten individual leaf petioles per sample day. Sampling was done monthly, from June to September 2017 (chapter 2, figure 2.9).

- **Glasshouse experiment °Brix (strawberry fruit)**

°Brix from strawberry fruits was measured from the hydroponic experiment. Five individual strawberry fruits from each treatment (silicon-treated and untreated) on two sample days were selected for °Brix measurements. Strawberries were sampled twice throughout the experiment, and their °Brix were measured by the researcher with a refractometer (figure 2.9b). Refer to chapter 2, section 2.5.1, for methods used in sampling. Tesco's method was also used for measurements in the hydroponic experiment.

5.7.6. Chlorophyll

Chlorophyll contents of strawberry leaves were measured weekly during the hydroponic experiment. Measurements were carried out using the chlorophyll meter SPAD-502Plus from Konica Minolta (figure 5.5). The chlorophyll meter SPAD-502Plus is a lightweight and water-resistant. The handheld device is used to measure and determine the relative amount of chlorophyll present in the plant. The device works by measuring the absorbance of the leaf content without causing it to the plant (Minolta, 2009). Figure 5.6 is a photo of the SPAD meter used for chlorophyll measurement in this experiment.
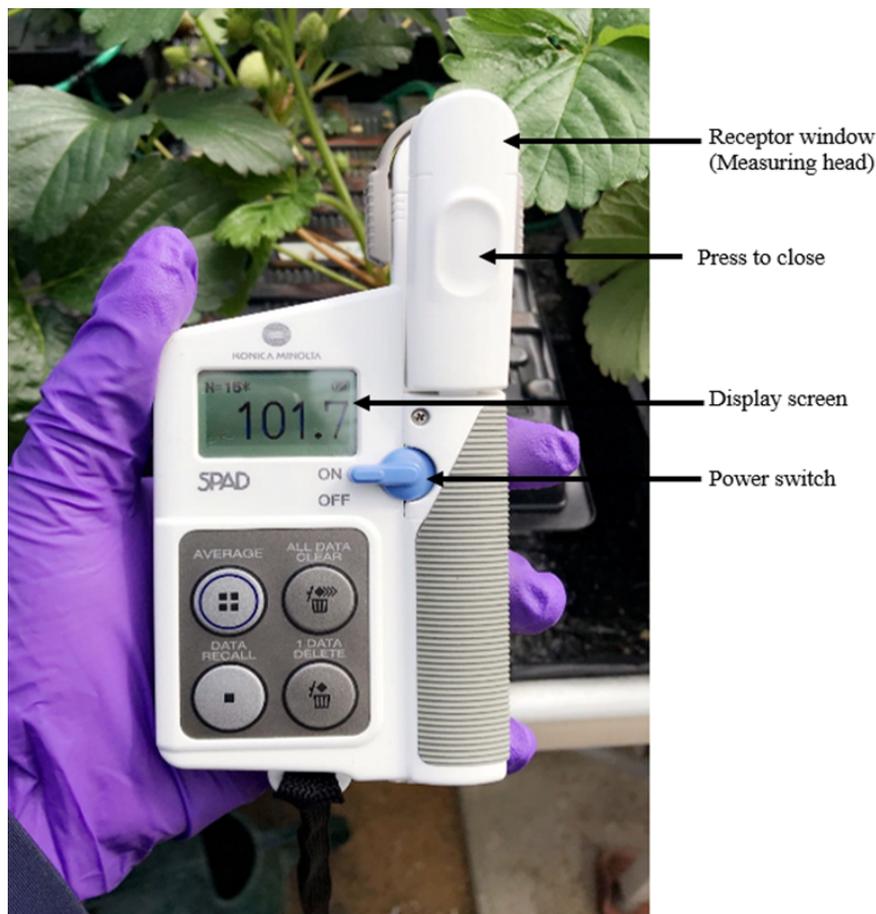
Figure 5. 6. The chlorophyll meter SPAD-502Plus. Lightweight and water-resistant.

Measurements were derived by inserting leaf blades (one leaflet at a time) into the receptor window (see figure 5.6) and closing the measuring head of the chlorophyll meter. Data collected were recorded in µmol/m2. Measurements were carried out for treated plants (with silicon nutrients) and untreated plants (without silicon nutrient). Strawberry leaves from each treatment were measured by randomly sampling ten leaves. Twenty measurements from untreated and silicon-treated plants were measured once a week. The averages of chlorophyll contents per treatment (untreated and silicon-treated) were recorded, and measurements were repeated each following week. The SPAD-502Plus has a memory space for up to 30 readings. Therefore, each piece of data can be recalled and saved after measurements (figure 5.6).

5.7.7. Fresh weight biomass

Whole strawberry plants (with leaves, leaf petioles, roots and runners still attached) were harvested at the end of the hydroponic experiment (figure 5.7). Each strawberry plant's fresh weight (g) from the silicon-treated and untreated were recorded.
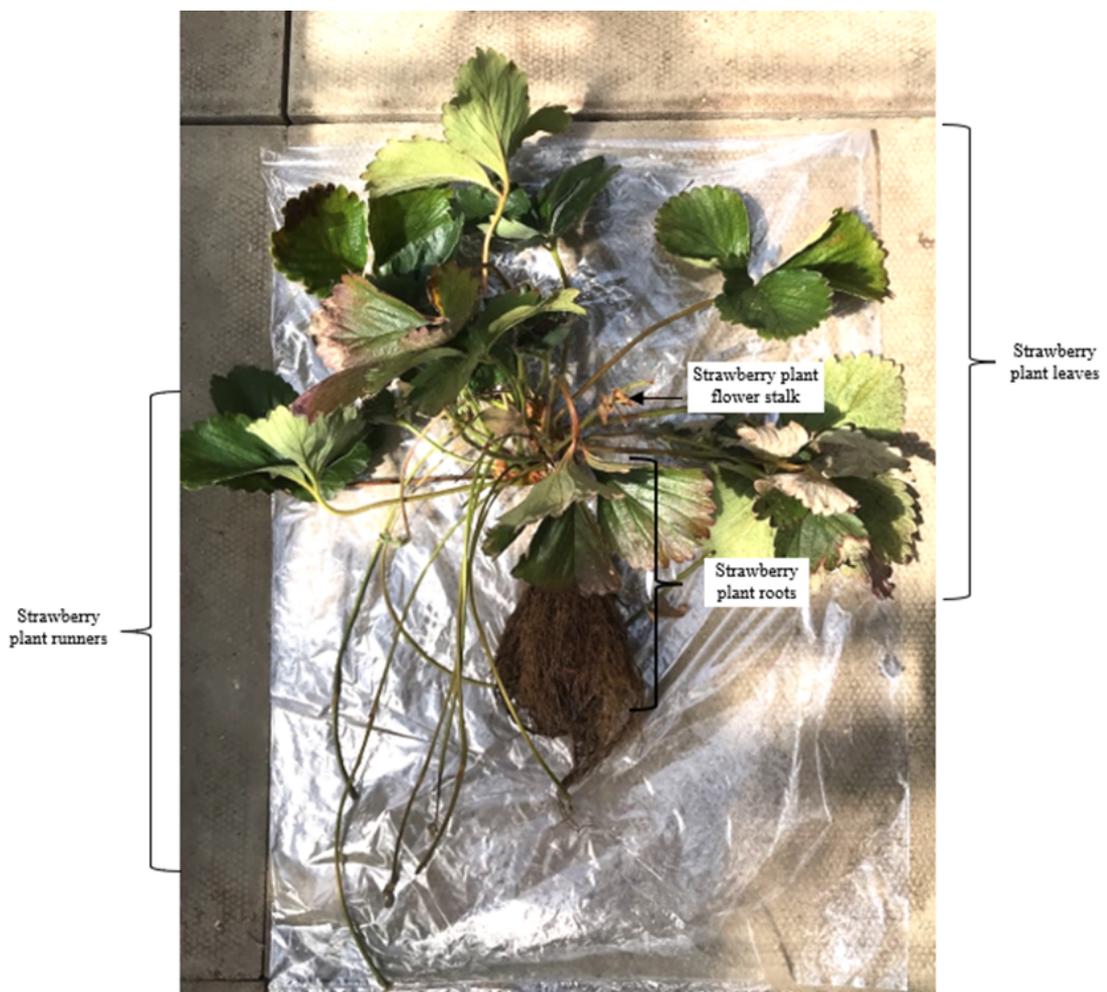
Figure 5. 7. A harvested strawberry plant from the hydroponic experiment at 22 weeks. End of experiment.

5.8. Results

The graph includes average values of °Brix strawberry fruits sampled from the silicon fertigation field experiment in 2017. °Brix levels were sampled on 20 of July 2017, 24 July, 27 of July 2017 and 1 of August 2017 only.

5.8.1. Mean values of 10 strawberry fruit °Brix per treatment from the silicon fertigation field experiment 2017
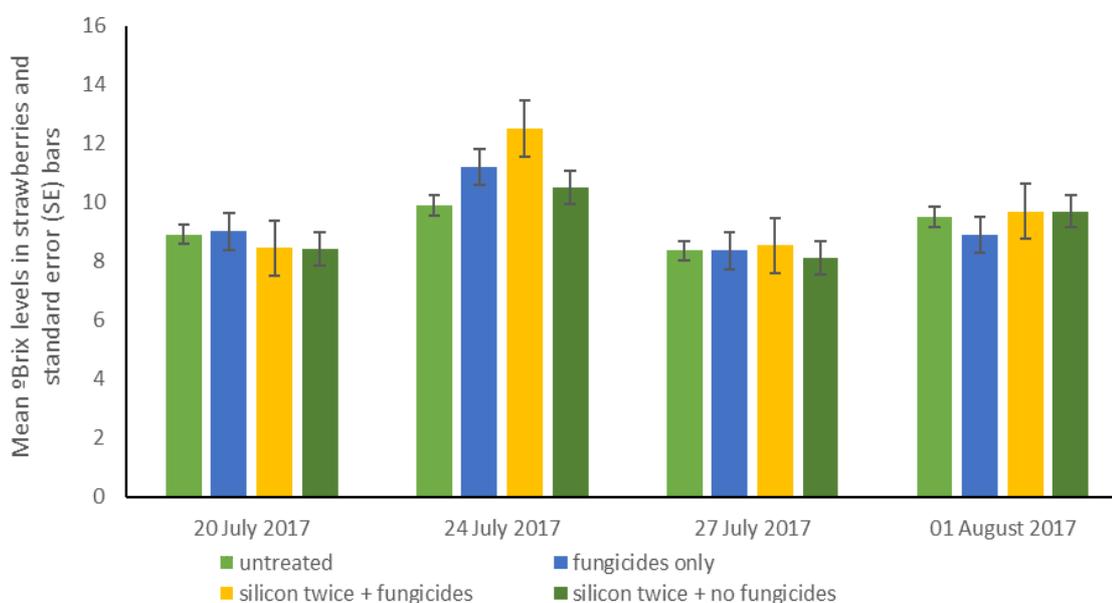
Figure 5. 8. Means of 10 strawberry fruits °Brix levels from the silicon field experiment 2017. The different bars are average values of strawberry fruits sampled from 20 July to 1 August 2017. Light green is untreated, the blue is the fungicides-only treatment, the yellow is silicon applied twice weekly + fungicides, and the dark green is silicon applied twice weekly without fungicides. Means from 10 strawberries per treatment were used to create the graph shown. The Paired Sample for Means was used to analyse the data collected as it was normally distributed.

°Brix levels of strawberry fruits from untreated and silicon-treated plants and analysis (appendix 64) are compared in the summary tables 5.3 and 5.4.

Table 5. 3 Summary of ºBrix levels from strawberry fruits from the 2017 field experiment

Sample	Treatment	Statistical test applied	Statistical findings (see appendix 64 for statistical workings)
ºBrix levels in strawberry fruits	Untreated control (no fungicides, no silicon) and silicon twice-a-week + fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + fungicides ºBrix levels.	P>0.05; no statistical increase was found between strawberry fruits ºBrix levels sampled from the silicon twice-a-week + fungicides treatment and untreated control sampled on 20 July 2017. The null hypothesis (HO) is accepted.
ºBrix levels in strawberry fruits	Untreated control (no fungicides, no silicon) and silicon twice + no fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + no fungicides ºBrix levels.	P>0.05; no statistical increase was found between strawberry fruits ºBrix levels sampled the silicon twice-a-week + no fungicides treatment and untreated sampled on 20 of July 2017. The null hypothesis (HO) is accepted.
ºBrix levels in strawberry fruits	Untreated control (no fungicides, no silicon) and silicon twice-a-week + fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + fungicides ºBrix levels.	P<0.05; there was a statistical increase in the strawberry fruits ºBrix levels sampled from the silicon twice-a-week + fungicides treatment compared to untreated sampled 24 of July 2017. The null hypothesis (HO) is rejected.
ºBrix levels in strawberry fruits	Untreated control (no fungicides, no silicon) and silicon twice a week + no fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + no fungicides ºBrix levels.	P<0.05; there was a statistical increase in the strawberry fruits ºBrix levels sampled from the silicon twice-a-week + no fungicides treatment compared to untreated sampled 24 of July 2017. The null hypothesis (HO) is accepted.

Table 5.3. shows an increase (P<0.05) in ºBrix levels found only in silicon twice + fungicides and no fungicides treatment compared to untreated fruits sampled on 24 of July 2017, but no increase (P>0.05) in ºBrix levels of fruits from the silicon twice-a-week + fungicides and no fungicides compared to untreated sampled 20 of July 2017. See table 5.4 for remaining ºBrix levels of fruit results sampled on other days.

Table 5. 4 Summary of ºBrix levels from strawberry fruits from the 2017 field experiment (continued)

Sample	Treatment	Statistical test applied	Statistical findings (see appendix 64 for statistical workings)
ºBrix levels in strawberry fruits	Untreated control (no fungicides, no silicon) and silicon twice-a-week + fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + fungicides ºBrix levels.	P>0.05; no statistical increase was found between strawberry fruits ºBrix levels sampled from the silicon twice-a-week + fungicides and untreated treatment sampled on 27 July 2017. The null hypothesis (HO) is accepted.
ºBrix levels in strawberry fruits	Untreated control (no fungicides, no silicon) and silicon twice a week + no fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + no fungicides ºBrix levels.	P>0.05; a statistical increase was found between strawberry fruits ºBrix levels sampled from the silicon + no fungicides treatment and untreated sampled 27 of July 2017. The null hypothesis (HO) is accepted.
ºBrix levels in strawberry fruits	Untreated control (no fungicides, no silicon) and silicon twice-a-week + fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + fungicides ºBrix levels.	P>0.05; no statistical differences were found between strawberry fruits ºBrix levels sampled from the silicon twice + fungicides treatment compared to untreated sampled on 01 of August 2017. The null hypothesis (HO) is accepted.
ºBrix levels in strawberry fruits	Untreated control (no fungicides, no silicon) and silicon twice a week + no fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + no fungicides ºBrix levels.	P>0.05; there was no statistical difference found between strawberry fruits ºBrix levels sampled from the silicon twice-a-week + no fungicides treatment compared to untreated sampled 01 of August 2017. The null hypothesis (HO) is accepted.

Conclusions from the summary in tables 5.3 and 5.4 show that °Brix levels of strawberry fruits in the silicon field experiments 2017 using the Paired t -Test Sample for Means showed that there was an increase (P<0.05) in strawberry fruits °Brix of the silicon twice + fungicides and no fungicides treatment compared to untreated sample on the 24 of July 2017 only and there was no increase (P>0.05) in strawberry fruit °Brix levels sampled on the 20 July, 27 of July 2017 and 1 of August 2017.

5.8.2. Means of 10 strawberry leaf petioles ºBrix per treatment from the silicon fertigation field experiment 2017

The graph includes average values of ºBrix leaf petioles sampled from the silicon fertigation field experiment in 2017. ºBrix levels were sampled on 20 June 2017, 18 July, 01 August 2017 and 19 September 2017 only.
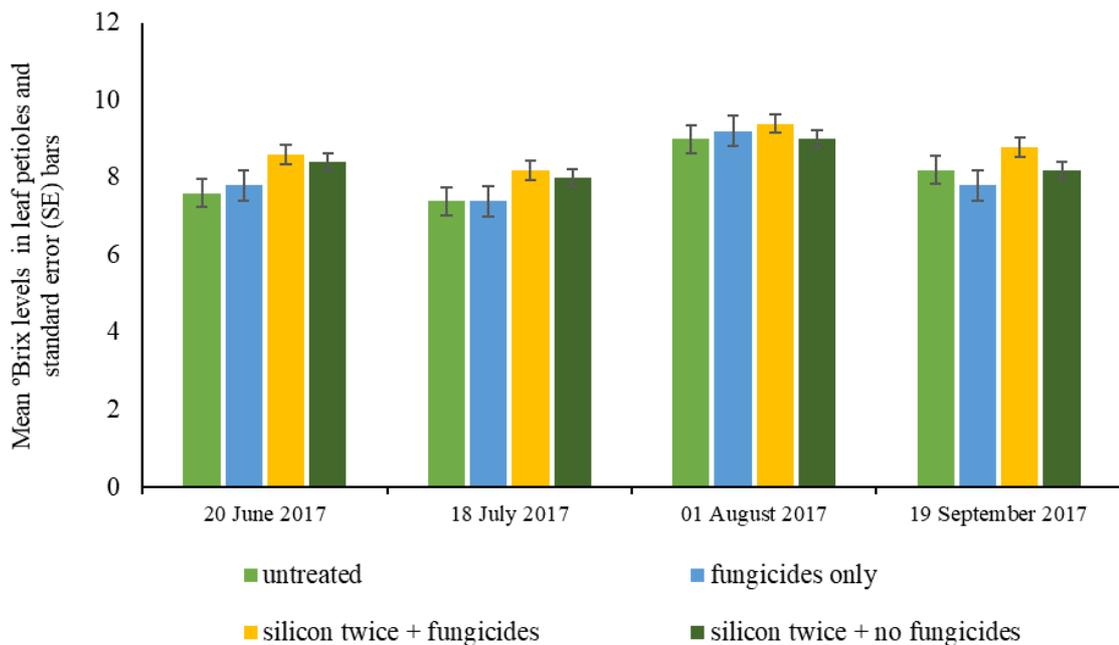
Figure 5. 9. Means of ºBrix levels in strawberry leaf petioles from the silicon fertigation 2017. The different bars are average values of leaf petioles sampled from 20 June 2017 to 19 September 2017. Light green is untreated, the blue is the fungicides-only treatment, the yellow is silicon applied twice weekly + fungicides, and the dark green is silicon applied twice weekly without fungicides. Means from 10 leaf petioles per treatment were used to create the graph.

Growers routinely measure the ºBrix of strawberry leaf petioles to understand the carbohydrates transported from the leaf petioles to the fruits (personal communication from grower). A summary of ºBrix levels of leaf petioles from untreated and silicon twice-a-week only with and without fungicides treated plants from the 2017 field experiment is in tables 5.5 and 5.6. Find statistical workings in appendix 65.

Table 5. 5 Summary of ºBrix levels from strawberry leaf petioles from the 2017 field experiment

Sample	Treatment	Statistical test applied	Statistical findings
ºBrix levels in strawberry leaf petioles	Untreated control (no fungicides, no silicon) and silicon twice-a-week + fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + fungicides ºBrix levels.	P>0.05; no statistical increase was found in the ºBrix levels of strawberry leaf petioles sampled from silicon twice-a-week + fungicides compared untreated treatment sampled 20 June 2017. The null hypothesis (HO) is accepted.
ºBrix levels in strawberry leaf petioles	Untreated control (no fungicides, no silicon) and silicon twice + no fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + no fungicides ºBrix levels.	P>0.05; no statistical increase was found between strawberry leaf petioles ºBrix levels sampled from the silicon twice-a-week + no fungicides treatment compared to untreated sampled 20 June 2017. The null hypothesis (HO) is accepted.
ºBrix levels in strawberry leaf petioles	Untreated control (no fungicides, no silicon) and silicon twice-a-week + fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + fungicides	P>0.05; no statistical increase was found in strawberry leaf petioles ºBrix levels sampled from the silicon twice-a-week + fungicides treatment compared to untreated 18 of July 2017. The null hypothesis (HO) is accepted.
ºBrix levels in strawberry leaf petioles	Untreated control (no fungicides, no silicon) and silicon twice-a-week + no fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice- a-week + no fungicides	P>0.05; no statistical increase was found in strawberry leaf petioles ºBrix levels sampled from the silicon twice-a-week + no fungicides treatment compared to untreated 18 of July 2017. The null hypothesis (HO) is accepted.
ºBrix levels in strawberry leaf petioles	Untreated control (no fungicides, no silicon) and silicon twice-a-week + fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + fungicides	P>0.05; no statistical increase was found in strawberry leaf petioles ºBrix levels sampled from the silicon twice-a-week + fungicides treatment compared to untreated sampled 01 of August 2017. The null hypothesis (HO) is accepted.
ºBrix levels in strawberry leaf petioles	Untreated control (no fungicides, no silicon) and silicon twice-a-week + no fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice-a-week + no fungicides	P>0.05; no statistical increase was found in strawberry leaf petioles ºBrix levels sampled from the silicon twice-a-week + no fungicides treatment compared to untreated sampled 01 of August 2017. The null hypothesis (HO) is accepted.

From 5.5 above there is no increase (P>0.05) found in ºBrix levels of leaf petioles between all treatments sampled from 20 June to 1 August 2017. See table 5.6 for samples collected in September 2017.

Table 5. 6 Summary of ºBrix levels from strawberry leaf petioles from the 2017 field experiment

Sample	Treatment	Statistical test applied	Statistical findings (see appendix 65 for statistical workings)
ºBrix levels in strawberry leaf petioles	Untreated control (no fungicides, no silicon) and silicon twice + fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice + fungicides ºBrix levels.	P>0.05; no statistical increase was found in strawberry leaf petioles ºBrix levels sampled from the silicon twice + fungicides treatment compared to untreated sampled 19 September 2017. The null hypothesis (HO) is accepted.
ºBrix levels in strawberry leaf petioles	Untreated control (no fungicides, no silicon) and silicon twice + no fungicides	Shapiro-Wilk test for normality and t-Test Paired Two Sample for Means to find the average values of differences between untreated and silicon twice + no fungicides ºBrix levels.	P>0.05; no statistical increase was found in strawberry leaf petioles ºBrix levels sampled from the silicon twice + no fungicides treatment compared to untreated sampled 19 September 2017. The null hypothesis (HO) is accepted.

From results shown in table 5.5 and 5.6 there was no increase or differences (*P*>0.05) between ºBrix levels of strawberry leaf petioles sampled from the silicon twice-a-week treatment compared to the untreated from the 2017 silicon fertigation field experiment.

5.8.3. Effects of silicon nutrient on average values of leaf number (per plant and treatment) in the hydroponic experiment

Results in figure 5.10 and appendix 66 revealed that the number of leaves in the hydroponic glasshouse experiment was enhanced from an average value of nine leaves (per plant and treatment) in untreated to 15 leaves (per plant and treatment) in the silicon-treated. Silicon-treated strawberry plants had a higher leaf number compared with untreated. This difference between the two treatments was statistically significant ($P<0.05$) as interpreted by the paired samples for means t-test analysis in appendix 66. Figure 5.10 shows average values of progress levels (leaves) between silicon-treated and untreated strawberry plants.
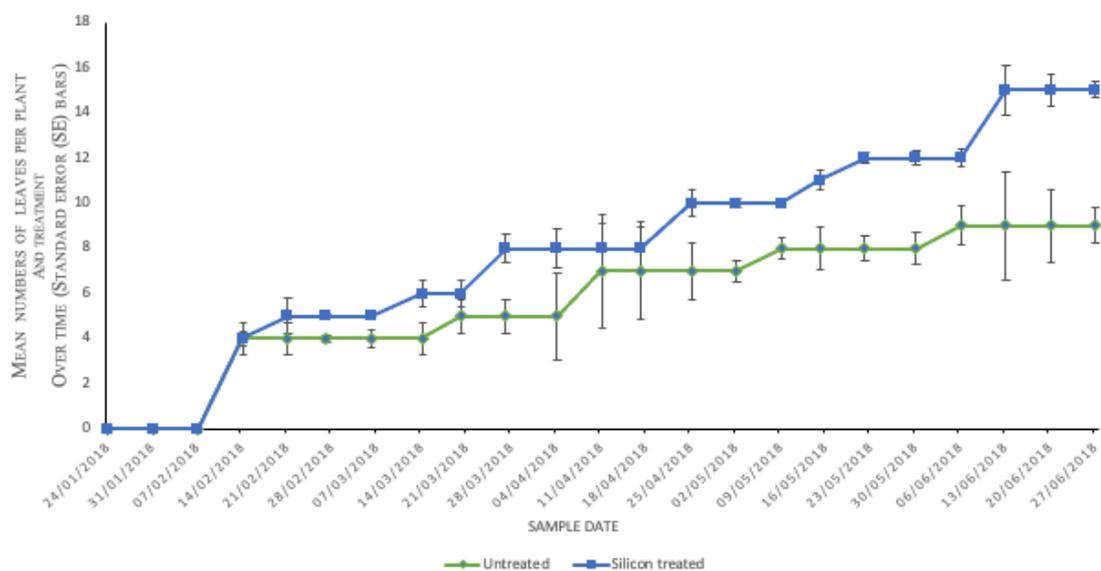
Figure 5. 10. Mean numbers of leaves per plant and treatment over time in the hydroponic glasshouse experiment 2018. The graph in figure 5.10 shows the increase in the number of leaves per plant in silicon-treated plants compared with untreated over time.

Mean numbers of leaves per plant between silicon-treated plants and untreated showed a statistical difference ($P<0.05$). The paired sample t-test for Means revealed an increase in the leaf number of silicon-treated plants compared to untreated plants. Refer to appendix 66 for statistical workings.

5.8.4. Effects of silicon nutrient on average values of the number of runners (per plant and treatment) in the hydroponic experiment

Figure 5.11 and appendix 67 showed that the average values of the number of strawberry runners increased from three runners per plant in untreated to 6 runners per plant in the silicon-treated over time. A statistically significant difference ($P< 0.05$) using the Wilcoxon Sign Ranked test was found between the silicon-treated strawberry

plants and untreated. Silicon-treated strawberry plants had a higher number of runners than untreated (figure 5.11 and appendix 67).
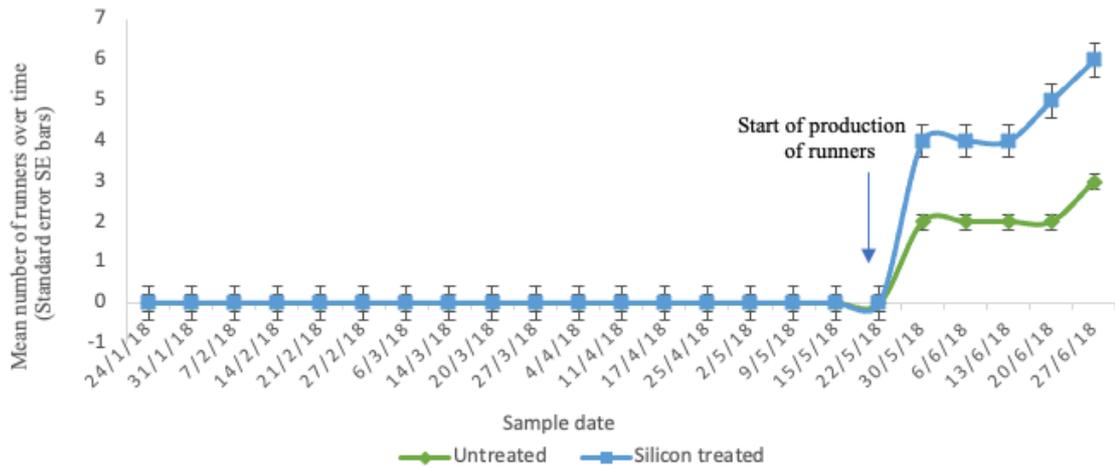
Figure 5. 11. Mean numbers of runners in the hydroponic experiment 2018. The graph displays the increase in runners in the hydroponic glasshouse experiment 2018. Mean number per plant and treatment in silicon-treated plants compared to untreated.

Mean numbers of runners per plant between silicon-treated plants and untreated showed a statistical difference (P<0.05). The Wilcoxon-Sign ranked test revealed an increase in the number of runners of silicon-treated plants compared to untreated plants. Refer to appendix 67 for statistical workings.

5.8.5. Effects of silicon nutrient on the number of flowers (per plant and treatment) in the hydroponic experiment

Flowering was first observed in silicon-treated plants. Two silicon-treated plants started (out of ten plants) flowering on 15 May 2018, a week before the untreated, which flowered on 22 May 2018. Flowering and fruiting ended in both treatments by 27 June 2018 (figure 5.12).

125
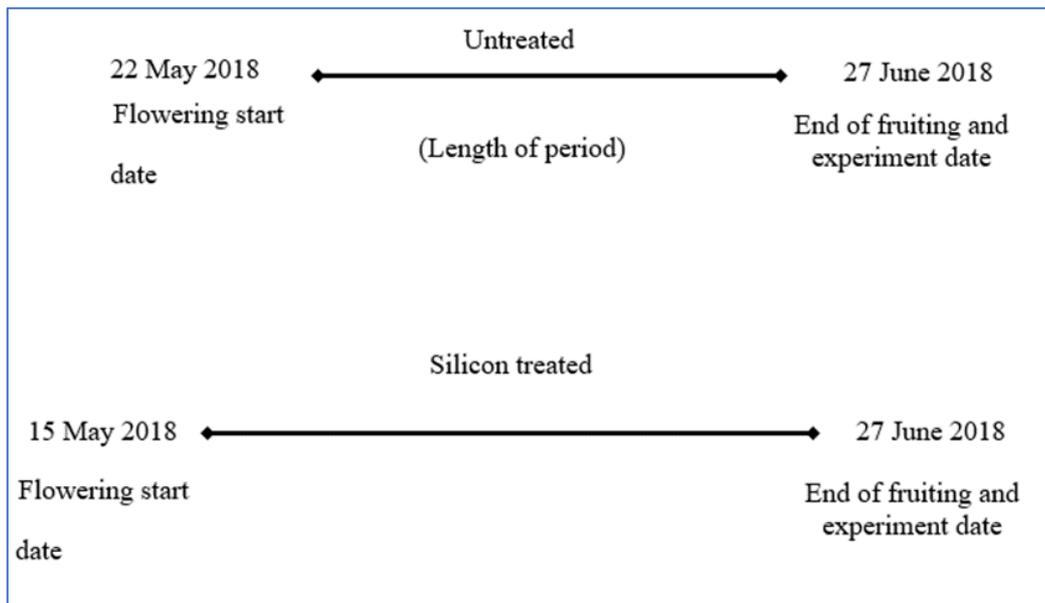
Figure 5. 12. Flowering to fruiting period in the hydroponic experiment 2018

5.8.6. Effect of silicon nutrient on the average values of the number of fruits in the hydroponic experiment

Statistical tests using the Wilcoxon-Sign Ranked test between average values of silicon-treated and untreated fruit numbers revealed a statistical difference (P<0.05) between both treatments. It suggests that there were more fruits in the silicon-treated plants than untreated. Refer to appendix 68 and 69 for statistical workings.

5.8.7. Effects of silicon nutrient on the °Brix levels of fruit (per treatment) in the hydroponic experiment

Appendix 70 shows individual °Brix levels readings in five different strawberry fruits from the silicon-treated and untreated. Statistics have not been applied to °Brix sampled from the hydroponics as these are individual readings and so not applicable. From the table in appendix 70, levels of fruit °Brix varied. No statistical difference is shown between the silicon-treated and untreated. Simple averages of °Brix levels from appendix 70 in untreated fruits sampled both on 13 June 2018 and 20 June 2018 read 13.5, and averages of °Brix levels in the silicon-treated fruits sampled both on 13 June 2018, and 20 June 2018 read 15. Silicon-treated fruits suggest a higher °Brix according to these averages but are not confirmed statistically.

5.8.8. Effects of silicon nutrient on average values of chlorophyll levels (per treatment) in leaves of plants grown hydroponically

Means levels of chlorophyll contents in the leaves of silicon-treated plants are compared to untreated using the paired sample for means test. Statistical tests in appendix 71 and 72 showed no statistical increase (P>0.05) in the silicon-treated plants compared to

untreated. Results showed that the chlorophyll contents in the leaves of silicon-treated strawberry plants were not significantly different (P>0.05) from untreated. Means of ten leaves per plant (ten silicon-treated and untreated measurements) were used to analyse data in untreated and silicon-treated (appendix 71 and 72).

5.8.9. Effects of silicon nutrient on the fresh weights (biomass) of whole strawberry plants grown hydroponically

Ten whole strawberry plants with leaves, roots and runners still attached from silicon-treated and untreated plants were weighed at the end of the hydroponic experiment, and the raw results are shown in appendix 73. No statistical test applied to this section as the results presented in appendix 73 are based on individual fresh weights of strawberry plants in both untreated and silicon treatment.

Conclusions from simple averages using Excel (2021) between both treatments (appendix 73) revealed silicon-treated plants seemed to have weighed (average = 169g) more than untreated (average = 144g). However, these results from the data presented here are not statistically proven. Figure 5.13 is only shown as an example of a strawberry plant that was treated with silicon (left side) and one that had no silicon treatment (right side). The photo in figure 5.13 shows any difference in the structure or appearance of a plant treated with silicon and without silicon grown in hydroponics. Table 5.7 below summarises the main findings from the hydroponic experiment in 2018.

Table 5. 7 Summary of findings in the hydroponic experiment 2018

Growth parameter	Treatments	Statistical test applied	Statistical findings (see appendix 66 to 73 for statistical workings)
Leaves	Untreated control (no silicon) and silicon-treated	Shapiro-Wilk normality test and parametric test; Paired Samples Test	There was a statistical increase ($P<0.05$) in the average values of the number of silicon-treated strawberry plant leaves compared to untreated. The null hypothesis (HO) is rejected.
Runners	Untreated control (no silicon) and silicon-treated	Shapiro-Wilk normality test and non-parametric test; Wilcoxon Signed Ranked Test	There was a statistical increase ($P<0.05$) in the average values of the number of silicon-treated plant runners compared to untreated. The null hypothesis (HO) is rejected.
Fruits	Untreated control (no silicon) and silicon-treated	Shapiro-Wilk normality test and non-parametric test; Wilcoxon Signed Ranked Test	There was a statistical increase ($P<0.05$) in the average values of numbers of silicon-treated plant fruits compared to untreated. . The null hypothesis (HO) is rejected.
Chlorophyll	Untreated control (no silicon) and silicon-treated	Shapiro-Wilk normality test and parametric test; Paired Samples Test	No statistical increase was ($P>0.05$) in the average values of numbers of chlorophyll contents of silicon-treated plants compared to untreated. The null hypothesis (HO) is accepted.

Table 5.7 showed the growth parameters of strawberry plants growing in hydroponics. The results revealed that silicon-treated plants had more leaves, runners and fruits ($P<0.05$) than untreated plants. Results also showed that silicon did not raise ($P>0.05$) the chlorophyll contents in the leaves of the silicon-treated strawberry plants compared to untreated. Figure 5.13 compares a silicon-treated plant and an untreated plant for visual purposes.

Figure 5. 13. Silicon-treated (left side) and Untreated (right side) individual strawberry plants from the hydroponic glasshouse experiment 2018.

The observation drawn from the sizes of the plants shown in figure 5.13 is that plants grown without silicon can be smaller than those grown with silicon. However, the significance of this photo (figure 5.13) is not quantified and cannot be generalised.

5.8.10. Observations on plants grown hydroponically without silicon and plants grown with silicon

There were no obvious symptoms, such as premature yellowing of leaves, deformed fruits or stunting in growth observed in untreated and silicon-treated strawberry plants throughout the length of the glasshouse hydroponic experiment. Strawberry plants grown without silicon were smaller in their numbers of leaves, runners and fruits compared to silicon-treated strawberry plants at the end of the experiment. The number of leaves on the plants grown without silicon was significantly lower than the number of leaves on the plants treated with silicon. See figure 5.10 for the graph of the production of leaves. The numbers of runners were significantly more in silicon-treated plants compared with untreated. See figure 5.11 for the graph of the production of runners. No significant differences ($P>0.05$) were found in chlorophyll levels, °Brix levels and fresh weights of strawberry plants from this experiment. Plants grown without silicon were smaller concerning their leaf and runner numbers.

5.9.   Discussion

5.9.1. Effects of silicon nutrient on the °Brix levels of fruits and leaf petioles in the silicon fertigation field experiment 2017 and fruits of the Hydroponic glasshouse experiment

In the field experiment, strawberry fruits in figure 5.8 showed an increase ($P<0.05$) in °Brix levels of strawberries sampled on 24 July 2017 only and no increase ($P>0.05$) in strawberries sampled on 20 July 2017, 27 July 2017, 1 and of August 2017. Leaf petioles °Brix results showed there was no significant increase ($P>0.05$) in levels of °Brix in leaf petioles samples on 20 June 2017, 18 July 2017, 01 August 2017 and 19 September 2017 (figure 5.9). Jin (2015) reported that the average values of °Brix of fruits of strawberries from a field experiment were significantly higher in silicon-treated plants than untreated. Liu (2016) also found a significant increase ($P<0.05$) in the levels of °Brix in leaf petioles and fruits with the use of silicon.

The glasshouse hydroponic experiment showed an elevation in the sample averages of strawberry fruits °Brix between both treatments, but statistics were not applicable. However, results from the field and glasshouse experiments varied (fruits and leaf petioles). Plants in the field experiment may have been stressed under different climate conditions compared with the glasshouse experiment, which was grown in a controlled environment. As mentioned, other authors have found that strawberry fruit and leaf petiole °Brix in a field experiment is enhanced with silicon use. The work reported here moved on from the field experiment to examine both field experiments and hydroponically grown strawberry plants °Brix.

A higher °Brix of strawberry fruit is important to growers as they satisfy and meet the demands of UK supermarkets. Each plant's sucrose (sugar) content may differ, and other factors, including ripening and direct sunlight, can affect sugar levels (Cao et al., 2015). The work reported in the chapter moved on further from the work reported by Jin (2015) from assessing °Brix levels in field experiments to assessing other growth parameters, such as numbers of leaves, runners, fruits, chlorophyll contents and fresh weights of plants in the hydroponic experiment.

5.9.2. Effects of silicon nutrient on the average values of the number of leaves in the hydroponic experiment

The first assessment in the glasshouse hydroponic experiment was to determine the number of leaves in silicon-treated and untreated strawberry plants as plants matured throughout the experiment. Results showed an increase in the average values of the number of strawberry leaves in silicon-treated strawberry plants compared with untreated (figure 5.10, appendix 66). Statistical analysis using the paired-sample t-test showed that both untreated and silicon-treated plants differed significantly ($P<0.05$). The production of leaves is one of the signs of a healthy growing plant. The more leaves, the more benefit to the plant as plants receive their energy and sugars from their leaves. More leaves in plants are considered a benefit as this can positively affect yield by fruit production and possibly higher °Brix.

An example of silicon enhancing the number of leaves was shown in a study conducted in Pakistan on Maize in a pot experiment, which showed that silicon significantly ($P<0.05$) increased the number of leaves on silicon-treated Maize crops compared with untreated crops (Amin et al., 2018). Dehghanipoodeh et al. (2018) also showed that silicon foliar application treatment had a bio-stimulative effect by increasing the leaf area in strawberry plants growing in a pot experiment under natural outdoor conditions. Zahedi et al. (2020) Showed an increase in the number of leaves of strawberry plants using the addition of foliar sprays treatment of Nanoparticles (NPs) containing silicon dioxide (SiO_2) and Selenium (Se) in the cultivar Gaviota.

5.9.3. Effects of silicon nutrient on the average values of the number of runners produced in the hydroponic experiment

The second assessment in this experiment was to determine if silicon positively affected the number of runners laid down by strawberry plants growing in the hydroponics. Both silicon-treated and untreated strawberry plants produced runners. However, more runners were produced in the silicon-treated plants compared to untreated (figure 5.11, appendix 67). Growers cut off runners from mother plants to preserve the plant's strength in producing more flowers and fruits. Runners may not be useful to growers

but are beneficial to plant propagators. Statistics (appendix 67) showed that silicon-treated plants had more runners than untreated and both treatments were statistically different ($P<0.05$). This also supports the previous work by Jin (2015), which showed that adding silicon to strawberry plants growing in a glasshouse pot experiment increased the weight of runners of silicon-treated plants compared to untreated plants. Previous work did not focus on the numbers of runners but on their weights (Liu, 2016).

5.9.4. Effects of silicon nutrient on flowering in the hydroponic experiment

Flowering (in two out of ten silicon-treated plants) was a week early in silicon-treated strawberry plants compared with untreated (figure 5.12). An example of early flowering was found in an experiment conducted on *Helianthus annuus* (Sunflower) plants, which showed that early flowering and increased flower quality occurred with optimum silicon treatment compared with untreated control (Kamenidou, Cavins and Marek, 2008). Although the literature has not shown early flowering in strawberry plants, this experiment suggests that silicon can boost flowering times in strawberry plants. Figure 5.12 shows the flowering to fruiting period in the glasshouse hydroponic experiment. Results suggest that silicon-treated plants, which flowered earlier, produced fruits quicker. The more fruits were picked, the more fruits they produced. The average values in this experiment, the fruiting period in silicon-treated plants, were longer than untreated. Therefore, if silicon application can stimulate strawberry plants to flower longer, it could encourage a longer fruit production time (figure 5.12).

5.9.5. Effects of silicon nutrient on the number of fruits produced in the hydroponic experiment

There was an increase in the average values of the number of strawberry fruits from the silicon-treated strawberry plants compared with untreated (appendix 69). Fully ripe fruits counted from the silicon-treated plants were more per plant ($P<0.05$) than untreated. This work supports (in hydroponics) the work done by Jin (2015) and Liu (2016), which also showed a significant increase ($P<0.05$) in fruit yield in silicon-treated crops in a glasshouse pot experiment compared with untreated. A study in India showed that foliar silicon application significantly improved soybean growth and yield (Shwethkumari et al., 2017). Another study by Artyszak (2018) showed an increase in the number of grains per cob in Maize. Field experiments conducted in Morocco using sugar beet showed that a silicon application systematically increased root yield and leaf yield by 40% (Prentice, 2017). Results from the hydroponic experiment encouraged early fruiting from silicon-treated plants that had their first flowering.

5.9.6. Effects of silicon nutrient on chlorophyll contents in leaves of plants grown hydroponically

Chlorophyll measurement was a part of the weekly assessment in the hydroponic experiment. Results from statistical analysis using the Paired Sample test in appendix 72 showed no significant increase ($P>0.05$) of chlorophyll contents in the leaves of silicon-treated plants compared with untreated. A study by Silva et al. (2012) showed that silicon had a beneficial effect on the chlorophyll contents in tomato cultivars. Xie et al., 2014 also reported a positive effect of silicon on chlorophyll contents in Maize (*Zea mays L.*). Their work showed that silicon application in a field experiment increased the total chlorophyll contents of Maize leaves, which shows that the photosynthetic efficiency of Maize was increased with the use of an adequate silicon application.

Work done by Dehghanipoodeh et al. (2018) showed that silicon significantly ($P<0.05$) improved the chlorophyll contents of strawberry plants in silicon-treated plants in comparison to untreated plants. Chlorophyll in photosynthesis help plants obtains energy from sunlight (Kobayashi and Masuda, 2019). The more chlorophyll in the leaves, the more sugars they can produce, which in turn may enhance °Brix levels leading to an overall benefit to the plant. More leaves and chlorophyll contents can encourage the production of sugars in leaves, which are transported to the fruit.

5.9.7. Effects of silicon nutrient on the fresh weights (biomass) of plants grown hydroponically

At the end of the experiment, fresh weights (averages, appendix 73) of strawberry plants suggests that silicon-treated plants weighed more than untreated plants. However, this was based on individual whole plants, and statistics were not applicable here and so were not confirmed. Jin (2015) found that both fresh and dry weights of whole strawberry plants (including roots, tops and runners) had greater biomass ($P<0.05$) in silicon-treated plants than untreated in a glasshouse experiment. Liu (2015) also showed higher strawberry crop biomass of dry and fresh weights in silicon-treated plants ($P<0.05$) compared with untreated in a glasshouse experiment. Li et al. (2018) reported that silicon significantly increased ($P<0.05$) the biomass of the cotton plant. Luyckx et al. (2017) showed that fresh leaf weight of hemp plants treated with silicon had larger biomass than untreated. Biomass is important for crop yield and development (Ayal et al., 2019).

Another example is a study conducted in India, which showed that silicon use on peppers significantly ($P<0.05$) increased plant height and biomass production (Artyszak, 2018). A study on potato cultivation in Israel showed that silicon fertilization increased the average tuber weight, dry tuber weight, tuber yield and larger fresh weight of potato plants (Vulavala et al., 2016). Amin et al. (2018) reported that silicon applied to Maize crops in a pot experiment increased the number of grains, length of cob and plant height (biomass).

5.9.8. Observations on the health of plants grown hydroponically with and without silicon

In the hydroponic experiment, observations from strawberry plants showed no symptoms of stunting in growth, yellowing of leaves or deformed fruits occurred in untreated plants. However, untreated plants had fewer leaves, fewer runners and fewer fruits. Statistics using the paired-sample t-test and Wilcoxon-Sign Ranked test revealed that silicon-treated strawberry plants significantly increased ($P<0.05$) in the numbers of silicon-treated leaves, runners and fruits compared to untreated. Still, no significant increase ($P>0.05$) was found in the chlorophyll contents of leaves compared with untreated. The results in table 5.8 showed the effects of silicon on plants growing in hydroponics in a glasshouse experiment. The symptoms seen of plants not given any silicon in the hydroponic experiment is that they were smaller, as shown by their leaf, runners and numbers of fruits.

5.10. Conclusion

This chapter showed that silicon improved the quality of strawberry plants growing in a hydroponic glasshouse experiment by increasing their numbers of leaves, and runners, stimulating early flowering and fruit number of silicon-treated plants compared with untreated control.

Previous work done by Jin (2015) and Liu (2016) on the benefits of silicon, particularly in strawberry plants, showed that silicon increased the °Brix levels in the fruits of silicon-treated strawberry plants compared with untreated. Disease reduction with silicon used in a two-year experiment and silicon deposition experiment in a one-year glasshouse experiment using fewer samples, which were not quantified, was reported by Jin (2015). However, cultivars explored the work reported in this thesis differently. Experiments were conducted in glasshouse (two years) and field (three years) experiments. A deposition experiment was replicated in a glasshouse and field experiment, which was quantified. Additionally, a hydroponic experiment was set up following these experiments to investigate the growth parameters of strawberry plants growing in Hoagland's solution. This experiment was specific to this thesis and did not carry on from work shown in Jin's thesis.

The work reported in this thesis chapter investigated further by assessing other growth parameters (besides fruit °Brix and biomass as shown by other authors) such as the numbers of leaves, runners, fruits, chlorophyll contents in the leaves, particularly in strawberry plants. As the literature shows, silicon does encourage growth parameters, such as °Brix and biomass. However, this chapter investigated strawberries' overall plant growth parameter and found other benefits to using silicon in growing strawberries, including fruit °Brix. The overall performance of the plant is enhanced. The aims and objectives that silicon can improve growth parameters of strawberry plants are met by the numbers of leaves, runners and fruits in silicon-treated plants. However, not by their chlorophyll contents of the leaves, °Brix of fruits and fresh weights biomass.

Chapter 6 - Overall discussion and conclusion

This chapter covers i) Overall discussion and conclusion of experimental chapters 3 to 5 and ii) Future work.

The key aim of this thesis was to examine the effects of the silicon nutrients delivered through the fertigation system on the development of strawberry powdery mildew (*Podosphaera aphanis*) disease levels in different strawberry plant cultivars in chapter 3, to examine the amounts and pattern of distribution of silicon in leaves, leaf petioles and roots of strawberry plants in a glasshouse and field experiments in chapter 4, to evaluate °Brix levels of silicon-treated fruits and leaf petioles with untreated fruits and leaf petioles, to measure growth parameters of strawberry plants in the absence and presence of silicon in a glasshouse hydroponic experiment in chapter 5. The research work reported in this thesis has shown that the application of silicon nutrients through the roots of strawberry plants once and twice a week via a fertigation system reduced disease severity in a different strawberry plant cultivar, Malling Centenary, in 2016. Secondly, the work revealed in chapter 4 that the silicon administered to strawberry plants is laid down regularly in the leaves, leaf petioles, and roots. This deposition was quantified (amounts) through fluorescence intensity measurements for two consecutive years in a glasshouse 2017 and field experiments 2017 and 2018. Lastly, the work reported in this thesis also found that silicon can enhance strawberry plant growth features, such as their leaves, runners and fruit number. This thesis's aims, objectives and findings are discussed in the various sections listed here.

6.1. Effects of silicon on powdery mildew disease *Podosphaera aphanis* on Malling Centenary and Amesti Cultivar

A fundamental part of the study was to examine whether the effects of silicon, when applied to different cultivars of strawberries, have similar effects of working shown by other authors mentioned in this thesis. Cultivars used in this study were the Malling Centenary plants in 2016 (figure 3.1) and Amesti in 2017 and 2018 (figures 3.4 and 3.6). The main findings from this experiment were that the use of silicon treatment as nutrients in the fertigation system caused a delay in the start of levels of disease by 29 days (figure 3.1, appendix 14). The work in this thesis revealed that silicon applied once and twice a week with and without fungicides reduced levels of disease ($P < 0.05$) in a 2016 fertigation field experiment, which meets the first aim of this thesis, to examine whether the addition of silicon through a fertigation system can reduce disease severity of strawberry powdery mildew *Podosphaera aphanis* on a different cultivar, Malling Centenary.

Examples of disease level reduction with the addition of silicon in strawberry plants were reported by Jin (2015) using different cultivars, such as Elegance, Alexandra, Shelley and Sonata. Another example is shown by Liu (2016), also using the strawberry cultivar Sonata, which caused a reduction in the severity of disease levels ($P < 0.05$). The work of Jin (2015) and Liu (2016), combined with the work in the thesis, have shown disease reduction in 5 consecutive years. This disease reduction has been shown on six different strawberry plant cultivars (Malling Centenary, Amesti, Elegance, Alexandra, Shelley and Sonata). Jin (2015) and Liu (2016) showed that in silicon-treated strawberry plants, there was a delay in the onset of disease development by more than two weeks. However, this experiment showed that adding silicon with and without fungicides caused disease reduction (2016) and a much longer delay in building disease levels by 29 days.

These consistent results suggest that using silicon nutrients in the fertigation tubes is a useful part of an integrated disease control programme showing both delays in the start of disease build-up and overall disease reduction. The research reported in the literature has used a variety of forms of silicon and a variety of methods of application. The 'Sirius' (see product description in chapter 2, section 2.1.3 and appendix 5) used in this thesis does not contain potassium or selenium. This bioavailable form of silicon can be used safely in fertigation tubes. Other positive effects of silicon in reducing diseases on other crops are found in chapter 1, table 1.7 and 1.8. Whilst most recorded disease reduction is in crops that are silicon accumulators, reduced levels of powdery mildew were also found in melon (a non-silicon accumulator) using potassium silicate (Barker and Pilbeam, 2006). Results from this experiment have shown that the aim and objectives of the chapter were met in 2016 with strawberry powdery mildew (*Podosphaera aphanis*) disease reduction using a different cultivar from previous work.

6.2. Silicon deposition in strawberry plants cultivar (passive defence pathway)

This research shows that silicon was preferentially deposited in the leaves, leaf petioles and roots of silicon-treated Malling Centenary strawberry plants in a glasshouse pot experiment. This deposition of silicon was laid down in a regular pattern, and the silicon quantification analysis revealed that more silicon ($P<0.05$) was deposited in the cuticle, epidermis, and palisade layer of treated strawberry leaves compared to untreated strawberry leaves. In the leaf petioles, more deposits of silicon ($P<0.05$) were laid down mainly in the xylem of the treated petioles compared to untreated petioles. In the roots, there were more deposits of silicon ($P<0.05$) accumulated in the xylem of silicon-treated roots compared to untreated roots (figure 4.2, appendix 25 to 33 and 60).

Previously, a glasshouse work reported by Jin (2015) showed that in the cultivar Shelley, there were more silicon deposits in the epidermis and vascular tissue of the leaf and leaf petiole of a silicon-treated plant. Jin (2015) reported this effect was not

replicated by cross-sections or quantified by the amounts of silicon deposited in the plants. The work was assessed by eye from a glasshouse experiment. The glasshouse experiment in this chapter was quantified and showed a consistent modification of the plant morphology using this silicon.

Furthermore, to the study reported from the glasshouse experiment, field experiments of silicon deposition assessments in 2017 showed that they were also laid down in a regular pattern in silicon-treated plants compared to untreated. The results showed that more deposits ($P<0.05$) of silicon were found in the cuticle, epidermis, and palisade layer of treated strawberry leaves compared to untreated leaves. More deposits of silicon ($P<0.05$) were laid down in the xylem of the treated petioles compared to untreated petioles. More silicon was also found laid down in the silicon-treated roots ($P<0.05$) compared to untreated (figure 4.3, appendix 34 to 42 and 61). The 2018 field study showed that more silicon deposits ($P<0.05$) were laid down in a regular pattern in the cuticle, epidermis, and palisade layer of treated strawberry leaves compared to untreated leaves. However, there was no significant difference ($P>0.05$) between the silicon-treated and untreated plants in the petioles and roots. (figure 4.4, appendix 43 to 51 and 62).

Debona et al. (2017) showed in an experiment using rice (silicon accumulator) on the disease rice blast. They hypothesized that the change in morphology caused by the silicon deposits reduced infection by rice blast. See diagram in chapter 1, figure 1.23. The work reported here and shown in chapter 4 suggests a similar modification in strawberry plants (a silicon non-accumulator). The disease reduction shown in chapter 3 could be caused by the morphological modifications induced by the silicon deposition (see future work section). This suggests that silicon can enhance the passive defence pathway of strawberry plants. These experiments reported demonstrate the pattern and quantification of silicon in the leaves, petioles and roots of strawberry plants both in a glasshouse and field experiments for 2 consecutive years, thus meeting the aims and objectives of the chapter in the 2017 glasshouse experiment and 2017 field experiment, which was to examine the amounts and pattern of distribution of silicon in leaves, leaf petioles and roots of strawberry plants. Future work should investigate the link between silicon enhancing the passive defence pathway and reducing disease susceptibility to strawberry powdery mildew *Podosphaera aphanis*.

6.3. Benefits of silicon nutrients use on strawberries

This research study also measured the effects of silicon on the growth parameters of strawberry plants growing in hydroponics. This experiment revealed that the numbers of leaves, runners and fruits of strawberry plants treated with silicon were significantly more than ($P<0.05$) untreated plants. An example of some benefits linked with silicon use is shown by Zahedi et al. (2020) by using foliar sprays of Nanoparticles (NPs) containing silicon dioxide (SiO_2) and Selenium (Se) (Se/SiO_2-NPs (100 mg L^{-1}) on

strawberry plants. These benefits included increased area and numbers of leaves, chlorophyll content, reduced cracked fruits, enhanced vitamin c content and fruit firmness. In this experiment, it is impossible to distinguish between the effects of silicon, selenium and the use of nanoparticles. However, the study reported here compared silicon-treated plants and untreated plants. The main findings of the experiment revealed that silicon-treated plants had increased ($P<0.05$) numbers of leaves (figure 5.10 and appendix 66), runners (figure 5.11 and appendix 67) and fruits (appendix 69) compared to untreated plants. Other improved factors included early flowering observed in some plants from the silicon-treated plants compared to untreated (figure 5.12). Fresh weights (averages) based on individual whole strawberry plants showed that silicon-treated plants seemed to have weighed more than untreated plants. However, this is not statistically proven (appendix 73). An increase in the yield of fruit, shelf-life and vegetative growth in the strawberry plants was reported by Peri-Felipo (2020).

Some growers have reported toxicity effects when using potassium silicate (personal communications from grower). This includes albino fruits. However, Maas (2004) only reports albino fruits with the excess use of potassium. At the concentration used (0.017%), no toxicity symptoms were observed in this thesis, with the bioavailable form of silicon (not containing potassium or sodium) used throughout the experiments. No toxicity symptoms were found in work reported by Jin (2015) and Liu (2016). Kamenidou, Cavins and Marek (2008) showed that growth abnormalities were observed when concentrations of 100 and 200 mg·L-1 Si were supplied as potassium silicate (KSiO3) in Gerbera and Sunflower. These high levels of potassium silicate were found to deform flowers.

The literature suggests that strawberry growers are advised not to use more than 22mg/L of silicon. Although growers are frightened of albinism, the academic literature also suggests that this whiteness in strawberries is caused by excess potassium, calcium and nitrogen (Lieten, Horvath and Asard, 2000) rather than silicon. This experiment suggests that the aim to measure the growth parameters of strawberry plants grown in the presence and absence of silicon was met through the increased numbers of leaves, runners and fruits of silicon-treated plants compared to untreated. The study revealed that the aims and objectives were met through the benefits of using silicon in growing strawberry plants in a hydroponic experiment in the presence and absence of silicon.

6.4. Conclusion

The effects of the silicon (bioavailable form) shown in this thesis include higher strawberry crop yield and quality and disease reduction probably mediated via the passive defence pathway. Therefore, the recommendation for growers from this thesis is to incorporate the regular use of silicon as a nutrient via fertigation as part of an integrated management plan to optimise strawberry production.

6.5. Future work

This research thesis has summarised following future work to follow on from experiments reported here and can investigate the following.

a) The link between silicon nutrients enhances the passive defence pathway and reduces disease susceptibility.

To elucidate silicon's effects in enhancing the passive defence pathway using light microscopy and electron microscopy analysis. The experiment would quantify the penetration and subsequent growth of *Podosphaera aphanis* in treated and untreated strawberry plants.

b) Effects of silicon on yield quality and shelf life of strawberries

Strawberry growers are under pressure to produce more class 1 fruits and reduce fruit waste, thus reducing the waste of class 2 fruits (there is no real market for class 2 fruits). This thesis and unpublished work suggest that using silicon nutrients may raise the number of class 1 fruits and increase shelf life. This work would evaluate strawberries harvested from silicon-treated and untreated plants for their shelf life and examine if they were less susceptible to post-harvest decay. Work can also examine whether silicon may affect the firmness of strawberry fruits, thus reducing fruit bruising concerning strawberry fruit picking and transport.

c) Further investigations of the cause of albinism in strawberries

Further work can examine the effects of silicon concentration on strawberries and the interaction of silicon, potassium, calcium and nitrogen. This experiment can investigate the causes of albinism as the literature suggests that there could be a link between high levels of silicon use potentially leading to whiteness in strawberries. The methods for this experiment will include growing strawberry plants in hydroponics containing Hoagland's solution in a glasshouse experiment.

As Hoagland's contains no silicon but contains all the necessary nutrients essential for optimal plant growth, treatment for the hydroponic experiments can include plants growing in Hoagland's alone (untreated) plants growing with a regular weekly dose of silicon application and plants growing with excessive amounts of silicon application, which could be applied once or twice-a-week. This experiment will monitor and observe the growth and development of the strawberry plants throughout their life cycle. During the fruiting season, strawberries will be harvested from all strawberry plants in the hydroponic experiment to assess their colour, flavour and firmness for signs of albinism.

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APPENDICES

Appendix 1 Silicon fertigation field trial 2016 (Ladybird field) Silicon fertigation field trial 2016 (Ladybird field)

Silicon Trial, Maltmas farm, Friday Bridge, Wisbech. 2016. Ladybird field. Amistl. start: 19.4.16
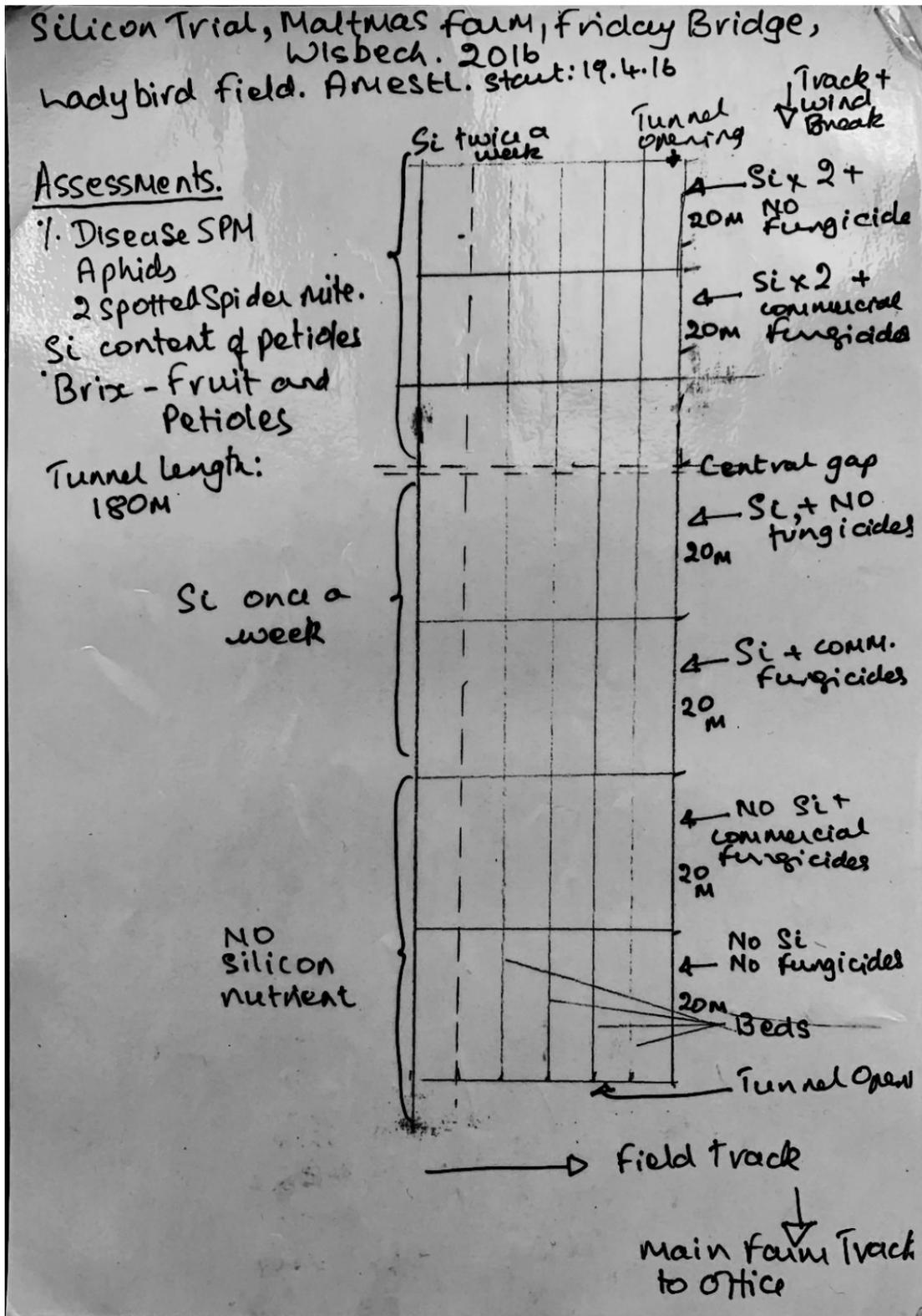
Appendix 2 Silicon fertigation field trial 2017 (Amelia field)

Silicon fertigation field trial, Mattmas farm, friday Bridge,

Amelia   Amesti Variety   Amelia field   2017

_Assessments
% Disease SPM   (silicon & prediction field trial)
· Silicon content of leaves
· Brix - fruit and petioles

Tunnel opening   End

Wind Break

Silicon Fertigation

Silicon Only 20 metres

Silicon + fungicides 70 metres

180 METRES

Central Isle

NO Silicon

Fungicide Only 70 metres

N

Untreated 20 metres

Beds   E · D   C   B · A

Farm   Tunnel opening   Start

Appendix 3 Silicon fertigation field trial 2018 Amelia field

Silicon Fertigation Field Trial 2018 - Amelia Field (Amesti)

Tunnel end

Silicon fertigation

180 Meters

No silicon

N
W — E
S

Silicon twice Weekly without fungicides

(20 Metres)

Silicon twice weekly + Fungicides

(70 Metres)

Central Gap

Fungicides

(70 Metres)

Untreated (Control)

(20 Metres)

Farm road

Tunnel entrance

Assessments:
-% cover SPM (fortnightly disease assessment)
-Silicon content in water
-Silicon extraction from leaves and fruit
-Silicon deposition in leaves and fruit
-Chlorophyll measurement in the fertigation field

Appendix 4 Strawberry powdery mildew disease assessment key by jin (2015)

40% 50% 60% 80%

20% 25% 30% 35%

1% 5% 10% 15%

Appendix 5 Safety data sheet
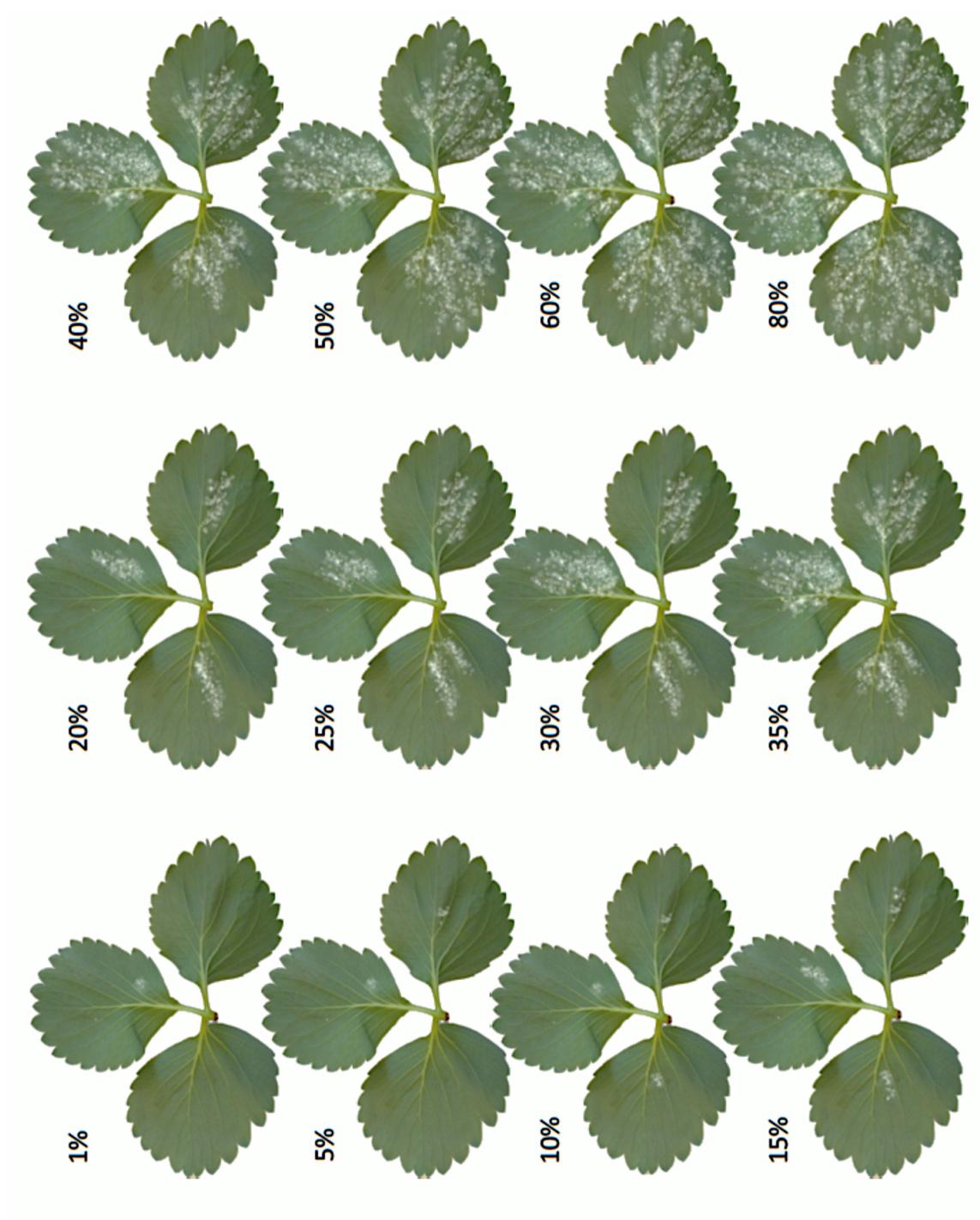
SAFETY DATA SHEET

Sirius

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.

Appendix 6 Safety data sheet cont'd

Sirius

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Wear suitable protective equipment.
Environmental precautions	Do not allow product to enter drains. Advise local authorities if large spills cannot be contained.
Clean up methods	For large spills: Absorb with inert, absorbent material. Transfer to suitable, labelled container. Clean spillage area thoroughly with plenty of water. For small spills: Flush down the drain with plenty of water.

7. HANDLING AND STORAGE

Handling	Wear suitable protective equipment. Avoid contact with eyes and skin.
Storage	Keep out of the reach of children. Store in correctly labelled containers. Store in original container. Keep containers tightly closed.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Exposure limits

Ethyl alcohol (Ethanol)	WEL 8-hr limit ppm: 1000 WEL 15 min limit ppm: -	WEL 8-hr limit mg/m3: 1920 WEL 15 min limit mg/m3: -
Respiratory protection	Wear suitable respiratory equipment when necessary.	
Hand protection	Chemical resistant gloves (plastic)	
Eye protection	Avoid contact with eyes. Provide eye wash station. Approved safety goggles.	
Protective equipment	Avoid contact with eyes and skin. Wash all contaminated clothing before reuse. Adopt best Manual Handling considerations when handling, carrying and dispensing.	

9. PHYSICAL AND CHEMICAL PROPERTIES

Description	Liquid.
Colour	Clear.
Odour	Slight.
pH	n/a
Flash point	35°C
Flammability limits	Flammable
Relative density	0.960 - 0.980
Viscosity	Kinematic Viscosity in 10-6 m²/s at 40°C (ISO 3219) - < 50 cps

10. STABILITY AND REACTIVITY

Stability	Stable under normal conditions.
Materials to avoid	Strong oxidising agents. Strong acids.
Hazardous decomposition products	Carbon oxides.

11. TOXICOLOGICAL INFORMATION

Acute toxicity	Ingestion is irritating to the respiratory tract and may cause damage to the central nervous system.
Corrosivity	May cause irritation to skin. Irritating to skin.
Repeated or prolonged exposure	Prolonged or repeated exposure may cause irritation to skin and mucous membranes.

Appendix 7 Maps of Maltmas farmMap A - Rural Land Register map of Maltmas Farm
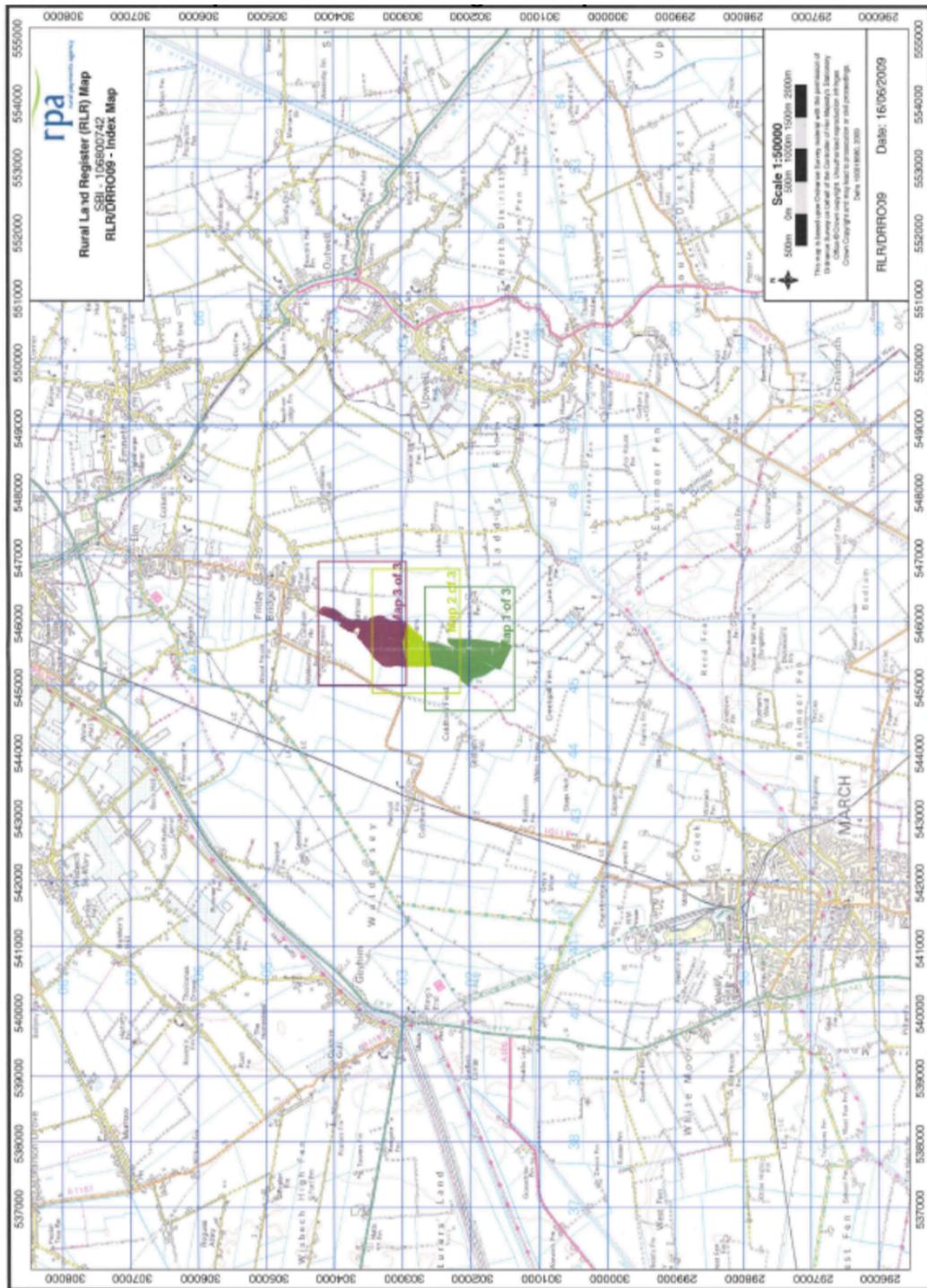
Appendix 8 Map B - Distribution of strawberry field in Maltmas Farm

The Amelia field and Ladybird field were areas covered in this thesis between 2016 and 2018
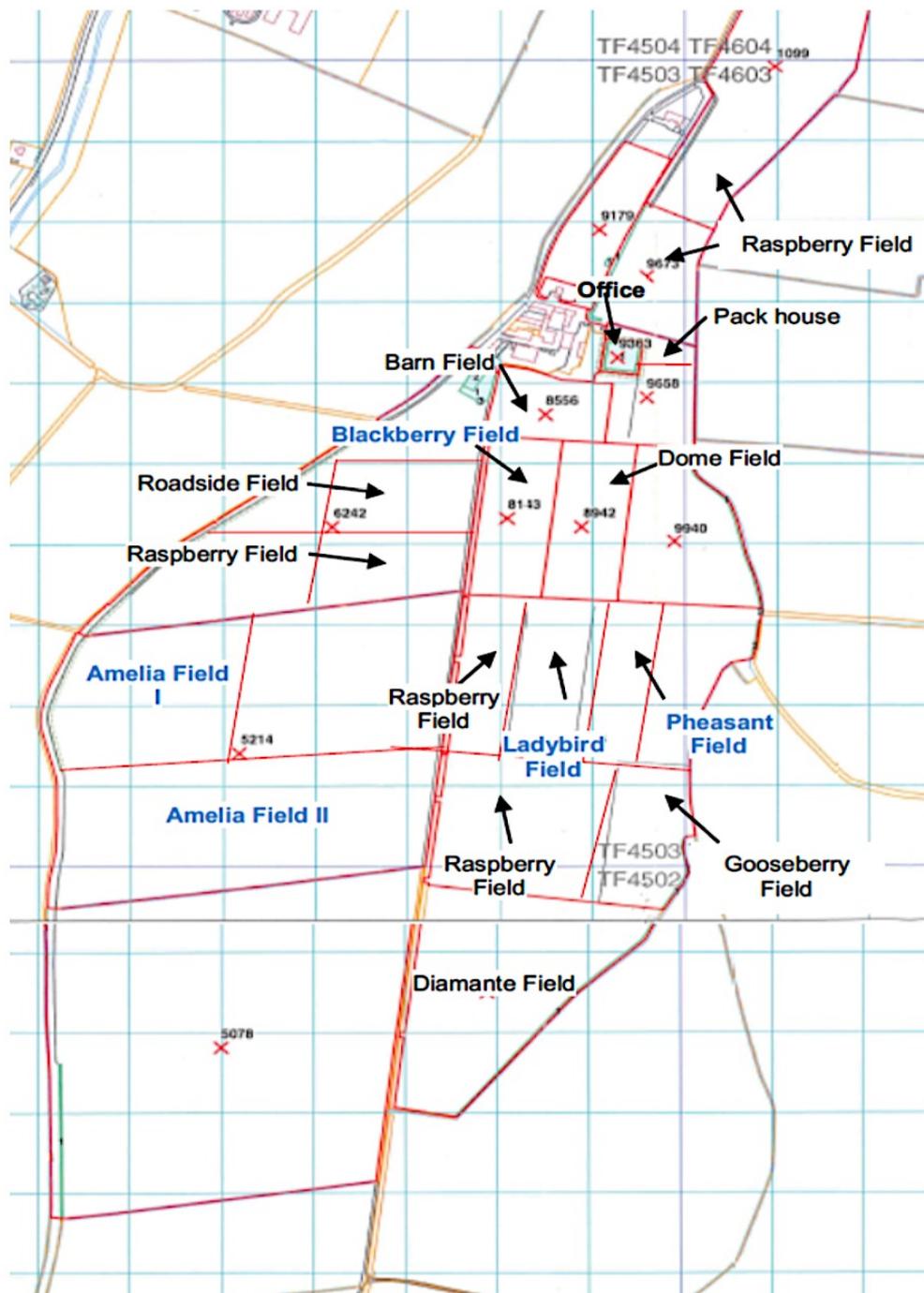
Appendix 9 List of fungicides for the silicon fertigation field trial 2016

MUDDY BOOTS
SOFTWARE LIMITED

Ancha I Commercial & Prediction Trial (handwritten)

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

5214, Strawberries, Sonata (1.00 ha)
(01-Oct-15 - 30-Sep-16) *Commercial Applications.* (handwritten)

Planned	Product	Area	Rate	Applied
12/04/16	TRIGGER 3	1.00	3.000 kg	N/C
29/04/16	SWITCH	1.00	1.000 kg	N/C
	SYSTHANE 20EW	1.00	0.330 lt	N/C
	SW7	1.00	100.000 unit	N/C
	MANZI	1.00	1.000 lt	N/C
	HALLMARK WITH ZEON TECHNOLOGY	1.00	0.100 ml	N/C
16/05/16	SIGNUM	1.00	1.250 kg	N/C
	SW7	1.00	100.000 unit	N/C
	MANZI	1.00	1.000 lt	N/C
	HALLMARK WITH ZEON TECHNOLOGY	1.00	0.100 ml	N/C
	HORTIPHYTE	1.00	2.000 unit	N/C
24/05/16	SWITCH	1.00	1.000 kg	N/C
	SW7	1.00	100.000 unit	N/C
	SYSTHANE 20EW	1.00	0.330 lt	N/C
	HORTIPHYTE	1.00	2.000 unit	N/C
	MANZI	1.00	1.000 lt	N/C
	SLUGGO	1.00	10.000 kg	N/C
01/06/16	HALLMARK WITH ZEON TECHNOLOGY	1.00	0.100 ml	N/C
	AMISTAR	1.00	1.000 lt	N/C
	STEM	1.00	1.000 unit	N/C
	SYSTHANE 20EW	1.00	0.330 lt	N/C
	HORTIPHYTE	1.00	2.000 unit	N/C
	SW7	1.00	100.000 unit	N/C
03/06/16	SIGNUM	1.00	1.250 kg	N/C
	SW7	1.00	100.000 unit	N/C
	SYSTHANE 20EW	1.00	0.330 lt	N/C
	HORTIPHYTE	1.00	2.000 unit	N/C
14/06/16	MANZI	1.00	1.000 lt	N/C
	HORTIPHYTE	1.00	2.000 unit	N/C
	ROVRAL WG	1.00	0.500 kg	N/C
	MANZI	1.00	1.000 lt	N/C
	SIGNUM	1.00	1.250 kg	N/C
17/06/16	SW7	1.00	100.000 unit	N/C
	MANZI	1.00	1.000 lt	N/C
	ROVRAL WG	1.00	1.000 kg	N/C
22/06/16	SW7	1.00	100.000 unit	N/C
	MANZI	1.00	1.000 lt	N/C
	TELDOR	1.00	1.000 kg	N/C
24/06/16	SW7	1.00	100.000 unit	N/C
	MANZI	1.00	1.000 lt	N/C
	SCALA	1.00	1.000 lt	N/C
27/06/16	MANZI	1.00	1.000 lt	N/C
	SW7	1.00	100.000 unit	N/C

27/06/16	PROLECTUS	1.00	1.000 kg	N/C
14/07/16	HALLMARK WITH ZEON TECHNOLOGY	1.00	0.100 ml	N/C
	SW7	1.00	100.000 unit	N/C
30/08/16	CORBEL	1.00	0.750 lt	N/C
	FORTRESS	1.00	0.250 lt	N/C
	SW7	1.00	100.000 unit	N/C
	HALLMARK WITH ZEON TECHNOLOGY	1.00	0.100 ml	N/C
	MASAI	1.00	0.750 kg	N/C

Handwritten notes:

− = mildew Action

Prediction Syst Applications

As commercial to 14th Jun

17 June — 300 mls Systhane/

2nd July — 1L Nimrod/Ha.

16 July — 300 mls Systhane

24 July — 1L Corbel.

08/Aug — 1L Nimrod.

30/08 — 1L Corbel + 250 mls Fortress

Print Date 05/09/2016   Page 1 of 1

Appendix 10 fungicides sprays used for the silicon fertigation field trial 2017 cont'd

MUDDY BOOTS
SOFTWARE LIMITED

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

5214, Strawberries, AMESTI (1.20 ha)
(01/10/2016 - 30/09/2017)

Planned	Product	Area	Rate	Applied
29/01/17	KERB FLO	0.60	2.000 lt	N/C
	DEVRINOL	0.60	5.000 lt	N/C
	STOMP 400 SC	0.60	3.300 lt	N/C
16/03/17	FENOMENAL	1.20	2.000 kg	N/C
27/03/17	VENZAR FLOWABLE	1.20	0.400 lt	N/C
	AMISTAR	1.20	1.000 lt	N/C
	ESCAR-GO 3	1.20	4.000 kg	N/C
10/04/17	REGLONE	0.60	2.000 lt	N/C
21/04/17	AMISTAR	1.20	1.000 lt	N/C
	HORTIPHYTE	1.20	2.000 unit	N/C
02/05/17	LUNA SENSATION	1.20	0.800 lt	N/C
	CALYPSO	1.20	0.250 lt	N/C
	MANZI	1.20	2.000 lt	N/C
15/05/17	MANZI	1.20	2.000 lt	N/C
	SIGNUM	1.20	1.250 kg	N/C
29/05/17	AMISTAR	1.20	1.000 lt	N/C
	CALYPSO	1.20	0.250 lt	N/C
02/06/17	LUNA SENSATION	1.20	0.800 lt	N/C
11/06/17	SCALA	1.20	1.000 lt	N/C
	SYSTHANE 20EW	1.20	0.330 lt	N/C
19/06/17	AMISTAR	1.20	1.000 lt	N/C
	SYSTHANE 20EW	1.20	0.330 lt	N/C
	TELDOR	1.20	1.000 kg	N/C
27/06/17	LUNA SENSATION	1.20	0.800 lt	N/C
06/07/17	NIMROD	1.20	1.000 lt	N/C
	TELDOR	1.20	1.000 kg	N/C
21/07/17	SYSTHANE 20EW	1.20	0.330 lt	N/C
	HALLMARK WITH ZEON TECHNOLOGY	1.20	0.100 ml	N/C
	TELDOR	1.20	1.000 kg	N/C
03/08/17	LUNA SENSATION	1.20	0.800 lt	N/C
	TRACER	1.20	0.150 lt	N/C
10/08/17	TELDOR	1.20	1.000 kg	N/C
	SYSTHANE 20EW	1.20	0.330 lt	N/C
	TRACER	1.20	0.150 lt	N/C
24/08/17	SIGNUM	1.20	1.250 kg	N/C
02/09/17	TRACER	1.20	0.150 lt	N/C
	TELDOR	1.20	1.000 kg	N/C

Appendix 11 fungicides sprays used for the silicon fertigation field trial 2018 cont'd

Appendix 12 fungicides sprays used for the silicon fertigation field trial 2018 cont'd

Appendix 13 Calculation of silicon nutrients (Sirius) application rate in the 2016 to 2018 silicon fertigation field trials at Maltmas Farm

The silicon fertigation field trials were set under polythene tunnels of 180 metres long. The trials consisted of 5 strawberry beds with 6 strawberry crops growing in coir bags (1 metre). Each field tunnel contained approximately 5,000 - 5300 strawberry plants. Water and silicon nutrient were delivered through 4 irrigation drippers per coir bag. Rate of irrigation was 2.2 L water per hour and each irrigation time was 5 minutes.

The suggested rate for silicon nutrient used in agriculture is 50 – 100ml in 100 – 600 L of water (Jin, 2015; Liu; 2016). The grower used 100ml of silicon nutrient 'Sirius' in 600 L per hectare. Which calculated as

100 mL in 600 L = 0.017% Sirius (v/v)
In 5 minutes, each irrigation tunnels received;

5 minutes/60 x 2.2 L / hour x 4 drippers x 180 m x 15 beds = 1584 Litre water

Therefore, the amount of silicon nutrient (0.017% Sirius v/v) for one irrigation tunnel

0.00017 x 1584 L = 0.269 L = approximately 270ml

Each strawberry plant received approximately 270ml

270ml / 5000 – 5300 plants x 2 tunnels = 0.025 – 0.027 ml Sirius per plant.

As Sirius consists of four different compounds, the actual amount of soluble silicon that was taken up by the plants may be lower than amounts calculated above.

Appendix 14 Chapter 3 - Results workings for disease levels of strawberry powdery mildew in the silicon fertigation field experiment 2016 (Malling Centenary)

Normality test for disease levels in the 2016 field experiment

(a)   **Normality Test**

Null and alternate Hypothesis:

HO: Difference between untreated and silicon + fungicides follow normal distribution

HA: Difference between untreated and silicon + fungicides does not follow normal distribution

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Difference	.170	12	.200[*]	.915	12	.246

*. This is a lower bound of the true significance.

If sig (P) value is less than critical value 0.05 we reject Ho. In the analysis sig value 0.246 greater than critical value 0.05 we accept Ho and conclude that the difference between untreated and silicon and fungicides follows normal distribution. The parametric test (Paired samples test) was used to test for significance difference.

(b)   **Null and Alternate Hypothesis**

Ho: There is no statistical significant difference between untreated and silicon + fungicides

Ha: There is statistical significant difference between untreated and silicon + fungicides-only

Paired Samples Test

	Paired Differences							
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Paired 1 – Untreated – Silicon_ Fungicides_	224.25000	254.42810	73.44707	62.59410	385.90590	3.053	11	.011

If sig (P) value is less than critical value 0.05 we reject Ho. In the analysis sig value.011 less than critical value 0.05 (0.011 <0.05) we reject Ho and conclude that there is statistical significant difference between untreated and silicon + fungicides treatment.

(c)   Normality Test

Null and alternate Hypothesis:

HO: Difference between untreated and silicon + no fungicides follow normal distribution

HA: Difference between untreated and silicon + no fungicides does not follow normal distribution

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Difference	.216	12	.126	.911	12	.222

a. Lilliefors Significance Correction

If sig value is less than critical value 0.05 we reject Ho. In the analysis sig value 0.222 is greater than critical value 0.05 we accept Ho and conclude that the difference between untreated and silicon + no fungicides follow normal distribution. The parametric test (Paired samples test) was used to test for significance difference.

(d)   Null and Alternate Hypothesis

Ho: There is no statistical significant difference between untreated and silicon + no fungicides

Ha: There is statistical significant difference between untreated and silicon + no fungicides.

Paired Samples Test

	Paired Differences							
			Std. Error Mean	95% Confidence Interval of the Difference				
	Mean	Std. Deviation		Lower	Upper	t	df	Sig. (2-tailed)
Pair 1 Untreated – Silicon_no_Fungicides	151.08333	297.17930	85.78828	-37.73539	339.90205	1.761	11	.106

If sig sig (*P*) value is less than critical value 0.05 we reject Ho. In the analysis sig value.106 is greater than critical value 0.05 (0.106 >0.05) we accept Ho and conclude that there is no statistical significance difference between untreated and silicon + no Fungicides.

(e)   Normality Test

Null and alternate Hypothesis:

HO: Difference between untreated and silicon twice + fungicides follow normal distribution

HA: Difference between untreated and silicon twice + fungicides does not follow normal distribution

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Difference	.169	12	.200[*]	.882	12	.094

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

If sig (P) value is less than critical value alpha 0.05 we reject Ho. In the analysis P value.094 greater than critical value 0.05 we accept Ho and conclude that data does follow normal distribution. The parametric test (Paired samples test) is used to measure the difference among the treatment.

(f)   Null and Alternate Hypothesis

Ho: There is no statistical significant difference between untreated and silicon twice + fungicides

Ha: There is statistical significant difference between untreated and silicon twice + fungicides.

Paired Samples Test

	Paired Differences							
			Std. Error	95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1 Untreated – Silicon twice_ Fungicides	251.08333	265.08882	76.52455	82.65393	419.51274	3.281	11	.007

If sig (P) value is less than critical value 0.05 we reject Ho. In the analysis sig value 0.007 less than critical value 0.05 (0.007 <0.05) we reject Ho and conclude that there is statistical significant difference between untreated and silicon twice + fungicides.

(g)   Null and alternate Hypothesis:

HO: Difference between untreated and silicon twice + no fungicides follow normal distribution

HA: Difference between untreated and silicon twice + no fungicides does not follow normal distribution

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Difference	.182	12	.200[*]	.869	12	.063

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

If sig (*P*) value is less than critical value alpha 0.05 we reject Ho. In the analysis *P* value.063 greater than critical value 0.05 we accept Ho and conclude that data does follow normal distribution. The parametric test (Paired samples test) was used to measure the difference among the treatment.

(h)   Null and Alternate Hypothesis

Ho: There is no statistical significant difference between Untreated and silicon twice + no Fungicides

Ha: There is statistical significant difference between untreated and silicon twice + no fungicides

Paired Samples Test

				Paired Differences				
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1 Untreated – Silicon twice_ no_ Fungicides	254.00000	254.50058	73.46799	92.29805	415.70195	3.457	11	.005

If sig (*P*) value is less than critical value 0.05 we reject Ho. In the analysis sig value 0.005 is less than critical value 0.05 (0.005 <0.05) we reject Ho and conclude that there is statistically significance difference between untreated and silicon twice + no fungicides.

Appendix 15 Raw data for silicon extraction from strawberry leaves in the silicon fertigation field trial 2016 (chapter 3)

Treatments	Sample date	Amount of silicon
Untreated	19/4/2016	0.42µg/mg
No silicon + fungicides only	19/4/2016	0.59µg/mg
Silicon + fungicides	19/4/2016	0.15µg/mg
Silicon + no fungicides	19/4/2016	0.15µg/mg
Silicon twice + fungicides	19/4/2016	0.18µg/mg
Silicon twice + no fungicides	19/4/2016	0.39µg/mg
Treatments	Sample date	Amount of silicon
Untreated	03/05/2016	0.42µg/mg
No silicon + fungicides only	03/05/2016	0.38µg/mg
Silicon + fungicides	03/05/2016	0.24µg/mg
Silicon + no fungicides	03/05/2016	0.33µg/mg
Silicon twice + fungicides	03/05/2016	0.41µg/mg
Silicon twice + no fungicides	03/05/2016	0.44 µg/mg
Treatments	Sample date	Amount of silicon
Untreated	07/06/2016	0.36µg/mg
No silicon + fungicides only	07/06/2016	0.32µg/mg
Silicon + fungicides	07/06/2016	0.51µg/mg
Silicon + no fungicides	07/06/2016	0.64µg/mg
Silicon twice + fungicides	07/06/2016	0.64µg/mg
Silicon twice + no fungicides	07/06/2016	0.68µg/mg
Treatments	Sample date	Amount of silicon
Untreated	05/07/2016	0.39µg/mg
No silicon + fungicides only	05/07/2016	0.89µg/mg
Silicon + fungicides	05/07/2016	0.49µg/mg

Appendix 16 Raw data for silicon extraction from strawberry leaves in the silicon fertigation field trial 2016 cont'd (chapter 3)

Silicon + no fungicides	05/07/2016	0.79μg/mg
Silicon twice + fungicides	05/07/2016	0.55μg/mg
Silicon twice + no fungicides	05/07/2016	0.42μg/mg
Treatments	Sample date	Amount of silicon
Untreated	02/08/2016	0.64μg/mg
No silicon + fungicides only	02/08/2016	0.30μg/mg
Silicon + fungicides	02/08/2016	0.89μg/mg
Silicon + no fungicides	02/08/2016	0.64μg/mg
Silicon twice + fungicides	02/08/2016	0.55μg/mg
Silicon twice + no fungicides	02/08/2016	0.59μg/mg
Treatments	Sample date	Amount of silicon
Untreated	06/09/2016	1.06μg/mg
No silicon + fungicides only	06/09/2016	1.32μg/mg
Silicon + fungicides	06/09/2016	1.79μg/mg
Silicon + no fungicides	06/09/2016	0.48μg/mg
Silicon twice + fungicides	06/09/2016	0.90μg/mg
Silicon twice + no fungicides	06/09/2016	2.92μg/mg

Appendix 17 chapter 3 results workings for silicon extraction between treatment in the 2016 field experiment

(a) **t-Test: Paired Two Sample for Means (Untreated and Silicon once + no fungicides)**

		Untreated	Silicon once +no fungicides
Mean		0.6125	0.6375
Variance		0.104758333	0.016025
Observations		4	4
Pearson Correlation		-0.854026446	
Hypothesized	Mean	0	
Difference			
df		3	
t Stat		-0.114477137	
P(T<=t) one-tail		0.458045687	
t Critical one-tail		2.353363435	
P(T<=t) two-tail		0.916091374	
t Critical two-tail		3.182446305	

Silicon extractions results are used as evidence that the silicon delivered via the fertigation system is taken up by the plants. Results from the table has shown that there was no statistical significant difference (*P*>0.05) between levels of silicon in untreated strawberry plants and strawberry plants treated with silicon once + no fungicides.

(b) t-Test: Paired Two Sample for Means

	untreated	Once silicon + fungicides
Mean	0.6125	0.442
Variance	0.03165	0.01967
Observations	4	4
Pearson Correlation	0.062925063	
Hypothesized Mean Difference	0	
df	3	
t Stat	-0.983507686	
P(T<=t) one-tail	0.190520022	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.381040044	
t Critical two-tail	2.776445105	

Results has shown that there was no statistical significant difference ($P>0.05$) between levels of silicon in untreated strawberry plant and once silicon + fungicides treatment.

(c) t-Test: Paired Two Sample for Means (Untreated and Silicon twice + fungicides)

	Untreated	Silicon twice + fungicides
Mean	0.6125	0.66
Variance	0.104758333	0.0274
Observations	4	4
Pearson Correlation	0.833085836	
Hypothesized Mean Difference	0	
df	3	
t Stat	-0.458708876	
P(T<=t) one-tail	0.338817331	
t Critical one-tail	2.353363435	

P(T<=t) two-tail	0.677634663	
t Critical two-tail	3.182446305	

Results from the table has shown that there was no statistical significant difference between (*P*>0.05) levels of silicon in untreated strawberry plants and strawberry plants treated with silicon twice + fungicides.

(d) t-Test: Paired Two Sample for Means

	Untreated	silicon twice + no fungicides
Mean	0.6125	1.375
Variance	0.021946667	0.17391
Observations	4	4
Pearson Correlation	0.705408461	
Hypothesized Mean Difference	0	
df	3	
t Stat	-5.436016697	
P(T<=t) one-tail	0.001428983	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.002857965	
t Critical two-tail	2.570581836	

Results has shown that there was a statistical significant difference (*P*<0.05) between levels of silicon in untreated strawberry plants and strawberry plants treated with silicon twice + no fungicides.

Appendix 18 Correlation analysis between silicon extracted and levels of disease in the 2016 field experiment

Before conducting correlation analysis, first of check whether the data collected is normal or not. This was done using the Shapiro Wilk test in table 3.4.

Null and alternate Hypothesis:

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Silicon_Extraction	.246	36	.000	.689	36	.000
Disease_Level	.360	36	.000	.435	36	.000
a. Lilliefors Significance Correction						

If the value of P is less than critical value alpha 0.05, reject Ho and conclude that data does not follow from normal distribution. In the analysis in table 3.4, the sig value of silicon extraction and disease level using the Shapiro wilk test is 0.000 less than critical value 0.05. Reject Ho and conclude that the data does not follow normal distribution. The sig value of Kolmogorov-Smirnov test is less than 0.05, reject Ho and conclude that data does not follow normal distribution. Since the data does not follow normal distribution, a nonparametric test was used to analyse this data. The Spearman's Rank correlation analysis was used to find the correlation (Relationship) between silicon extracted and disease levels in the silicon fertigation field experiment 2016. See table 3.5 for the correlation analysis.

Nonparametric Correlations

Correlations

			Silicon_Extraction	Disease_Level
Spearman's rho	Silicon_Extraction	Correlation Coefficient	1.000	.323
		Sig. (2-tailed)	.	.055
		N	36	36
	Disease_Level	Correlation Coefficient	.323	1.000
		Sig. (2-tailed)	.055	.
		N	36	36

The Spearman's Rank correlation analysis measured the strength of relationship between silicon extraction and level of infection in the silicon fertigation field experiment 2016. If the correlation is greater than 0.50 the relationship is strong. Although there was flooding in the silicon field tunnel in 2016 (These results presented here includes the flooded beds) the analysis shown in table 3.5, correlation value 0.323, suggests that there is a positively weak to moderate relationship between silicon extracted and levels of disease in the 2016 silicon fertigation field experiment.

However,

If the *P* value is less than critical value, reject Ho. In the analysis shown in table 3.5, the *P* value.055 is greater than critical value 0.05, accept Ho and conclude that the relationship between silicon extraction and disease level is moderate, but is not statistically significant (*P*>0.05*).*

Appendix 19 Raw data for silicon extraction from strawberry leaves in the silicon fertigation field trial 2017 (Chapter 3)

Treatments	Sample date	Amount of silicon
Untreated	9/4/2017	0.17µg/mg
No silicon + fungicides only	9/4/2017	0.25µg/mg
Silicon twice + fungicides	9/4/2017	0.56µg/mg
Silicon twice + no fungicides	9/4/2017	0.66µg/mg
Treatments	Sample date	Amount of silicon
Untreated	23/05/2017	0.20µg/mg
No silicon + fungicides only	23/05/2017	0.32µg/mg
Silicon twice + fungicides	23/05/2017	0.62µg/mg
Silicon twice + no fungicides	23/05/2017	0.55µg/mg
Treatments	Sample date	Amount of silicon
Untreated	06/06/2017	0.15µg/mg
No silicon + fungicides only	06/06/2017	0.19µg/mg
Silicon twice + fungicides	06/06/2017	0.72µg/mg
Silicon twice + no fungicides	06/06/2017	0.76µg/mg
Treatments	Sample date	Amount of silicon
Untreated	04/07/2017	0.22µg/mg
No silicon + fungicides only	04/07/2017	0.28µg/mg
Silicon twice + fungicides	04/07/2017	0.60µg/mg
Silicon twice + no fungicides	04/07/2017	0.75µg/mg
Treatments	Sample date	Amount of silicon
Untreated	01/08/2017	0.17µg/mg
No silicon + fungicides only	01/08/2017	0.24µg/mg
Silicon twice + fungicides	01/08/2017	0.84µg/mg
Silicon twice + no fungicides	01/08/2017	0.79µg/mg
Treatments	Sample date	Amount of silicon
Untreated	05/09/2017	0.26µg/mg
No silicon + fungicides only	05/09/2017	0.42µg/mg
Silicon twice + fungicides	05/09/2017	0.51µg/mg
Silicon twice + no fungicides	05/09/2017	0.44µg/mg

Appendix 20 Chapter 3 Results workings for silicon extraction in the silicon fertigation field experiment 2017

t-Test: Paired Two Sample for Means (Untreated and fungicides-only)

(a) t-Test: Paired Two Sample for Means

	Untreated	fungicides-only
Mean	0.195	0.128333333
Variance	0.00587	0.005336667
Observations	4	4
Pearson Correlation	0.559229726	
Hypothesized Mean Difference	0	
df	3	
t Stat	0.23218173	
P(T<=t) one-tail	0.412800395	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.825600791	
t Critical two-tail	2.570581836	

Results from appendix 20 a shown that there were no differences between levels of silicon in both treatment

(b) t-Test: Paired Two Sample for Means (Untreated and Silicon twice + fungicides)

	Untreated	Silicon twice + fungicides
Mean	0.195	1.055
Variance	0.0023	0.038966667
Observations	4	4
Pearson Correlation	0.605615032	
Hypothesized Mean Difference	0	
df	3	
t Stat	-9.963692477	
P(T<=t) one-tail	0.001075601	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.002151202	
t Critical two-tail	3.182446305	

Results from the table appendix 20 b has shown that there was a statistical significant difference ($P<0.05$) between levels of silicon in untreated strawberry leaves and strawberry leaves treated with silicon twice + fungicides.

(c) t-Test: Paired Two Sample for Means (Untreated and Silicon twice + no fungicides)

	Untreated	Silicon twice + no fungicides
Mean	0.195	0.8125
Variance	0.0023	0.072691667
Observations	4	4
Pearson Correlation	0.69991024	
Hypothesized Mean Difference	0	
df	3	
t Stat	-5.1777685	
P(T<=t) one-tail	0.00699142	
t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.01398284	
t Critical two-tail	3.18244631	

Results has from appendix 20 c has shown that there was a statistical significant difference ($P<0.05$) between levels of silicon in untreated strawberry leaves and strawberry leaves treated with silicon twice + no fungicides. The differences in silicon levels at the end of the season in the 2017 (Figure 3.5) compared with the 2016 silicon fertigation field experiment (Figure 3.4) may have contributed to the fact that the same age leaves were sampled in this revised silicon fertigation field experiment for 2017.

Appendix 21 Raw data for silicon extraction from strawberry leaves in silicon fertigation field trial 2018 (chapter 3)

Treatments	Sample date	Amount of silicon
Untreated	3/4/2018	0.35µg/mg
No silicon + fungicides only	3/4/2018	0.43µg/mg
Silicon twice + fungicides	3/4/2018	0.59µg/mg
Silicon twice + no fungicides	3/4/2018	0.52µg/mg
Treatments	Sample date	Amount of silicon
Untreated	08/05/2018	0.51µg/mg
No silicon + fungicides only	08/05/2018	0.45µg/mg
Silicon twice + fungicides	08/05/2018	0.58µg/mg
Silicon twice + no fungicides	08/05/2018	0.45µg/mg
Treatments	Sample date	Amount of silicon
Untreated	19/06/2018	0.40µg/mg
No silicon + fungicides only	19/06/2018	0.57µg/mg
Silicon twice + fungicides	19/06/2018	0.58µg/mg
Silicon twice + no fungicides	19/06/2018	0.59µg/mg
Treatments	Sample date	Amount of silicon
Untreated	03/07/2018	0.47µg/mg
No silicon + fungicides only	03/07/2018	0.47µg/mg
Silicon twice + fungicides	03/07/2018	0.70µg/mg
Silicon twice + no fungicides	03/07/2018	0.67µg/mg

Appendix 22 Chapter 3 Results workings for silicon extraction in the silicon fertigation field experiment 2018

(a) t-Test: Paired Two Sample for Means

	Untreated	Fungicides only
Mean	0.4325	0.642
Variance	0.066357143	0.357390476
Observations	4	4
Pearson Correlation	0.694210849	
Hypothesized Mean Difference	0	
df	4	
t Stat	-0.17323258	
P(T<=t) one-tail	0.434082502	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.868165005	
t Critical two-tail	2.446911851	

No statistical differences (P>0.05) were found between untreated and fungicides-only treatment

(b) t-Test: Paired Two Sample for Means (Untreated and Silicon twice + fungicides)

	Untreated	Silicon twice + fungicides
Mean	0.4325	1.035
Variance	0.00509167	0.031033333
Observations	4	4
Pearson Correlation	-0.3168856	
Hypothesized Mean Difference	0	
df	3	
t Stat	-5.7386375	
P(T<=t) one-tail	0.0052537	

t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.01050739	
t Critical two-tail	3.18244631	

Results has shown that there was a statistical significant difference (*P*<0.05) between levels of silicon in untreated strawberry leaves and strawberry leaves treated with silicon twice + fungicides. This shows that there was more silicon in silicon-treated plants compared to untreated plants.

(c) t-Test: Paired Two Sample for Means (Untreated and Silicon twice + no fungicides)

	Untreated	Silicon twice + no fungicides
Mean	0.4325	0.9125
Variance	0.005091667	0.06869167
Observations	4	4
Pearson Correlation	-0.577931972	
Hypothesized Mean Difference	0	
df	3	
t Stat	-3.10811476	
P(T<=t) one-tail	0.02647963	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.05295926	
t Critical two-tail	3.182446305	

Results from figure 3.7 and table appendix 22 c has showed a border line (P>0.05) between untreated strawberry leaves and strawberry leaves treated with silicon twice + no fungicides. As the two-tail value is 0.052, it is greater than 0.05. Therefore, the difference between silicon contents in both treatment is not significant.

Appendix 23 Protocol for LysoTracker Yellow HCK-123 fluorescence dye staining (Chapter 4)

The LysoTracker Yellow HCK-123 fluorescence dye was used to stain cross-sections separately on slides or petri dishes covered from direct light for 2 hours. The Vectashield mounting medium was used in drops per slide to preserve samples from drying out while staining.

After staining (2 hours) cross-sections are viewed using the GXML3201 LED fluorescence microscope. Silicon fluorescence density was estimated using the software program ImageJ, which measures total fluorescence within a given sample.

Protocol

One vial contains 1µM (50µL) of (stock) Lyso-Tracker dye

De-ionized water (9 mL)

Vectashield (in drops)

1. Dilute 1µM (50µL) of dye into 9 mL of de-ionized water

2. Transfer dye solution into a dark protective storage tube

3. Vortex (invert) solution

4. Pipette dye onto sample on slide or petri dish

5. Pipette drops of Vectashield on sample and coverslip to allow mounting medium to disperse over the entire section.

6. Allow samples to stain for 2 hours in the dark. Store dye in a cool dry place after use.

Appendix 24 Silicon fluorescence intensity quantification using ImageJ (Chapter 4)

Download free software program "ImageJ" and select the platform suitable to computer. Preferably Windows version.

1. RUN program (ImageJ);
   To allow ImageJ toolbar display on screen (Top centre of screen)

2. Drag and drop sample images (one at a time) unto toolbar on screen;
   Image is now ready for measurements

3. Click on "Analyze" and click "set measurement" from the drop-down menu;
   Tick (mark) Integrated density ("Int Density") which measures total fluorescence and "Area" (Un-mark all other set measurements not needed)

4. Click on the free-hands tool from the toolbar (yellow tracing line);
   Trace the fluorescing areas of the cell (sample). Double-click to erase or re-draw

5. Click on Analyze → Measure (or Ctrl 'M');
   ImageJ will then produce measurements in a result table

6. Copy results from table and paste in Microsoft Excel;
   In Excel, re-name samples i.e strawberry leaf petiole1 (Untreated control) etc.

7. Using the free-hands tool, measure a blank for each sample, i.e areas without fluorescence (Unknown background area) The blank is the black background.
   Measure only a small area for blank. Click Analyze → Measure (or Ctrl 'M'). Copy blank measurements from the results table displayed and paste unto a separate column or new spreadsheet on Microsoft Excel for calculations.

The blank (Unknown area) of cell subtracted from the Integrated density (Total fluorescence) of cell equals total quantity of fluorescence (without background fluorescence). The total fluorescence (Integrated density) given divided by the Area of cell then equals actual quantity of fluorescence per given sample.

Therefore;

Integrated density (Total fluorescence) – Blank (Unknown area of cell)

= Fluorescence of cell ÷ Area of cell

= Actual quantity of fluorescence

Counts per second (cps) is a unit for fluorescence and each value of actual fluorescence can be rounded off to a one or two decimal place value.

Appendix 25 Cross-sections (Replicates) of strawberry leaves in the glasshouse experiment 2017 (x40 and x400 magnifications) Chapter 4

Untreated leaves   **Silicon treated leaves**
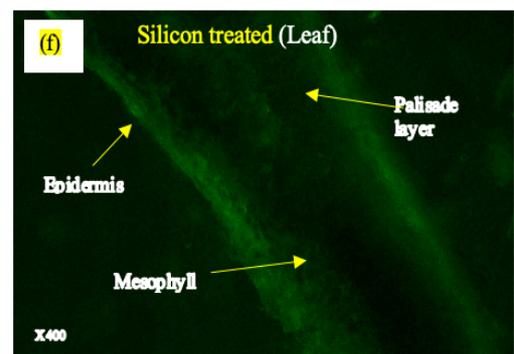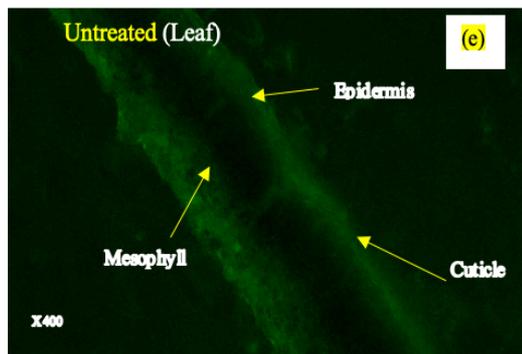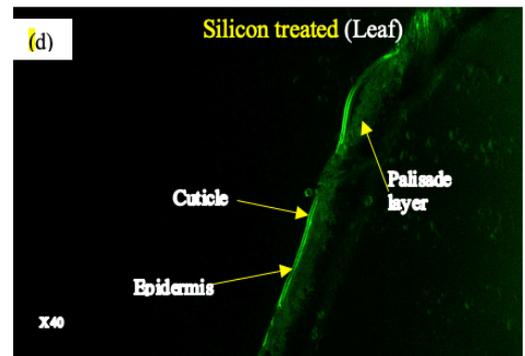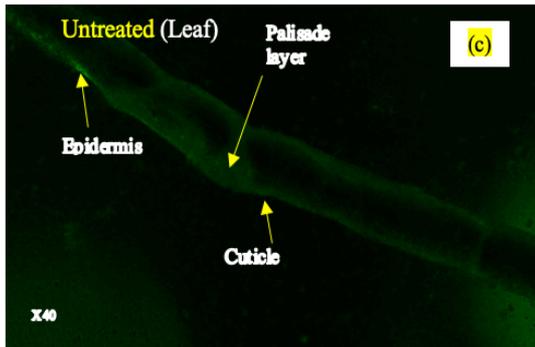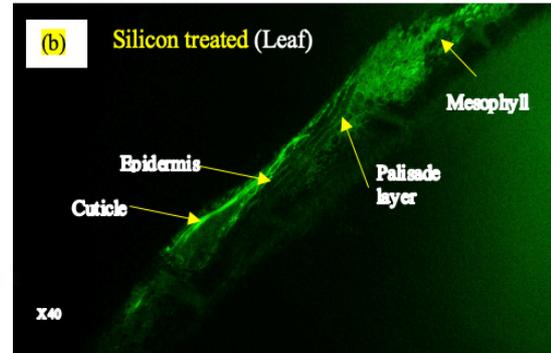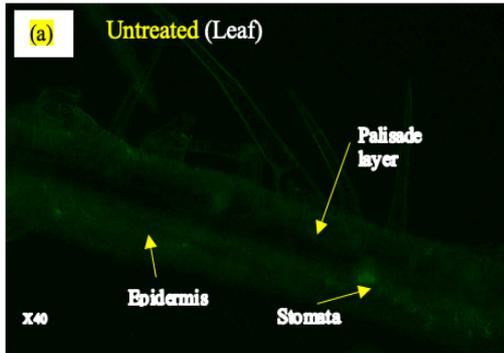
Appendix 26 Cross-sections (Replicates) of strawberry leaves in the glasshouse experiment 2017 (x40 and x400 magnifications) cont'd, Chapter 4

Untreated leaves   **Silicon-treated leaves**
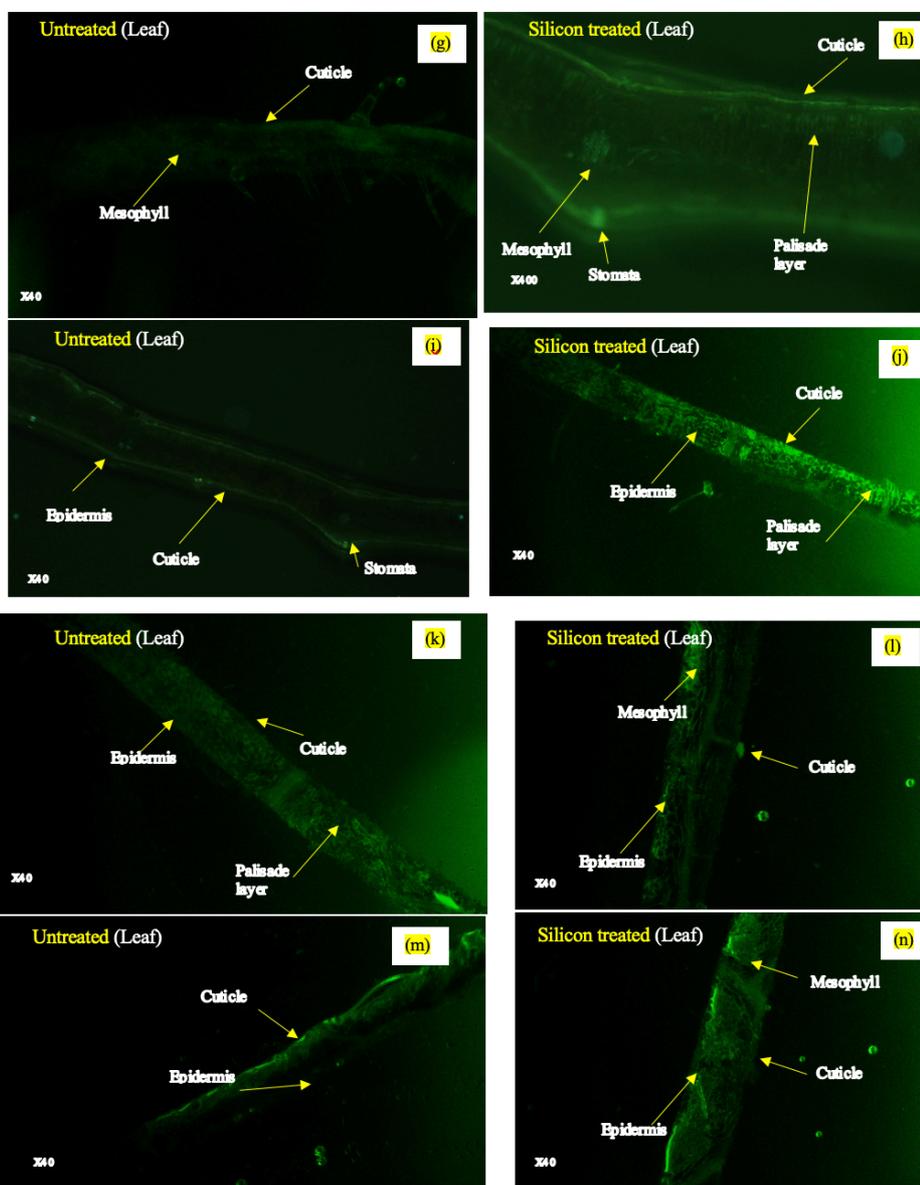
Images presented through fluorescence images in this appendix indicated that more deposits of silicon is found laid down in a regular manner and not in an amorphous form in the silicon treated plants presented on the right hand side compared to the untreated on the left hand side (Viewed at x40 and x400) throughout. The yellow arrows indicate the areas silicon has been found to accumulate more in this thesis hence the term, use ' Regularity in manner of deposition'. This was not always the case but mostly.

Appendix 27 : Cross-sections (Replicates) of strawberry leaves in the glasshouse experiment 2017 (x40 and x400 magnifications) cont'd, Chapter 4

Untreated leaves   **Silicon-treated leaves**
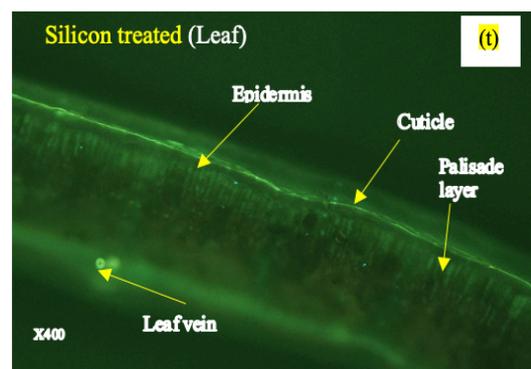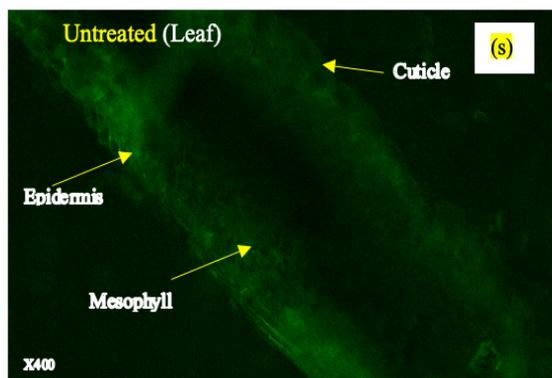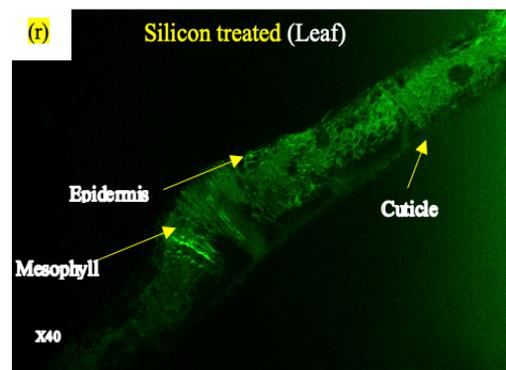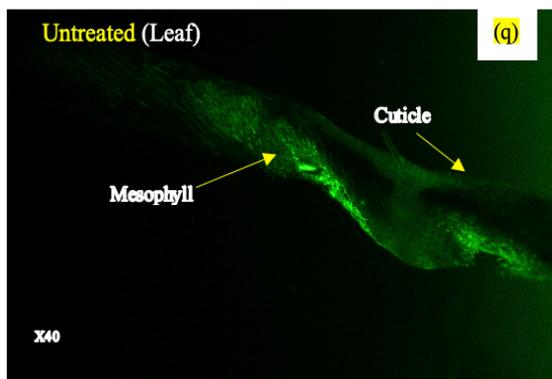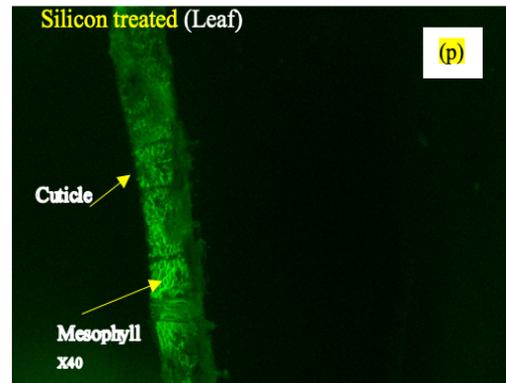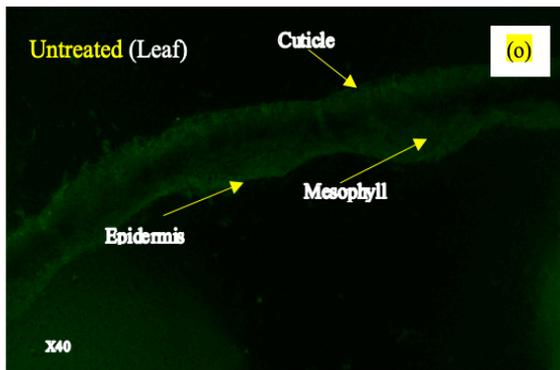
Appendix 28 Cross-sections (Replicates) of strawberry leaf petioles in the glasshouse experiments 2017 (x40 and x400 magnifications) Chapter 4
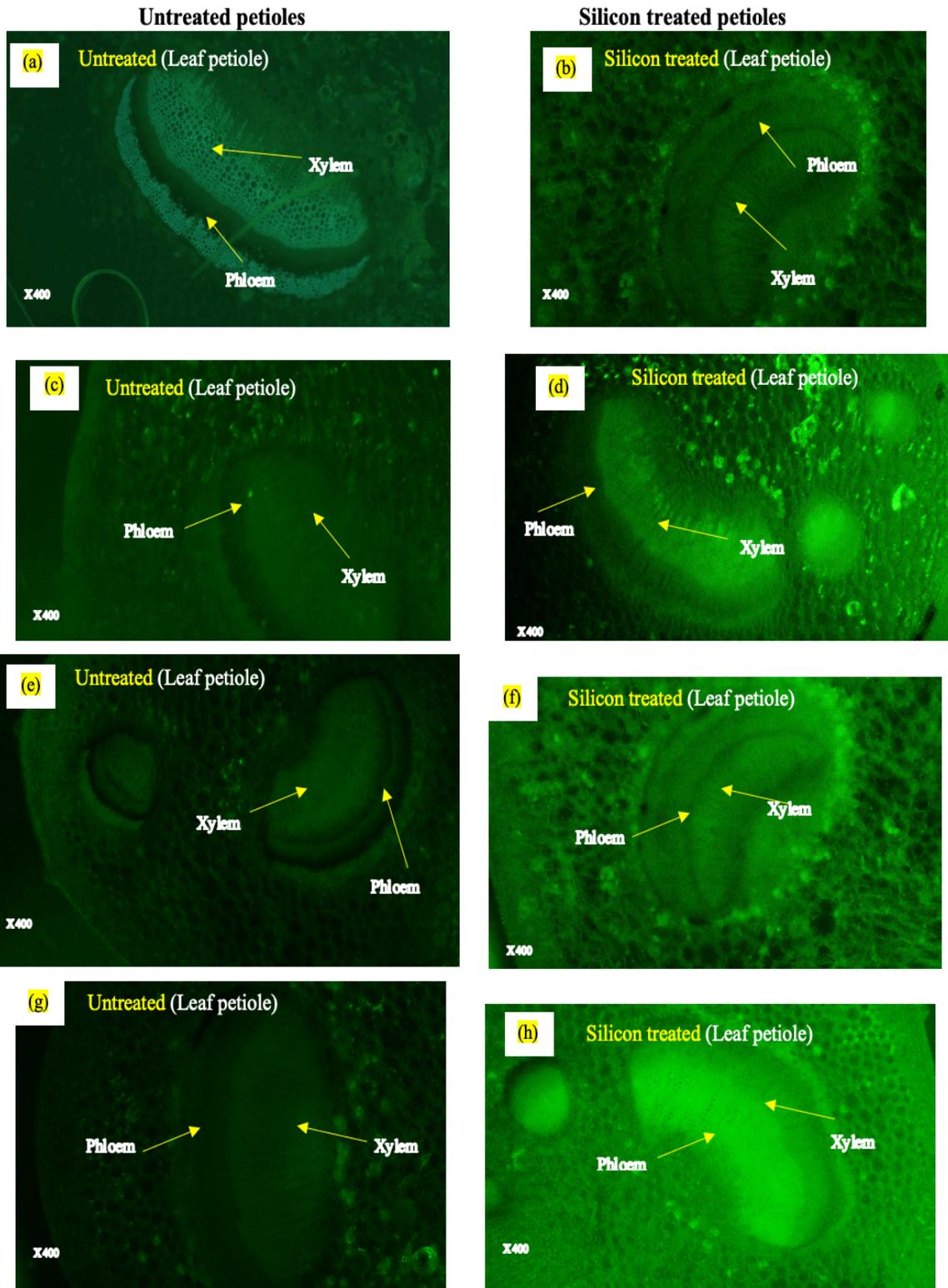
Appendix 29 Cross-sections (Replicates) of strawberry leaf petioles in the glasshouse experiments 2017 (x40 and x400 magnifications) Chapter 4

Untreated petioles   **Silicon-treated petioles**
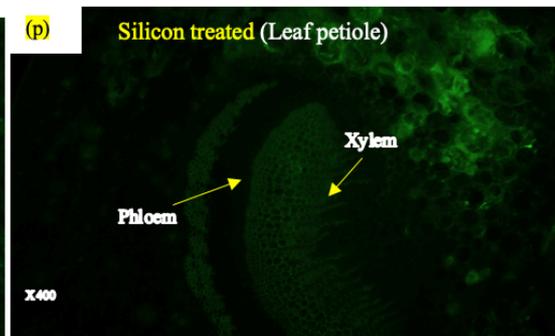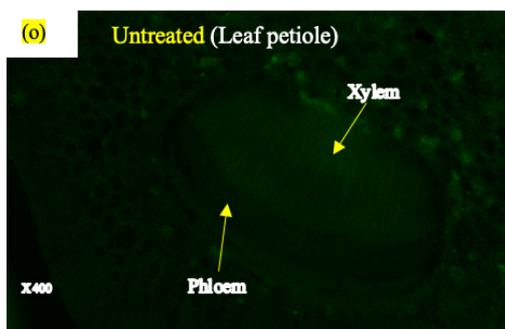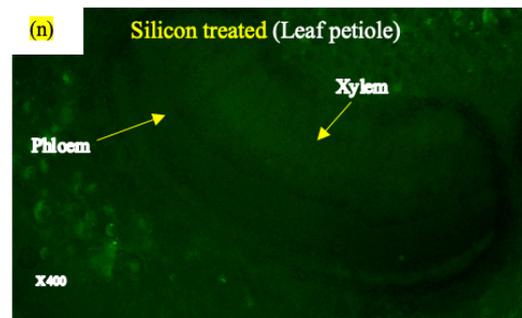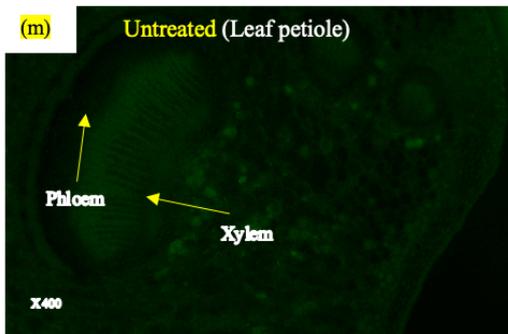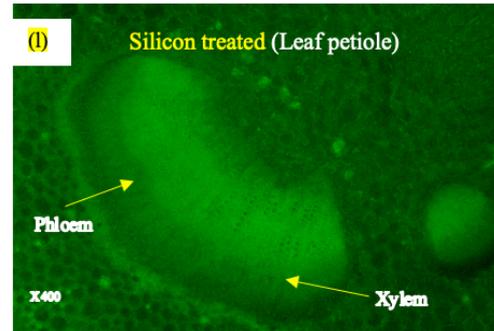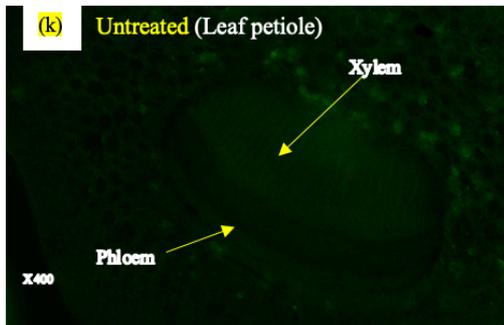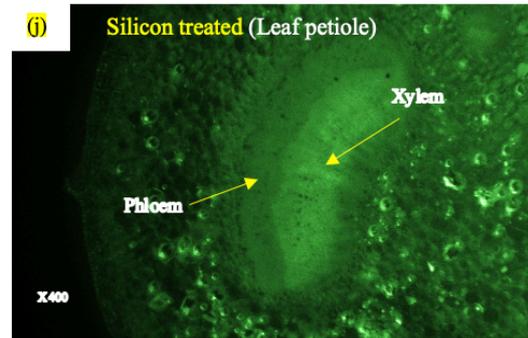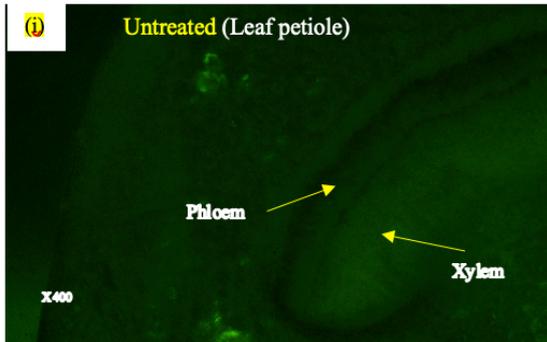
Appendix 30 Cross-sections (Replicates) of strawberry leaf petioles in the glasshouse experiments 2017 (x40 and x400 magnifications) cont'd, Chapter 4

Untreated petioles   **Silicon-treated petioles**
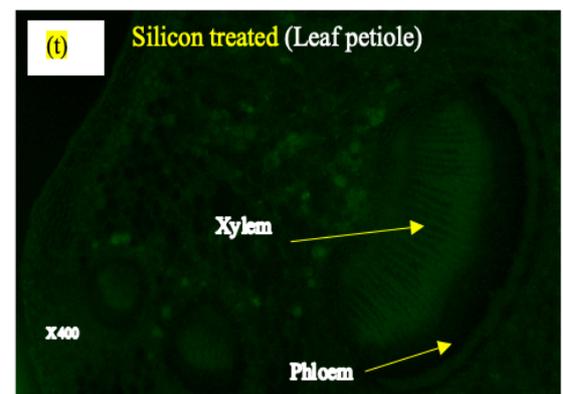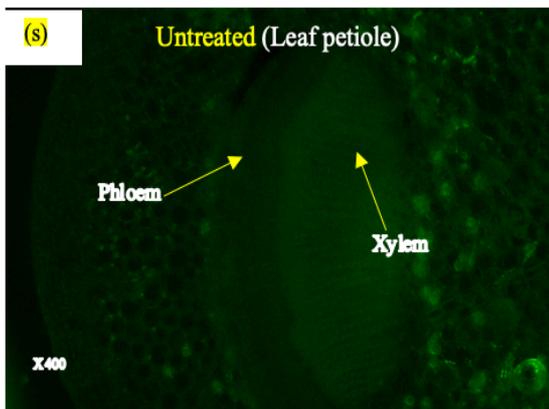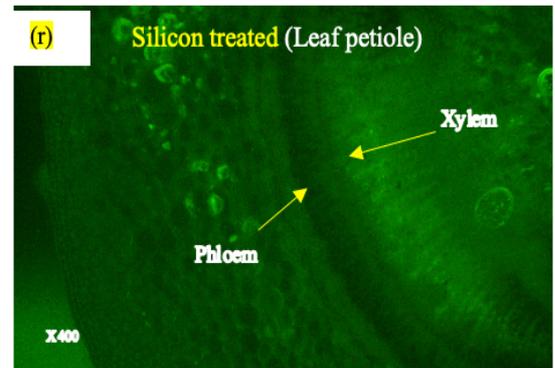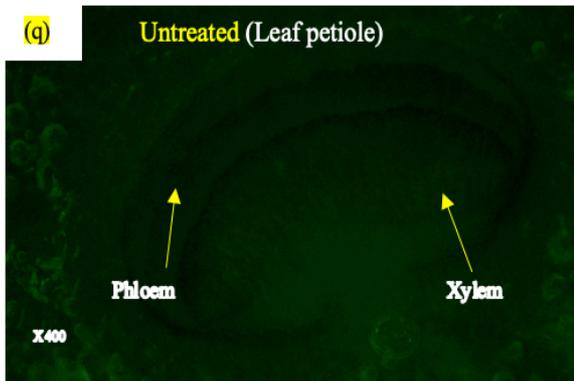
Appendix 31 Cross-sections (Replicases) of strawberry roots in the glasshouse experiments 2017 (x40 and x400 magnifications) Chapter 4

Untreated   **Silicon-treated roots**
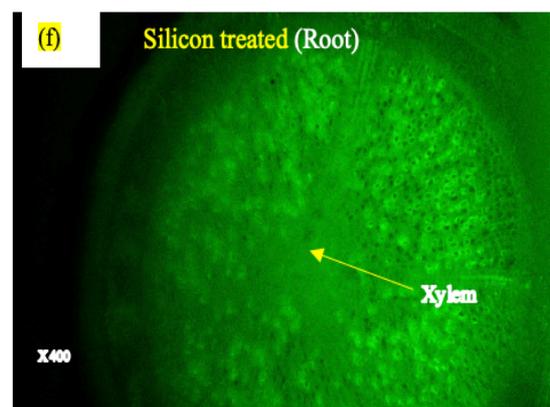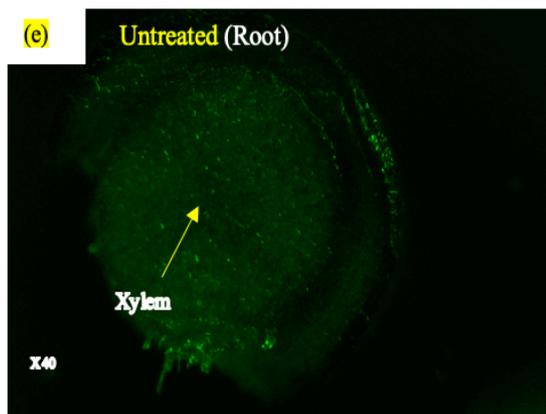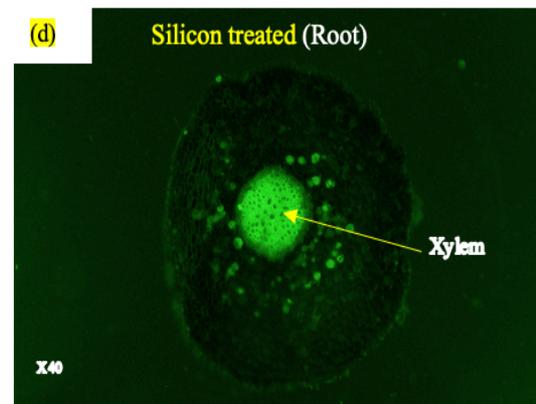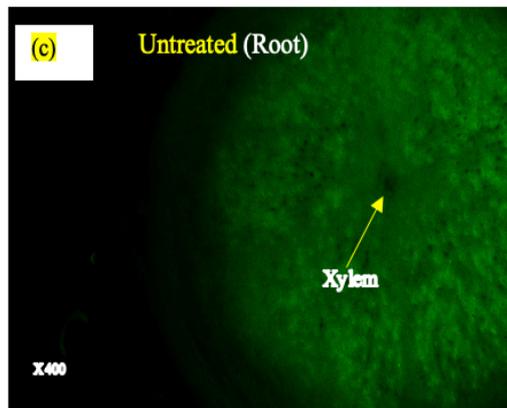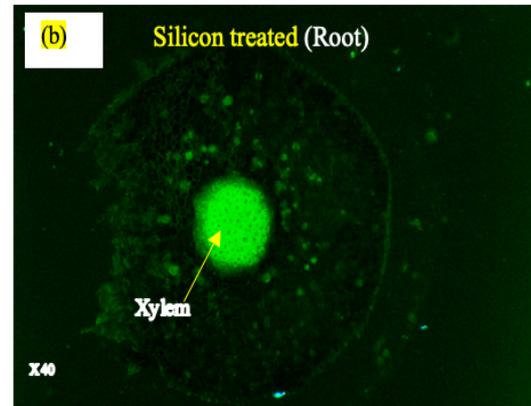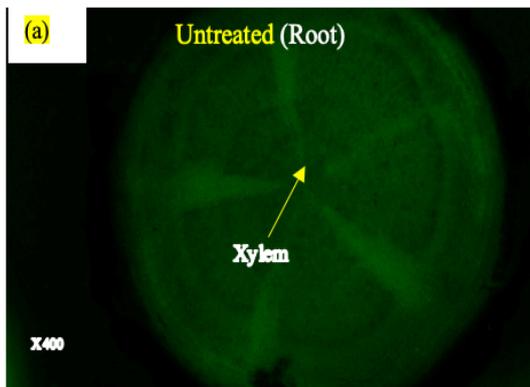
Appendix 32 Cross-sections (Replicases) of strawberry roots in the glasshouse experiments 2017 (x40 and x400 magnifications) cont'd, Chapter 4

Untreated   **Silicon-treated roots**
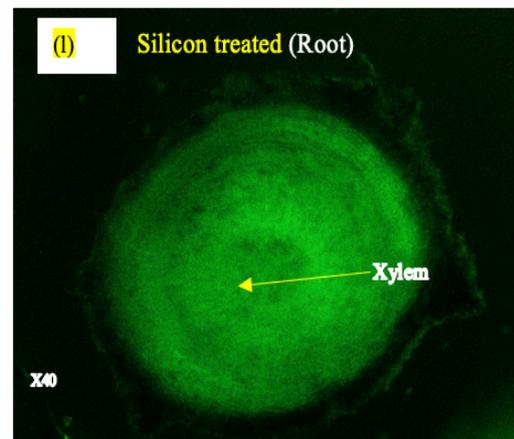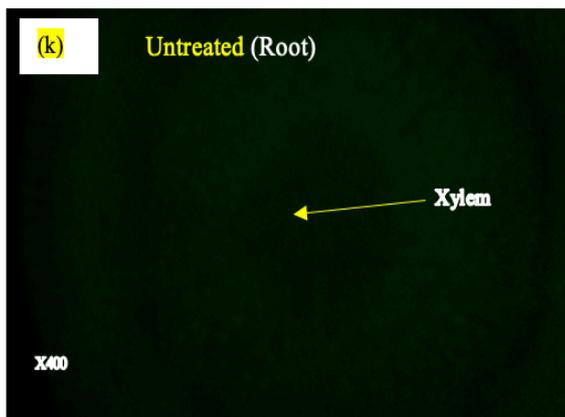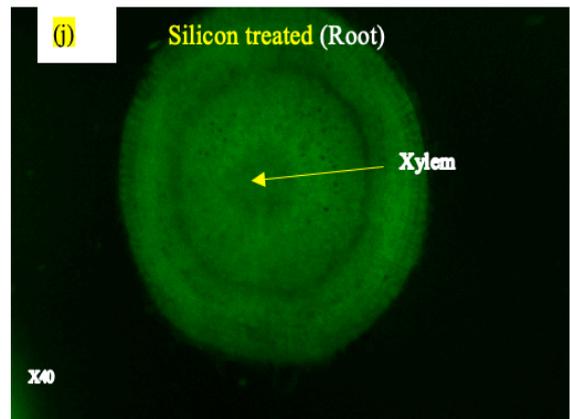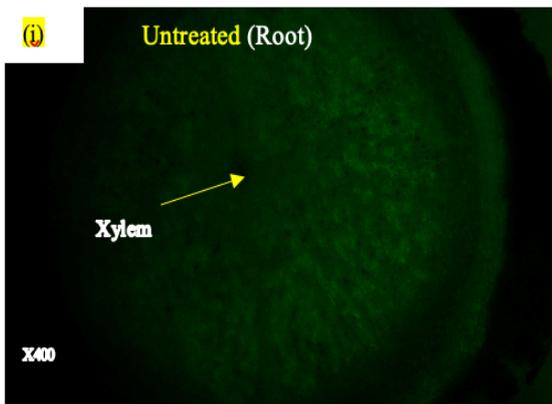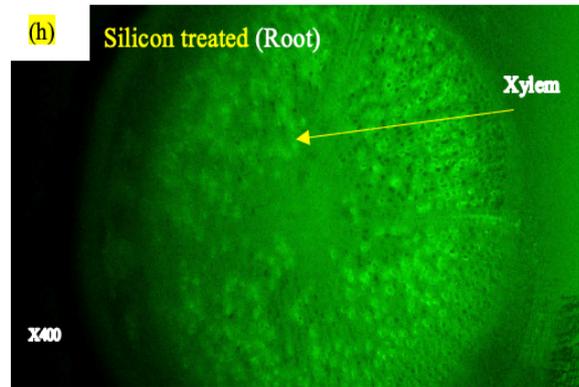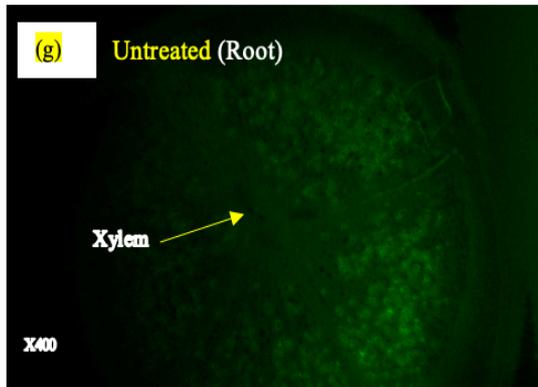
Appendix 33 Cross-sections (Replicases) of strawberry roots in the glasshouse experiments 2017 (x40 and x400 magnifications) cont'd, Chapter 4

Untreated   **Silicon-treated roots**
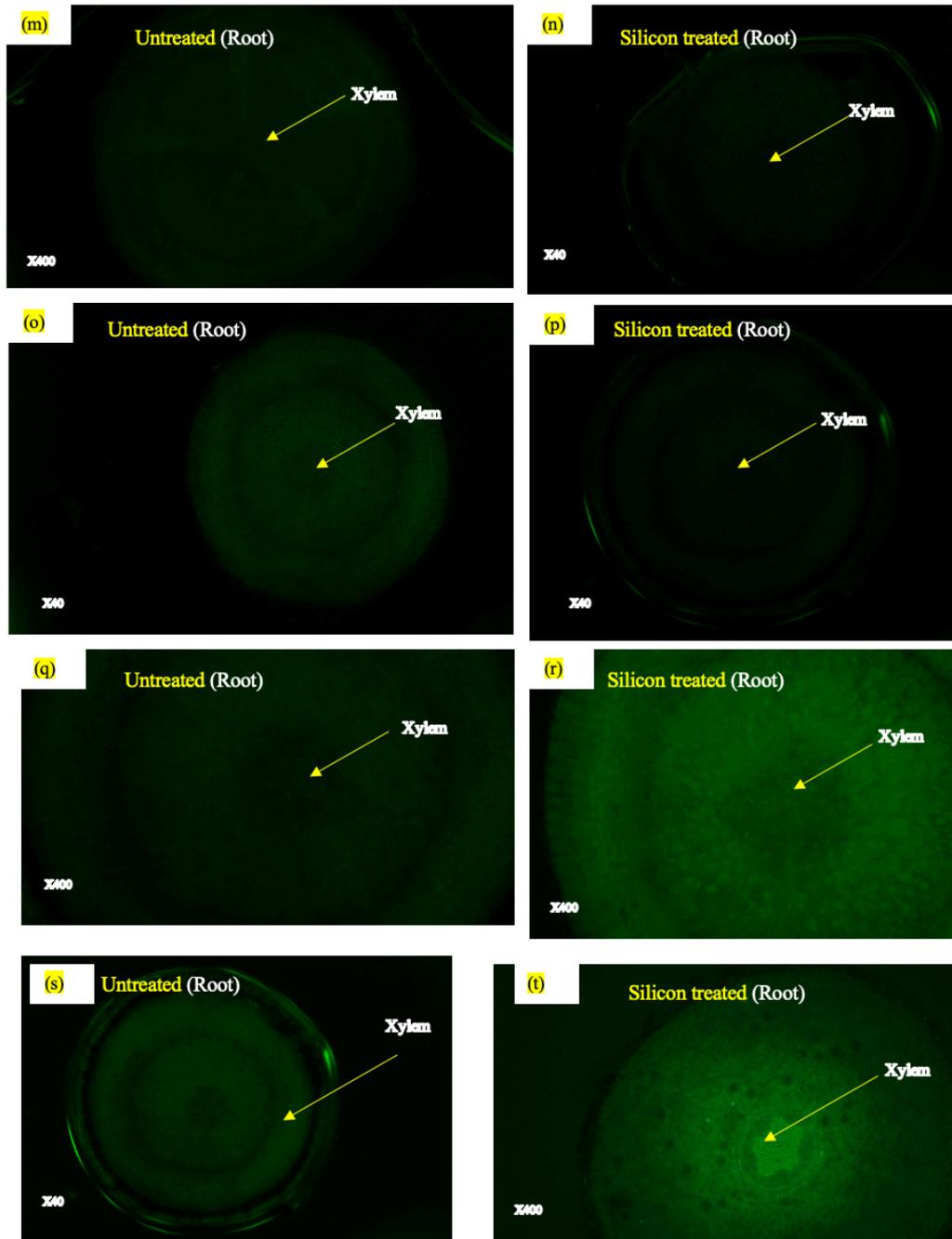
Appendix 34 Cross-sections (Replicates) of strawberry leaves in the silicon fertigation field 2017 (x40 and x400 magnifications) Chapter 4

Untreated leaves

Silicon treated leaves (Silicon twice only)
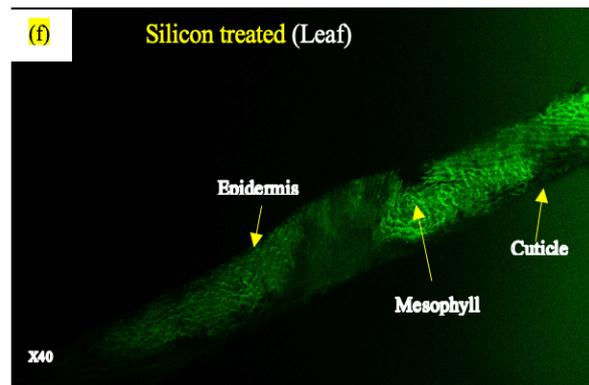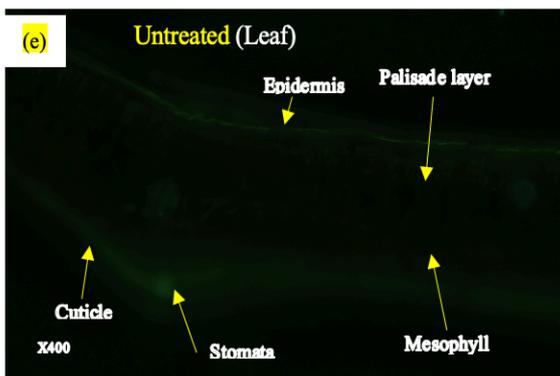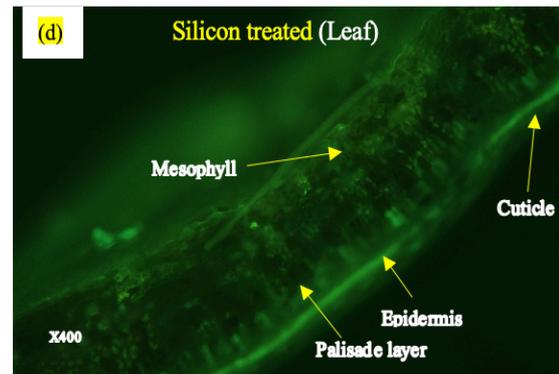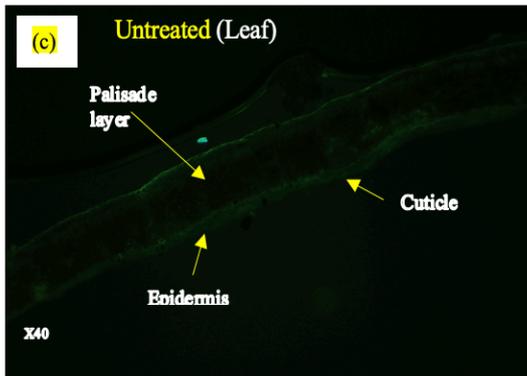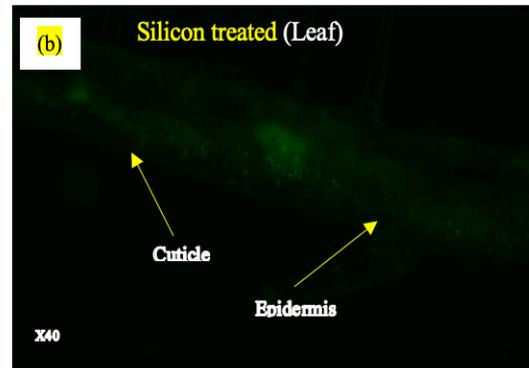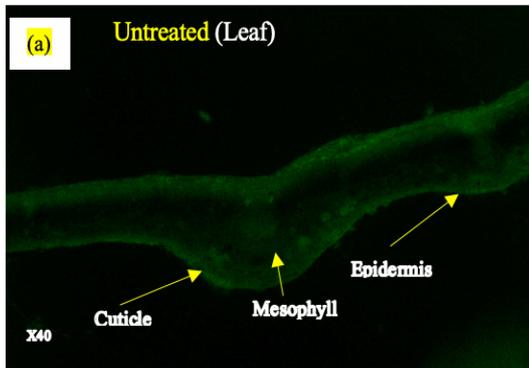
Appendix 35 Cross-sections (Replicates) of strawberry leaves in the silicon fertigation field 2017 (x40 and x400 magnifications) cont'd Chapter 4

Untreated   **Silicon-treated leaves (twice only)**
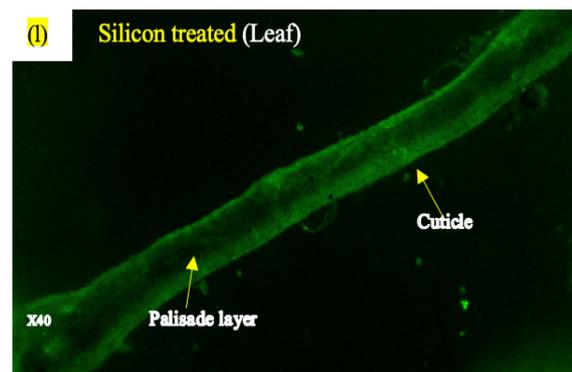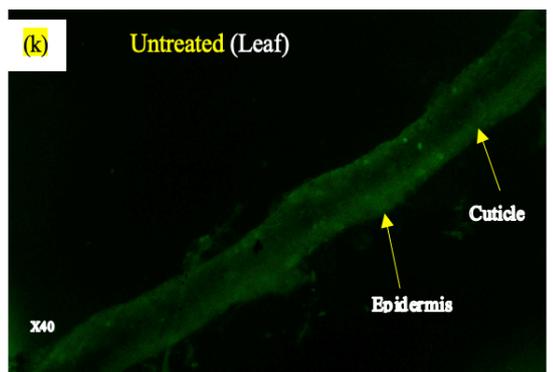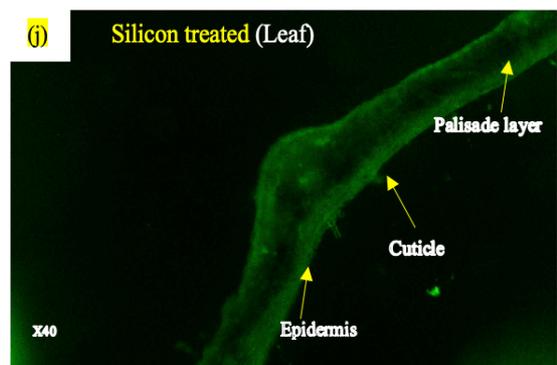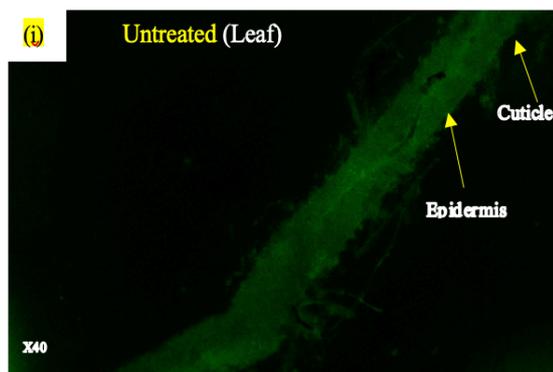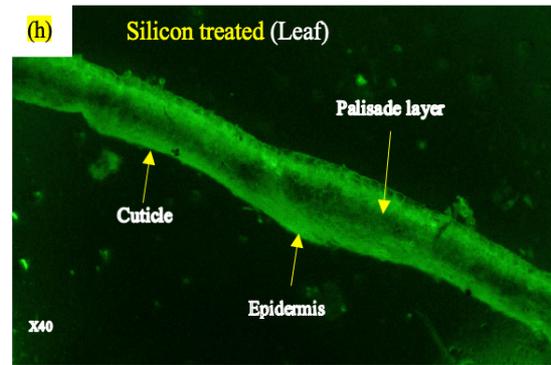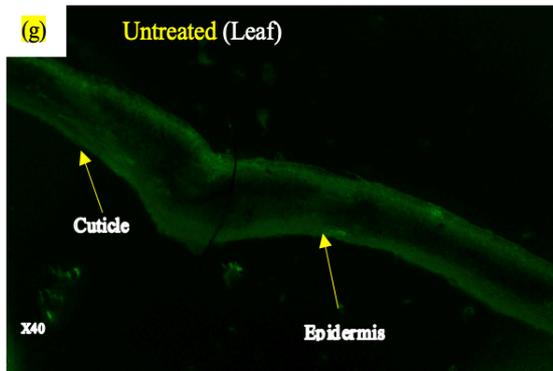
Appendix 36 : Cross-sections (Replicates) of strawberry leaves in the silicon fertigation field 2017 (x40 and x400 magnifications)

Untreated   **Silicon-treated leaves (silicon twice only)**
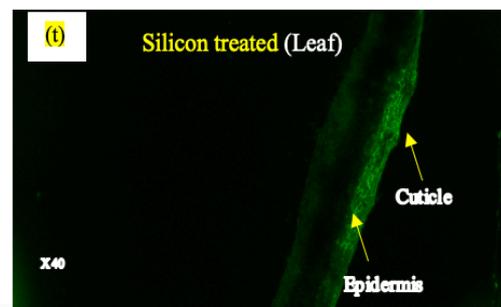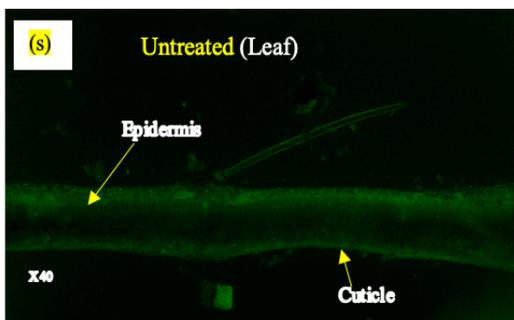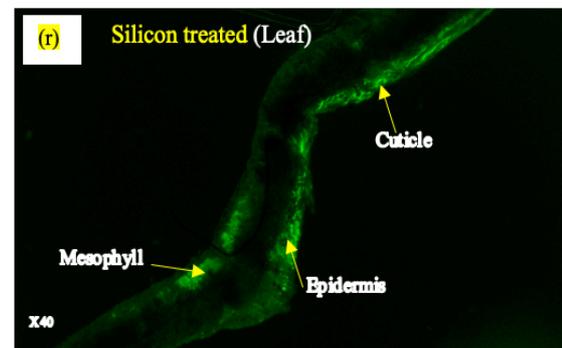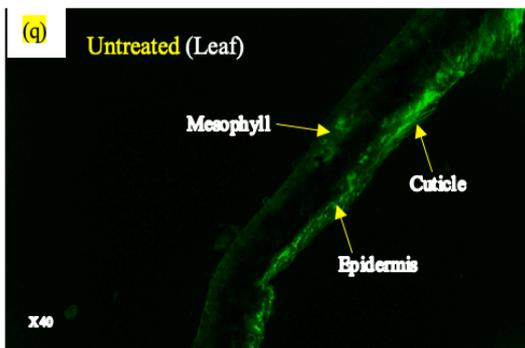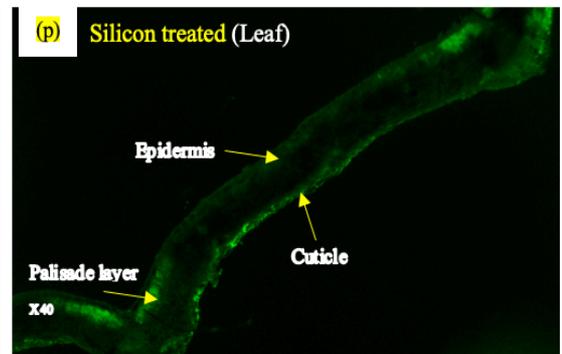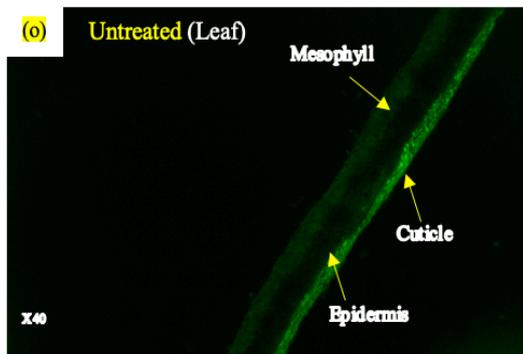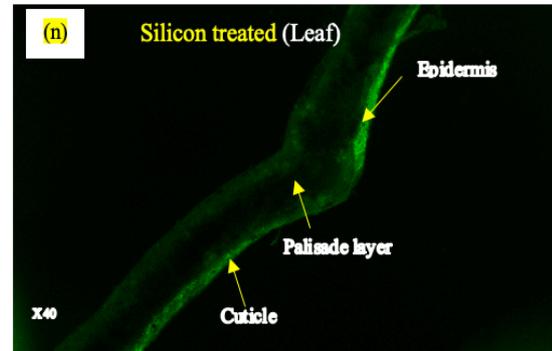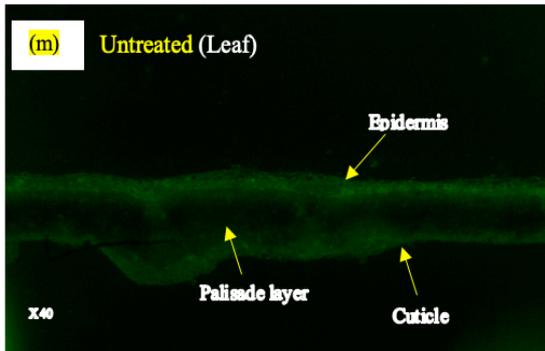
Appendix 37 Cross-sections (Replicates) of strawberry leaf petioles in the silicon field experiments 2017 (x40 and x400 magnifications) Chapter 4

Untreated   **Silicon-treated petioles (Silicon twice only)**
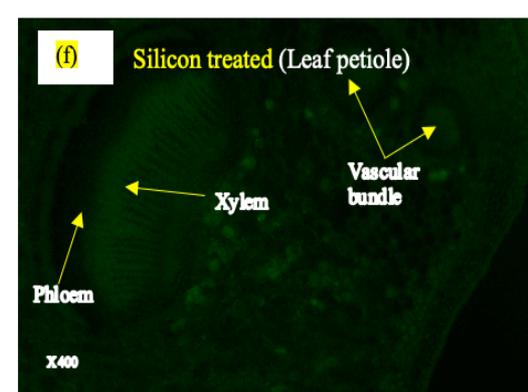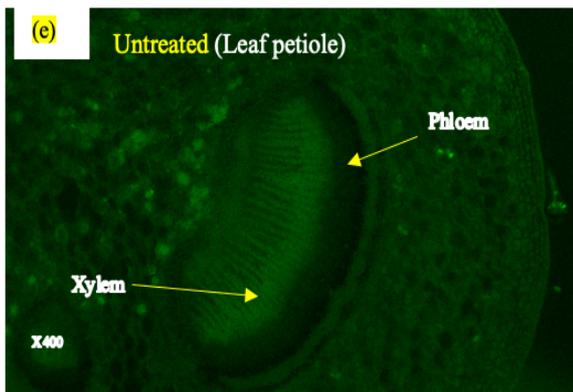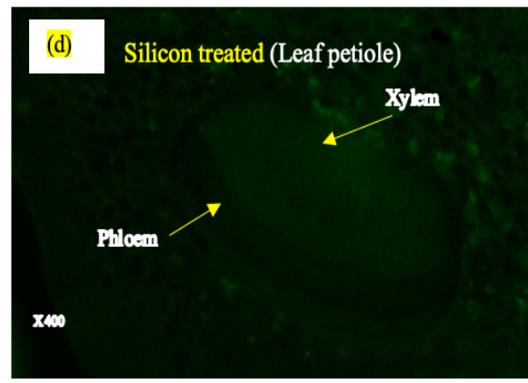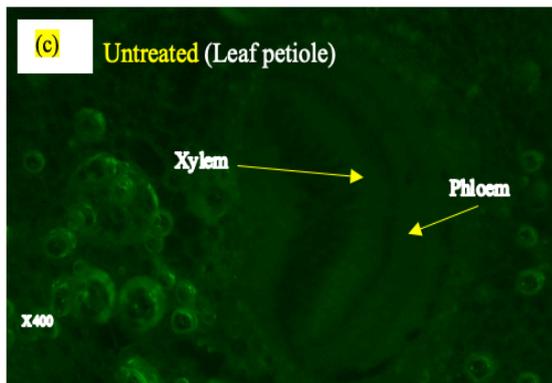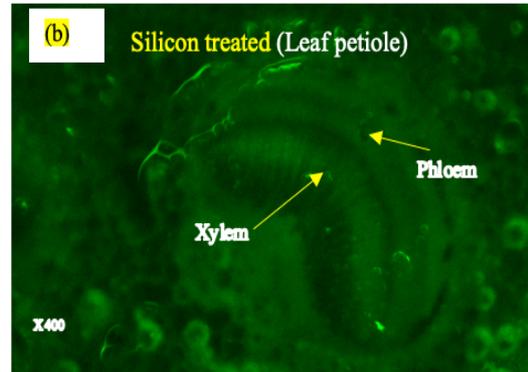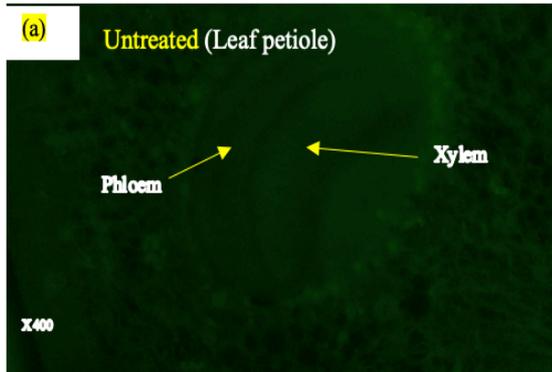
Appendix 38 Cross-sections (Replicates) of strawberry leaf petioles in the field experiment 2017 (x40 and x400 magnifications) cont'd, Chapter 4

Untreated   **Silicon-treated petioles (Silicon twice only)**
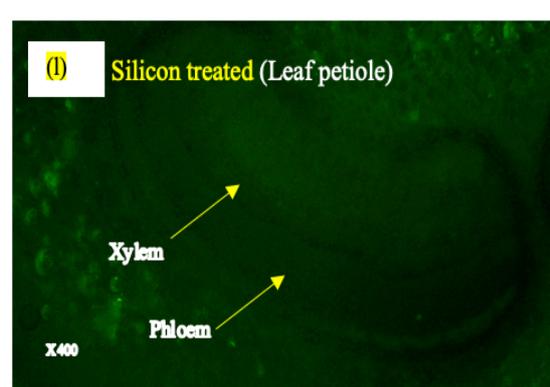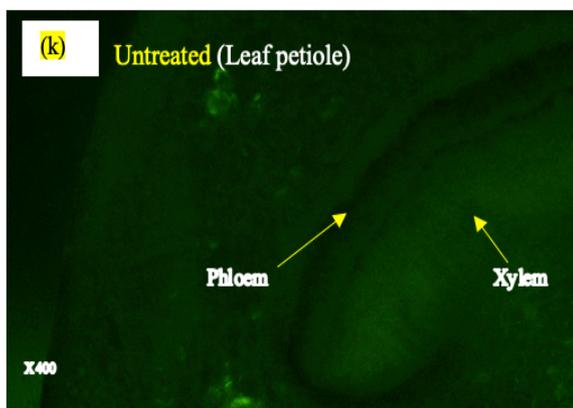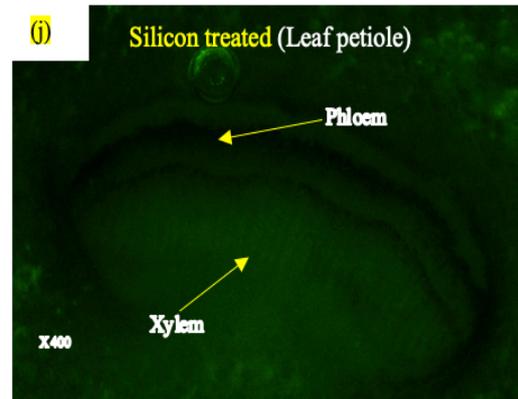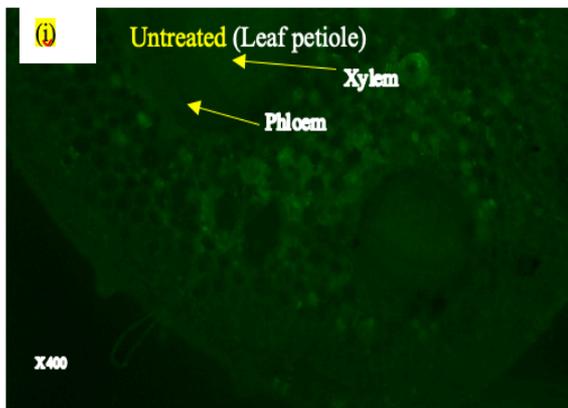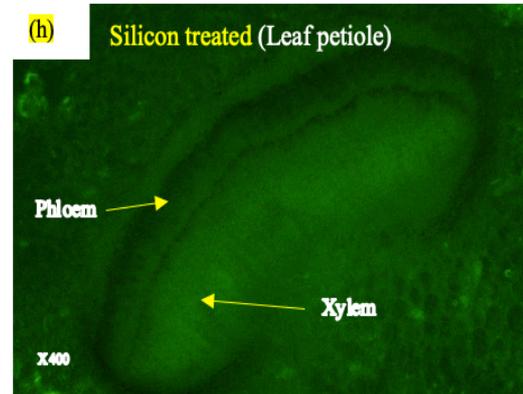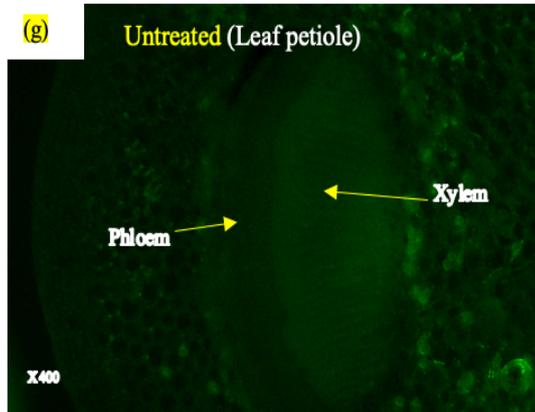
Appendix 39 Cross-sections (Replicates) of strawberry leaf petioles in the silicon field experiment 2017 (x40 and x400 magnifications) cont'd, Chapter 4

Untreated   **Silicon-treated petioles (Silicon twice only)**
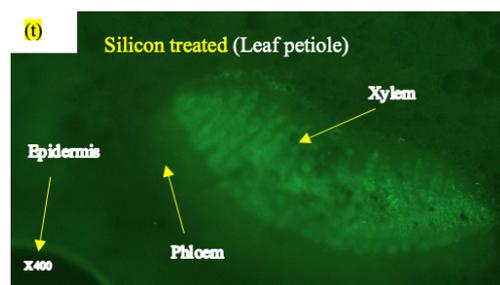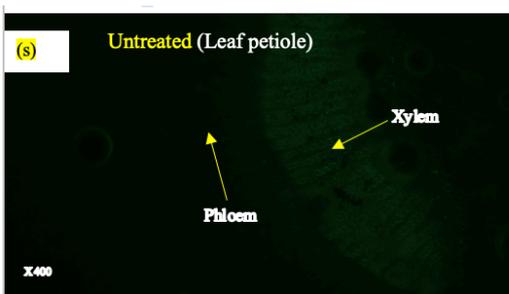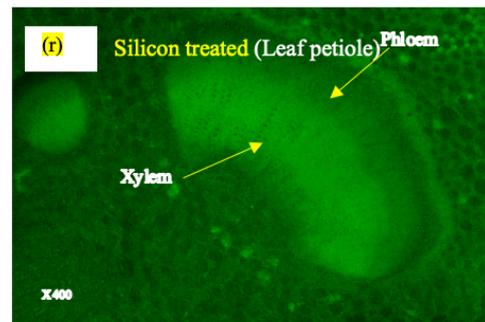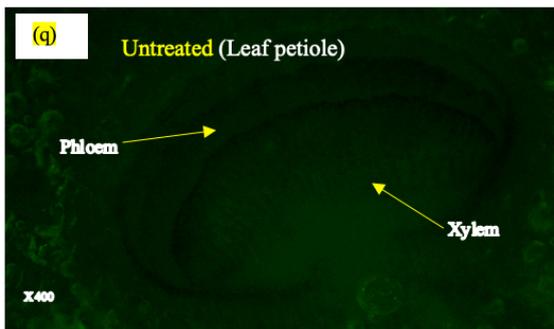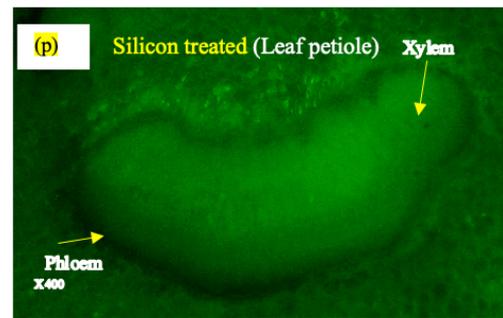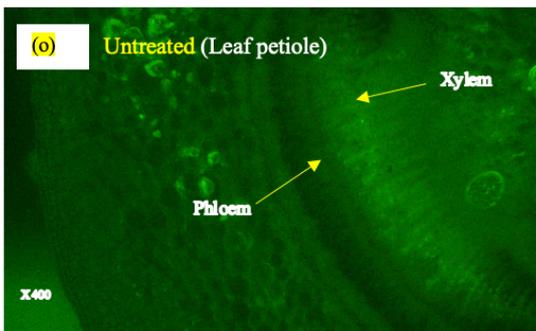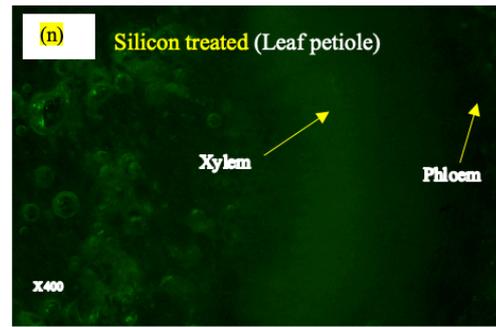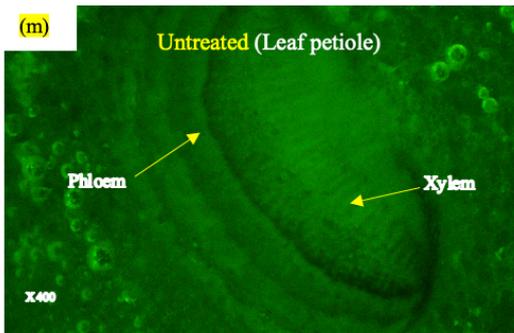
Appendix 40 Cross-sections (Replicates) of strawberry roots in the field experiment 2017 (x40 and x400 magnifications) Chapter 4

Untreated   **Silicon-treated roots (Silicon twice only)**
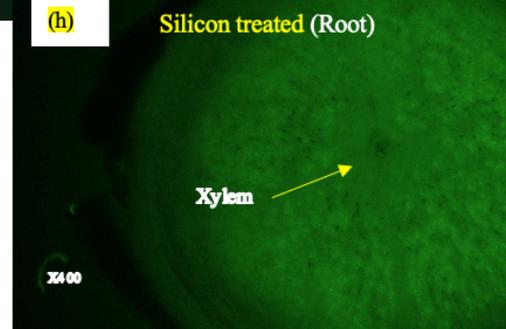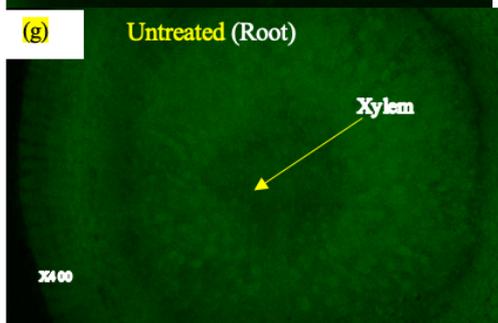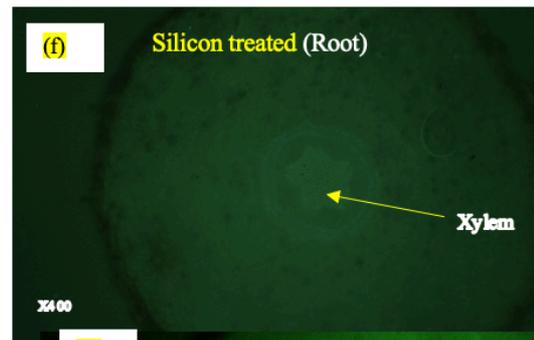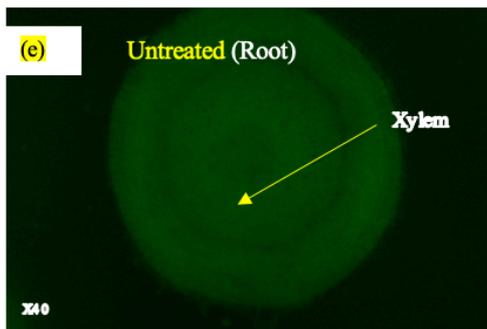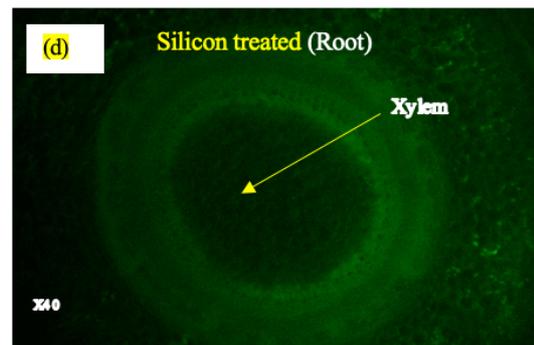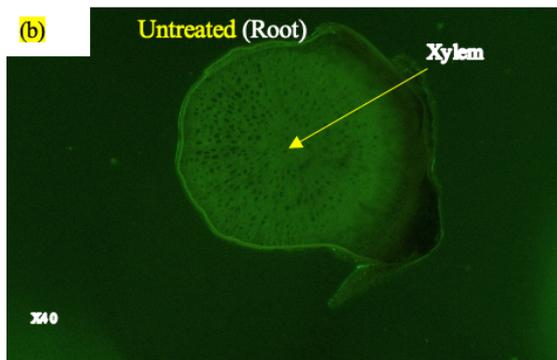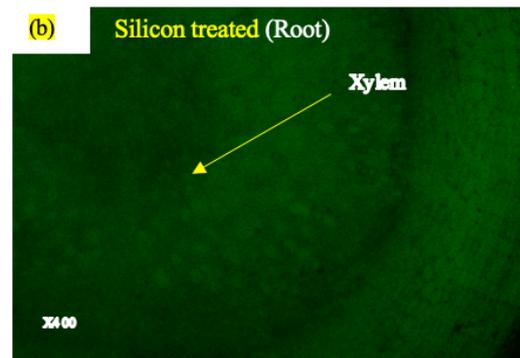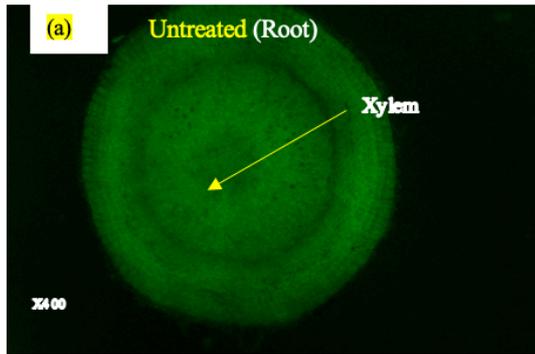
Appendix 41 Cross-sections (Replicates) of strawberry roots in the field experiment 2017 (x40 and x400 magnifications) cont'd, Chapter 4

Untreated   **Silicon-treated roots (Silicon twice only)**
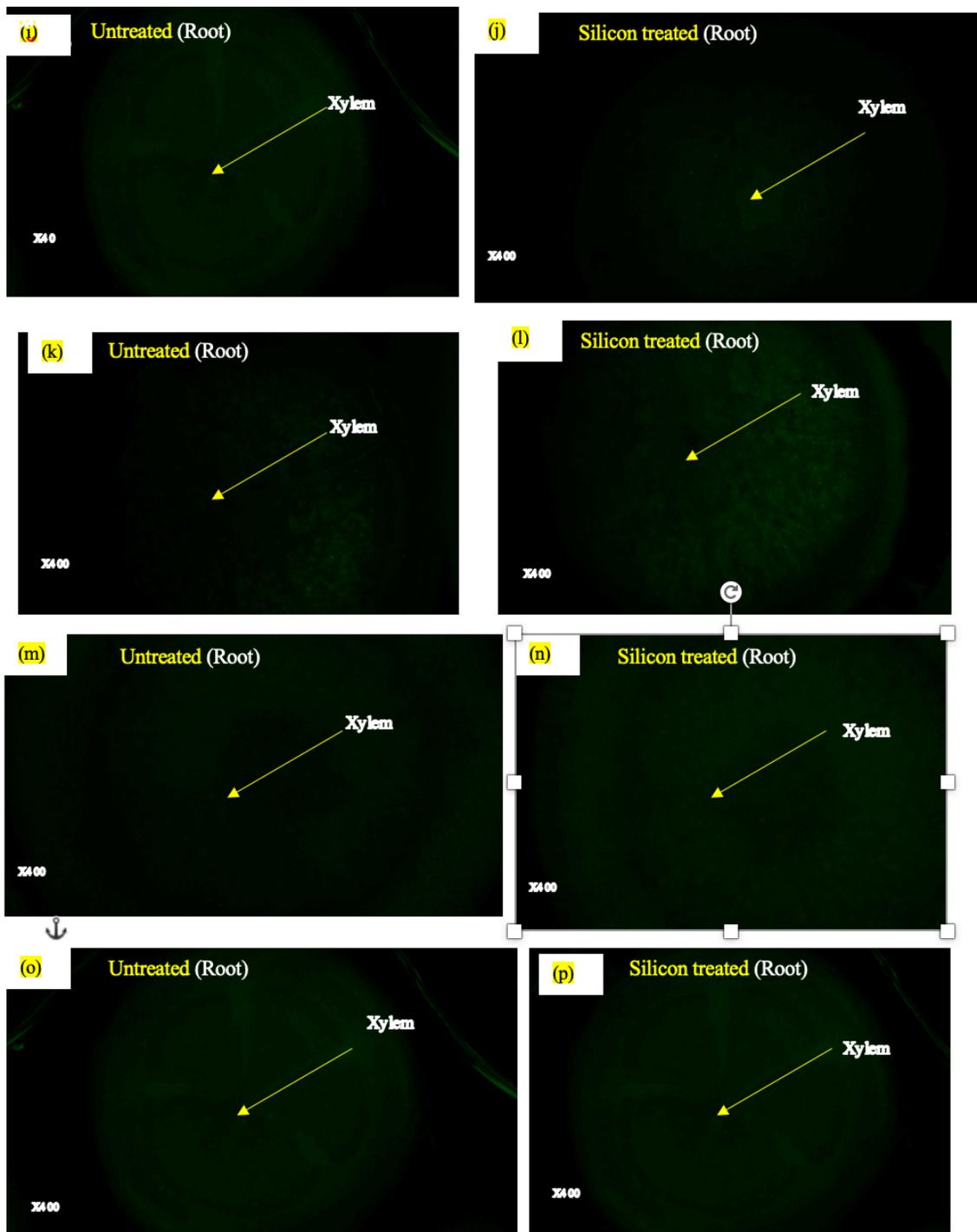
Appendix 42 Cross-sections (Replicates) of strawberry roots in the field experiment 2017 (x40 and x400 magnifications) cont'd, Chapter 4

Untreated   **Silicon-treated roots (Silicon twice only)**
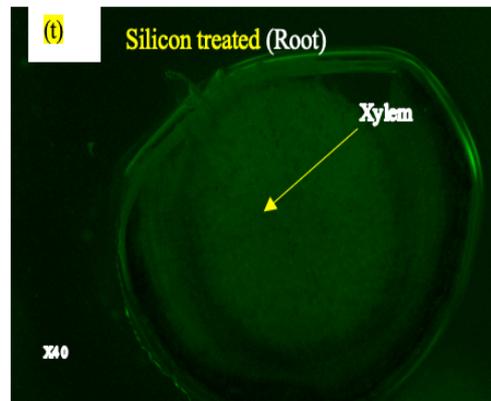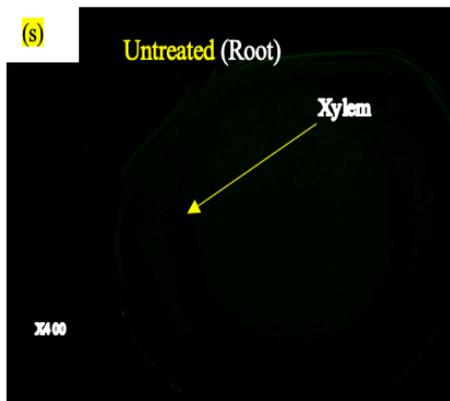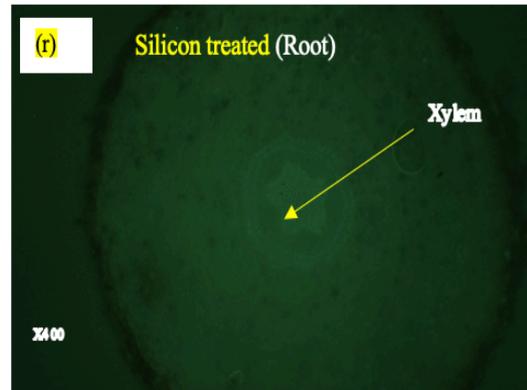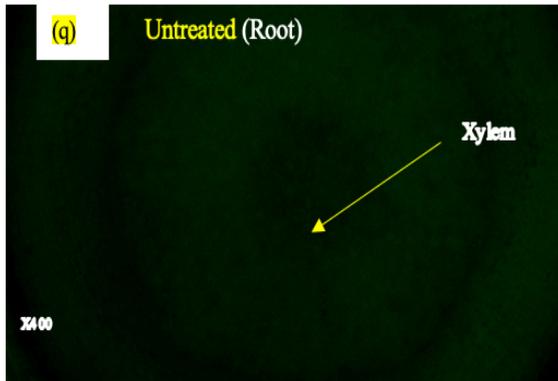
Appendix 43 Cross-sections (Replicates) of strawberry leaves in the silicon fertigation field 2018 (x40 and x400 magnifications) Chapter 4

Untreated leaves   **Silicon treated leaves (Silicon twice only)**
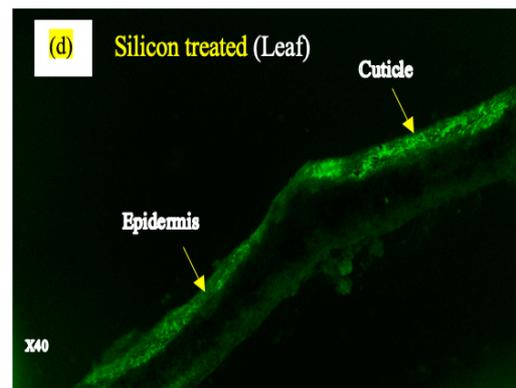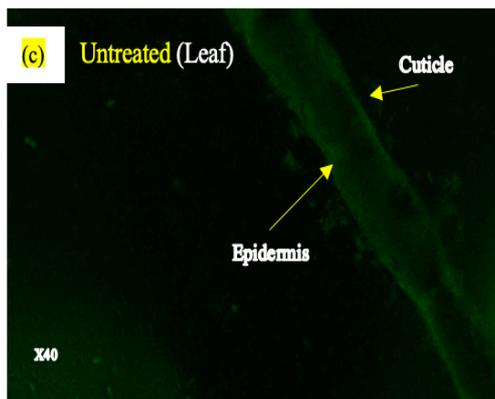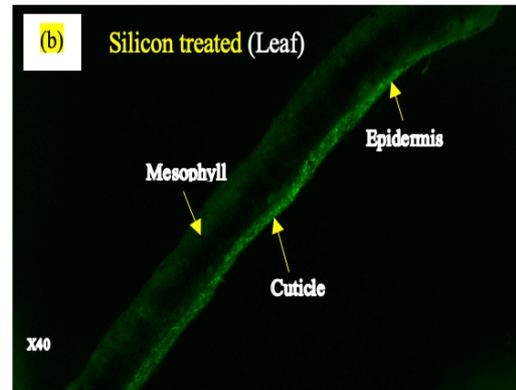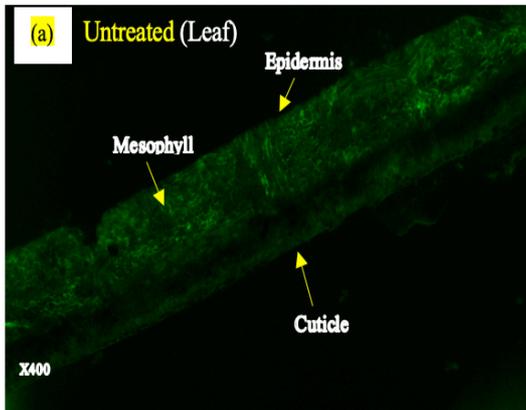
Appendix 44 : Cross-sections (Replicates) of strawberry leaves in the silicon fertigation field 2018 (x40 and x400 magnifications) cont'd, Chapter 4

Untreated leaves   **Silicon-treated leaves (Silicon twice only)**
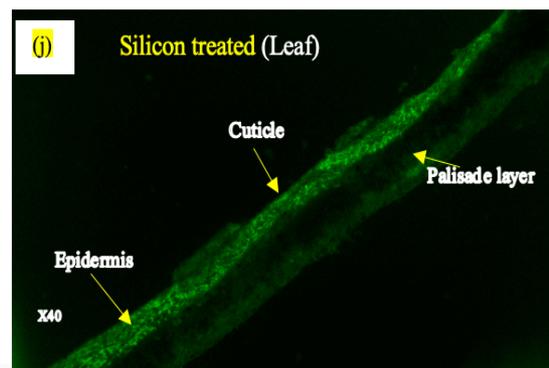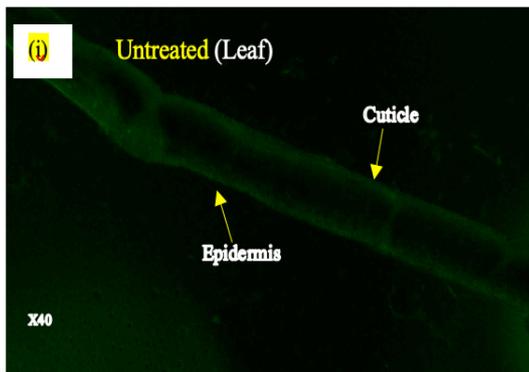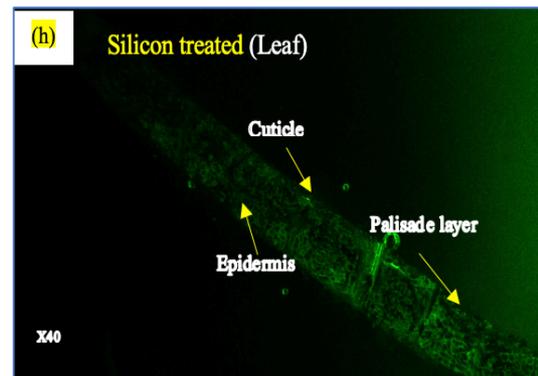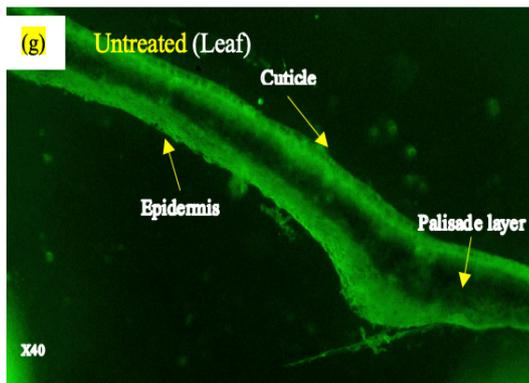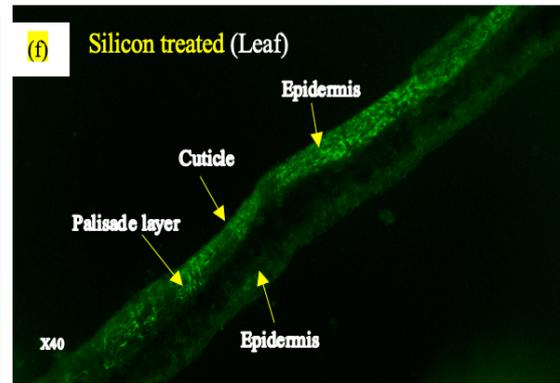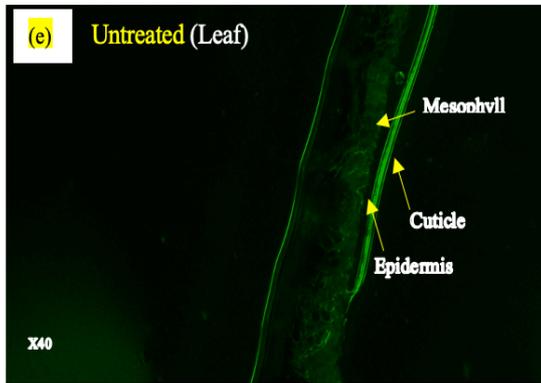
Appendix 45 Cross-sections (Replicates) of strawberry leaves in the silicon fertigation field 2018 (x40 and x400 magnifications) cont'd, Chapter 4

Untreated leaves   **Silicon-treated leaves (Silicon twice only)**
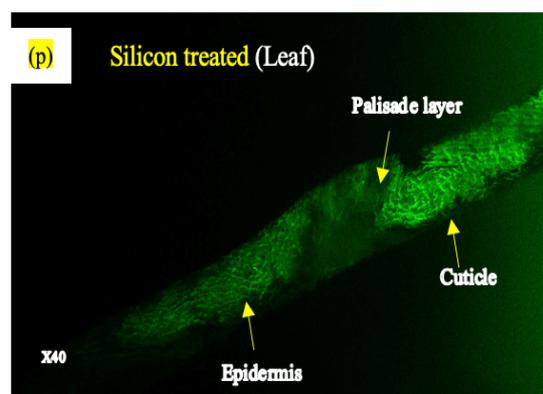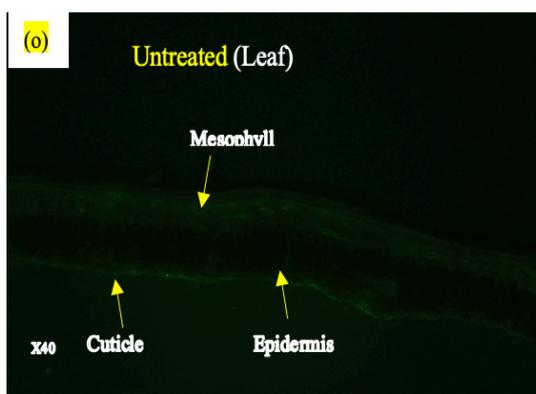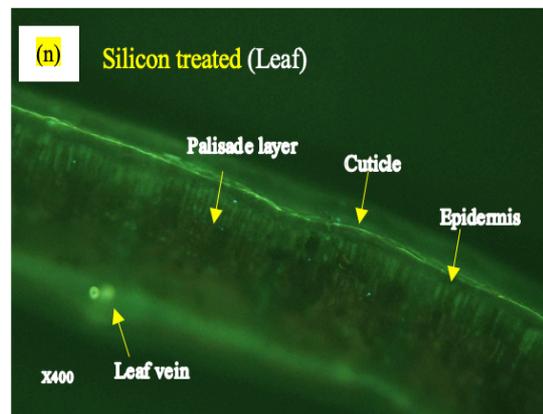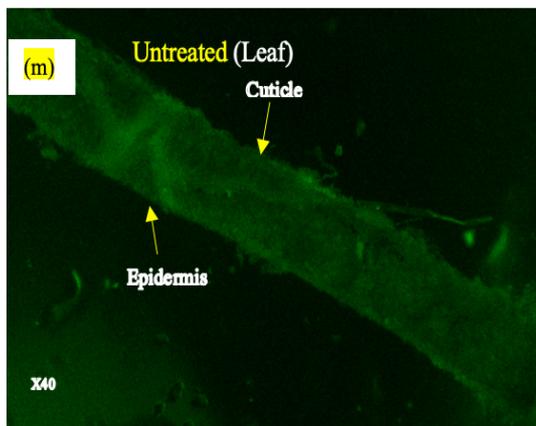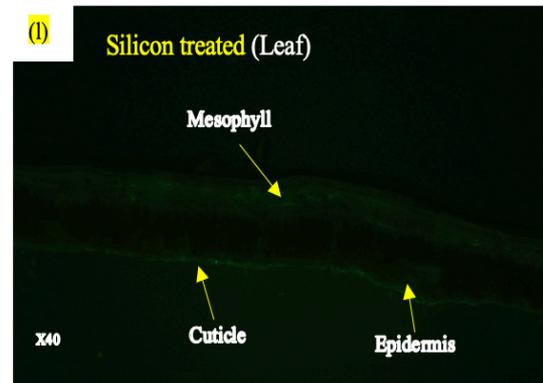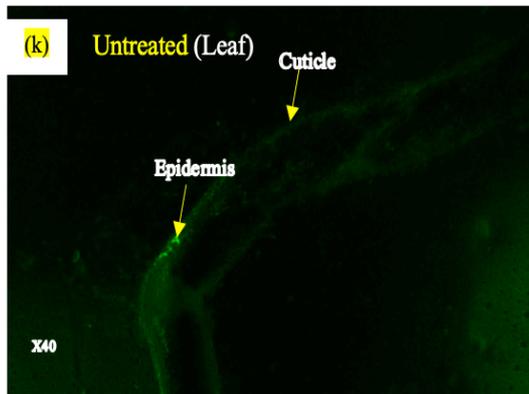
Appendix 46 Cross-sections (Replicates) of strawberry leaves in the silicon fertigation field 2018 (x40 and x400 magnifications) cont'd

Untreated leaves **Silicon-treated leaves (Silicon twice only)**
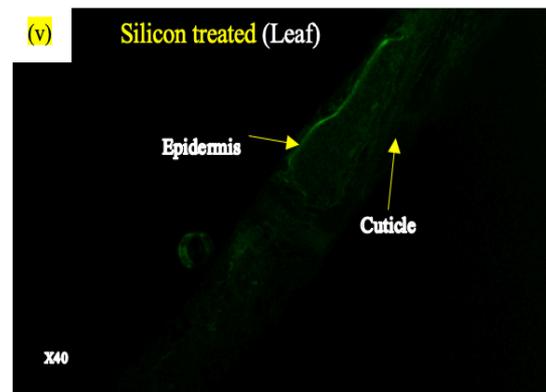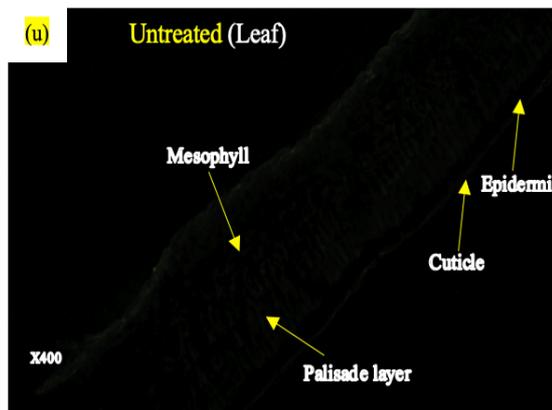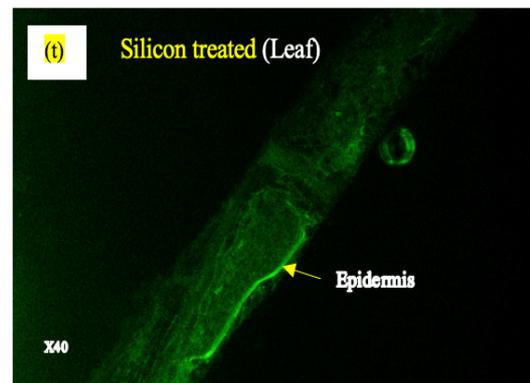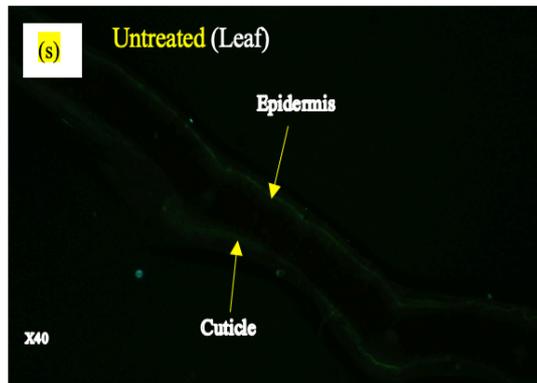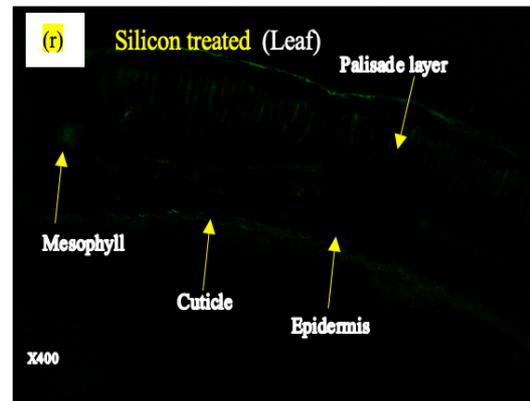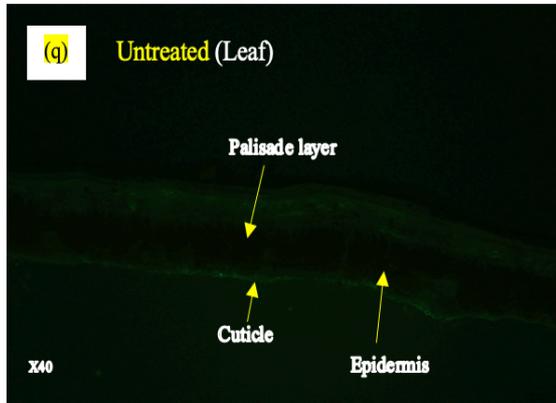
Appendix 47 Cross-sections (Replicates) of strawberry leaf petioles in the silicon fertigation field 2018 (x40 and x400 magnifications) Chapter 4

Untreated petioles   **Silicon-treated petioles (Silicon twice only)**
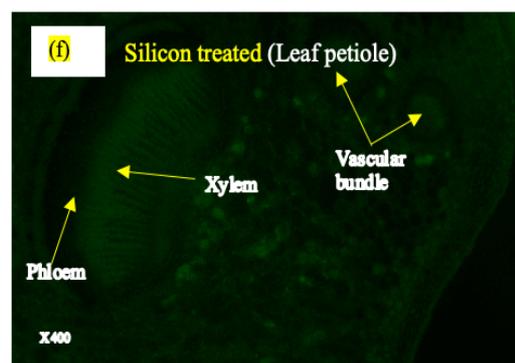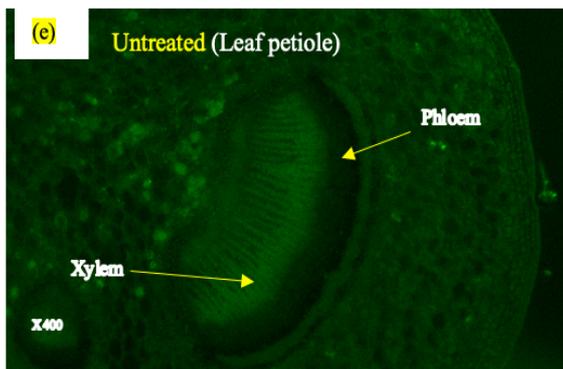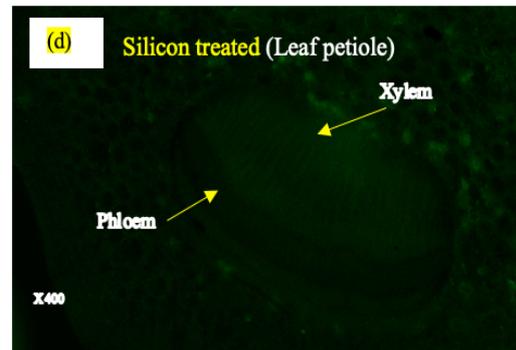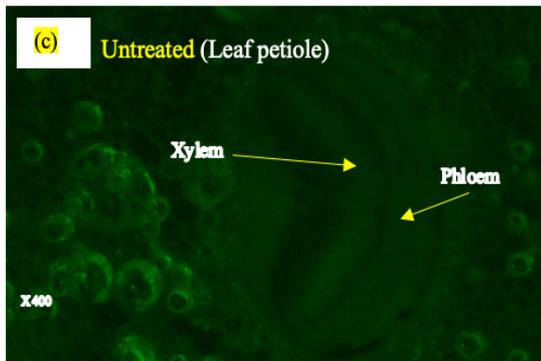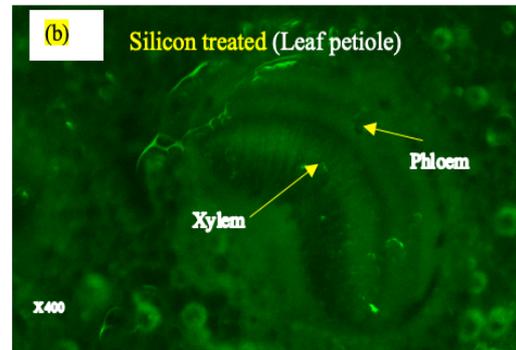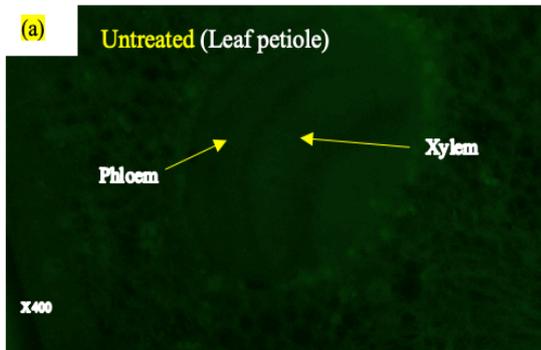
Appendix 48 Cross-sections (Replicates) of strawberry leaf petioles in the silicon fertigation field 2018 (x40 and x400 magnifications) cont'd Chapter 4

Untreated petioles   **Silicon-treated petioles (Silicon twice only)**
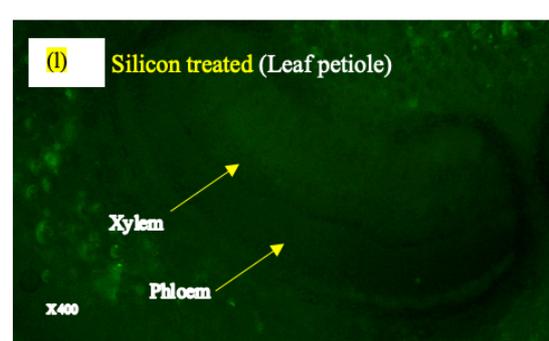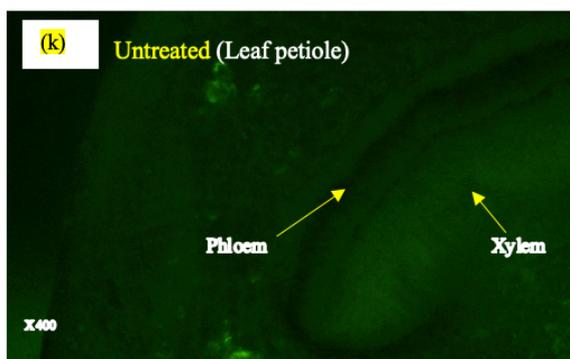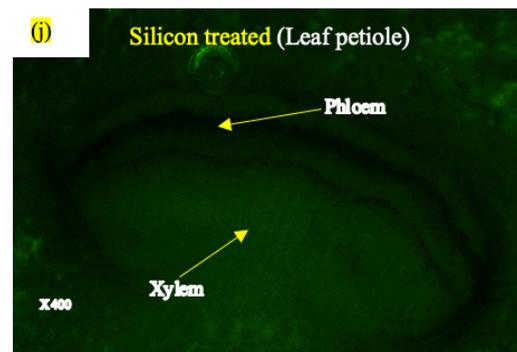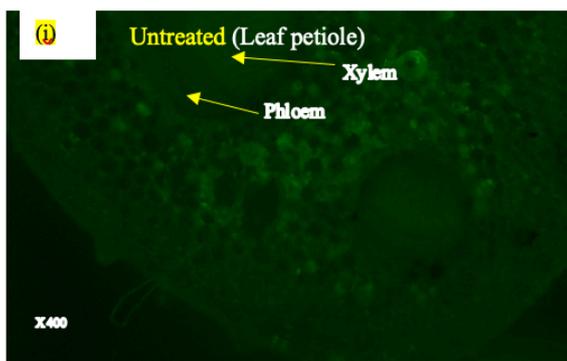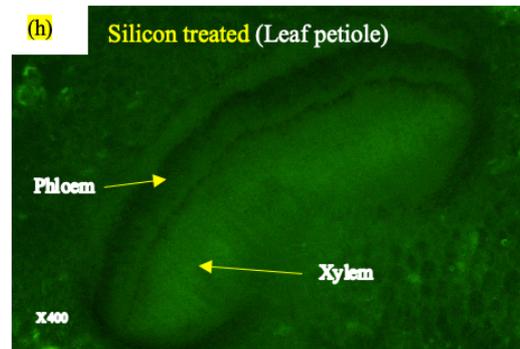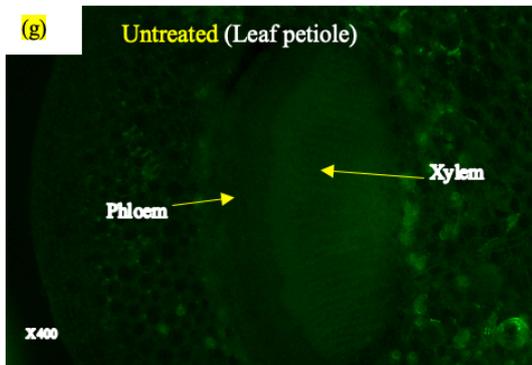
Appendix 49 Cross-sections (Replicates) of strawberry leaf petioles in the silicon fertigation field 2018 (x40 and x400 magnifications) cont'd Chapter 4

Untreated petioles   **Silicon-treated petioles (Silicon twice only)**
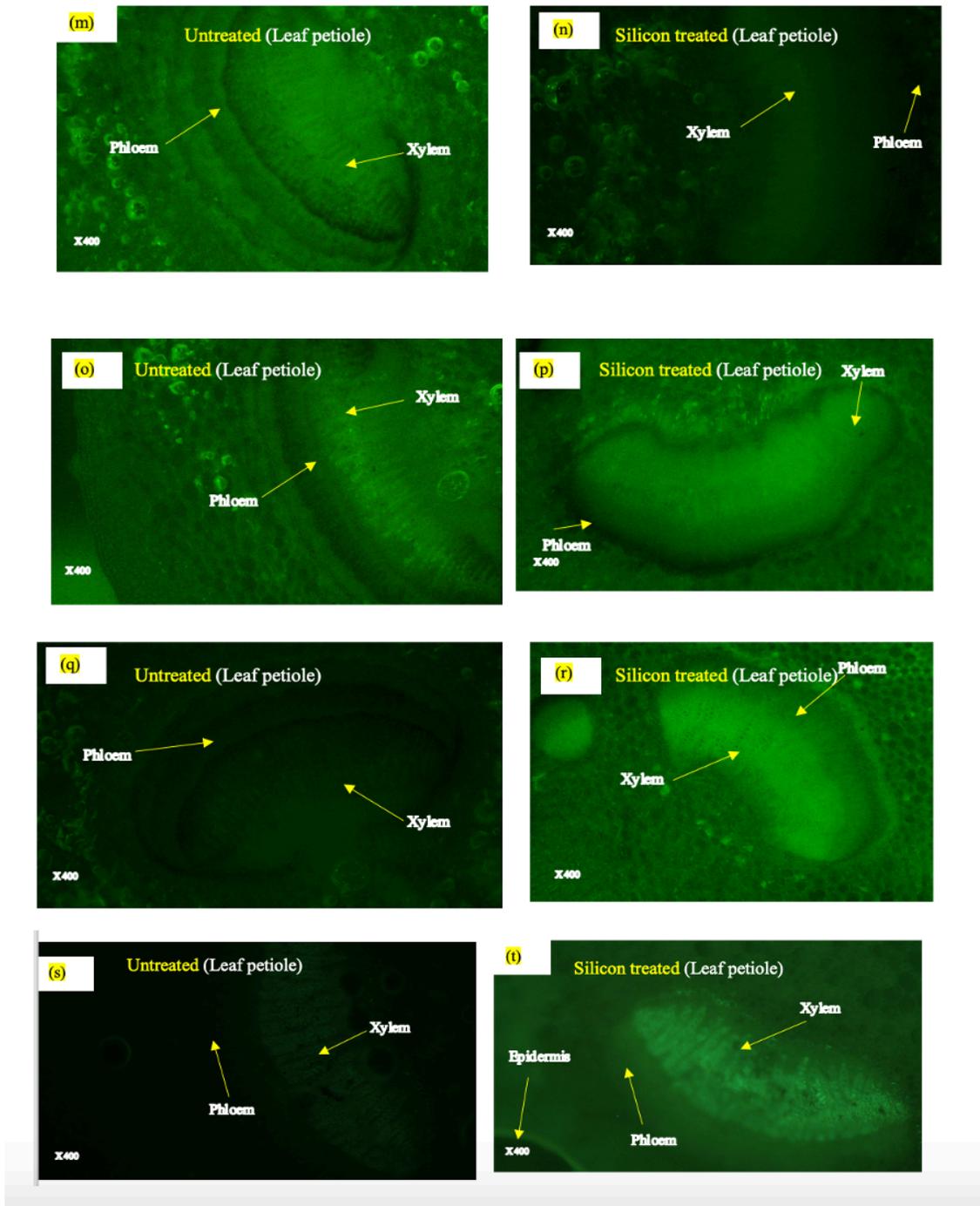
Appendix 50 Cross-sections (Replicates) of roots in the silicon fertigation field 2018 (x40 and x400 magnifications) Chapter 4

Untreated roots   Silicon-treated roots (Silicon twice only)
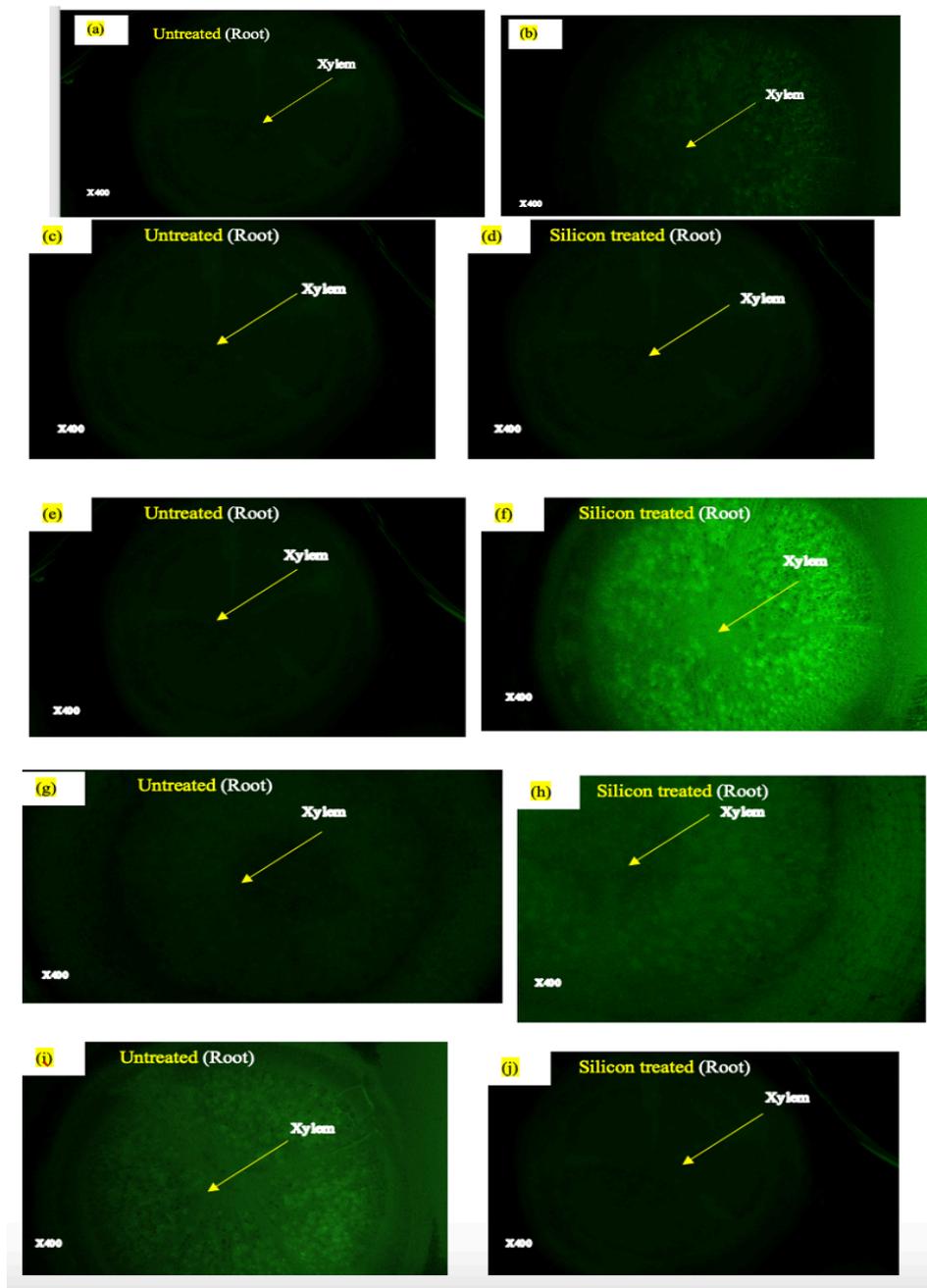
Appendix 51 Cross-sections (Replicates) of roots in the silicon fertigation field 2018 (x40 and x400 magnifications) cont'd Chapter 4

Untreated roots   **Silicon-treated roots (Silicon twice only**
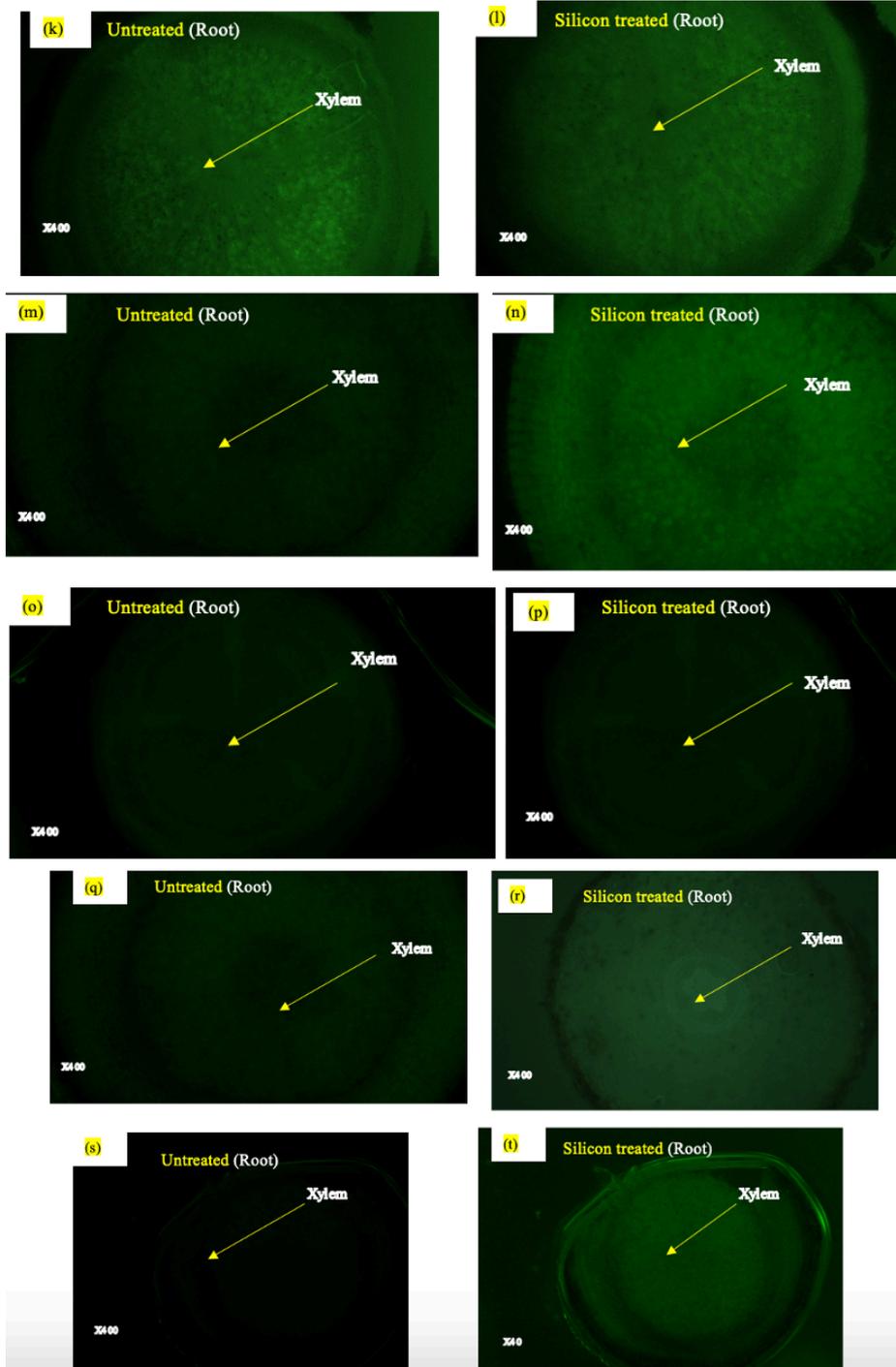
Appendix 52 Fluorescence images of untreated and silicon-treated flower stalks and strawberry fruit, from 2018 silicon field, Chapter 4

Silicon deposition in the silicon fertigation field experiment 2018. Silicon deposits in green fluorescence. Viewed at x40 and x400 magnification.

Silicon deposition in the silicon fertigation field experiment 2018. Silicon deposits in green fluorescence. viewed at x 400 magnification only.
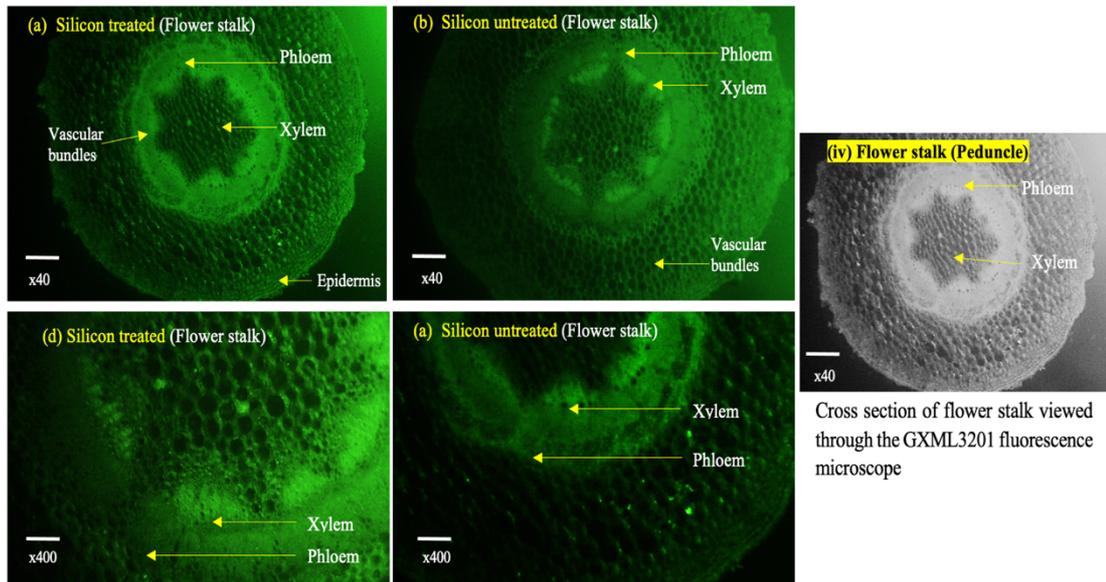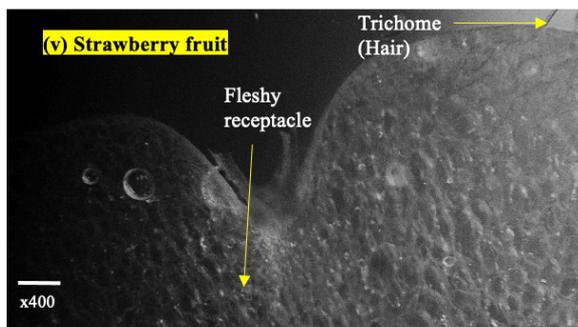
Cross section of strawberry fruit viewed through the GXML3201 fluorescence microscope

217

Appendix 53 Fluorescence images of untreated and silicon-treated only Achenes of strawberry plants, from 2018 silicon field, Chapter 4
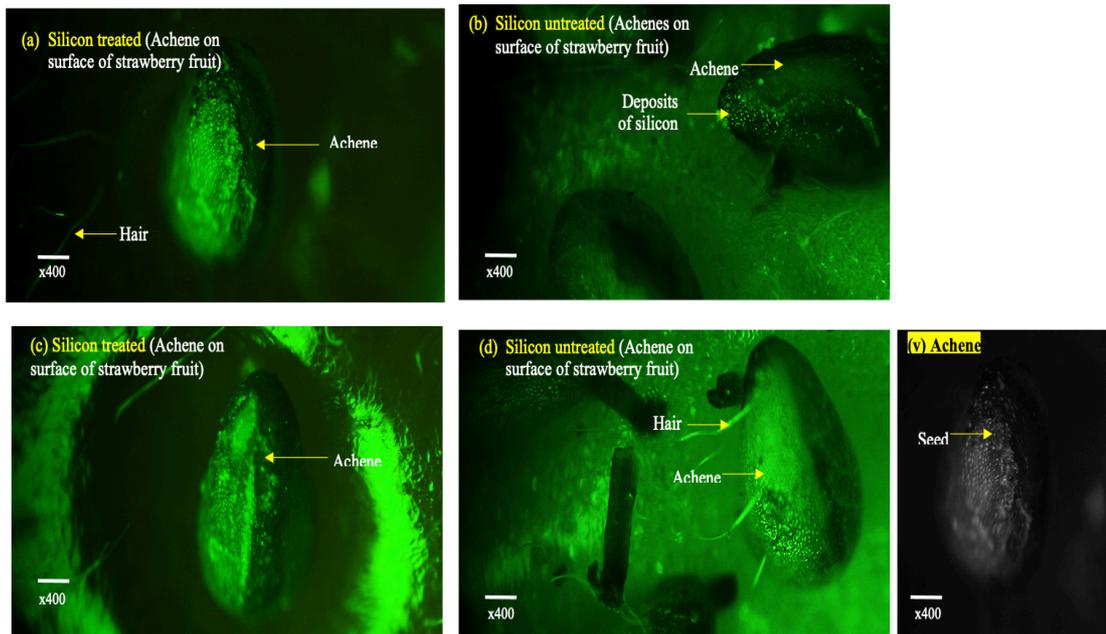
Silicon deposition in the silicon fertigation field experiment 2018.
Silicon deposits in green fluorescence. Viewed at x400 magnification only

Achene (Seed) viewed through UV light under the GXML3201 fluorescence microscope

Appendix 54 (ImageJ data sheet) Raw fluorescence intensities from Chapter 4

	Area	Mean	IntDen	RawIntDen	
			Results- Fluorscence intensities 2019-1.csv		
1	40282	35.740	1439671.000	1439671.000	
2	2657	3.021	8028.000	8028.000	
3	44391	36.327	1612600.000	1612600.000	
4	1593	2.968	4728.000	4728.000	
5	87136	36.880	3213545.000	3213545.000	
6	60646	48.754	2956763.000	2956763.000	
7	2036	3.183	6481.000	6481.000	
8	58404	56.169	3280496.000	3280496.000	
9	5626	5.111	28753.000	28753.000	
10	10524	47.311	497906.000	497906.000	
11	20036	55.895	1119914.000	1119914.000	
12	23575	38.981	918983.000	918983.000	
13	17060	47.137	804162.000	804162.000	
14	11573	54.687	632895.000	632895.000	
15	5688	51.875	295065.000	295065.000	
16	27636	14.851	410434.000	410434.000	
17	3687	2.752	10148.000	10148.000	
18	4076	4.353	17743.000	17743.000	
19	53550	40.668	2177773.000	2177773.000	
20	33597	46.124	1549638.000	1549638.000	
21	9814	3.805	37339.000	37339.000	
22	3913	2.638	10323.000	10323.000	
23	50478	19.244	971403.000	971403.000	
24	3109	1.383	4300.000	4300.000	
25	51780	49.485	2562345.000	2562345.000	
26	1295	6.521	8445.000	8445.000	
27	44482	39.830	1771719.000	1771719.000	
28	24434	55.611	1358805.000	1358805.000	
29	2167	5.919	12827.000	12827.000	
30	259	15.564	4031.000	4031.000	
31	28956	50.188	1453234.000	1453234.000	
32	4048	20.954	84823.000	84823.000	
33	709	3.292	2334.000	2334.000	
34	48889	42.242	2065162.000	2065162.000	
35	28666	97.091	2783217.000	2783217.000	
36	55503	39.749	2206196.000	2206196.000	
37	231053	33.791	7807411.000	7807411.000	
38	14040	107.665	1511611.000	1511611.000	
39	1818	54.742	99521.000	99521.000	
40	3353	64.434	216046.000	216046.000	
41	3176	67.107	213132.000	213132.000	

Appendix 55 (ImageJ data sheet) Raw fluorescence intensities from Chapter 4

	Area	Mean	IntDen	RawIntDen	
41	3176	67.107	213132.000	213132.000	
42	2128	72.492	154262.000	154262.000	
43	2217	11.499	25494.000	25494.000	
44	308	12.162	3746.000	3746.000	
45	139	4.676	650.000	650.000	
46	1488	81.108	120689.000	120689.000	
47	4682	113.854	533066.000	533066.000	
48	4519	46.538	210305.000	210305.000	
49	4519	46.538	210305.000	210305.000	
50	430	53.251	22898.000	22898.000	
51	717	23.471	16829.000	16829.000	
52	2900	106.479	308790.000	308790.000	
53	3966	43.431	172248.000	172248.000	
54	9017	44.137	397987.000	397987.000	
55	9110	29.369	267549.000	267549.000	
56	4568	40.825	186487.000	186487.000	
57	3155	29.289	92407.000	92407.000	
58	1372	26.878	36877.000	36877.000	
59	1827	49.624	90663.000	90663.000	
60	3240	15.949	51674.000	51674.000	
61	296	25.811	7640.000	7640.000	
62	4038	57.488	232135.000	232135.000	
63	2539	100.273	254593.000	254593.000	
64	2347	55.149	129434.000	129434.000	
65	3815	27.331	104266.000	104266.000	
66	676	88.809	60035.000	60035.000	
67	676	88.809	60035.000	60035.000	
68	5994	98.967	593209.000	593209.000	
69	2519	100.773	253847.000	253847.000	
70	4010	36.218	145235.000	145235.000	
71	1810	12.742	23063.000	23063.000	
72	4038	36.081	145694.000	145694.000	
73	2588	29.172	75496.000	75496.000	
74	1313	24.969	32784.000	32784.000	
75	1948	29.407	57285.000	57285.000	
76	1668	40.923	68260.000	68260.000	
77	3606	39.818	143585.000	143585.000	
78	1860	22.355	41580.000	41580.000	
79	1876	32.436	60849.000	60849.000	
80	713	34.363	24501.000	24501.000	
81	4321	33.934	146630.000	146630.000	

Appendix 56 (ImageJ data sheet) Raw fluorescence intensities; Chapter 4

	Results- Fluorscence intensities 2019-1.csv				
	Area	Mean	IntDen	RawIntDen	
82	6551	19.318	126551.000	126551.000	
83	2255	14.957	33728.000	33728.000	
84	2924	61.148	178796.000	178796.000	
85	1386	17.317	24001.000	24001.000	
86	920	18.291	16828.000	16828.000	
87	4251	17.558	74638.000	74638.000	
88	2701	13.889	37513.000	37513.000	
89	2322	14.037	32594.000	32594.000	
90	14715	31.751	467215.000	467215.000	
91	1036	64.259	66572.000	66572.000	
92	2302	51.682	118972.000	118972.000	
93	4902	19.062	93440.000	93440.000	
94	2815	52.831	148718.000	148718.000	
95	4148	60.899	252607.000	252607.000	
96	2480	23.956	59410.000	59410.000	
97	5409	39.674	214599.000	214599.000	
98	2384	62.877	149899.000	149899.000	
99	1944	72.166	140291.000	140291.000	
100	5492	22.547	123827.000	123827.000	
101	11103	61.526	683125.000	683125.000	
102	4653	42.755	198939.000	198939.000	
103	2849	15.802	45019.000	45019.000	
104	2325	43.623	101423.000	101423.000	
105	2931	56.184	164674.000	164674.000	
106	2329	32.933	76701.000	76701.000	
107	3634	38.574	140177.000	140177.000	
108	4804	64.424	309494.000	309494.000	
109	5352	18.458	98786.000	98786.000	
110	1569	68.651	107713.000	107713.000	
111	1569	24.465	38385.000	38385.000	
112	14901	14.231	212053.000	212053.000	
113	3695	38.734	143123.000	143123.000	
114	5455	58.667	320029.000	320029.000	
115	5557	117.589	653444.000	653444.000	
116	5344	83.075	443955.000	443955.000	
117	5513	62.385	343931.000	343931.000	
118	5206	31.180	162325.000	162325.000	
119	5557	52.031	289134.000	289134.000	
120	5557	50.381	279968.000	279968.000	
121	4949	26.176	129543.000	129543.000	
122	4181	55.367	231488.000	231488.000	

Appendix 57 (ImageJ data sheet) Raw fluorescence intensities; Chapter 4

	Area	Mean	IntDen	RawIntDen	
121	4949	26.176	129543.000	129543.000	
122	4181	55.367	231488.000	231488.000	
123	5557	42.107	233991.000	233991.000	
124	210	15.386	3231.000	3231.000	
125	1120	25.438	28490.000	28490.000	
126	2591	17.764	46027.000	46027.000	
127	1842	56.015	103180.000	103180.000	
128	1540	47.056	72467.000	72467.000	
129	4917	40.169	197511.000	197511.000	
130	2719	41.121	111807.000	111807.000	
131	3700	35.744	132254.000	132254.000	
132	3700	35.744	132254.000	132254.000	
133	2786	41.413	115378.000	115378.000	
134	3418	37.319	127556.000	127556.000	
135	6798	26.133	177655.000	177655.000	
136	5141	47.514	244271.000	244271.000	
137	5141	14.255	73283.000	73283.000	
138	2278	47.303	107757.000	107757.000	
139	5536	35.574	196937.000	196937.000	
140	5414	30.594	165635.000	165635.000	
141	17660	12.654	223465.000	223465.000	
142	17660	47.058	831052.000	831052.000	
143	34719	27.916	969227.000	969227.000	
144	8687	29.059	252432.000	252432.000	
145	4562	25.451	116106.000	116106.000	
146	16257	52.237	849211.000	849211.000	
147	5818	25.731	149703.000	149703.000	
148	1797	24.200	43487.000	43487.000	
149	15467	41.330	639250.000	639250.000	
150	5559	48.645	270417.000	270417.000	
151	5014	25.794	129332.000	129332.000	
152	5978	48.292	288689.000	288689.000	
153	5978	52.086	311372.000	311372.000	
154	3865	47.790	184709.000	184709.000	
155	3865	47.790	184709.000	184709.000	
156	5429	25.232	136987.000	136987.000	
157	1846	27.645	51032.000	51032.000	
158	3518	99.807	351120.000	351120.000	
159	6786	37.725	255999.000	255999.000	
160	7004	31.623	221489.000	221489.000	
161	7004	21.084	147670.000	147670.000	

Appendix 58 ImageJ data sheet) Raw fluorescence intensities; Chapter 4

	Area	Mean	IntDen	RawIntDen	
			Results- Fluorscence intensities 2019-1.csv		
161	7004	21.084	147670.000	147670.000	
162	3534	40.630	143588.000	143588.000	
163	4750	35.340	167867.000	167867.000	
164	4750	26.617	126430.000	126430.000	
165	5412	44.717	242009.000	242009.000	
166	5412	89.868	486366.000	486366.000	
167	4285	113.072	484514.000	484514.000	
168	6778	33.676	228257.000	228257.000	
169	311	39.006	12131.000	12131.000	
170	2584	23.439	60567.000	60567.000	
171	4204	26.344	110750.000	110750.000	
172	9859	51.011	502916.000	502916.000	
173	16615	49.074	815367.000	815367.000	
174	4243	38.660	164034.000	164034.000	
175	5488	25.835	141780.000	141780.000	
176	713	30.302	21605.000	21605.000	
177	7144	36.987	264236.000	264236.000	
178	7459	109.554	817163.000	817163.000	
179	7459	109.554	817163.000	817163.000	
180	1735	32.086	55670.000	55670.000	
181	5039	25.588	128936.000	128936.000	
182	4917	22.559	110921.000	110921.000	
183	5664	24.909	141082.000	141082.000	
184	5664	24.090	136446.000	136446.000	
185	8158	105.220	858384.000	858384.000	
186	5281	25.459	134450.000	134450.000	
187	353	44.691	15776.000	15776.000	
188	1756	36.952	64887.000	64887.000	
189	1211	71.141	86152.000	86152.000	
190	1525	69.990	106735.000	106735.000	
191	92	20.685	1903.000	1903.000	
192	3018	63.712	192283.000	192283.000	
193	3193	52.062	166234.000	166234.000	
194	4343	58.165	252610.000	252610.000	
195	568	50.533	28703.000	28703.000	
196	1809	14.457	26153.000	26153.000	
197	1767	26.906	47543.000	47543.000	
198	1093	47.191	51580.000	51580.000	
199	880	61.183	53841.000	53841.000	
200	3947	38.626	152458.000	152458.000	
201	478	30.084	14380.000	14380.000	

Appendix 59 (ImageJ data sheet) Raw fluorescence intensities; Chapter 4

	Results- Fluorscence intensities 2019-1.csv				
	Area	Mean	IntDen	RawIntDen	
178	7459	109.554	817163.000	817163.000	
179	7459	109.554	817163.000	817163.000	
180	1735	32.086	55670.000	55670.000	
181	5039	25.588	128936.000	128936.000	
182	4917	22.559	110921.000	110921.000	
183	5664	24.909	141082.000	141082.000	
184	5664	24.090	136446.000	136446.000	
185	8158	105.220	858384.000	858384.000	
186	5281	25.459	134450.000	134450.000	
187	353	44.691	15776.000	15776.000	
188	1756	36.952	64887.000	64887.000	
189	1211	71.141	86152.000	86152.000	
190	1525	69.990	106735.000	106735.000	
191	92	20.685	1903.000	1903.000	
192	3018	63.712	192283.000	192283.000	
193	3193	52.062	166234.000	166234.000	
194	4343	58.165	252610.000	252610.000	
195	568	50.533	28703.000	28703.000	
196	1809	14.457	26153.000	26153.000	
197	1767	26.906	47543.000	47543.000	
198	1093	47.191	51580.000	51580.000	
199	880	61.183	53841.000	53841.000	
200	3947	38.626	152458.000	152458.000	
201	478	30.084	14380.000	14380.000	
202	739	71.622	52929.000	52929.000	
203	3153	68.751	216773.000	216773.000	
204	3153	64.734	204106.000	204106.000	
205	1978	47.892	94730.000	94730.000	
206	919	65.252	59967.000	59967.000	
207	374	61.037	22828.000	22828.000	
208	204	71.431	14572.000	14572.000	
209	346	38.760	13411.000	13411.000	
210	286	21.545	6162.000	6162.000	
211	683	52.919	36144.000	36144.000	
212	797	78.881	62868.000	62868.000	
213	797	78.881	62868.000	62868.000	
214	2676	66.557	178106.000	178106.000	
215	1215	51.583	62673.000	62673.000	
216	1215	51.583	62673.000	62673.000	
217	626	71.158	44545.000	44545.000	
218	1390	48.004	66726.000	66726.000	

Appendix 60 Chapter 4 - Results workings Means of 10 fluorescence intensities (integrated density) in the leaf, leaf petiole and root from a glasshouse experiment 2017

Normality test (a) and (b) One Way Anova in cross-sections of untreated and silicon-treated leaves in a glasshouse experiment 2017

A.   Normality test, Null and alternate Hypothesis: leaves

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Response	.145	20	.200[*]	.907	20	.057

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Since the sig value.057 is greater than critical value, accept Ho and conclude that data does follow normal distribution. The One Way Anova was then used to determine the average values of differences between fluorescence intensities of untreated leaves and silicon-treated leaves. See table 4.2 b for the One Way Anova test used to evaluate the cross-section average values.

B.   One Way Anova

ANOVA

Response

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	22.898	1	22.898	7.142	.016
Within Groups	57.710	18	3.206		
Total	80.608	19			

b between silicon-treated leaves and untreated leaves, shows that since the sig value.016 is less than critical value 0.05, reject Ho and conclude that there is a statistical significant difference between the average values of silicon-treated leaves and untreated leaves in the glasshouse experiment. This shows that silicon-treated plants had more silicon deposits (Fluorescence intensity) than untreated.

(a) Normality (b) One Way Anova test in cross-sections of untreated and silicon-treated leaf petioles in a glasshouse experiment 2017

A. Normality test, Null and alternate Hypothesis: Leaf petioles

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Response	.177	20	.102	.909	20	.060

a. Lilliefors Significance Correction

The test for normality between untreated and silicon-treated leaf petioles in table 4.3 (a) show that since the sig value.060 is greater than critical value, accept Ho and conclude that the data does follow normal distribution. Therefore, the One Way Anova was used to determine average values of differences between leaf petioles from untreated and silicon-treated plants. See figure b for the One Way Anova test used.

B. One Way Anova, Null and Alternate Hypothesis: Leaf petioles

Ho: There is no statistical significance difference among the treatment average values

Ha: There is statistical significance difference among the treatment average values

ANOVA

Response

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	81.205	1	81.205	22.604	.000
Within Groups	64.665	18	3.593		
Total	145.870	19			

From table (b) since sig the value.000 is less than critical value 0.05, reject Ho and conclude that there is a statistical significant difference among the treatment average values of leaf petioles of untreated and silicon-treated plants. This shows that there was more silicon deposits (Fluorescence intensity) in the silicon-treated plants compared to untreated.

Table (a) Normality test and (b) One Way Anova in cross-sections of untreated and silicon-treated roots in a glasshouse experiment 2017

A.   Normality test, Null and alternate Hypothesis: Roots

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Response	.086	20	.200*	.991	20	.999

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

From table a of untreated and silicon-treated roots, since the sig value.999 is greater than critical value, accept Ho and conclude that the data does follow normal distribution. The One Way Anova (Table b) was used to evaluate the differences of average values of cross-sections of the roots between untreated and silicon-treated.

B.   One Way Anova, Null and Alternate Hypothesis

Ho: There is no statistical significant difference among the treatment average values

Ha: There is statistical significant difference among the treatment average values

ANOVA

Response

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	116.162	1	116.162	31.283	.000
Within Groups	66.838	18	3.713		
Total	183.000	19			

In table b, the sig value.000 is less than critical value 0.05, reject Ho and conclude that there is a statistical significance difference among the treatment average values of roots of untreated and silicon-treated strawberry plants. The One Way Anova showed that average values of silicon-treated plant roots is statistical different from untreated plant roots. Table b shows that there was more silicon deposits (Fluorescence intensity) in the silicon-treated roots compared to untreated roots.

Appendix 61 Chapter 4 - Results workings Means of 10 fluorescence intensities (integrated density) in the leaf, leaf petiole and root from a field experiment 2017

Table a Normality test and b Two Way Anova in cross-sections of untreated and silicon twice only treatment leaves, leaf petioles and roots in a field experiment 2017

A.   Normality test, Null and alternate Hypothesis: Leaves, leaf petioles and roots
Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Response	.118	120	.306	.930	120	.502

a. Lilliefors Significance Correction

From table a for untreated and silicon twice only treatment for the leaves, leaf petioles and roots, since the sig value.502 is greater than critical value, accept Ho and conclude that data does follow normal distribution. Therefore, the Two Way Anova was used to determine the difference between the average values of cross-sections from both treatment. The Two Way Anova in table b was used to test for the average values of differences between untreated and silicon twice only treatment in the leaves, leaf petioles and roots of the 2017 field experiment.

B.   Two Way Anova
Null and alternate Hypothesis:

Ho: Treatment average values of are not statistically significantly different

Ha: Treatment average values of are statistically significantly different

Ho: There is no statistical significant difference between block average values

Ha: There is statistical significant difference between block average values

Tests of Between-Subjects Effects

Dependent Variable: Response

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	283.544[a]	11	25.777	3.825	.000
Intercept	6442.778	1	6442.778	955.918	.000
Treatment	203.141	3	67.714	10.047	.000
Block	21.143	2	10.572	1.569	.213
Treatment * Block	59.260	6	9.877	1.465	.197
Error	727.907	108	6.740		
Total	7454.229	120			
Corrected Total	1011.451	119			

a. R Squared = .280 (Adjusted R Squared = .207)

Appendix 62 Chapter 4 - Results workings Means of 10 fluorescence intensities (integrated density) in the leaf, leaf petiole and root from a field experiment 2018

Table (a) Normality test and (b) Two Way Anova in cross-sections of untreated and silicon twice only treatment leaves, leaf petioles and roots of the field experiment 2018

A.   Normality test, Null and alternate Hypothesis: Leaves, leaf petioles and roots

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Response	.061	120	.200[*]	.978	120	.051

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

From table a for untreated and silicon twice only treatment cross-sections, since the sig value 0.051 is greater than 0.05 accept Ho and conclude that data follows normal distribution. The Two Way Anova was used to find the differences between the average values of untreated and silicon twice only treatment from table b.

B.   Two Way Anova

Null and alternate Hypothesis:

Ho: Treatment average values of are not statistically significantly different

Ha: Treatment average values of are statistically significantly different

Ho: There is no statistically significant difference between block average values

Ha: There is statistically significant difference between block average values

Tests of Between-Subjects Effects

Dependent Variable: Response

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	70.205^a	11	6.382	2.220	.018
Intercept	2109.498	1	2109.498	733.690	.000
Treatment	6.419	3	2.140	.744	.528
Block	45.815	2	22.907	7.967	.001
Treatment * Block	17.970	6	2.995	1.042	.403
Error	310.521	108	2.875		
Total	2490.224	120			
Corrected Total	380.725	119			

From results in table 4.8b, the Two Way Anova revealed that there was a statistical significant difference between leaves of silicon-treated plants and untreated plants ($P<0.05$). However, no statistical differences ($P>0.05$) was found between silicon-treated and untreated leaf petioles and roots in the silicon fertigation field experiment 2018.

4.7b, the sig value of treatment average values shows that there is a statistical significant difference among treatment average values because sig value.000 is less than critical value 0.05. Since the sig value.213 shows that there is no statistical significant difference among block average values. The conclusion from the Two Way Anova conducted between fluorescence intensities of cross-sections values showed that there was a statistical significant difference between cross-sections of leaves in silicon-treated leaves compared to untreated leaves. The results also showed that there was no statistical significance difference between cross-sections of leaf petioles and roots in silicon-treated plants compared to untreated. Data from the Two Way Anova analysis has shown that silicon-treated fluorescence deposits were higher in silicon-treated cross-sections compared to untreated in the 2017 field experiment.

Appendix 63 Hoagland's solution recipe (chapter 5)

To make up Hoagland's stock solution the following procedures were carried out in order

Each stock component was weighed and transferred into separate plastic storage bottles with appropriate labels.

Each made up stock component was pipetted to 800 mL de-ionized water and then filled to 1 Litre.

The solution was mixed thoroughly and transferred into 10 Litre plastic storage tanks.

10 L x 4 plastic storage tanks containing made up Hoagland's solution was added to 5 L plastic boxes in preparation for the planting of the Malling Centenary strawberry plants.

pH and EC was measured with a pH and EC metre before planting the strawberry plants. The pH was between 5.5 to 6.0 and EC was between 1.0dS/m to 1.3dS/m.

Hoagland's solution was topped in hydroponic tubs twice-a-week as levels reduced as plants grow in size. Forty Litres of Hoagland's were prepared prior to top ups

Appendix 64 Chapter 5 - Results workings - Means of 10 strawberry fruits ºBrix levels from the silicon field experiment 2017

i. **(i) Test for normality and (ii) & (iii) t-Test Paired Sample for Means Strawberry ºBrix, Untreated and Silicon twice + fungicides and silicon twice + fungicides (20 of July 2017)**

ii.   Normality Test

Null and alternate Hypothesis:

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Response	.069	36	.200[*]	.986	36	.911

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

In table (i) if the sig value is less than critical value 0.05, reject Ho. In the analysis for samples assessed on 20 of July 2017, the sig value 0.911 is greater than critical value 0.05. Accept Ho and conclude that the data follows a normal distribution. The t-Test Paired Two Samples for Means was used to determine the differences between average values of strawberries sampled on 20 of July 2017.

Table (ii) showed that the average values of strawberries ºBrix sampled on 20 of July 2017 from the silicon twice + fungicides treatment was not statistically ($P>0.05$) different from untreated. There was no increase in silicon-treated fruit ºBrix compared to untreated.

iii.   **t-Test: Paired Two Sample for Means: Strawberry ºBrix, Untreated and Silicon twice + no fungicides (20 of July 2017)**

	Untreated	Silicon x 2 + no fungicides
Mean	9.866666667	9.355555556
Variance	0.6075	1.832777778
Observations	9	9

Pearson Correlation	0.182037825
Hypothesized Mean Difference	0
df	8
t Stat	1.069333782
P(T<=t) one-tail	0.15805972
t Critical one-tail	1.859548038
P(T<=t) two-tail	0.316119439
t Critical two-tail	2.306004135

Table (iii) showed that the average values of strawberries ºBrix sampled on 20 of July 2017 from the silicon twice + no fungicides treatment was not statistically ($P>0.05$) different from untreated. Conclusions from table a to c showed that ºBrix levels of strawberry fruits sampled on the 20 of July 2017 in the silicon-treated plants was not statistically different ($P>0.05$) from untreated.

(iv) Test for normality and (v) & (vi) t-Test Paired Sample for Means Strawberry ºBrix, Untreated and Silicon twice + fungicides and silicon twice + fungicides (24 of July 2017)

 iv. **Normality Test**

Null and alternate Hypothesis:

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Response	.161	40	.010	.927	40	.013

a. Lilliefors Significance Correction

As the sig value.013 in table (iv) is less than the critical value 0.05, reject Ho and conclude that data does not follow normal distribution, so the t-Test Paired Two Samples for Means was used to test for the significance difference between untreated and silicon twice + fungicides treatment sampled 24 of July 2017.

v.t-Test: Paired Two Sample for Means: Strawberry °Brix Untreated and Silicon twice + fungicides (24 of July 2017)

	Untreated	Silicon x 2 + fungicides
Mean	8.32	10.94
Variance	2.166222222	4.936
Observations	10	10
Pearson Correlation	0.166568057	
Hypothesized Mean Difference	0	
df	9	
t Stat	-3.378778344	
P(T<=t) one-tail	0.004071372	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.008142743	
t Critical two-tail	2.262157163	

Table (v) showed that the average values of strawberries ºBrix sampled on 24 of July 2017 from the silicon twice + fungicides treatment was statistically ($P<0.05$) different from untreated. ºBrix levels of strawberry fruits sampled from the silicon twice + fungicides was higher than untreated.

235

vi. t-Test: Paired Two Sample for Means: Strawberry °Brix Untreated and Silicon twice + no fungicides (24 of July 2017)

	Untreated	Silicon x 2 + no fungicides
Mean	8.32	11.72
Variance	2.166222222	9.308444444
Observations	10	10
Pearson Correlation	-0.355915667	
Hypothesized Mean Difference	0	
df	9	
t Stat	-2.807032325	
P(T<=t) one-tail	0.010238031	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.020476062	
t Critical two-tail	2.262157163	

Table (vi) showed the average values of strawberries ºBrix sampled on the 24 of July 2017 from untreated and silicon twice + no fungicides was statistically different ($P<0.05$). Conclusions from table i to vi showed that silicon-enhanced ºBrix levels of strawberry fruits sampled from the silicon-treated plants on 24 of July 2017 compared to untreated. Both silicon treatment were higher than untreated on the 24 of July 2017.

vii. Test for normality and (viii) & (ix) t-Test Paired Two Sample for Means Untreated and Silicon twice + fungicides and silicon twice + fungicides (27 of July 2017)

vii. Normality Test

Null and alternate Hypothesis:

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Response	.125	40	.117	.937	40	.027

From table (vii) since the sig value.027 is less than critical value 0.05, reject Ho and conclude that data (Means of strawberries sampled 27 of July 2017) does not follow normal distribution. The t-test Paired Sample for Means was then used to test for significance differences between untreated and silicon twice + fungicides treatment.

viii.   t-Test: Paired Two Sample for Means: Strawberry °Brix Untreated and Silicon twice + fungicides (27 of July 2017)

	Untreated	Silicon x 2 fungicides
Mean	9.9	10.94
Variance	2.533333333	4.936
Observations	10	10
Pearson Correlation	0.076667901	
Hypothesized Mean Difference	0	
df	9	
t Stat	-1.249560426	
P(T<=t) one-tail	0.121488767	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.242977533	
t Critical two-tail	2.262157163	

Table (viii) showed that the average values of strawberries °Brix sampled on 27 of July 2017 from the silicon twice + fungicides treatment was not statistically ($P>0.05$) different from untreated.

ix. t-Test: Paired Two Sample for Means: Strawberry °Brix Untreated and Silicon twice + no fungicides (27 of July 2017)

	Untreated	Silicon x 2 + no fungicides
Mean	9.9	10.45
Variance	2.533333333	6.138333333
Observations	10	10
Pearson Correlation	-0.364039577	
Hypothesized Mean Difference	0	
df	9	
t Stat	-0.511926395	
P(T<=t) one-tail	0.310513352	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.621026704	
t Critical two-tail	2.262157163	

Table (ix) showed that the average values of strawberries sampled on the 27 July from untreated and silicon twice + no fungicides was not statistically different ($P>0.05$). Conclusions from table (vii, viii and ix) showed that on the 27 of July 2017, Strawberries from the silicon twice + fungicides and silicon twice + no fungicides treatment were not statistically ($P>0.05$) different from untreated.

(xi) Test for normality and (xii) & (xiii) t-Test Paired Two Sample for Means Untreated and Silicon twice + fungicides and silicon twice + fungicides (01 of August 2017)

xi. Normality Test

Null and alternate Hypothesis:

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Response	.103	40	.200[*]	.963	40	.207

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

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n table (xi) the sig value.207 is greater than the critical value.05, accept Ho and conclude that data (Means of strawberries sampled 01 of August 2017) follows a normal distribution. The t-Test Paired Two Sample for Means was used to test for significant differences between treatment average values of strawberries sampled on 01 of August 2017.

xii. t-Test: Paired Two Sample for Means: Strawberry ºBrix Untreated and Silicon twice + fungicides (01 of August 2017)

	Untreated	Silicon x 2 + fungicides
Mean	9.47	9.66
Variance	2.042333333	3.633777778
Observations	10	10
Pearson Correlation	-0.301492806	
Hypothesized Mean Difference	0	
df	9	
t Stat	-0.222092719	
P(T<=t) one-tail	0.414599768	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.829199535	
t Critical two-tail	2.262157163	

Table (xii) showed that the average values of strawberries ºBrix sampled on 01 of August 2017 from the silicon twice + fungicides treatment were not statistically ($P>0.05$) different from untreated.

xiii. t-Test: Paired Two Sample for Means: Strawberry ºBrix Untreated and Silicon twice + no fungicides (01 of August 2017)

	Untreated	Silicon x 2 + no fungicides
Mean	9.47	9.73
Variance	2.042333333	1.382333333
Observations	10	10
Pearson Correlation	-0.365755989	
Hypothesized Mean Difference	0	
df	9	
t Stat	-0.381127712	
P(T<=t) one-tail	0.35597482	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.711949641	
t Critical two-tail	2.262157163	

Table (xiii) showed that there was no significant difference ($P > 0.05$) of average values of ºBrix levels of strawberries sampled 01 of August 2017 between untreated and silicon twice + no fungicides treatment. Conclusions from table (xi, xii, xiii) showed that silicon did not increase ºBrix levels of strawberries sampled on 01 of August 2017.

Appendix 65 Chapter 5 - Results workings - Means of 10 strawberry leaf petioles ºBrix levels from the silicon field experiment 2017

(xiv) Test for normality and (xv) and (xvi) t-test: Paired Two Samples for Means: Leaf petioles ºBrix, Untreated and silicon twice + fungicides and silicon twice + no fungicides (20 June 2017)

xiv. Normality Test

Null and alternate Hypothesis:

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Untreated	.152	10	.200[*]	.955	10	.732
Fungicides	.194	10	.200[*]	.916	10	.324
Silicon_x_2Fungicides	.197	10	.200[*]	.927	10	.418
Silicon_x_2_no_Fungicid es	.172	10	.200[*]	.934	10	.488

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

If the sig value in table (xiv) is less than the critical value 0.05, reject Ho and conclude that data does not follow normal distribution. Since the sig value is greater than the critical value 0.05 accept the Ho and conclude that data follows normal distribution. The t-Test Paired Two Sample for Means was used to test for significance difference between treatment (Untreated, silicon twice + fungicides and silicon twice + no fungicides) average values ofs for leaf petioles sampled 20 June 2017 (table xv).

xv. t-Test: Paired Two Sample for Means: Leaf petioles ºBrix Untreated Silicon twice + fungicides (20 June 2017)

	Untreated	Silicon x 2 + fungicides
Mean	8.75	9.86
Variance	1.542777778	3.464888889
Observations	10	10
Pearson Correlation	0.196555163	
Hypothesized Mean Difference	0	
df	9	
t Stat	-1.7337872	
P(T<=t) one-tail	0.05849371	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.116987421	
t Critical two-tail	2.262157163	

Table (xv) showed that the average values of strawberry leaf petioles ºBrix sampled on 20 June 2017 from the silicon twice + fungicides treatment was not statistically ($P>0.05$) different from untreated.

xvi. t-Test: Paired Two Sample for Means: Leaf petioles °Brix Untreated and Silicon twice + no fungicides (20 June 2017)

	Untreated	Silicon x 2 + no fungicides
Mean	8.75	8.49
Variance	1.542777778	2.387666667
Observations	10	10
Pearson Correlation	-0.344747179	
Hypothesized Mean Difference	0	
df	9	
t Stat	0.358705056	
P(T<=t) one-tail	0.36404313	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.72808626	
t Critical two-tail	2.262157163	

Table (xvi) showed that the average values of strawberry leaf petioles °Brix sampled on 20 June 2017 from the silicon twice + no fungicides treatment was not statistically (*P*>0.05) different from untreated. Conclusions from table (xiv, xv, xvi) showed that silicon did not elevate °Brix levels of leaf petioles sampled 20 June 2017.

(xvii) Test for normality and (xviii) and (xix) t-test: Paired Two Samples for Means: Leaf petioles ºBrix, Untreated and silicon twice + fungicides and silicon twice + no fungicides (18 of July 2017)

xvii. Normality test

Null and alternate Hypothesis:

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Untreated	.186	10	.200[*]	.958	10	.759
Fungicides	.139	10	.200[*]	.932	10	.464
Silicon_x_2Fungicides	.179	10	.200[*]	.904	10	.240
Silicon_x_2_no_Fungicides	.249	10	.079	.868	10	.096

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

From table (xvii) if the sig value is less than critical value, reject Ho and conclude that data does not follow normal distribution. Since the sig value is greater than critical value 0.05 accept Ho and conclude that data follows normal distribution. The t-Test Paired Two Sample for Means was used to test for significance differences between untreated and silicon twice + fungicides (Table xviii).

xviii. t-Test: Paired Two Sample for Means: Leaf petioles °Brix Untreated and Silicon twice + fungicides (18 of July 2017)

	Untreated	Silicon x 2 + fungicides
Mean	8.45	7.9
Variance	2.613888889	1.684444444
Observations	10	10
Pearson Correlation	-0.311360065	
Hypothesized Mean Difference	0	
df	9	
t Stat	0.734640524	
P(T<=t) one-tail	0.240630197	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.481260393	
t Critical two-tail	2.262157163	

Table (xviii) showed that the average values of strawberry leaf petioles °Brix sampled on 18 of July 2017 from the silicon twice + fungicides treatment was not statistically ($P>0.05$) different from untreated.

xix. t-Test: Paired Two Sample for Means: Leaf petioles °Brix Untreated and Silicon twice + no fungicides

	Untreated	Silicon x 2 + no fungicides
Mean	8.45	8.84
Variance	2.613888889	1.556
Observations	10	10
Pearson Correlation	0.226989804	
Hypothesized Mean Difference	0	
df	9	
t Stat	-0.68364964	
P(T<=t) one-tail	0.255705985	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.511411971	
t Critical two-tail	2.262157163	

Table (xix) showed that the average values of strawberry leaf petioles °Brix sampled on 18 of July 2017 from the silicon twice + no fungicides treatment was not statistically (P>0.05) different from untreated. Table (xvii, xviii, xix) suggests that silicon did not increase °Brix levels of leaf petioles in silicon-treated plants sampled 18 of July 2017.

(xxi) Test for normality and (xxii) and (xxiii) t-test: Paired Two Samples for Means: Leaf petioles ºBrix, Untreated and silicon twice + fungicides and silicon twice + no fungicides (01 of August 2017)

xxi. Normality test

Null and alternate Hypothesis:

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Untreated	.298	10	.012	.863	10	.083
Fungicides	.223	10	.172	.921	10	.369
Silicon_x_2Fungicides	.186	10	.200[*]	.940	10	.550
Silicon_x_2_no_Fungicides	.159	10	.200[*]	.964	10	.833

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

In table (xxi) If sig value is less than critical value, reject Ho and conclude that data does not follow normal distribution. Since the sig value is greater than critical value 0.05 we accept Ho and conclude that data follows normal distribution. The t -Test: Paired Two Sample for Means was used to test for significant difference between untreated, silicon twice + fungicides and silicon twice + no fungicides (Table xxii and xxiii).

xxii. t-Test: Paired Two Sample for Means:

Strawberry Leaf petioles ºBrix Untreated and Silicon twice + fungicides (01 of August 2017)

	Untreated	Silicon x 2 + fungicides
Mean	8.99	8.86
Variance	3.016555556	1.387111111
Observations	10	10
Pearson Correlation	0.447908735	
Hypothesized Mean Difference	0	
df	9	
t Stat	0.256373744	
P(T<=t) one-tail	0.401713182	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.803426364	
t Critical two-tail	2.262157163	

Table (xxii) showed that the average values of strawberry leaf petioles ºBrix sampled on 01 of August 2017 from the silicon twice + fungicides treatment was not statistical higher ($P>0.05$) and different from untreated.

xxiii. t-Test: Paired Two Sample for Means: Strawberry Leaf petioles ºBrix Untreated and Silicon twice + no fungicides (01 of August 2017)

	Untreated	Silicon x 2 + no fungicides
Mean	8.99	7.11
Variance	3.016555556	1.289888889
Observations	10	10
Pearson Correlation	-0.344672099	
Hypothesized Mean Difference	0	
df	9	
t Stat	2.497531922	
P(T<=t) one-tail	0.016999616	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.033999232	
t Critical two-tail	2.262157163	

Table (xxiii) showed that the average values of leaf petioles ºBrix sampled on 01 of August 2017 from the silicon twice + no fungicides treatment was not statistical higher ($P>0.05$) and different from untreated. Table (xxi, xxii, xxiii) showed that silicon did not elevate ºBrix levels of leaf petioles sampled 01 of August 2017.

(xxiv) Test for normality and (xxv) and (xxvi) t-test: Paired Two Samples for Means: Leaf petioles ºBrix, Untreated and silicon twice + fungicides and silicon twice + no fungicides (19 September 2017)

xxiv**. Normality test**

Null and alternate Hypothesis:

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Level of significance = 0.05

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Untreated	.174	10	.200[*]	.947	10	.631
Fungicides	.158	10	.200[*]	.978	10	.951
Silicon_x_2Fungicides	.216	10	.200[*]	.908	10	.268
Silicon_x_2_no_Fungicides	.197	10	.200[*]	.928	10	.425

*. This is a lower bound of the true significance.

From table (xxiv) If the sig value is less than critical value, reject Ho and conclude that data does not follow normal distribution. However, since the sig value is greater than critical value 0.05, accept Ho and conclude that data follows normal distribution. The Paired Two Sample for Means was then used to find the differences between untreated and silicon twice + fungicides in table xxv.

xxv. t-Test: Paired Two Sample for Means:

Strawberry Leaf petiole ºBrix Untreated and Silicon twice + fungicides (19 September 2017)

	Untreated	Silicon x 2 + fungicides
Mean	8.52	9.13
Variance	1.832888889	3.909
Observations	10	10
Pearson Correlation	-0.517883354	
Hypothesized Mean Difference	0	
df	9	
t Stat	-0.661080749	
P(T<=t) one-tail	0.262560136	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.525120271	
t Critical two-tail	2.262157163	

Table 5.12(xxv) showed that the average values of leaf petioles ºBrix sampled on 19 September 2017 from the silicon twice + fungicides treatment was not statistically ($P>0.05$) different from untreated.

xxvi. t-Test: Paired Two Sample for Means: Strawberry Leaf petiole ºBrix Untreated and Silicon twice + no fungicides (19 September 2017)

	Untreated	Silicon x 2 + no fungicides
Mean	8.52	9.42
Variance	1.832888889	3.806222222
Observations	10	10
Pearson Correlation	0.26737835	
Hypothesized Mean Difference	0	
df	9	
t Stat	-1.384342366	
P(T<=t) one-tail	0.099805679	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	0.199611358	
t Critical two-tail	2.262157163	

Table (xxvi) showed that in the average values of leaf petioles ºBrix levels sampled from 19 September 2017 there was no statistical difference ($P>0.05$) between untreated and silicon twice + no fungicides treatment. Conclusions from table (xxiv, xxv, xxvi) showed that silicon did not raise ºBrix levels of the leaf petioles sampled 19 September 2017.

Appendix 66 Chapter 5 - Results workings - Means number of leaves from the hydroponic experiment 2018

Table (i) Test for normality and (ii) Paired Samples Test – Mean leaf number per plant (Untreated and silicon-treated)

i. **Normality Test**

HO: Difference between untreated and silicon-treated follows normal distribution

HA: Difference between untreated and silicon-treated does not follows normal distribution

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Difference	.179	23	.054	.925	23	.084

a. Lilliefors Significance Correction

From table (i) If the sig value is less than critical value 0.05, reject Ho. In the analysis in figure 5.14a the sig value 0.084 is greater than critical value 0.05, accept Ho and conclude that the difference between untreated leaves and silicon-treated leaves follows a normal distribution. So the parametric test was used to test for significance difference between both treatment (Table ii).

ii. **Null and Alternate Hypothesis**

Ho: There is no statistically significant difference between untreated and silicon-treated.

Ha: There is statistically significance difference between untreated and silicon-treated.

Parametric Test: (Paired Samples Test)

Paired Samples Test

		Paired Differences							
				Std. Error Mean	95% Confidence Interval of the Difference				
		Mean	Std. Deviation		Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	Untreated - Silicon_Treated	-2.30435	1.98711	.41434	-3.16364	-1.44506	-5.561	22	.000

From table (ii) If the sig value is less than critical value 0.05, reject Ho. The Paired Samples test conducted between average values of number of leaves per plant sig value.000 is less than critical value 0.05 (0.00 <0.05) reject Ho and conclude that there is a statistical significance difference between untreated leaves and silicon-treated leaves. This showed that there is a significant increase in the average values of number of leaves from the silicon-treated plants compared with untreated in the hydroponics over time

Appendix 67 Chapter 5 - Results workings - Means number of runners from the hydroponic experiment 2018

Table (iii) Test for normality and (iv) non-parametric test; Wilcoxon Signed Ranked Test – Mean runner number per plant (untreated and silicon-treated)

iii. Normality Test

Null and alternate Hypothesis:

Ho: Data follows normal distribution.

Ha: Data does not follow normal distribution.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Difference	.437	23	.000	.582	23	.000

a. Lilliefors Significance Correction

In the analysis (Table iii) the sig value of difference represent paired difference also does not follow normal distribution. Therefore, to test for significance difference the non-parametric test; Wilcoxon Sign Ranked Test is used in table iv to test the differences between untreated and silicon-treated plants.

iv. Non-Parametric Test

Wilcoxon Sign Ranked Test

Null and Alternate Hypothesis

Ho: There is no statistically significant difference between untreated and silicon-treated.

Ha: There is statistically significance difference between untreated and silicon-treated.

Test Statistics[a]

	Silicon_Treated - Untreated
Z	-4.000[b]
Asymp. Sig. (2-tailed)	.000

a. Wilcoxon Signed Ranks Test

b. Based on negative ranks.

The Wilcoxon Sign Ranked Test conducted between silicon-treated runners and untreated runners showed that the sig value.000 is less than the critical value 0.05 (0.00 <0.05) which concludes that the differences between untreated runners and silicon-treated runners is statistically different. Silicon-treated plants had more runners compared to untreated ($P<0.05$).

Appendix 68 Mean numbers of fruits per plant tubs counted at two sample dates

Sample date	Untreated	Silicon-treated
13/06/2018	2	5
13/06/2018	1	3
13/06/2018	3	2
13/06/2018	1	4
Fruits were not produced in all plants from the experiment on the 13/06/2018	From the 13 June 2018, fruits were sampled from only 4 plant tubs.	From the 13 June 2018, fruits were sampled from only 4 plant tubs
From the 20 June fruits were sampled from all 5 plants tubs	From the 20 June 2018, fruits were sampled from all 5 plant tubs	From the 20 June 2018, fruits were sampled from all 5 plant tubs
20/06/2018	2	5
20/06/2018	1	3
20/06/2018	2	3
20/06/2018	1	4
20/06/2018	2	3

Appendix 69 Chapter 5 - Results workings - Mean number of strawberry fruits from the hydroponic experiment 2018

Table (vi) Test for normality and (vii) Non-parametric test; Wilcoxon Signed Ranked Test – Mean number of fruits per plant (Untreated and silicon-treated)

vi. Normality test

Null and alternate Hypothesis

HO: Difference between untreated and silicon-treated follows normal distribution

HA: Difference between untreated and silicon-treated does not follows normal distribution

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Difference	.237	9	.156	.820	9	.035

a. Lilliefors Significance Correction

According to Shapiro Wilk Test in table (vi) the sig value.035 is less than critical value 0.05, reject Ho and conclude that difference between the average values of number of untreated fruits and silicon-treated fruits does not follows normal distribution. The non-parametric test; Wilcoxon Sign Ranked Test was used to find the differences between the two treatment variables (vii).

vii. Non-Parametric Test

Wilcoxon Sign Ranked Test

Null and Alternate Hypothesis

Ho: There is no statistically significant difference between Untreated and silicon-treated.

Ha: There is statistically significance difference between untreated and silicon-treated.

Test Statistics[a]

	Silicon_Treated - Untreated
Z	-2.461[b]
Asymp. Sig. (2-tailed)	.014

a. Wilcoxon Signed Ranks Test

b. Based on negative ranks.

From table (vii) the test showed that the sig value.014 is less than critical value of 0.05, reject Ho and conclude that there is a statistical significant difference ($P<0.05$) between the average values of number of silicon-treated fruits compared with the number of untreated fruits.

Appendix 70 Strawberry fruit °Brix from 5 individual strawberries

°Brix per plant	Untreated	Silicon-treated
13/06/2018	9	14
	10	14
	17	20
	10	16
	16	13
20/06/2018	16	12
	10	15
	16	19
	15	13
	16	14

Appendix 71 Mean of 10 leaves, per plant (chlorophyll content) from untreated and silicon-treated plants

Sample date	Untreated	Silicon-treated
06/03/2018	33.9	30.9
14/03/2018	21.0	51.8
20/03/2018	32.5	97.3
27/03/2018	40.0	88.4
04/04/2018	40.5	55.9
11/04/2018	43.2	11.2
17/04/2018	20.0	13.2
25/04/2018	49.5	44.5
02/05/2018	55.7	53.2
09/05/2018	33.0	15.9
15/05/2018	28.9	41.3
22/05/2018	23.8	55.4
30/05/2018	33.5	57.7
06/06/2018	40.0	55.4
13/06/2018	36.4	57.3
20/06/2018	39.0	43.9
27/06/2018	22.3	46.5

Appendix 72 Chapter 5 - Results workings - Means number of chlorophyll contents of the leaves from the hydroponic experiment 2018

Table (x) Test for normality and (xi) Paired Samples Test – Mean chlorophyll content per plant (Untreated and silicon-treated)

x. Normality Test

HO: Difference between untreated and silicon-treated follows normal distribution

HA: Difference between untreated and silicon-treated does not follows normal distribution

Tests of Normality

	Kolmogorov-Smirnov^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Difference	.084	23	.200*	.976	23	.825

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

In the Shapiro wilk test (Table x) done between average values of number of silicon-treated and untreated chlorophyll content of strawberry leaves, the sig value.825 is greater than the critical value 0.05, accept Ho and conclude that the difference between untreated chlorophyll content and silicon-treated chlorophyll content follows a normal distribution. So the parametric test (Table xi) was used to test for significance difference.

xi. Null and Alternate Hypothesis

Ho: There is no statistically significant difference between Untreated and silicon-treated.

Ha: There is statistically significance difference between untreated and silicon-treated.

Paired Samples Test

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	Untreated - Silicon_Treated	-8.24348	25.16054	5.24633	-19.12371	2.63675	-1.571	22	.130

The Paired Sample Test shown in table (xi) assessed the difference between average values of number of chlorophyll content of untreated and silicon-treated. Since the sig value.130 is greater than critical value 0.05, accept Ho and conclude that there is no statistical significant (*P*>0.05) difference between untreated and silicon-treated chlorophyll contents in the leaves.

Appendix 73 fresh weights biomass (No fruits were included) of 10 individual whole strawberry plants (Untreated and silicon-treated)

The table (xii) is drawn from individual fresh weights of strawberry plants from the hydroponics at the end of the experiment.

(xii)

Untreated plants (g)	Silicon-treated plants (g)
120	148
151	155
128	190
134	164
160	189
138	178
173	168
147	177
140	196
156	129

Untreated (average) = 144g (approx.) and silicon-treated plants (average) = 169g (approx.)

Appendix 74 COSHH FORMS (Field experiments)

Substance(s) information						
Description of Activity	Maltmas farm (Wisbech) – Silicon nutrients (Sirius) application a) fertigation system b) Spray bottles (Hand sprayers)					
Risk Level for this Activity	LOW					
Location of Activity	Maltmas farm, Wisbech, Friday Bridge, Cambridgeshire, UK					
Product Name	Composition	Quantity	Concentration	CAS Number	UN Number	
Strawberry Leaves	N/A	160 - 200 leaves per sample date	-	-	-	
Silicon nutrient (Sirius) "Si(OH)4"	N/A	3mls (2.5mls) of silicon nutrients in 1.25L spray bottles	0.25%	-	-	
Silicon nutrients (Sirius) "Si(OH)4"	N/A	Silicon nutrients twice weekly via fertigation system	0.017%	-	-	
Schedule 5 CTSA?		Explosive regulations 2014?		Chemical Weapons Convention?		Drug Precursors?

Hazard Classification *(tick as appropriate)*

Please complete appendix if using more than 3 chemicals or dealing with any flammable, oxidising or explosive substance

			extremely flammable				Highly flammable
			Flammable				Explosive
			Dangerous to the environment				Corrosive
			Acutely Toxic				Toxic
		✔	Irritant				Harmful
			Sensitising, mutagenic, carcinogenic, allergenic, etc.				Oxidising
			Biohazard				Radioactive

		Extremely temperature	Low			Other	

Route(s) of Entry *(tick as appropriate)*

Skin Contact		Inhalation	✔	Eye Contact	✔	Ingestion	✔	Other *(specify)*	

Use of Substance(s)

How should the substance be used?	Silicon nutrients (Sirius) used on the farm are; - Hand sprayed by students or researchers - Applied through the fertigation system by growers.
Who will be exposed to the substance?	Students, researchers and growers
How much is used during the activity?	**Silicon nutrients (Sirius) application** - Silicon nutrients (Sirius) in fertigation system (N/A) - Silicon sprays (Sirius) - 1.25L C.
Duration exposed to the substance(s)?	**Silicon nutrients (Sirius) application** - Silicon nutrients in fertigation system – 30 minutes - Silicon sprays (Sirius) - 30 minutes – 1 hour D.
What are the consequences of exposure?	See page 3-4
Does the substance pose additional risks to certain individuals *(e.g. expectant mothers)*	Immunocompromised Individuals or individuals with predisposing factors; e.g. pregnant, long-term antibacterial antibiotics treatment, organ transplant patients, etc. from micro-organisms and chemicals.
Will the substance(s) or composition change during the activity and have these changes been considered during this assessment?	No.

Control Measures

	Yes	No
Does the Substance(s) require authorisation to retain? (i.e. is it used very infrequently or does age pose additional risks)		✔
Can a less hazardous substance be used to do the same job? *(if you do not know, please contact your supplier for additional information) [use the hierarchy of controls]*		✔

What controls are required for this substance, other than Personal Protective Equipment (PPE)? *(E.g. well ventilated areas, not in spray/mist form, local exhaust ventilation, authorised persons only)*

Authorised persons only (i.e. researcher and laboratory technicians. Do not use excess volumes of chemicals; replace chemicals back into appropriate storage once completed work. Don't leave large quantities of leaves out on bench.

Is any Personal Protective Equipment required, when using this substance?						Yes	No
						✓	

	Hearing protection *(State type required)*		Gloves *(State type required)*		Overalls/clothing *(State type required)*
	N/A		Latex/ Nitrile Gloves		Laboratory Coat
	Mask/respirator *(State type required)*		Other *(State type required)*		Eye Protection *(State type required)*
	N/A		No Open Shoes		Protective glasses, when handling chemicals

Are any PPE combinations contra indicated?	N/A

Other Precautions and Emergency Procedures

Handling & Storage & Transport	- All chemicals listed are segregated and stored away from combustible materials in the laboratory. - Use correct PPE, when handling chemical substances. - Carefully carry chemicals, with lids on, don't carry more than can safely manage.

Have persons undertaking this activity and using this substance(s) been provided with information and training in its use? *(Ensure that the findings in this risk assessment are communicated to all involved with this activity and that they are aware of the required controls).*		Yes	No
		✓	

First Aid Measures	**Sirius (Silicon nutrient):** In contact with eyes: Rinse immediately with plenty of water for 15 minutes holding the eyelids open. Seek medical attention. • If inhaled: Move the exposed person to fresh air. Seek medical attention. • If ingested: Do not induce vomiting. Rinse mouth thoroughly. Drink 1 to 2 glasses of water. • Signs and symptoms of exposure: Irritating eyes, respiratory tract, nausea. If irritation or symptoms persist, seek medical advice.
Fire Fighting Measures	**Sirius (Silicon nutrient)** - Product is not flammable. - Use appropriate media for adjacent fire. - Use flooding quantities of water to cool containers, keep away from common metals.

	Emergency procedure in case of fire: Sound alarm, evacuate, leave all belongings, taking nearest exit, don't return to the building until instructed to do so.
Accidental Release Measures	**Liquid Spill:** Sirius (Silicon nutrient) – Absorb with inert, absorbent material. Transfer to suitable, labelled container. Clean spillage area thoroughly with plenty of water.
Disposal	**Liquids**: Sirius (Silicon nutrient) – dispose of as special waste in compliance with local and national regulations. Empty containers can be cleaned with water or deionized water and sent for disposal or recycling.

		Yes	No
Health Surveillance required?			✓

Workplace Exposure Limits Ref: EH40			
Is/are the substance(s) used subject to the referenced in the latest edition of the HSE's EH40 document? (If yes, then please refer to the UH Workplace Exposure Limits Guidance and complete Appendix)		Yes	No
			✓

DSEAR Risk Assessment			
Is/are the substance(s) used subject to the Dangerous Substances & Explosive Atmospheres Regulations? (If yes, then please complete Appendix)		Yes	No
			✓

Assessment of Risk			
Are all the controls detailed currently in place?		Yes	No
		✓	

If these controls are not in place or additional controls are required, please state action to be taken.
(Please note – COSHH substances must NOT be used if adequate controls measures are not in place).

Remedial Actions Required	Date for completion
N/A	

Are all Hazards to health adequately controlled with all control measures?		Yes	No
		✓	

Are there any hazards not relating to substances that need to be considered for this activity?

Hazard	What harm	Who	Controls
Use of Sharps (Scalpel, scissors)	⚠️ Cuts, self-inoculation	Researcher, Technical Staff	Use with care, don't run in the laboratory
Use of Pipettes	⚠️ Repetitive movement, self-inoculation	Researcher, Technical Staff	Use with care, if using for long period of time, take regular breaks.
Use of Glassware (inc. slides)	⚠️ If broken, cuts, self-inoculation	Researcher, Technical Staff	Handle glass with care and do not drop. If broken glass, dispose of in appropriate sharps container.
Sitting at bench or cabinet to use microscope	⚠️ Physical/ ergonomical-sitting a long time	Researchers	Take regular breaks to move and stretch
Electrical Laboratory equipment	⚠️ Electric shock and burns from contact with live parts	Researchers, Other members of the lab	Ensure the equipment is not damaged before use and is full plugged in. Ensure socket is turned off before inserting plug. Electrical equipment should be regularly PAT tested.
Slips/trips/falls	⚠️ Injury from falling over	Researchers, all members of the laboratory	Do not have loose wires and cables on the floor. Immediately clean up any spillages on the ground. Be careful on uneven ground.
Fire	⚠️ Burns	Researchers, all members of the laboratory	Follow local procedures.
Allergy to the spores	☣️ Allergic Reaction	All members of the laboratory	Don't have lots of leaves open on the workbench. Clean bench after assessment. In event of allergic reaction, seek local first aider.
Use of Spectrophotometer	⚠️ UV	Researchers/ Laboratory	Do not use with lid open

		technicians	
Temporary blinding due to levels of illumination being set too high	Damage to eyes	Researchers	Reduce light intensity immediately. Take regular breaks from using the microscope.
Eye strain and long-term ocular problems	Damage to eyes	Researcher	Take regular breaks from using the microscope.
Falling objects- microscope falling of bench	Physical- heavy object falling on person	Researcher, other members of the laboratory	Ensure microscope is away from the edge of the bench
Physical injury to head and eyes being pushed into microscope	Physical	Researcher	Be aware of positioning of microscope to not hit self against it

Appendix 75 COSHH forms (Laboratory and Glasshouse experiments)

Substance(s) information	
Description of Activity	**Laboratory experiment:** ➢ Silicon extraction from strawberry leaves from a silicon fertigation field trial. ➢ Silicon localization in strawberry plants (Strawberry leaf, petiole and root cross- section for fluorescence microscopy) **Glasshouse experiment:** ➢ Silicon deposition in strawberry plants (Cross-sections for confocal microscopy)
Risk Level for this Activity	Low
Location of Activity	**Laboratory experiments:** ➢ New science building (Room 2J013) College lane, University of Hertfordshire. **Glasshouse experiments:** mpus, Lower Hatfield road, Hertford mpus

Product Name	Composition	Quantity	Concentration	CAS Number	UN Number
Strawberry leaves	N/A	Approx. 100 per sample	N/A	N/A	N/A
Chemicals listed	N/A	Quantity listed	N/A	N/A	N/A

Schedule 5 CTSA?		Explosive regulations 2014?		Chemical Weapons Convention?		Drug Precursors?	

Hazard Classification *(tick as appropriate)*

			extremely flammable Please complete appendix 2				Highly flammable Please complete appendix 2
		✓	Flammable Please complete appendix 2				Explosive Please complete appendix 2
			Dangerous to the environment				Corrosive
			Acutely Toxic				Toxic
		✓	Irritant				Harmful

266

☒ IRRITANT	⚠	✓	Irritant	☒ HARMFUL	⚠		Harmful
☒	☣	✓	Sensitising, mutagenic, carcinogenic, allergenic, etc.	🔥	🔥		Oxidising *Please complete appendix 2*
☣	⚠		Biohazard	☢	☢		Radioactive
❄	⚠		Extremely Low temperature				Other

Route(s) of Entry *(tick as appropriate)*							
Skin Contact	✓	Inhalation	✓	Eye Contact	✓	Ingestion	✓	Other *(specify)*

Use of Substance(s)

How should the substance be used?	• Chemicals used for silicon extractions are to be pipetted and titrated • Chemicals used for silicon localization should be pipetted and stained
Who will be exposed to the substance?	Researchers and Laboratory technicians
How much is used during the activity?	**Laboratory experiment - Silicon extraction:** ➢ Dry strawberry leaves sample – 100mg (0.1mg) ➢ Sirius (Polyether modified polysioxane, Ethanol tetraethyl silicate and Alkyloxypoly ethyleneoxy ethanol) – Less than 100ml per treatment ➢ Nitric Acid – 1ml ➢ Mounting fluid + Glycerine +lactic acid – 0.0% ➢ H_2O_2 – 2mls ➢ NaOH – 2mls ➢ Boric acid – 0.075mls ➢ Ammonium molybdate – 0.25mls ➢ Tartaric acid – 0.25mls ➢ Reducing solutions (1-amino-2-mapthol-4 sulfonic acid + $NaHSO_3$ + Deionized water) – 0.25mls **Laboratory experiment – Silicon deposition:** ➢ Vectashield mounting medium (for fluorescence) – in drops per slide (One drop per slide) ➢ Lyso tracker Yellow HCK-123 (Fluorescence dye) - $1\mu M$ ➢ De-ionized water – 9mls **Glasshouse experiment:** ➢ Sirius- 0.017% and 0.25% ➢ Bug clear (for fruit and vegetables) contains 17g/L rapeseed oil – Maximum number of treatment – 3 per crop

	➢ Bug clear (for fruit and vegetables) contains 17g/L rapeseed oil – Maximum number of treatment – 3 per crop ➢ Chicken poo (Manure) – Less than a hand full per crop ➢ Tomorite (Liquid fertilizer) contains seaweed extracts – 1 Litre ➢ De-ionized water
Duration exposed to the substance(s)?	**Silicon extraction (Laboratory):** Leaves in incubator – 2 – 3 days Autoclave – 1 hour Titration – 2 hours **Silicon deposition (Laboratory):** Staining (cross-sections) – 2 hours
What are the consequences of exposure?	See page 6-7
Does the substance pose additional risks to certain individuals *(e.g. expectant mothers)*	Immune system compromised people, e.g. pregnant woman, long-term antibacterial antibiotics treatment, organ transplant patients etc. From microbiological and some chemicals.
Will the substance(s) or composition change during the activity and have these changes been considered during this assessment?	N/A

Control Measures		

	Yes	No
Does the Substance(s) require authorisation to retain? (i.e. is it used very infrequently or does age pose additional risks)		✓
Can a less hazardous substance be used to do the same job? *(if you do not know, please contact your supplier for additional information) [use the hierarchy of controls]*		✓

What controls are required for this substance, other than Personal Protective Equipment (PPE)? *(E.g. well ventilated areas, not in spray/mist form, local exhaust ventilation, authorised persons only)*

Authorised persons only (i.e the researcher and laboratory technicians)

	Yes	No
Is any Personal Protective Equipment required, when using this substance?		

	Hearing protection *(State type required)*		Gloves *(State type required)*		Overalls/clothing *(State type required)*
	N/A		**Latex gloves**		**Lab coat**

	Mask/respirator *(State type required)*		Other *(State type required)*		Eye Protection *(State type required)*
	N/A		Cover shoes or safety shoes		**Eye goggles**

Are any PPE combinations contra indicated?	N/A

Other Precautions and Emergency Procedures	

Handling & Storage & Transport	All chemicals listed are segregated and stored away from combustible materials in the laboratory.

Have persons undertaking this activity and using this substance(s) been provided with information and training in its use? *(Ensure that the findings in this risk assessment are communicated to all involved with this activity and that they are aware of the required controls).*	Yes	No
	✓	

First Aid Measures	J. **SIRUS**: In contact with eyes: Rinse immediately with plenty of water for 15minutes holding the eyelids open. Seek medical attention.

- In contact with skin: If inhaled: Move the exposed person to fresh air. Seek medical attention.

- If inhaled: Move the exposed person to fresh air. Seek medical attention.

- If ingested: Do not induce vomiting. Rinse mouth thoroughly. Drink 1 to 2 glasses of water.

- Signs and symptoms of exposure: Irritating eyes, respiratory tract, nausea. If irritation or symptoms persist, seek medical advice

K.

L. NITRIC ACID, TARTARIC ACID, BORIC ACID:

- In contact with eyes: Contact rapidly causes severe damage. Symptoms include eye burns, watering eyes. Permanent damage to cornea may result. In case of eye contact, rinse with plenty of water and seek medical attention immediately.

- In contact with skin: Severe and rapid corrosion from contact. Extent of damage depends on duration of contact. Symptoms include burning, itching, redness, inflammation and/or swelling of exposed tissues. Harmful if absorbed through skin. Immediately flush with plenty of water at least 15 minutes while removing contaminated clothing and wash using soap. Get medical attention.

	- <u>If ingested</u>: Do not induce vomiting. Severe and rapid corrosive burns of the mouth, gullet and gastrointestinal tract will result if swallowed. Wash out mouth with water and give a glass of water or milk. Get medical attention immediately. - Signs and symptoms of exposure: burning, coking, nausea, vomiting, severe pain, coughing, wheezing, laryngitis, shortness of breath and headache. - Is hospitalization required? Yes. For Nitric acid in eyes. - If ingested: Inhalation of mists can cause corrosive action on mucous membranes. Move casualty to fresh air and keep at rest. May be fatal if inhaled, may cause delayed pulmonary edema. Get medical attention. M. TRYPAN BLUE: - In contact with eyes: flushes eyes with water as a precaution. - In contact with skin: wash off with soap and plenty of water. Consult with 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 and seek urgent medical A.D. Signs and symptoms of exposure: Not thoroughly investigated (according to Sigma MSDS). Persons at special risk. All persons at potential risk. N. BUG CLEAR (Protection during/in use - Keep off skin, keep away from eyes, wash off splashes immediately. - Do not breathe spray. - Wash hands and exposed skin after use - IF SWALLOWED: Call a POISON CENTRE or doctor/physician if you feel unwell. O. Rinse mouth; - If medical advice is needed, have product container or label at hand. - Dispose of content/ content to a household waste recycling centre as hazardous waste except for empty containers, which

	can be disposed of by recycling - Contact your local council for details. **TOMORITE (Liquid fertilizer)** • If inhaled; unlikely to cause a problem • If in contact with skin; wash off with water • If in contact with eyes; irrigate with water, seek medical advice if irritation persists. • If ingested, Obtain medical advice P.
Fire Fighting Measures	Types of fire extinguisher to be used: use water spray alcohol-resistant foam, dry chemical or carbon dioxide. Nitric acid, tartaric acid and boric acid- Flammable. Use extinguishing media appropriate to the surrounding fire conditions; Sirius - Product is not flammable. Use appropriate media for adjacent fire. Use flooding quantities of water to cool containers, keep away from common metals. Emergency procedure in case of fire: Wear self-contained, approved breathing apparatus and full protective clothing, including eye protection and boots. Material can react violently with water (splattering and misting) and react with metals to produce flammable hydrogen gas.
Accidental Release Measures	**Solid spill:** Trypan blue- without raising any dust, mop up spill with tissue paper **Liquid Spill:** Sirius – Absorb with inert, absorbent material. Transfer to suitable, labelled container. Clean spillage area thoroughly with plenty of water. **Nitric acid**- Cautiously add water to spill, taking care to avoid splashing and splattering. Neutralize diluted spill with soda ash or lime. Absorb neutralized spill with vermiculite or other inert absorbent material, then place in a suitable container for disposal. Clean surfaces thoroughly with water to remove residual contamination **Trypan blue**- absorb spill into tissue.
Disposal	**Solids**: **Strawberry leaves** – Segregate and collect into sealed bin bags for plants disposal.

	Liquids: Sirius – dispose of as special waste in compliance with local and national regulations. Empty containers can be cleaned with water or deionized water and sent for disposal or recycling. **Nitric acid, Tartaric acid, Boric acid**- Carefully dilute with water, neutralize spill. Neutralized material may be flushed to sewer or disposed of through a licensed contractor. Users should review their operations in terms of the applicable federal/nation or local regulators and consult with appropriate regulatory agencies before discharging or disposing of waste materials. Containers, if thoroughly cleaned, preferably by rinsing three times and handling the rinse water as waste residues, may be disposed of or recycled as non-hazardous waste. Users should review their operations in terms of the applicable federal/national or local regulations and consult with appropriate regulatory agencies before discharging or disposing at waste material. **Trypan Blue**- Put in a hazardous waste bag and send out via yellow bin disposal system **Solvents:** Biohazard: Autoclaves microbiological wastes. As long as they do not contain toxic materials.

		Yes	No
Health Surveillance required?	Does this substance(s) require any user health surveillance?, if yes, please state, which type & level, and, who it is conducted by:		✓

Workplace Exposure Limits Ref: EH40

	Yes	No
Is/are the substance(s) used subject to the referenced in the latest edition of the HSE's EH40 document? (If yes, then please see workplace exposure limits guidance note)		✓

DSEAR Risk Assessment

	Yes	No
Is/are the substance(s) used subject to the Dangerous Substances & Explosive Atmospheres Regulations? (If yes, then please complete Appendix 2)		✓

Assessment of Risk

	Yes	No
Are all the controls detailed currently in place?	✓	

If these controls are not in place or additional controls are required, please state action to be taken.
(Please note – COSHH substances must NOT be used if adequate controls measures are not in place).

Remedial Actions Required	Date for completion
Please state any further controls that have not been outlined in the assessment that may be required?	

	Yes	No
Are all Hazards to health adequately controlled with all control measures?		

		✓	

Are there any hazards not relating to substances that need to be considered for this activity?

Hazard	What harm	Who	Controls
Sirius (Polyether modified polysioxane, Ethanol tetraethyl silicate and Alkyloxypoly ethyleneoxy ethanol)	Is an irritant and may cause damage eyes if in contact.	Researchers	Q. R. S. <u>In contact with eyes</u>: Rinse immediately with plenty of water for 15minutes holding the eyelids open. T. Seek medical attention.
Nitric acid, Boric acid, Ammonium molybdate, Tartaric acid and reducing solutions (1-amino-2-mapthol-4 sulfonic acid)	Contact with combustible material may cause fire and severe burns. <u>In contact with eyes</u> Contact rapidly causes severe damage. Symptoms include eye burns, watering eyes. Permanent damage to cornea may result. <u>In contact with skin:</u> Severe and rapid corrosion from contact. Extent of damage depends on duration of contact. Symptoms include burning, itching, redness, inflammation and/or swelling of exposed tissues. Harmful if absorbed through skin.	Researchers	<u>In contact with eyes</u>: In case of eye contact, rinse with plenty of water and seek medical attention immediately. <u>In contact with skin</u>: Immediately flush with plenty of water at least 15 minutes while removing contaminated clothing and wash using soap. Get medical attention.
Reducing solutions (1-amino-2-	Irritating eyes, respiratory tract, nausea. If irritation or symptoms persist, seek medical advice	Researcher	<u>If inhaled</u>: Move the exposed person to fresh air. Seek medical attention.

mapthol-4 sulfonic acid + NaHSO₃)			If ingested: Do not induce vomiting. Rinse mouth thoroughly. Drink 1 to 2 glasses of water.
Bug clear	Is an irritant and may cause damage eyes if in contact.	Researcher	In contact with eyes: Rinse immediately with plenty of water for 15minutes holding the eyelids open.
Vectashield mounting medium (for fluorescence)	This product has been classified as non-hazardous based on the physical and/or chemical nature and/or concentration of ingredients. (Non-hazardous substance or mixture)	Researcher	Inhalation: Remove to fresh air. Skin contact: Wash thoroughly with soap and water. Eye contact: Flush eyes with water as a precaution. Ingestion: Do not induce vomiting. Wash out mouth with water.
TOMORITE - (Liquid fertilizer)	This product has been classified as non-hazardous based on the physical and/or chemical nature and/or concentration of ingredients. (Non-hazardous substance or mixture)	Researcher	If inhaled; unlikely to cause a problem If in contact with skin; wash off with water If in contact with eyes; irrigate with water, seek medical advice if irritation persists. If ingested, Obtain medical advice
Trypan blue	Irritating eyes, respiratory tract, nausea. If irritation or symptoms persist, seek medical advice	Researcher	If inhaled: Move the exposed person to fresh air. Seek medical attention. If ingested: Do not induce vomiting. Rinse mouth thoroughly. Drink 1 to 2 glasses of water.

Appendix 76 List of posters

Poster I

Poster II

University of Hertfordshire
UH

REDUCING EPIDEMICS OF STRAWBERRY POWDERY MILDEW USING SILICON NUTRIENT TO GIVE REDUCED SUSCEPTIBILITY TO *PODOSHPEARA APHANIS*

Authors: Asiana ,I ., Hall A.M., Davies K
University of Hertfordshire, College Lane, Hatfield AL10 9AB, UK

Introduction

❖ The most important disease of protected strawberries in the UK is powdery mildew caused by the fungus *Podosphaera aphanis* which has to be controlled by the frequent use of fungicides. (Dodgson et al., 2008).

❖ Work carried out at the University of Hertfordshire has shown that silicon nutrient (Sirius, OrionFT) is taken up more effectively through the roots of strawberries than from the foliage. Also the weekly use of a silicon nutrient in the fertigation tubes at a commercial strawberry farm resulted in reduced susceptibility to powdery mildew and some pests. The work reported here aimed to quantify the effect of the use of a silicon nutrient in reducing susceptibility to strawberry powdery mildew.

Aim

To quantify the effect of a silicon nutrient on strawberry plants resulting in reduced susceptibility to *Podosphaera aphanis*.

Materials and methods

❖ There were six treatments in this trial: untreated control (no silicon, no fungicide) silicon nutrient once a week and no fungicides; silicon nutrient twice a week and no fungicides; silicon nutrient once a week with fungicides; silicon nutrient twice a week with fungicides; fungicides with no silicon nutrient.

❖ Five beds were in each treatment. The silicon (Sirius from OrionFT) was applied through the fertigation tubes at the rate of 0.017% once or twice a week.

❖ Strawberry powdery mildew was monitored fortnightly from April to September 2016 by taking 15 leaves per bed per treatment, and assessing the disease levels using a dissection microscope. An assessment key was used to give the percentage cover of mycelium on the sampled leaves. In addition silicon was extracted from the sampled leaves using the AID (Autoclave Induced Digestion) method.

Polythene
Bed A
Bed B
Bed C
Bed D

Figure 1: North-view of the 2016 silicon field polythene tunnel (Entrance)

Polythene
Bed A
Bed B
Bed C
Bed D

Figure 2: South-view of the 2016 silicon field polythene tunnel (Far-end)

Result

The results are shown in Table 1 and demonstrate that the silicon nutrient reduces susceptibility to strawberry powdery mildew in all treatments where it is used. It also shows that the silicon nutrient alone reduces disease susceptibility when compared with the untreated control. Part of the tunnel used for this experiment was flooded after exceptional heavy rain in June 2016. The flooding resulted in high humidity for several weeks which resulted in higher than expected disease levels in some parts of the tunnel, particularly in the 'No silicon plus fungicides treatment'. The silicon extraction figures are in proportion to the doses of silicon nutrient applied through the fertigation tubes.

Figure 3: Silicon field trial epidemic curve

Discussion and Conclusion

The silicon trial controlled the disease with the application of silicon from the period of low disease pressure to the period of high disease pressure. However, if growers adopt the silicon nutrient in use with fungicides, it would encourage better crop yields, a more effective control of strawberry powdery mildew and economic benefits to farmers.

Future work

Further work will examine the use of fungicides without a tank mix of the silicon nutrient and also fungicides with a non silicon wetter. This will be compared with the use of silicon in the fertigation tubes.

Acknowledgement

Special thanks to Henry & Harriet Duncalfe, Maltmas farm for the provision of the field trial, Gidon Bahiri (OrionFT) for providing Sirius for the silicon field trial and also my supervisors Dr Avice. M. Hall and Dr Keith Davies for accepting to provide me with all the support I will need all throughout my research journey.

Table 1: Area Under the Disease Progress Curve (AUDPC)and silicon extraction levels in the silicon fertigation field trial 2016

Table 1. *Area Under the Disease Progress Curve (AUDPC) and silicon extraction levels in the silicon fertigation field trial 2016*

Treatments	AUDPC	Silicon Extractions
Untreated – no fungicide, no silicon	3,423	0.36 mg L^{-1}
No silicon nutrient + fungicides	2,825	0.32 mg L^{-1}
Silicon nutrient + fungicides	732	0.51 mg L^{-1}
Silicon nutrient + no fungicides	1610	0.51 mg L^{-1}
Silicon nutrient twice + fungicides	410	0.64 mg L^{-1}
Silicon nutrient twice + no fungicides	375	0.68 mg L^{-1}

References

Dogdson J, Hall A, Parker S. 2008. Control of strawberry powdery mildew under protection (project SF 62 & SF 62a). Factsheet 17/08. spp. Stoneleigh, Warwickshire: HDC.

Poster III

University of Hertfordshire UH 25

AN INVESTIGATION INTO A SILICON LOCALIZATION IN STRAWBERRY PLANTS LINKED TO REDUCTION IN DISEASE SUSCEPTIBILITY TO *PODOSPHAERA APHANIS*

Authors: Asiana ,I ., Hall A.M., Davies K.
University of Hertfordshire, College Lane, Hatfield AL10 9AB, UK

Introduction

❖ The most important disease of protected strawberries in the UK is strawberry powdery mildew caused by *Podosphaera aphanis,* which has to be controlled by the frequent use of fungicides. (Dodgson, Hall & Jin 2016).

❖ All plants contain silicon but work carried out at the University of Hertfordshire has shown that the weekly use of a silicon nutrient in the fertigation tubes at a commercial strawberry farm results in reduced susceptibility to this disease. Silicon can only be taken up in a bioavailable form and the nutrient used in this experiment is a bioavailable form of silicon.

Aim

❖ To examine the effect of a silicon nutrient applied through the roots of strawberry plants in reducing strawberry powdery mildew.

Materials and methods

❖ The silicon fertigation field trial had 6 treatments and samples were collected every fortnightly for disease assessment. For treatments see table 2.
❖ The silicon localization experiment in the glasshouse had 12 treated and 12 untreated strawberry plants in a glasshouse. 0.017% silicon nutrient (as in the fertigation field trial) was delivered for 8 weeks into two ways;
a). Through the root application
b). Hydroponically
❖ Cross-sections of strawberry leaves, petioles and roots were stained with a fluorescence dye (Basic amine Lyso tracker yellow HCK -123), final concentration 1μM (Shetty et al.,2012).
❖ Examination of sections was conducted using a confocal microscope at x400 magnification and wavelength 450nm.

Results

Figure 1 and Table 1 shows the largest epidemic took place in the untreated and the silicon nutrient reduced the epidemic even in the absence of the fungicide. The results in figures 2-7 showed that in the leaf, silicon was found in the cuticle, epidermis, palisade layer, stomata and vascular tissue. In the petiole, the silicon was found in the epidermis and xylem and in the roots, the silicon was found in the xylem only. The fluorescence intensity (Table 2) of the cross sections was quantified and this shows that the silicon was 5 times higher in the treated plants than the untreated. In addition, the silicon fertigation field trial has shown that plants with higher levels of silicon are less susceptible to the disease (Figure 1 and Table 1).

Cuticle
Palisade layer
Lower epidermis
Stoma
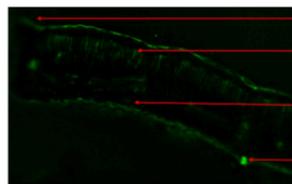
Figure 2; Strawberry leaf section with a silicon nutrient under a confocal microscope. Magnification x400.
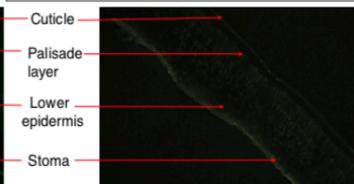
Figure 3; Strawberry leaf section without a silicon nutrient under a confocal microscope. Magnification x400.

Epidermis
Phloem
Xylem
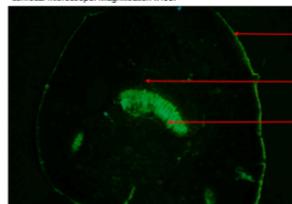
Figure 4; Strawberry petiole cross section with a silicon nutrient under a confocal microscope. Magnification x400.
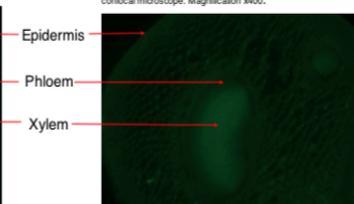
Figure 5; Strawberry petiole cross section without a silicon nutrient under a confocal microscope. Magnification x400.

Xylem
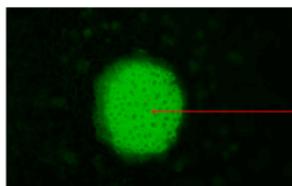
Figure 6; Strawberry root cross section with a silicon nutrient under a confocal microscope. Magnification x400.
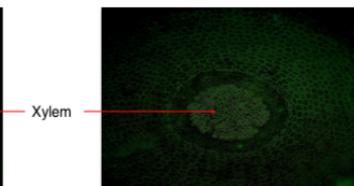
Figure 7; Strawberry root cross section without a silicon nutrient under a confocal microscope. Magnification x400.

Figure 1; Silicon fertigation field trial epidemic curve

SILICON TRIAL EPIDEMIC CURVE
Untreated — No silicon + fungicides — Silicon + fungicides
Silicon + no fungicides — Silicon twice + fungicides — Silicon twice + no fungicides
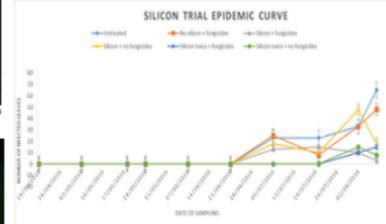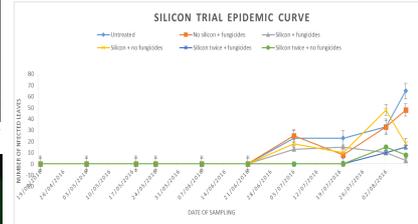
Table 1; Area under the disease progress curve (AUDPC)

Treatments	AUDPC
Untreated - no fungicides, no silicon nutrient	3,423
No silicon nutrient + fungicides	2,825
Silicon nutrient + fungicides	732
Silicon nutrient + no fungicides	1,610
Silicon nutrient twice + fungicides	410
Silicon nutrient twice without fungicides	375

Table 2; Fluorescence Integrated density

Section	Untreated Fluorescence intensity	Treated Fluorescence intensity
Leaf	2.209W/m^2	7.923W/m^2
Petiole	1.913W/m^2	7.770W/m^2
Root	1.266W/m^2	11.594W/m^2

Acknowledgement

Discussion and conclusion

In treated plants, the silicon was found in the vascular tissue throughout the plant, in the leaf deposited in the epidermis, palisade layer and stomata. Additionally there was more silicon in the treated than the untreated plants and level of silicon correlates with reduced disease susceptibility.

Future work

To compare silicon and its localization applied through a spray and applied through the roots of plants in a glasshouse, on a commercial strawberry farm and on a field plot.

References

Dogdson J, Hall A, Jin S. 2016. Control of strawberry powdery mildew under protection (project SF 62 & SF 62a). Factsheet 17/02. spp. Stoneleigh, Warwickshire: AHDB horticulture.
Shetty, R.,Jensen, B.,Shetty, N.P.,Hansen,M.,Hansen,C.W.,Starkey,K.R.&Jorgensem, H.J.L.(2012). Silicon induced resistance against powdery mildew of roses caused by *Podosphaera pannosa*. Plant Pathology.61,120-131.

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ARE STRAWBERRIES EVER DEFICIENT IN SILICON ?

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Introduction

The most important disease of protected strawberries in the UK is strawberry powdery mildew caused by *Podosphaera aphanis,* which has to be controlled by the frequent use of fungicides (Dodgson,Hall & Jin 2016).

Work carried out at the University of Hertfordshire has shown that the weekly use of silicon nutrient in the fertigation tubes at a commercial strawberry farm results in reduced susceptibility to this disease. Previous work done at the university has also shown that the use of silicon nutrient enhances the constitutive defence mechanism of the plant to infection and additional crop benefits (Jin, 2015 & Liu, 2017). Different soils have different levels of silicon and most strawberries are grown in coir which has little bioavailable silicon. This could lead to silicon deficiency in most strawberry plants.

Aim

To characterize the symptoms of silicon deficiency in strawberry plants and to evaluate what occurred when grown without silicon.

Material and methods

A hydroponic experiment was set up for 22 weeks (24 January – June 2018) in plastic tubs containing Hoagland's solution (Jones, 2016). All work was carried out in plastic (to exclude glass) See figure 1. Tubs were wrapped with black polythene bags to reduce light penetration. Bare roots Malling Centenary strawberry plants were planted in tubs with black lids. Plants were spaced apart to minimize crowding in tubs. Air supply was provided to the roots of the plants by means of aeration pumps.

Treatment was a weekly application of silicon nutrient 50mls per tub. The nutrient used was "Sirius", a bioavailable form of silicon "Si(OH)4" (Polyether modified Polysioxane, Ethanol, tetraethyl silicate and alkloxypoly Ethyleneoxy ethanol) v/v 0.25% for the treated. The untreated (control) used de-ionized water only. Additionally, they were topped up with Hoagland's solution twice weekly.

Results

Results in table 1 found that there were significantly more leaves ($P < 0.05$), significantly more runners ($P < 0.05$), significantly more fruits ($P < 0.05$), significantly higher °Brix levels ($P < 0.05$) and significantly more chlorophyll ($P < 0.05$) in 'Sirius' treated strawberries compared with the untreated. See table 1.

Additionally, there was an increase in weight, size and biomass in the treated than the untreated plants (see table 1, figure 2 and 3). Flowering was a week earlier in the treated strawberries compared to the untreated. See table 1. (Data was analysed using Analysis of Variance (ANOVA), Regression statistics and the "Unpaired" t test respectively).

Results from statistics and table 1 show a summary of additional benefits of using silicon nutrient in growing strawberries.

Table 1; Cumulative results from the hydroponic deficiency experiment January – June 2018

Strawberry plants	Untreated (no silicon nutrient) de-ionized water	Treated (silicon nutrient) v/v 0.25%
Leaves	142 (Counted weekly for 22 weeks)	195 (Counted weekly for 22 weeks)
Runners	32 (Counted weekly for 22 weeks)	48 (Counted weekly for 22 weeks)
Flowering dates	22 May 2018 (First sight of flowering)	15 May 2018 (First sight of flowering)
Number of Fruits	15 (Counted during fruiting period)	32 (Counted during fruiting period)
°Brix	13 (Measurements taken after each sample)	15 (Measurements taken after each sample)
Weight of fruit (g)	12.9 (Fruits weighed after each sample)	20.6 (Fruits weighed after each sample)
Size (centimetres)	1.18cm (Fruit size was measured after sample)	1.38cm (Fruit size was measured after sample)
Chlorophyll (µmol/m²)	909 (Measured by SPAD once weekly)	1099.86 (Measured by SPAD once weekly)
Fresh weight biomass (g)	144 (At the end of experiment)	169 (At the end of experiment)

Figure 1: Hydroponic tubs on glasshouse bench after three weeks of planting

Figure 2: First week of planting. Strawberry roots forming new roots. Old mature roots are brown and new roots are whitish in colour.

Figure 3: After three weeks of planting. Strawberry roots forming new roots. Old mature roots are brown and new roots are whitish in colour.

Acknowledgement

References

Dodgson J, Hall A, Jin S. (2016). Control of strawberry powdery mildew under protection (project SF 62 & SF 62a). Factsheet 17/02. spp. Stoneleigh, Warwickshire: AHDB horticulture.
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Jones Jr, J. B. (2016). *Hydroponics: a practical guide for the soilless grower.* CRC press.
Liu B (2017) *Sustainable strawberry production and management including control of strawberry powdery mildew.* Unpublished doctoral thesis. Hatfield: University of Hertfordshire

Discussion and conclusion

Whilst the results from the hydroponic deficiency experiment showed no deficiency symptoms, the leaves, runners, fruits and chlorophyll of silicon treated strawberries were significantly higher than the untreated. The concentration of silicon nutrient used significantly increased productivity.

The results suggests that though silicon is not an essential element, it is probably a limiting factor in strawberry productivity. It is therefore recommended that growers use silicon nutrient throughout the growing season, particularly when growing in coir.