The effect of silicon on strawberry plants and it's role in reducing infection by *Podosphaera aphanis*

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Abstract

Podosphaera aphanis which causes powdery mildew of strawberry is of economic importance in strawberry production in United Kingdom as it affects yield and fruit quality. Silicon has been shown to reduce the severity of a number of plant diseases. In strawberry, the mechanism of suppression against powdery mildew remains uncertain. Therefore, it has been suggested that supplying silicon would help strawberry plants to absorb silicon and improve resistance against the pathogen. The silicon based wetter Omex SW7 was used and three different concentrations of silicon wetter were applied on the leaves of the strawberry plants (foliar application). Each treatment was applied at three different timings. Enhanced level of silicon was quantified by the Autoclave Induced Digestion (AID) method. Whilst the main study has used the variety Elsanta, other varieties have also been used. It was observed that the weekly application (total 5 sprays) of 3 different concentrations of Omex SW7 on leaves showed significantly ($P<0.05$) higher silicon concentrations compared to application 3 times in weeks 1, 3 and 5 or twice in weeks 1 and 5. Microscopic observations showed that weekly application of standard, high and very high concentrations of Omex SW7 significantly ($P<0.05$) increased the number and length of leaf hairs on both the upper and lower surfaces of strawberry leaves. The different varieties of strawberry used showed morphological changes in the leaves with regard to the density and length of leaf hairs. Untreated leaves from Rhapsody had no hairs on the upper leaf surface, but a low density of hairs was observed after treatment with the high concentration of Omex SW7.

In this study, Omex SW7, a silicon based wetter was applied to the roots of strawberry plants (root application) and silicon accumulation and physical changes in leaves were assessed. Strawberry variety Elsanta was used in this experiment. Results revealed that weekly application (total 5 applications) of 2 different concentrations (standard and high) of Omex SW7 to roots showed significantly ($P<0.05$) higher silicon concentrations compared to application 3 times in weeks 1, 3 and 5 or twice in weeks 1 and 5. It was found that the weekly application of two different concentrations of Omex SW7 showed significantly higher ($P<0.05$) leaf hair numbers and significantly ($P<0.05$) longer leaf hair length on both the upper and lower surfaces of leaves compared to application 3 times in weeks 1, 3 and 5 or twice in weeks 1 and 5.

The effect of foliar application of silicon and potassium carbonate to limit *P. aphanis* infection was examined in a field trial under polythene tunnel at Wisbech. Treatments were arranged in a randomised block design of 3 replicates. There were a total of 18 plots. There were six treatments and all treatments were applied to the strawberry variety Sonata. Results revealed that application of silicon based wetter Omex SW7 onto the leaf surface does result in accumulation of silicon in the leaves. The application of Omex SW7 has stimulated an increase in the number and length of leaf hairs in strawberry plants. Results showed that germinating ascospores and colonies were present in all plots before the trial was sprayed. Treatments with standard and high Omex SW7 significantly ($P<0.05$) reduced the number of germinating ascospores and colonies in this trial. However, Potassium carbonate alone gave some reduction in the number of colonies and germinating ascospores. Moreover, potassium carbonate mixed with silicon based wetter Omex SW7 significantly ($P<0.05$) reduced the number of germinating ascospores and colonies.

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Chapter-1: General Introduction

1.1. Introduction

Strawberries are one of the most popular fruit in the United Kingdom (UK) and the production of strawberries has been expanding rapidly to meet rising consumer demand (Defra, 2008). During the past 18 years, total area has decreased from around 5600 hectares (1988/89) to 3800 hectares (2005/2006) (Dodgson, 2008). However during the same period, gross production has risen from approximately 43,000 tonnes to 64,000 tonnes. The use of improved varieties and production techniques helps to increase productivity and enhance fruit quality. In addition, emphasis on quality control, marketing and sales promotion has helped to increase sales of fresh fruit. Due to the high demand for these crops, methods were devised to harvest them over a longer period. Strawberry production under polythene tunnels is a method which helps to extend the strawberry harvest to 26 weeks (CALU, 2007; Dodgson, 2008).

The use of polythene tunnels, however, tends to create the environmental conditions (15^0C to 30^0C and 95% humidity, Dodgson *et al*., 2008) which are conducive to epidemics of powdery mildew (*Podosphaera aphanis*). This has had a major impact on strawberry production. The control of powdery mildew, which occurs sporadically during the growing season, is one of the greatest challenges faced by the UK growers. Powdery mildews are one of the most common diseases in many crops and fruit including wheat, barley, grape, apple, strawberry and a number of vegetables and ornamental plants. Despite extensive research on their pathogenesis, epidemiology and control, powdery mildew infections remain among the most important plant pathological problems worldwide (Belanger and Labbe, 2002). Fungicides are generally used by growers to control powdery mildew infections. Due to the adverse effect of fungicides, there is research effort to find new, non-chemical methods of control powdery mildew (Anon., 2005). This includes alternative methods such as biocontrol agents or natural products to control powdery mildews (Pertot *et al*., 2008; Kiss, 2003).
In plant pathology, the term biological control and its abbreviated synonym biocontrol refers to the suppression of diseases by the use of microbial antagonists (Pal and Gardener, 2006). In a broader sense, the term biological control means the use of the

natural products extracted or fermented from various sources. The use of biocontrol agents in reducing disease has increased dramatically within the last 10 years. These are still a small fraction in comparison with chemical fungicides which are used in field crops and also in glasshouse crops. Biocontrol agents are very sensitive to environmental conditions. Biocontrol agents are more effective in glasshouses and polytunnels because temperature and relative humidity are more easily controlled in the glasshouses and polytunnels than in the field and for this reason, more uses of biological agents are observed in glasshouses in comparison with the field (Paulitz and Belanger, 2001). There are limited numbers of biocontrol agents which are effective in reducing the strawberry powdery mildew. Most of the studies focus on the application of *Ampelomyces quisqualis* which parasitizes and kills the powdery mildew fungus (Falk *et al*., 1995). *Ampelomyces quisqualis* requires high relative humidity and sometimes paraffin oil mixed with it for achieving good disease control.

Several alternative control agents, i.e. soluble silicon, oils, salts and plant extracts have been tested against powdery mildew on different crops (Falk *et al*., 1995; Kiss, 2003, Pertot *et al*., 2008). The benefits of silicon have been well documented in plants. Although silicon has not been recognised as an essential element for higher plants, the beneficial effects have been demonstrated in many plants. These include enhanced productivity and tolerance to various biotic and abiotic stresses (Ma and Yamaji, 2006). Beneficial effects of silicon have been particularly well documented in rice and cucumber (Kim *et al*. 2002; Samuels *et al*., 1993). Silicon fertilization is now routinely applied to rice and sugarcane to enhance growth and yield (Kim *et al*., 2002; Ma *et al*., 2002). One of the most thoroughly studied beneficial effects of silicon on plant health is its role in reducing susceptibility to fungal diseases.

Besides this, the addition of silicon has also been shown to have beneficial effects on strawberry plants. Foliar applications of silicon induce metabolic changes in strawberry plants (Wang and Galletta, 1998). Wang and Galletta (1998) also reported that foliar applications of silicon increased chlorophyll and organic acid content and enhanced plant growth. In strawberry, Miyake and Takahashi (1986) in their experiment found that fruit production is related to silicon fertilization. They indicated that silicon helps to increase pollen fertility and also increases the production of fruit (Miyake and

Takahashi, 1986). The above study showed that although strawberry is not a silicon accumulator, silicon does have beneficial effects on strawberry plants. However, in strawberry, the mechanism of suppression of powdery mildew remains unclear. Therefore, enhancing the plant's natural resistance by optimizing the mineral fertilization could be a useful contribution to a sustainable control of *Podosphaera aphanis*. Silicon supply might be a tool to achieve this goal since the positive effects of silicon on plant health have been widely demonstrated.

1.2. Literature Review

1.2.1. Strawberry Plant

Strawberries are perennial, dicotyledonous, non-deciduous plants. The modern strawberry, *Fragaria x ananassa* Duchesene (family Rosaceae) is a hybrid species that dominates commercial production worldwide (Hokanson and Maas, 2001). The modern strawberry *Fragaria ananassa* resulted from a cross between *Fragaria chiloensis* (L) Duchesene and *Fragaria virginia* Duchesene. Following further hybridizations since 1850 the modern *Fragaria x ananassa* developed with the large, fragrant and tasty fruit that is common today (Maas, 1998). The genus *Fragaria* consists of approximately 20 species, with a basic chromosome number X=7. To distinguish between species, the number of pairs of these chromosomes must be determined, some strawberry plant species are diploid, meaning they have two sets of the seven chromosomes (14 total); others are tetraploid (4 pairs, 28 total), hexaploid (6 pairs, 42 total), octoploid (8 pairs, 56 total). The cultivated strawberry (*Fragaria x ananassa*) is octoploid (Marta *et al*., 2004). Classification of the strawberry plant is shown below.

Scientific Classification

Kingdom : Plantae
Class : Magnoliopsida
Order : Rosales
Family : Rosaceae
Genus : *Fragaria*
Species : *ananassa*

Classification of strawberry plant within the kingdom Plantae (Anon, 2012a).

The main stem of the strawberry plant is the much shortened stem called a crown. Strawberry plants produce leaves and flowering stems from a woody crown in a spiral arrangement (Figure 1). Each leaf has three or more leaflets depending on the variety; leaflets are oval to oblong with coarsely toothed edge (Pritts and Handley, 1998).

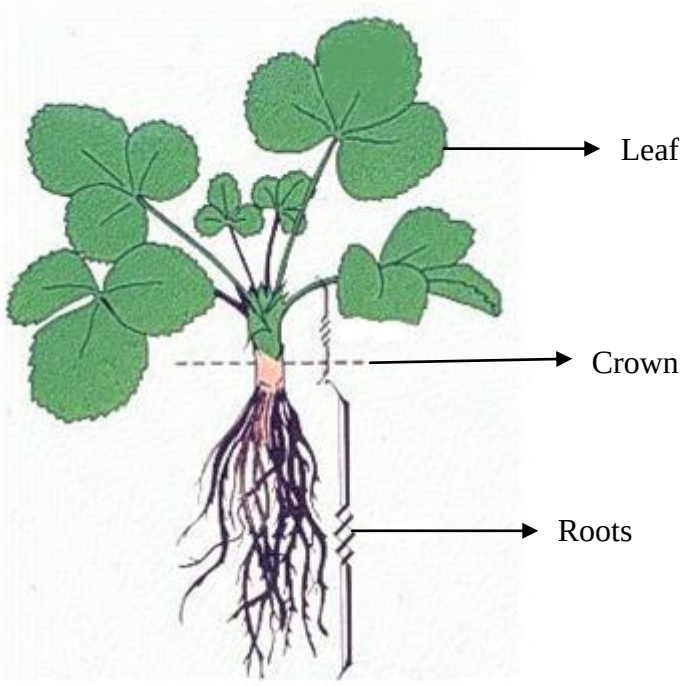


Figure 1: Diagram of a strawberry plant. Leaves, crown and roots of a strawberry plant are shown in this diagram (Source: strawberrygardenproject.blogspot.com).

Roots develop from the crown where the crown tissue comes into contact with the soil (Figure 2). They extend several inches into the soil and form numerous lateral roots, which are the primary means of taking in water and nutrients. Lateral roots usually survive one or two years. The largest concentration of roots occurs in the upper three inches of the soil. Length and number of roots formed depends upon soil conditions and plant density (Darrow, 1999).

Strawberry plants also produce stolons, known as runners (Figure 2). These runners produce roots when they come into contact with soil and then can be detached from the parent plant. Runner development is stimulated by long day lengths and warm temperatures. Therefore runners emerge mostly during the summer months. Runner plants are the primary means of propagating strawberries commercially. Runners which have rooted over the summer are dug up late in the autumn and stored in a cool

place at about 0^0C (32^0F) until spring for planting (Handley, 1998). In crop production, removal of runners is expensive and labour intensive, so cultivars that produce fewer runners are favoured.

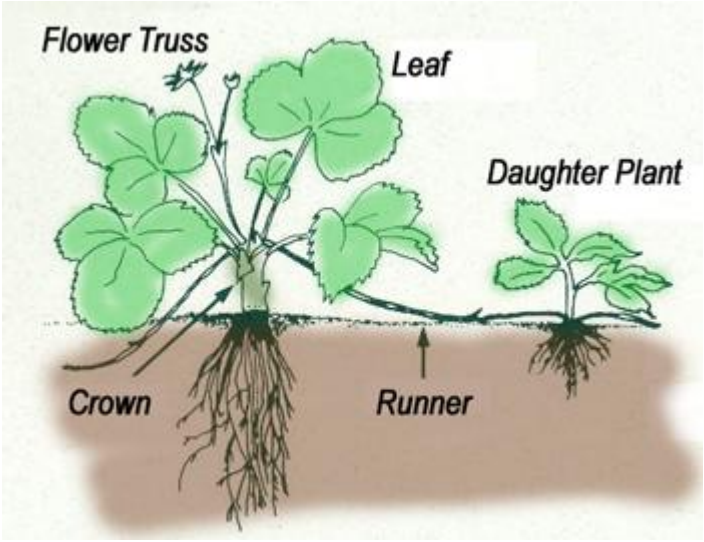


Figure 2: Diagram of a strawberry plant and daughter plant. Leaf, crown, runner and daughter plant of a strawberry plant are shown in this diagram (Source: strawberrygardenproject.blogspot.com).

The strawberry inflorescence is a modified stem called cyme terminated by a primary flower. Following the primary flower, there are typically two secondary, four tertiary and depending on the variety and time of season, eight quaternary flowers (Handley, 1998). An individual flower typically has 10 green sepals, five white petals, 20 to 30 stamens and 60 to 600 pistils (Figures 3A and B). The greatest number of pistils occurs on the primary flower and decreases successively down the inflorescence. Each pistil contains a single ovary (Figure 3A) that develops into an achene (Handley, 1998, Darnell *et al*., 2003). Achenes are the true fruits of the strawberry. Together with the receptacle they form an aggregate, which is referred to as a berry (Figure 3A), but it is not a true berry in the botanical sense. Development of fruit from pollination to ripeness takes from 20 to 60 days, depending on the cultivar and weather conditions. While all strawberry plants share common characteristics, they are also extremely variable according to variety and the environment in which they are grown.

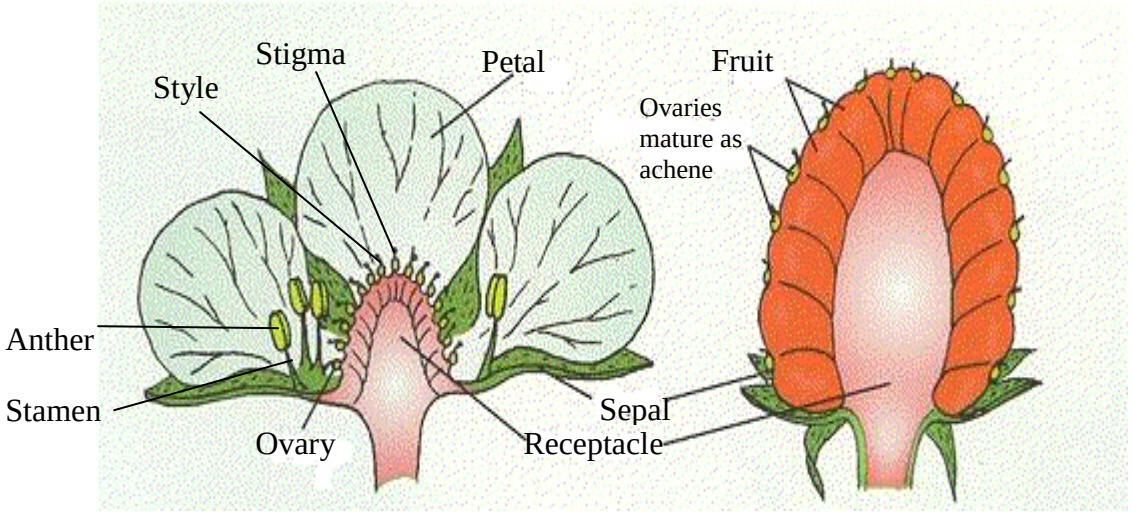


Figure 3A: Different parts of a strawberry flower are shown on the left and on the right a strawberry fruit showing the swollen end of the flower stalk which becomes red and fleshy and bears the ripened pistils over its surface (Source www.tripod.com).

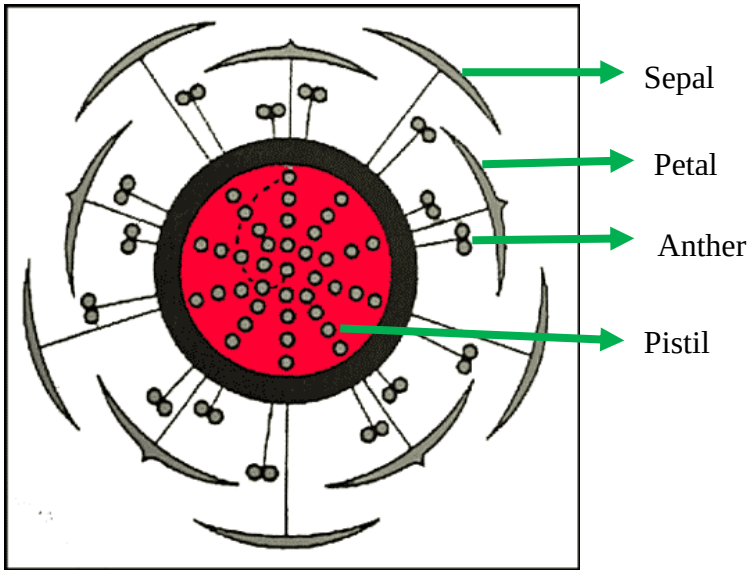


Figure 3B: Floral diagram for strawberry (Source www.the wild classroom.com)

1.2.2. Polythene tunnel production of strawberries

Growing strawberries under polythene tunnels is one of the success stories of the British soft fruit industry (Figure 4). In the UK, the growers started to use polythene tunnels in 1993 (Fletcher, 2006; Anon., 2012b). Strawberries and raspberries, British-grown soft fruit, have become an important and successful rural business. In the last four years sales of home grown berries have increased 130% in the UK supermarket (Anon., 2012b). The plastic structures (polythene tunnels) are not permanent, so they

can be moved to cover the crop as required. Polythene tunnels were developed from similar designs used by farmers in Spain to protect their winter salad crops.

More than fourteen years ago it was very difficult to grow strawberries in Britain due to the unpredictable weather conditions and disease. Summer rain not only prevents harvesting, but also produces poor quality berries. Most were used for the production of jam and other fruit products most notably because the berries were not of high quality. The farmers of Spain, France and America used polythene tunnels and as a result they produced high quality fruit and dominated the UK market with imports. Since the use of polythene tunnels in the UK the level of imports has dropped. Currently 80% of strawberries sold in supermarkets are produced under polythene tunnels in Britain (Anon., 2012b).

Figure 4: Strawberry plants under polythene tunnel.

The advantages of using tunnels are:

* The season of strawberry harvest can be extended from 6 weeks (June and July) to 26 weeks (May to November) (CALU, 2007; Dodgson, 2008). Prior to the introduction of polythene tunnels in the UK, British strawberries were only available for the short 6 weeks season in June and July. The use of polythene tunnels has enabled British soft fruit to be successfully grown from early May to November. This has dramatically reduced the amount of imported soft fruit from Spain, France and America.

* Production of class 1 grade (supermarket saleable fruit) can be improved from 55-70% to 80-90% (CALU, 2007; Phillips and Reid, 2008). The use of polythene tunnels protects the fruit from rain damage and provides temperature more conducive to strawberry production than external temperatures, at the start and end of the season. In the UK, 50% of the soft fruit yield was grade 1 fruit before the use of polythene tunnel; now it is nearer 90% (Anon., 2012b).

* Reduction in the use of pesticides by up to 50% against *Botrytis*, Downy mildew and Black spot (CALU, 2007; Anon., 2012b). Soft fruit's biggest enemy is *Botrytis* and due to the infection of the fruit by *Botrytis cinerea* yield was significantly reduced. Under tunnels, the fruit are kept dry which significantly reduces the moisture related diseases such as *Botrytis*, Downy mildew and Black spot. Thus the polythene tunnel protects the fruit from moisture which reduces the need to spray with chemicals.

1.2.3. Strawberry cultivation methods

The extension of British strawberry production by the use of polythene tunnels needs different varieties and cultivation methods to produce fruit at different times of the season.

1.2.3.1. Varieties

Strawberry varieties are grouped according to their harvesting periods. Strawberry varieties are divided into June-bearers, Everbearers and Day-neutral types. June-bearer strawberry varieties initiate flowers under short day conditions and are tremendously popular and common. Most June-bearing strawberry varieties produce the largest strawberries around the month of June. Everbearing strawberry varieties initiate flowers under long day conditions and generally produce fruit twice a year, once in the spring and again in the autumn. In general, there are fewer runners in ever bearing strawberry varieties in comparison with June-bearing varieties (Anon., 2012a). Everbearer varieties are grown commercially under Spanish tunnels in Scotland but are much more difficult to grow than June-bearers.

Day-neutral strawberry varieties produce fruit regardless of day length and produce a good yield in the first year they are planted. In general day-neutral varieties produce smaller strawberries than do the June-bearing and everbearing varieties (Hancock and Handley, 1998). Generally growers have several early, mid season and everbearer fields. They are managed so that fruit production by the farm continues throughout the season (Dodgson, 2008). The strawberry growing season is broken down into 3 main cropping periods, which are shown schematically in Figure 5. This shows the period when each crop is covered and subsequently picked. The early crop is often a second season crop that has over wintered in the ground. The main crop is planted at the start of the season (March to early May) and forced to produce fruit (a 60 day crop). June-bearer varieties and day-neutral varieties are used to produce the early crop and main crop. The late crop is produced using special everbearer varieties which produce fruit over a longer period of time (Dodgson, 2008).

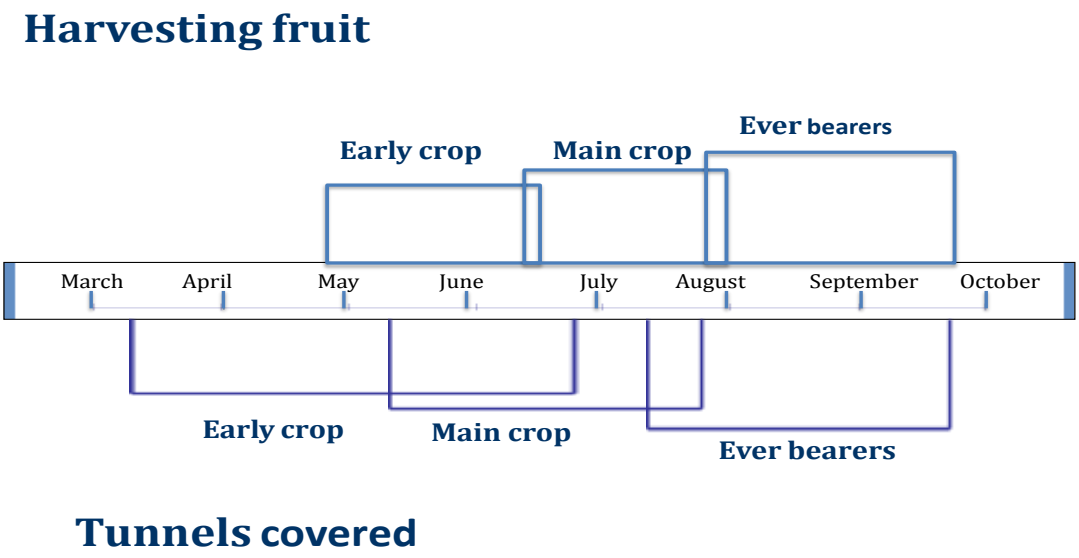


Figure 5: Diagram shows the relationship between harvesting and crop cover use polythene tunnels (Source Dodgson, 2008).

1.2.3.2. Planting

Commercial strawberries can be grown on the flat or in raised beds (Figure 6A). The raised bed is covered with a plastic mulch, to prevent weeds growing and help protect the ripe fruit (Figures 6A and B). The irrigation system is placed under the mulch (Figure 6A). Alternatively, strawberries can be grown in containers, troughs or bags filled with potting mix on the top of the ground (Figure 6C). The troughs can be either on the ground or placed on raised platforms to ease picking. Raised beds and troughs require more irrigation but reduce the risk of soil borne disease considerably. Strawberry plants can be planted any time from February through to July. When strawberry plants are planted in the summer, sometimes they need to be misted by over head sprinklers, in addition to the irrigation system within the strawberry bed (Figure 6B).The strawberry plants for commercial production are produced by specialist growers referred to as propagators (Dodgson, 2008). They bulk up the numbers of strawberry runners until there are enough to be harvested and sold to the growers.

All the plants produced by propagators are grown in open fields. Propagation of strawberry plants is highly regulated as the asexual development of new plants could lead to the transmission of viruses to the daughter plants. Usually the propagation of strawberry plants and the production of commercial fruit are carried out at completely different locations (Dodgson, 2008). When harvested, runners are graded for size then stored at 0^0C over the winter in a cold store before being planted the following season by strawberry growers. The plant health propagation scheme (PHPS) provides healthy planting material to growers. Nuclear Stock association provides a scheme that reduces the risk of viruses being passed to growers via plants (Anon., 2006).

Figure 6A: Preparing raised bed for planting. Irrigation pipe under the raised bed (as indicated by arrow).

Figure 6B: Planting in raised bed. Temporary irrigation pipe used to mist plant (when planted in summer) in addition to the irrigation system. This is only done for a maximum of two to three weeks after planting.

Figure 6C: Plants grown in bags filled with peat placed on raised bed.

1.2.3.3. Spacing

Newly received runners should be planted as soon as possible in raised beds (Figure 6B) or troughs. Runners should be planted with the crown of the plant just level with the soil surface. Runners should be planted approximately 35-45 cm apart within rows and 65-75 cm between rows. It is important not to leave the roots exposed or to bury the crowns (Lewis, 2006). Firm the soil gently round plants with heel or knuckles.

1.2.3.4. Watering/Irrigation

After planting, strawberry plants should be watered regularly to encourage root development. Watering may need to be continued under dry conditions (CALU, 2007). Water is always delivered through irrigation pipes. When the weather gets cool and the plants are transpiring less, irrigation frequency will be reduced. Once fruit set has occurred, it is essential not to over water strawberries. Use of excess water may encourage the development of grey mould (*Botrytis cinerea*) which results in fruit rotting.

1.2.3.5. Continuing care

In spring, as growth begins, dead leaves, aphids, and other pests in the crown and elsewhere can create problems for fruit production. For this reason, growers remove dead leaves regularly. To reduce disease and pests, growers generally use recommended pesticides. From June onwards plants will begin to produce rapidly extending runners. These runners will produce more fruit but of generally smaller size. It may also increase the likelihood of disease. For this reason, all runners are removed regularly unless used to fill gaps in the rows (Anon., 2012g).

1.2.3.6. Polythene tunnel management

The conditions inside polythene tunnels need to be carefully managed, to optimize the fruit production of the plants (Dodgson, 2008; Rowley and Drost, 2010). Soft, white, non woven, UV established polypropylene material permeable to air and water called horticultural fleece is sometimes used to cover the field. Horticultural fleece protects the plants from late frost and encourages the plants to produce flowers. The most important point about using tunnels is to protect the fruit and flowers from frost injury in the early and late parts of the year. The second important point is to keep the temperature in the tunnel warm enough for the plants to continue growing (Rowley and Drost, 2010). Temperatures in tunnels can become too hot in day time, especially when the sun is shining. In order to avoid high temperature, tunnels can be vented by pushing the polythene sheets up the sides of the tunnels (Figure 7A).

On the other hand when the temperature is cool or during the night the sheets can be lowered to keep warm air inside the tunnels (Figure 7B). A maximum and minimum thermometer is a valuable tool to help growers determine when to vent and when to close their tunnels (Figure 7C). At the start of the season tunnels are only vented when necessary and the sheets are lowered at night. During this time the tunnels are fitted with doors and plastic sheet at the edge where the polythene sheet does not reach the ground, so that the tunnel is completely enclosed (Dodgson, 2008). When the weather gets hotter these can be removed. As the season progresses to summer the sheets are

fixed in the open position until the conditions cool down again towards the end of the season.

Figure 7A: Tunnel with the polythene sheet pulled down (as indicated by the arrows)

Figure 7B: Tunnel with the polythene sheet pushed up (as indicated by the arrow). Plants grown in raised bed.

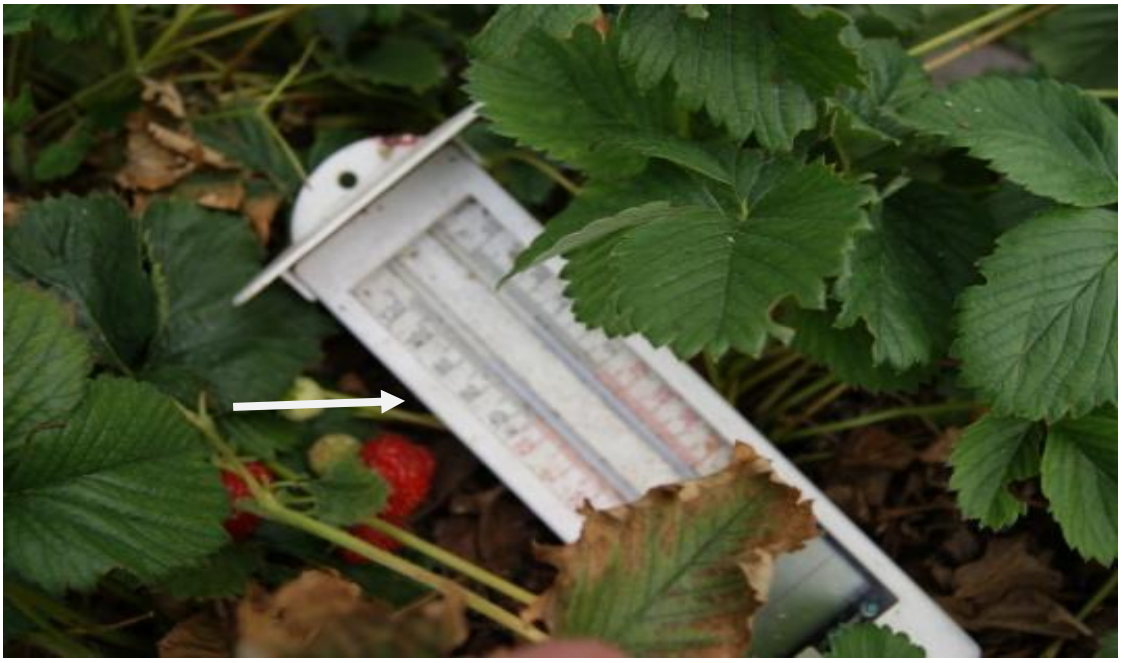


Figure 7C: Maximum and minimum thermometer (indicated by arrow) in the raised bed, under polythene tunnel used by growers.

1.2.3.7. Fertilizer

Plant nutrition is best accomplished through fertigation or applying fertilizer in the irrigation water. Fertigation will help growers to apply fertilizers in small amounts every time plants are watered. Fertilizer rates vary among soil types according to nutrient holding capacity (Rowley and Drost, 2010). The optimal pH range for strawberries is 5.8-6.5. Granular fertilizer (N-P-K) can be broadcast over each raised bed prior to laying plastic mulch. Although, the growers choose not to apply fertilizer this way, they can apply a soluble fertilizer through the irrigation system (Lewis, 2006). It is very important not to apply too much nitrogen to strawberry plants. Excessive nitrogen will encourage the growth of branch crowns. Too many branches in crowns significantly reduce the average fruit size in the strawberry plants (Lewis, 2006).

1.2.3.8. Pollination

Pollination is the transfer of pollen from the anthers (male floral part) to the stigma (female floral part) of a flower. Most fruit crops require pollination. Pollen from the anther adheres to the sticky surface of stigma and grows down the style and unites with the female cell in the ovary. This enables fertilization which results in development of seeds from the flower (Warmund, 2002). Strawberry flowers need to be pollinated to

produce fruit like other fruit bearing plants. In the spring, strawberry plants start to produce flowers. The new flowers mostly rely on the wind to circulate pollen among the plants. However, strawberry plants grown in the glasshouses or tunnels require bees to pollinate the plants. Honey bee hives are generally used to enhance pollination and fruit set. Honey bees which help pollinate strawberries can help increase fruit size up to 40 percent. Alternatively, Bumble bee hives (supplied by Biological control companies) may also be used. Bumble bees are more expensive than honey bees but are generally more active in cold, dull weather early in the year (Lewis, 2006).

1.2.3.9. Harvesting

Strawberries are very delicate so strawberries must be picked and handled very carefully. The fruit must be firm, well coloured and free from rot. The optimum time to harvest strawberries is the early morning when the fruits are cool (CALU, 2007). When harvested at the right time and handled properly strawberries will remain in good condition for many days.

1.2.3.10. Pests and diseases in strawberry crops

The worldwide most common diseases of strawberry plants are listed by Maas (1998) shown in Table 1 and those which are significant in UK strawberry production are also shown in this table.

Table 1: Infectious diseases of strawberry plants (worldwide and UK) caused by the different causal organisms (Maas, 1998; Anon., 2004).

Infectious diseases of strawberry plants		
Types of diseases	Number of species (Worldwide)	Number of species (UK)
Bacterial diseases (leaf)	2	1
Fungal diseases (fruit)	17	4
Fungal diseases (leaf)	20	5
Fungal diseases (root and crown)	23	3
Aphid transmitted viruses	6	1
Nepoviruses	5	1
Other viruses and virus like diseases	5	0
Leafhopper transmitted diseases	6	1
Phytoplasmas (mycoplasmas)	2	1

The two most common diseases of strawberries under polythene tunnels are powdery mildew and grey mould. Powdery mildew diseases will be described in section 1.2.4. Grey mould caused by *Botrytis cinerea* is severe in cloudy, cool and humid conditions. Grey mould is a fluffy fungal growth on the fruit or flower stalks making the fruit unmarketable. Excessive foliage from too much nitrogen fertilization can also trigger grey mould problems (Rowley and Drost, 2010).

Table 2: Name and number of different pest species affecting strawberry production in the UK (Anon., 2004).

Name of the pests	Number of species
Aphid	4
Whitefly	1
Two- spotted spider mite	2
Tarnished plant bug	1
Thrips	1
Weevil	3
Caterpillars	2
Slugs	1
Beetle	1
Nematodes	1

Table 2 shows the pests which affect strawberry production. The two most damaging pests are Two-spotted spider mites and Aphids. There are also many arthropod and mollusc pests of strawberries and a large proportion of these can act as vectors for pathogens. Adult spider mites hibernate in the winter period and in early spring move on to host plants to start laying eggs. The young nymphs and adults feed on the underside of the leaves, where cell contents have been sucked out. If not controlled, the mite population multiplies rapidly and whole leaf turns bronze. A range of different aphid species attack strawberry plants. Different species of aphids transmit a range of viruses into strawberry plants including Yellow edge and Crinkle virus which result in crinkling, stunting or yellow mottling in leaves of affected plants. Early control of aphids helps prevent population build up and the spread of sooty mould and viruses (Lewis, 2006). Identification of virus diseases that affected cultivated strawberries were first noted in the 1920s. The viruses affecting strawberries are disseminated through seed or pollen, vegetative propagation, vectors (aphids, thrips, mites, whiteflies, leafhoppers and nematodes) (Tzanetakis and Martin, 2013). In general growth and yield of

strawberry plants are not affected by a single virus infection. However infections by multiple viruses have the potential to reduce the yield. For this reason, it is important to minimise viruses in strawberry propagation through the use of certified virus free runners.

1.2.4. Strawberry powdery mildew

1.2.4.1. Classification

Strawberry powdery mildew is a fungal disease caused by the pathogen *Podosphaera aphanis*. This biotrophic fungus is in the family Erysiphaceae (Powdery mildews), a member of the class Ascomycetes (Kirk *et al.*, 2011). There are many species and genera of angiosperms that are hosts for the biotrophic parasitic powdery mildew fungi. Taxonomy and identification of the Erysiphales have traditionally been based on the teleomorph (sexual stage) and the morphology of the ascocarp and its appendages. Current taxonomic research in powdery mildews includes anamorphic (asexual stage) morphology and molecular approaches (Glawe, 2008). The morphology based taxonomy has been extensively revised on the basis of DNA sequence data. Identification of powdery mildew according to new taxonomy incorporates characteristics of the whole fungus (anamorph plus telemorph i.e holomorph) (Glawe, 2008). Powdery mildew genera are now grouped into five tribes, and some genera have been added or merged. There has been confusion about the name of the causal agent, whether strawberry and hop powdery mildew are caused by the same fungus *Sphaerotheca macularis* (Smith *et al.,* 1988). The study of Braun (2002) provides the confirmation that the causal agents of strawberry and hop powdery mildew are different. The causal pathogen of strawberry powdery mildew is *Podosphaera aphanis* and the fungus that infects hop is *Podosphaera macularis* (Braun, 2002). According to Braun (2002) *Podosphaera* and *Sphaerotheca* both produce chasmothecia containing only a single ascus. The main difference between *Podosphaera* and *Sphaerotheca* is that ascocarp appendages are dichotomously branched in *Podosphaera* and ascocarp appendages are myceloid in *Sphaerotheca* (Braun, 2002; Webster and Weber, 2007). Braun (2002) differentiates *Podosphaera* and *Sphaerotheca* on the basis of the sexual stage and the morphology of the chasmothecium. In order to reconstruct the phylogeny

of *Podosphaera* and to determine evolutionary relationships between *Podosphaera and Sphaerotheca*, molecular examinations were carried out by Saenz and Taylor (1999) and Takamatsu *et al*., (2000). Saenz and Taylor (1999) stated that *Sphaerotheca* spp. could be changed to *Podosphaera* and demonstrated that *Podosphaera* and *Sphaerotheca* do not form distinct clades. A comprehensive examination was carried out by Takamatsu *et al*., (2000) and fully confirmed the outcome of the experiments carried out by Saenz and Taylor (1999). The chasmothecia of *P. aphani*s contain a single ascus and the ascus usually contains 8 ascospores. According to Mukerji (1968) the cleistothecia (chasmothecia) of *P. aphanis* (referred to as *Sphaerotheca macularis*) are gregarious or scattered, or caespitose, 60-125 µm diameter, dark brown to black, smooth and with numerous hyphal appendages from the lower half and each contains one ascus. The classification of *P. aphanis* is provided below:

Kingdom	Fungi
Phylum	Ascomycota
Class	Ascomycetes
Order	Erysiphales
Family	Erysiphaceae
Genus	*Podosphaera*
Species	*aphanis*

Classification of *Podosphaera aphanis* within the kingdom Fungi (Webster and Weber, 2007; Kirk *et al*., 2011).

1.2.4.2. Economic loss caused by strawberry powdery mildew

Powdery mildew of strawberry is one of the most destructive diseases causing significant losses in strawberry production (Peries, 1962a; Belanger and Labbe, 2002). Serious damage to the foliage results in reduction of photosynthesis due to the dense mycelium coverage, which can lead to necrosis and eventual defoliation. When foliage infections are severe, flowers and fruit may also be infected. Powdery mildew is a polycyclic disease and thus many infection cycles occur throughout the season (Lucas, 1998). When a polycyclic pathogen completes each life cycle quickly then disease levels build up rapidly. Every species develops at different rates. For this reason, the length of each cycle depends on the species of pathogen. Peries (1962b) described that a *P. aphanis* spore takes 120 hours to germinate and form a mature colony. According to

Dodgson (2008) spores of *P. aphanis* require 144 hours to germinate and develop a mature colony. The incidence of powdery mildew infection is increasing rapidly, causing damage to fruit and leading to crop losses. The disease can result in yield losses of between 20-70% of the crop potential. At 20% losses, this could contribute to decreases in industry volume of 12,800 tonnes or market value of £25 million (Dodgson, 2008).

1.2.4.3. Symptoms of infection

Powdery mildew can be recognized easily on most varieties by the white powdery mycelial and spore growth that forms on both sides of leaves, sometimes on flowers and fruits and on shoots (Amsalem *et al.,* 2006). Symptoms of *P. aphanis* cause a progression of symptoms on the leaves and fruit. A healthy strawberry leaf is flat and green. When they are infected by the fungus *P. aphanis*, the leaves of strawberry begin to cup upwards exposing the underside of the leaf (Dodgson, 2008). If infection progresses, mycelium can be first seen on the lower leaf surface and then on the upper surface. After this stage purple reddish blotches appear on the upper surface of the leaves. When foliage infections are severe, flowers and fruit may also be infected (Blanco *et al.,* 2004; Dodgson *et al.,* 2007). Infected fruit become hard, covered by white mycelium and unmarketable. Symptoms of powdery mildew infections are shown in Figure 8.

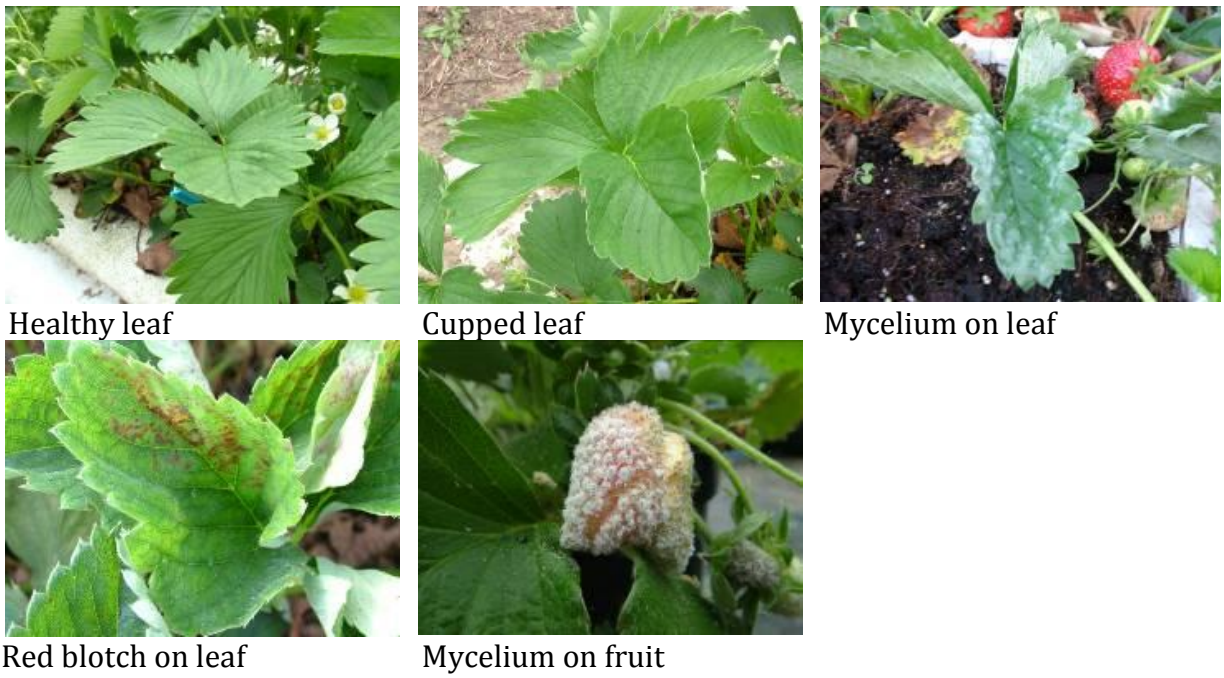


Figure 8: Symptoms of strawberry powdery mildew (modified from Dodgson, 2008).

1.2.4.4. Reproduction of powdery mildew

Powdery mildews are biotrophic parasites and they require living plant tissue to grow. Powdery mildew fungi including *Podosphaera aphanis* reproduce by means of asexual spores, known as conidiospores (conidia) or by means of sexual spores, known as ascospores. The life cycle of strawberry powdery mildew (Figure 9) is dependent on the influence of temperature and humidity on growth from spore germination through to mycelium (Figures 10C and D) development and finally to spore production. When conditions are favourable, the fungi produce asexual spores that are called conidia (Figure 10A). Conidia are genetically similar and produce in enormous numbers during the growing season, under disease conducive conditions. The rapid rate of asexual reproduction can lead to exponential growth of powdery mildew populations which can initiate epidemic. These enable a rapid build up of the epidemic. Conidia are smooth walled and barrel shaped (Figure 10B) 25 to 38 µm long and 15 to 23 µm wide with whorl-patterned ends (Mukerji, 1968; Braun *et al*., 2002).

Pathogens exploit every possible pathway to enter their host, although individual species of pathogen tend to have a preferred method. Rusts and powdery mildews are both biotrophic fungi, though they use different pathways to enter the host plants. Rusts usually enter the host plants through natural openings like stomata. Once a spore has anchored itself to the plant and the germ tube emerges, the germ tube eventually locates stomata by a specific surface sensing mechanism. This involves growing towards a ridge between the epidermal cells, followed by a perpendicular growth which ends at the stomata (Dickinson, 2003). The powdery mildews use the direct penetration pathway to enter the host (Carver *et al*., 1995). This requires adhesion of pathogen to the plant surface, followed by the application of pressure and then enzymatic degradation of the cuticle and cell wall (Nicholson and Epstein, 1991; Francis *et al*., 1996). Germination of the powdery mildew conidia leads to the development of the germ tube which extends over the leaf surface. The germ tube searches for a favourable site for penetration and the tip of the germ tube swells to form an appressorium (Kunoh *et al*., 1979; Lucas, 1998; Green *et al*., 2002). The spherical structure of the appressorium increases the attachment between the fungus and the host. The

appressorium produces a fine needle-like penetration peg, which applies huge pressure to the host cuticle and cell wall (Green *et al.*, 2002).

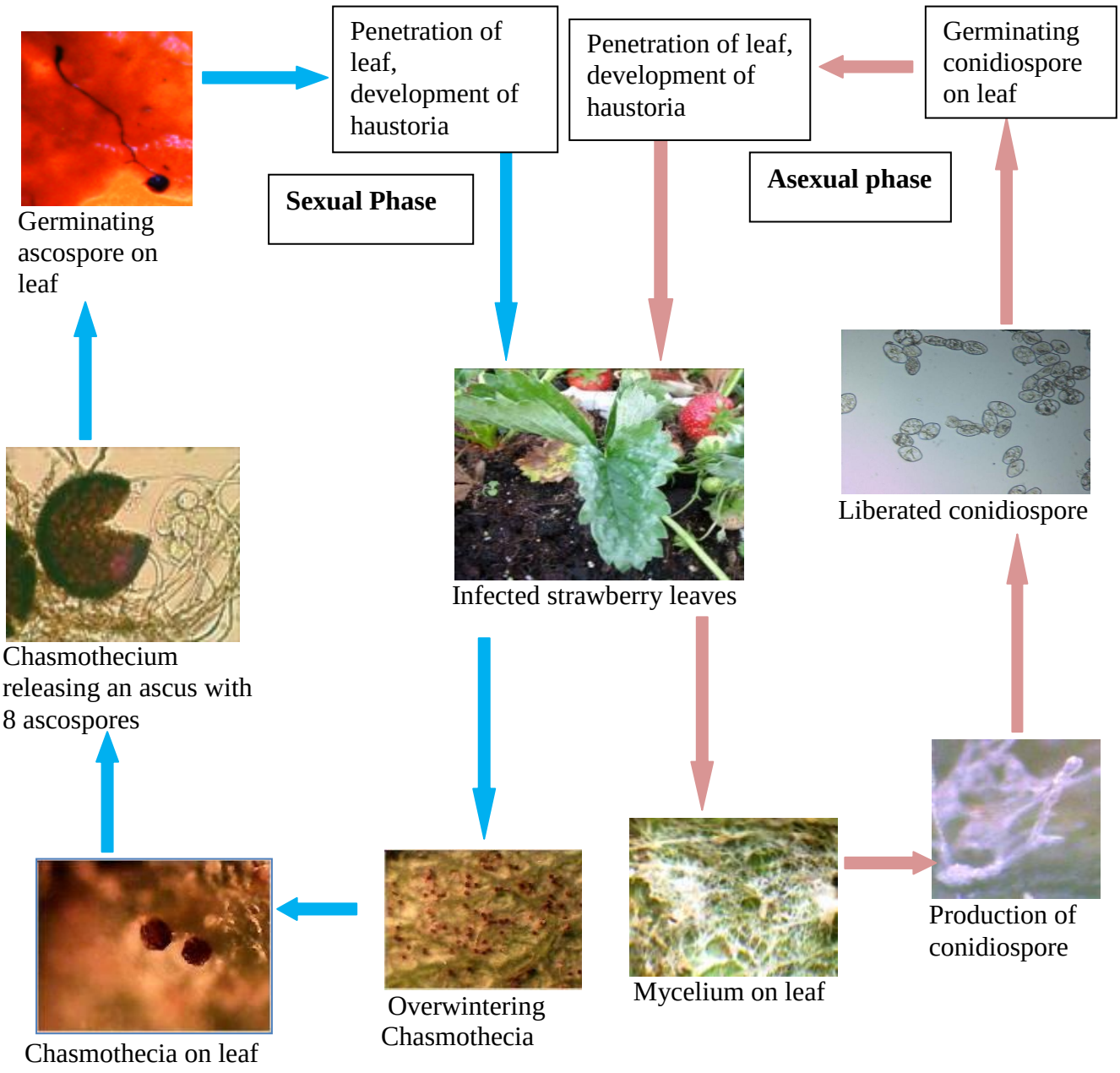


Figure 9: Life cycle of powdery mildew of strawberry caused by *Podosphaera aphanis.* Infected strawberry leaves, (Source Dodgson, 2008). Overwintering chasmothecia, (X10) (Source Fatema, 2011). Chasmothecia on Leaf (X30) (Source Fatema, 2011). Chasmothecium releasing an ascus with 8 ascospores (X400) (Source Jin, 2012). Germinating ascospore on leaf (X100) (Source Fatema, 2011). Liberated conidiospore (X400) (Source Gadoury *et al.*, 2007). Production of conidiospore (X200) (Source Jin, 2012). Mycelium on leaf (X100) (Source Fatema, 2011).

Molecular and genetic techniques showed that several fungi are known to produce cutinase an enzyme able to degrade cutin and facilitate penetration of the outer layer of the host surface by the fungus (Keon *et al*., 1987; Lucas, 1998; Green *et al*., 2002). Now it is known that combinations of enzymatic (cutinase and cellulase) and mechanical forces are required for penetration of the host (Carver *et al*., 1995; Green *et al.,* 2002). Pathogen development is influenced by temperature, humidity, nutrient availability and pH. The temperature range of germination of *P. aphanis* conidia is 15^0C to 25^0C (Peries, 1962a; Jhooty and McKeen, 1965). This is supported by Dodgson *et al*., (2008) who found that germination rate was similar over the temperature range 15^0C to 25^0C.

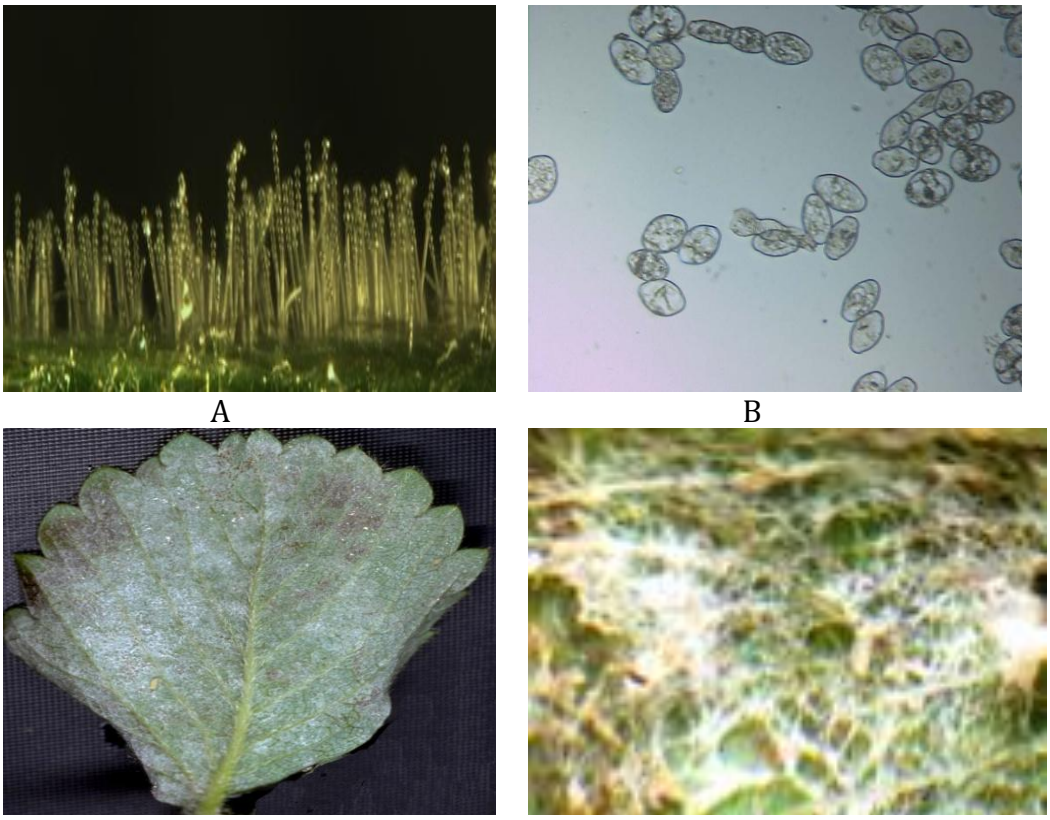


Figure 10: Different stages of asexual life cycle of *Podosphaera aphanis*. A) Conidia (X100) of *P. aphanis* born in chain. These stalk-like conidiophores give mildew colonies their powdery appearance (Source Gadoury *et al*., 2007). B) Conidia (X400) (Source Gadoury *et al*., 2007). C) Mycelium (web like growth) growth on the underside of the leaflet (X30) (Source Fatema, 2011). D) Mycelium on the underside of the leaf (X100) (Source Fatema, 2011).

Jhooty and McKeen (1965) found that maximum infection of the host occurs at 20^0C when the relative humidity is 100%. Given optimum conditions, conidia on strawberry leaves germinate 4 to 6 hours after inoculation and form indistinct appresoria (Braun *et*

al., 2002) within 12 hours (Jhooty and McKeen, 1965). All the germ tubes are fully developed after 48 hours and the development of haustoria in the host cell takes 48 hours. Conidiophores and conidia are developed between 3 to 5 days post inoculation (Peries, 1962b; Jhooty and McKeen, 1965). Conidiation is visible to the naked eye from 6 days post inoculation.

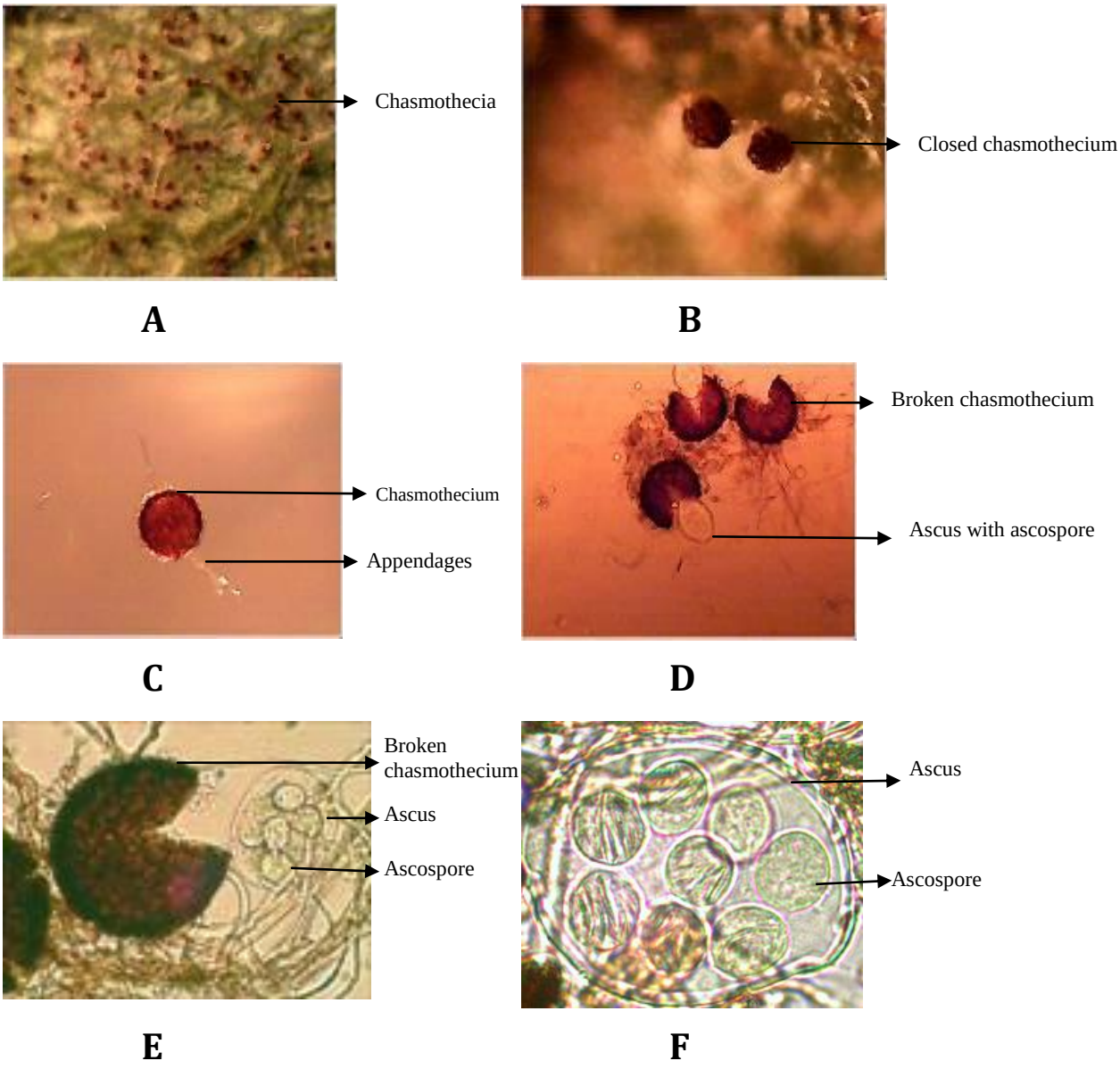


Figure 11: A) Chasmothecia on strawberry leaf (X10) (Source Fatema, 2011). B) Close up of chasmothecia on leaf (X30) (Source Fatema, 2011). C) A chasmothecium with appendages (X100) (Source Fatema, 2011). D) Broken chasmothecia each releasing an ascus (X100) (Source Ferrari, 2007). E) Broken chasmothecia releasing an ascus (X400) (Source Jin, 2012). F) Close up of an ascus containing 8 ascospores (X800) (Source Jin, 2012).

Sexual reproduction of the fungus results in the formation of ascospores which are contained in a fruiting body called chasmothecium. Initiation, development and maturation of chasmothecia followed an orderly process. According to Gadoury *et al.,* (2010) *P. aphanis* is a heterothallic fungus and is composed of two mutually exclusive mating type hypha. Asalf *et al*., (2013) confirmed that the fungus *P. aphanis* is heterothallic. However they (Asalf *et al*., 2013) stated that the initiation of chasmothecia development was not only dependent upon the presence of isolates of both mating types but they observed that initiation of chasmothecia development was suppressed at temperatures above 13^0C. For this reason they (Asalf *et al*., 2013) stated that *P. aphanis* chasmothecia development depends on heterothallism and also on temperature. Chasmothecium development is initiated when fusion occurs between hypha of two different mating types. Chasmothecia are produced in low light intensity or short days, high humidity and relatively low temperature during winter.

According to Gadoury *et al*., (2010) initiation, development of chasmothecia and maturation of germinable and infectious ascospores progressed through a lengthy process over the winter and takes about 4 weeks. Chasmothecia are nearly spherical, 60 to 120 µm in diameter, dark brown and smooth with many hyphal appendages (Figure 11C) (Mukerji, 1968). They are scattered or clumped on the leaf surface (Figures 11A and B) and each chasmothecium contains an ascus with eight ascospores (Figures 11D, E and F). Overwintered chasmothecia release ascospores at the end of the winter or early in the spring and visible mildew colonies can be observed within 7 to 10 days in the renewed plant growth in spring.

Ascospores are genetically dissimilar and they are the long term survival structure of the fungus (Moseman, 1966; Amsalem *et al*., 2006). Genetic recombination resulting from sexual reproduction can produce acospores of new genotypes that display greater virulence than parental genotypes. Ascospores are hyaline, oval, 10 to 50 µm long and 8 to 30 µm wide (Belanger and Labbe, 2002). Some researchers have concluded that they do not play an essential role in the life history of *P. aphanis* (Peries, 1962a; Maas, 1998). However, Hall *et al*., (2007) reported that in winter, chasmothecia developed and survived and ascospores initiate new infections in the spring after conducive conditions develop in the tunnels. Dodgson *et al*., (2005) strongly suggested that initial infection in

tunnels comes from disease that has over wintered in the field and not from conidiospores blown into the tunnels. Gadoury *et al.,* (2010) reported that chasmothecia of *P. aphanis* are functional survival structures and are the source of primary inoculum for strawberry powdery mildew epidemics.

1.2.4.5. Environmental requirements of the fungus *P. aphanis*

Throughout the life cycle, the fungi *P. aphanis* are affected by the environment in which they are surrounded.

1.2.4.5.1. Effect of temperature

Podosphaera aphanis has a range of temperatures or a specific temperature for optimal life cycle progression. The optimum temperature range for germination of the conidia is 18^0C to 22.5^0C described by Peries (1962a). Jhooty and McKeen (1965) observed in their study that maximum germination of conidia occurred near 20^0C. Amsalem *et al.,* (2006) described that the temperature for disease development ranges from 15^0C to 25^0C with an optimum of approximately 20^0C. However, temperatures below 10^0C and above 30^0C have been shown to have a detrimental effect on the development of strawberry powdery mildew (Peries, 1962a; Jhooty and McKeen, 1965). According to Dodgson *et al*., (2008) optimum temperature for germination and infection is 15-25^0C and optimum temperature for sporulation is 20^0C. All the conditions affecting the germination and growth of *P. aphanis* are summarised in Table 3.

The wide range of temperature tolerance indicates that the powdery mildew pathogen is able to survive from season to season. Jhooty and McKeen (1965) showed that conidia attached to mildewed leaves can survive longer than 40 days at 0^0C. During their experiment, about 1-3% of the conidia germinated at 0^0C. Schnathorst (1965) described that conidia will survive for several days when exposed to below freezing temperatures, therefore enabling the fungus to overwinter in this form.

Table 3: Summary of conditions that affect the life cycle of *Podosphaera aphanis* (data obtained from laboratory observations). (Peries, 1962a)[1], (Jhooty and McKeen, 1965)[2], (Amsalem *et al*., 2006) [3], (Dodgson, 2008)[4]

Variable		Germination	Infection	Sporulation
Temperature (O°C)	Minimum Optimum Maximum	2[1,4], 3[2], 5[3] 18-22.5[1], 15-25[2,3,4], 30-35[1,4], 38[2], 35[3]	5[1,2,4] 18-30[1,4] 30[1,2,4]	13[1,4] 20[2,4] 35[2,4]
Relative Humidity (%)	Minimum Optimum Maximum	12[1] 97[1], 100[3] 100[1,2,3,4]	No effect[1,4] No effect[1,4] No effect[1,4]	No effect[1,4] No effect[1,4] No effect[1,4]
Presence of free water (immersion time hours)	Minimum Optimum Maximum	NA[4] 0,NA[4] 24[1], NA[4]	No effect[1,4] No effect[1,4] No effect[1,4]	No effect[1,4] No effect[1,4] No effect[1,4]
Time of day (hours)	Minimum Optimum	No effect[1,4] No effect[1,4]	No effect[1,4] No effect[1,4]	20.00-8.00[1,4] 12.00-16.00[1,4]

1.2.4.5.2. Effect of humidity

Humidity, another component of the environment also has an effect on conidial germination and development. Jhooty and McKeen (1965) found that maximum development of the disease occurred at 100% RH. They also found very low levels of disease development at 18 and 8% RH. On the other hand RH levels of 70-75% and 80-85% gave a good result in conidiation. Amsalem *et al.,* (2006) also found that the production of conidia significantly decreased when the RH was above 95%. Dodgson *et al.,* (2008) reported that 97-100% relative humidity is optimum for germination of conidia. However, Peries (1962a) did not observe any effect of RH on the production of conidia in the RH range of 12-100%. Peries (1962a) also found that RH does not affect the development of the fungus after germination. Schnathorst (1965) in his review paper reported that free moisture is inhibitory to powdery mildew.

1.2.5. Control of powdery mildew

1.2.5.1. Chemical control

The vast majority of strawberry growers still use fungicides to control powdery mildew. Dodgson (2008) stated in his thesis that some growers use as many as 20 fungicides for control of just *P. aphanis* within a season. The growers apply fungicides regularly throughout the season and especially during fruit production. Table 4 lists all of those fungicides currently approved for use in strawberries, which provide some effective control of powdery mildew. The use of numerous fungicides over the decades has resulted in the development of fungicides resistance in *P. aphanis.* The occurrence of fungicide resistance has been increasing gradually since the introduction of systemic fungicides in the early 1970s.

When the growers use same mode of action of fungicides in multiple applications this can lead to fungicide resistance developing faster than if products with different modes of action are used. The increasing concerns for public health and the environment because of the use of pesticides, as well as the development of powdery mildew strains resistant to different fungicides motivate growers to seek alternative control methods (Pertot *et al*., 2008) For these reasons, a need has arisen for new and effective means of disease control that pose less risk to human health and the environment.

Table 4: Summary of fungicides currently approved for use in strawberry with recommendations for powdery mildew control (Anon., 2012i; Dodgson *et al*., 2008).

Mode of Action	Active ingredient	Trade name	Activity	Harvest Interval	Max. Number of applications/year & other information
Nucleic acid synthesis	Bupirimate	Nimrod	Protectant, Eradicant, Systemic	1day	3 applications. Outdoor and protected crops.
Respiration	Boscalid & Pyraclostrobin	Signum	Protectant, Systemic	3 days	Max total dose: 3.6kg/ha/year. Outdoor and protected crops.
	Kresoxim-methyl	Storby WG	Protectant	14 days	3 applications. Outdoor and protected crops. Do not use consecutive applications.
Signal transduction	Quinoxyfen	Fortess	Protectant, Systemic	14 days	Max total dose: 0.5 litres/ha/year. Outdoor and protected crops.
Sterol biosynthesis in membrane	Myclobutanil	Systhane20 EW Systhane 6 FLO	Protectant, Eradicant, Systemic	3 days	Max total dose: 2.7 litres/ha/year. Outdoor and protected crops.
	Penconazole	Topas Agrovista Penco	Protectant	3 days	4 applications. Outdoor and protected crops.
	Fenpropimorph	Corbel	Protectant, Eradicant, Systemic	14 days	3 applications. Outdoor crops only.
Not classified	Potassium hydrogen carbonate	Potassium bicarbonate	Eradicant	Nil	Unlimited. Max. Ind. dose: 20 g per litre. Max total dose: 60kg/ha/year. Outdoor and protected crops. Use food grade only. Adjuvants may be used.
Multi site contact activity	Sulphur	Kumulus DF	Protectant	Nil	Unlimited. Outdoor and protected crops.

1.2.5.2. Integrated control methods

Integrated pest management (IPM) is an effective and environmentally sensitive approach to control insects and other crop pests. IPM programmes use current, comprehensive information on the life cycles of pests and their interaction with the

environment. This information is used to manage pest control by the most economical means and with the least possible hazard to people, property and the environment. IPM can also be referred to as integrated control that uses a variety of complementary strategies including mechanical devices, physical devices, genetic, biological, cultural management and chemical management (Cross and Berrie, 2006). IPM can be used wherever pest damage occurs. Historically, the main focus of IPM programmes was on a horticultural insect pest. IPM programmes can be applied to diseases, weeds and other pests. According to the US Environmental Protection Agency, there are 6 basic components in IPM programmes:

1) Identify the pest
2) Understand the biology and life cycle of pests
3) Monitor pests and natural controls
4) Establish action threshold level (economic, health or aesthetics)
5) Select an appropriate combination of management practice (cultural, biological or chemical control)
6) Evaluate the programme

An integrated approach to disease control does not remove all pesticides, but it aims to reduce the frequency and the amount of pesticides used. This can be achieved in part by using alternative products where appropriate. Homma *et al.,* (1981) showed that sodium bicarbonate has an inhibitory effect on cucumber powdery mildew. The infection of powdery mildew in cucumber was significantly reduced by the foliar application of a mixture of Riboflavin and Methionine (Kang, 2008). Potassium bicarbonate has been shown to inhibit the powdery mildew of sweet red pepper and cucurbit (Fallik *et al.,* 1997, McGrath and Shishkoff, 1999). Potassium bicarbonate also has a good control effect on powdery mildew on soft fruit such as strawberry (Cross and Berrie, 2006; Dodgson, 2008). Potassium bicarbonate is an odourless, slightly basic, salty taste, white crystalline powder. It is stable under normal conditions. Potassium bicarbonate is soluble in water; however it is not soluble in alcohol. It is used as a contact fungicide in a variety of crops such as grapevine, pome and stone fruit, berries and soft fruit, vegetables and cereals (Homma *et al.,* 1981; Sawant and Sawant, 2008).

Potassium bicarbonate was found to be very effective in controlling established infection. Bicarbonate works by contact, so good leaf coverage is necessary to achieve the optimum control. Bicarbonate does not have a harvest interval, so it is an ideal product to use at the time fruit is being picked (Dodgson *et al*., 2008).

1.2.5.3. Biocontrol agents to control powdery mildew

Biological control is an alternative way to protect crops from pests and diseases including powdery mildew disease. Powdery mildew is one of the most common diseases in strawberry. UK strawberry growers are under pressure from retailers to produce high quality fruit while reducing the amount of chemicals applied to control pathogens. There are no strawberry varieties with acceptable fruit quality and resistance to other important diseases that are also resistant to powdery mildew (Pertot *et al*., 2008). Pertot *et al.,* (2008) described in their paper that beneficial arthropods are affected by fungicides, especially sulphur containing fungicides which are used to control powdery mildew. Moreover, fungicides also have a detrimental effect on the environment. All these constraints associated with the use of fungicides and resistant cultivars have led to the search for new alternatives such as, natural products or biocontrol agents (Kiss, 2003; Pertot *et al.,* 2008).

The term biological control has been used in entomology and plant pathology. In entomology, it has been used to describe the suppressing of the populations of different pest insects by the use of live predatory insects, nematodes or microbial pathogens. In plant pathology, the term applies to the use of microbial antagonists to suppress diseases. In both cases, the biological control agent (BCA) used refers to the organism that suppresses the pest or pathogen (Pal and Gardener, 2006). Moreover, the term biological control has also been applied to the use of natural products extracted or fermented from various sources. In general, the term biological control means the suppression of harmful activities of one organism by one or more other organisms. Nowadays, it is shown that suppression can be accomplished in many ways including cultural activities such as rotations and planting of disease resistant cultivars (Pal and Gardener, 2006). In plant pathology, biological control of plant pathogens is now an

established sub discipline. The amount of research in this area has increased dramatically over the last 20 years. The use of biocontrol agents in reducing diseases has also increased, but these are still small fractions that are used in the field in comparison with chemical fungicides (Belanger and Labbe, 2002).

Strawberry powdery mildew has been known in the UK for over 100 years and it is still an important disease in all parts of the world where strawberries are grown (Peries, 1962a; Maas, 1998). Powdery mildews cause significant diseases on many crops which reduce yield and causes important economic losses. For these reasons powdery mildews have received considerable attention from researchers to develop both conventional (chemical) and non conventional (biological) methods of control. Moreover, it is necessary that biological alternatives must offer special advantages to control powdery mildew worldwide. Table 5 presents a list of biological products that are known to have anti powdery mildew activity.

According to Belanger and Labbe, (2002), the non-chemical methods for powdery mildew control can be classified in two ways 1) Microorganisms, which protect plants through parasitism or antibiosis 2) Products or microorganisms which protect plants by inducing host defence mechanisms. The fungus *Ampelomyces quisqualis* has the capacity to parasitize powdery mildew pathogens (Kiss, 2003).

Ampelomyces quisqualis is now used commercially as a biocontrol agent against powdery mildews. A formulated product (AQ10) which uses *Ampelomyces quisqualis* was registered as a biocontrol agent in the late 1980's in Australia. However, the high humidity requirements of *A. quisqualis* have hampered its effectiveness (Kiss, 2003). Ecogen Inc. has developed a new formulation and use another strain of *A. quisqualis* which is effective at lower humidity. The growth of fungus is slow and it requires the presence of powdery mildew colonies for its development. For this reason, the use of *A. quisqualis* as a preventive measure makes a contradiction from an ecological point of view (Kiss, 2003; Belanger and Labbe, 2002).

Table 5: Biological products or agents with anti-powdery mildew activity (modified from Belanger and Labbe, 2002).

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

Verticillium lecanii, is a fungus and it has been reported to parasitize arthropods, rust and powdery mildew fungus. The use of fungal antagonists as a biocontrol agent is not without problems. One of the major problems is that they lose their effectiveness below 85-90% relative humidity. Another problem is that sometimes they cannot work without the addition of an additive (Belanger and Labbe, 2002). Kiss (2003) described that *V. lecanii, P. flocculosa* and *T. pallescens* work efficiently with the use of additive oils to control the powdery mildew of cucumber. AQ10 biofungicides are recommended to be used with a wetting agent. In addition, to increase the effectiveness of biocontrol agents paraffin oils are also used. Some oils alone can give better control against powdery mildew. For this reason, there is more understanding necessary to distinguish the efficiency of adjuvant and bio control agent in controlling disease. Soluble silicon has been used to protect powdery mildews on a variety of crops including monocotyledons and dicotyledons. However, it is still not clear the exact mode of action of silicon in reducing disease in plants. In monocotyledons, it was found that absorbed silicon accumulates in the leaf cells and creates a mechanical barrier against the penetration of the hyphal peg.

In dicotyledons such as cucumber, Miyake and Takahashi (1983) found that addition of soluble silicon reduces disease development. In strawberry, Wang and Galletta (1998) reported that foliar application of silicon induces metabolic changes in plants. Kanto *et al.,* (2004) in their experiment revealed that application of a silicon fertilizer reduces powdery mildew infection in strawberries. However, the exact mode of action of silicon in the reduction of powdery mildew disease in strawberry remains unclear. The control of powdery mildew disease in strawberry remains a challenge for future research and development.

1.2.6. Role of silicon in plants

Silicon (Si) is one of the most abundant elements on the surface of the earth and is also abundant in most soils (Epstein, 1999). It is readily taken up by plants and is often present in relatively high concentrations in plant tissues (Epstein, 1999). In some plants the concentration of silicon sometimes exceeds the concentrations of nitrogen and potassium (Epstein, 1994). However even taken up in high amounts it is the only

element that is not harmful for plants (Takahashi *et al*., 1990). Although it is not considered to be an essential nutrient for terrestrial plants in general (Epstein, 1999), silicon is often a major constituent of plant tissue.

Silicon has been shown to be a beneficial element for many and under certain conditions, perhaps most terrestrial plants (Epstein, 1994). Silicon has been used to prevent plant disease for centuries (Belanger *et al*., 1995). A number of studies have indicated that silicon application can reduce the severity of fungal diseases. For example, application of potassium silicate to soil reduces powdery mildew in strawberry by 86% in the first year and 60% in the second year (Kanto *et al.,* 2006). Root application of 1.7 mM silicon reduced the severity of powdery mildew disease in wheat by as much as 80% (Guevel *et al.,* 2007). The mechanism by which silicon protects plants from fungal attack is not well understood. Although silicon has for centuries been used for preventing diseases in agriculture, it's possible role in plant physiology and in disease prevention needs more understanding and more research. Other beneficial effects of silicon include increased growth in some plants and greater tolerance of environmental stresses such as cold, heat, drought, salinity, mineral toxicity or deficiency.

1.2.6.1. Silicon uptake in plants

Silicon content in plants greatly varies with species and differences in silicon content between plant species are related to differences in the silicon uptake mechanism. Three different modes of silicon uptake have been proposed for plants having different degrees of silicon accumulation, that is, active, passive and rejective uptake (Takahashi *et al.,* 1990). Plants with an active mode of uptake take up silicon faster than water and are classified as silicon accumulators. Plants with a passive mode of uptake take up silicon at a rate that is similar to the uptake rate of water and are classified as an intermediate. Plants with a rejective mode of silicon uptake take up silicon to a lower degree than water (Takahashi *et al.,* 1990). Plants take up silicon in the form of monosilicic acid (H_4SiO_4) and it accumulates in leaves and other plant tissue primarily as amorphous silicates (Epstein, 1994). Hydrated, amorphous silica is deposited in cell lumens, cell walls and intercellular spaces. It also accumulates in external layers below and above the cuticle of leaves. However, among plant species there are large

differences in the magnitude of silicon uptake, for example in rice the amount of silicon taken up from cortical cells to the xylem differs from that of cucumber and tomato. Silicon is present in roots, leaves, and the inflorescence bracts of cereals, especially in rice, wheat, oat and barley (Epstein, 1999). The uptake of silicon by plants is not well understood, but appears to be influenced by a number of soil and climatic factors.

1.2.6.2. Effects of silicon on plant growth and development

Silicon has been shown to play an important role in the development and growth of plants. Silicon enhances plant growth rates and increases yield by balancing nutrient uptake, transport and distribution. Even though from a scientific point of view silicon was not proven to be essential for higher plants, some investigators have shown the beneficial role of silicon in cucumber, tomato and strawberry plants (Epstein, 1999). Rice is considered to be a silicon accumulator and a relatively large amount of plant available silicon appears to be very important for both robust growth and fungal disease resistance of rice (Datnoff *et al*., 1997). An experiment conducted by Ma *et al*., (2002) showed that the addition of silicon had an effect on growth and development of rice plants. Adatia and Besford (1986) found that addition of silicon had an effect on cucumber leaves. They found that rigidity of mature leaves increased, the leaves had a rougher texture and were held more horizontally and the lower leaves on the high silicon plant were darker green and senescence was delayed. Epstein (1994) reported that deposition of silicon in the plant tissue decreases the transpiration rate and increases stem thickness and strength. According to Ma and Takahashi (2002) foliar application of silicon, increases accumulation of silicon in leaves, thus increases photosynthesis rates and increases yield.

1.2.6.3. Silicon and disease resistance in plants

One of the most thoroughly studied beneficial effects of silicon on plant health is its role in reducing susceptibility of some plants to fungal diseases. This effect of silicon has been particularly well documented in rice and cucumbers. Neck blast and brown spot are two major fungal diseases which have an effect of reducing rice yield in Florida (Datnoff *et al*., 1991). Datnoff *et al*., (1991) found that the amount of neck blast disease reduced 73-86% and the amount of brown spot reduced 58-75% when silicon was

added during 1987 and 1988 growing season. Remarkably, this degree of disease control was not significantly different from that achieved by fungicides such as benomyl (Datnoff *et al.,* 1997). According to Datnoff *et al*., (1991) rice yield also increased 56-88% and they concluded that it might be due to addition of silicon which helps to reduce the disease. As with rice, addition of silicon in greenhouse grown cucumber has also been shown to decrease fungal diseases (Balanger *et al*., 1995; Menzies and Belanger, 1996). Adatia and Besford (1986) found that addition of silicon has an effect on reducing the powdery mildew infection on cucumber. Menzies *et al*., (1991) investigated the effect of different rates of silicon (Potassium silicate) on powdery mildew infection. The investigators found that silicon fertilization reduced the leaf area covered by powdery mildew by as much as 98% (Menzies *et al.,* 1991). Shetty *et al*., (2012) found that rose powdery mildew caused by *Podosphaera pannosa* was significantly reduced by the application of silicon. Potassium silicate was used as the source of silicon and 100 ppm was applied to the roots of rose plants. Application of silicon increased the silicon content two to four folds compared to control plants. Silicon application reduced the disease severity by up to 48.9%.

Despite the wide range of beneficial roles of silicon in managing disease susceptibility there is a scientific debate, as to how silicon reduces disease in plants. One possible mechanism is that the application of soluble silicon accumulates in the apoplast, particularly in the epidermal cell wall. The silicon deposition in the cell wall inhibits fungal disease by physically inhibiting fungal germ tube penetration in the epidermis (Epstein, 1994; Samuels *et al.,* 1993). In cucumber, Samuels *et al.,* (1993) reported that on silicon treated plants, germinating conidia had shorter hyphae. They also showed that at the early stage of infection, the presence of silicon was correlated with a decreased fungal colony. Besides these, silicon fertilization has also been shown to decrease fungal disease susceptibility in strawberry. Kanto *et al*., (2007) examined the effect of silicon fertilization on germination of conidia of strawberry powdery mildew and also the effect of silicon on the leaf cuticle. In this experiment, silicon was added in the hydroponic solution and strawberry leaves were inoculated with powdery mildew conidia. Leaves were observed with a scanning electron microscope 1-2 days after inoculation showed that the germ tube and the length of secondary hyphae were shorter and fewer branches in comparison with the control. They (Kanto *et al*., 2007)

also reported that germination rate of powdery mildew conidia was 49.7% in silicon treated leaves and 67.2% on untreated control leaves. In strawberry Shen *et al.,* (2010) investigated the effect of potassium silicate on the growth of five soil borne fungi which slows plant growth, reduces yield and causes economic loss to the strawberry industry. Results showed that the growth of the four fungi (*Rhizoctonia solani, Pestalobiopsis clavispora, Fusarium oxysporum and Fusarium oxysporum f. sp. fragariae*) were significantly ($P<0.05$) inhibited on potassium silicate amended PDA plates. However the reduction of pH in the potassium silicate amended PDA plates was not effective to inhibit the fungus growth. In summary, investigation indicated that reduction of fungal diseases in strawberry plants is probably not due to the fungistatic effect of silicon, but rather due to other mechanisms such as silicon acting as a physical barrier against pathogen penetration or silicon induced defence response in plants.

Another mechanism through which silicon affects disease susceptibility could be the metabolic role of silicon. Belanger *et al.,* (2003) explained that in wheat exogenously supplied silicon stimulated the accumulation of phenolic compounds in infected cells and protected wheat plants from powdery mildew disease caused by *Blumeria graminis f. sp. tritici*. Microscopic and ultra structural observations have described the presence of phenolic materials associated with degraded powdery mildew haustoria in epidermal cells of silicon treated wheat leaves (Belanger *et al*., 2003). Borel *et al*., (2005) indicated that silicon treated wheat plants produce an antifungal compound in response to powdery mildew infection. The phenolic compounds are highly toxic to the pathogen and fungal hyphae are destroyed by the action of phenolic compounds. As a consequence, the pathogen failed to colonize and for this reason disease severity decreased (Epstein, 1994).

1.2.6.4. Effect of silicon on diseases caused by abiotic stresses

In addition to inhibiting fungal diseases (biotic stress) silicon has the potential to significantly decrease the diseases caused by abiotic stresses in plants (Figure 12). Chemical stresses include salt, metal toxicity, nutrient imbalance and physical stresses include lodging, drought, radiation, high temperature, freezing, UV radiation and many others (Epstein, 1999; Ma, 2004; Epstein, 2009).

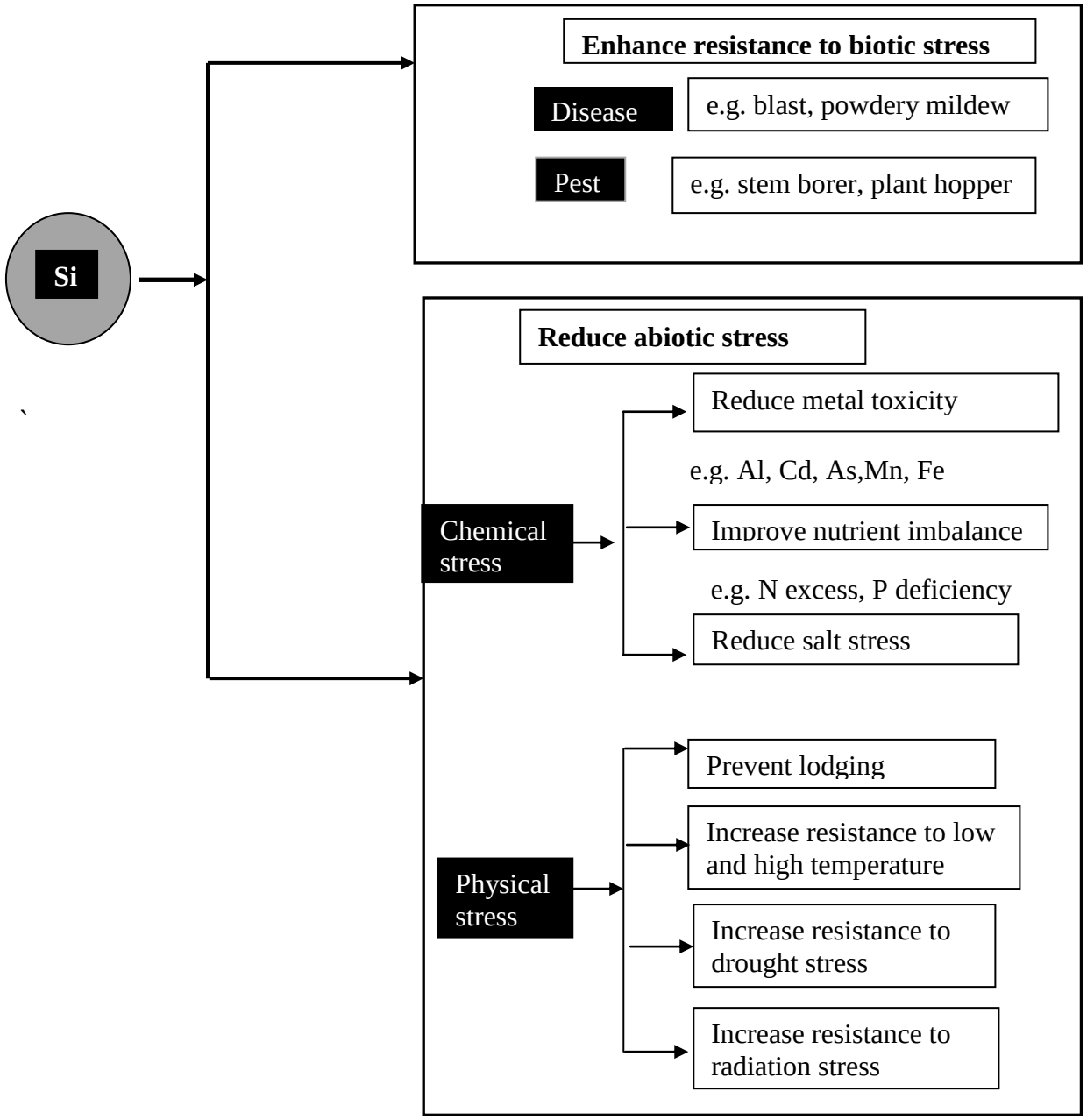


Figure 12: Beneficial effects of silicon on plant growth in relation to biotic and abiotic stresses. Modified from (Ma and Yamaji, 2006).

Most of these beneficial effects are also attributed to silicon deposition in cell walls of roots, leaves, stems, and hulls. Silicon has been shown to reduce manganese (Mn) and iron (Fe) toxicity and also has beneficial effects on aluminium (Al) toxicity (Marshner, 1995). An early and striking demonstration of the significance of silicon in plant nutrition was given by Williams and Vlamis (1957). They found that barley plants grown in standard nutrient solutions in the absence of silicon developed necrotic spots on their leaves. Necrotic spots increased when Mn concentration increased and decreased when Mn concentration lowered. Necrotic spots disappeared with the addition of silicon. It was also observed that the leaves of the silicon treated plants were healthy and green and took up more Mn than the leaves of the non silicon treated plants. The leaves of non silicon treated plants were heavily affected by necrotic spots and took up less Mn compared to the silicon treated leaves. For this reason the authors thought that addition of silicon to the solution did not reduce the Mn uptake, but led to a homogeneous distribution of Mn in the leaf blade whereas in the absence of silicon, Mn tends to be distributed non homogenously and accumulates to toxic levels in leaves which help to build up the necrotic spots on the leaves. However, the exact mechanism of homogeneous distribution of Mn by silicon has not been known yet.

According to Epstein (1999) deposition of silicon in leaves, culms and hulls enhances the strength and rigidity of cell walls and reduces transpiration from the cuticle and develops resistance to UV and drought stresses, varying temperature and lodging. The rate of transpiration of silicon deficient rice plants increased by about 30% over the rate of control plants (Yoshida *et al*., 1962). This observation suggests that silicon has a role in transpiration. The rate of transpiration is influenced by the deposition of the silicon on the cell wall. Silicon deposited in the cell wall forms a protective layer reducing transpiration through the outer cells. Silicon has been shown to reduce high transpiration caused by drought or heat stress. Water is lost faster through transpiration when the temperature is more than 90^0F. This results in harmful increase in intercellular mineral concentrations that inhibit plant function. Deposit of silicon in the cell wall reduced transpiration loss caused by high temperatures thus allowing continued metabolic functions at high temperature.

1.2.7. Leaf hairs

Trichomes and bristles generally known as leaf hairs are derived from specialized epidermal cells on leaf or stem. These hairs are produced as a result of the outward growth of one or more epidermal cells. The morphology and density of leaf hairs vary considerably among plant species and may also vary among populations and within individual plants (Werker, 2000). Trichomes serve a variety of functions such as decrease of water loss by transpiration and lower plant temperature. They also provide a defence against insects. Some trichomes have glands which release poisonous substances that trap insects and other organisms. Dalin *et al*., (2008) showed that glandular trichomes contribute to plant resistance against herbivorous animals, especially insects. The structure of trichomes can range from unicellular to multi cellular and the trichomes can be straight, spiral, hooked, branched or unbranched (Werker, 2000). Larger trichomes may play a role as a structural defence against the pathogen to prevent mycelia penetration and infection (Bonos *et al.,* 2004). Bonos *et al*., (2004) investigated dollar spot, a major disease that affects the performance of creeping bentgrasss on golf greens and fairways. In their investigation they found that resistant clones have 42 to 64% larger trichomes than susceptible clones. Therefore they stated that trichome size may play a role in resistance of creeping bentgrass to the dollar spot fungus. This study aimed to investigate the relationship between density of leaf hairs and reduction of infection by *Podosphaera aphanis*, the causal agent of strawberry powdery mildew.

1.2.8. Silicon wetter Omex SW7

Omex SW7 is a silicon based wetter, designed to give enhanced uptake of foliar nutrients. It also supplies silicon to the crop, when it is used at a higher rate. Omex SW7 is routinely used by growers to apply potassium carbonate. In order to achieve a good spread of potassium carbonate, Omex SW7 is used as a wetter in the application of potassium carbonate to reduce the infection of strawberry powdery mildew. Silicon is widely recognised as a beneficial element for many crops. Omex SW7 can be applied to a wide range of agricultural and horticultural crops, including soft fruit, field vegetables,

potatoes and ornamental and nursery plants. Both hard and soft water are suitable for making the dilution. After application to the crops, it is transported in the xylem and deposited in the epidermal cells. It acts as a physical strengthener when it forms a complex with calcium in the cell wall (Anon., 2012h).

1.2.9. Potassium carbonate (K50)

Potassium carbonate is a concentrated inorganic formulation containing potassium and carbonate. K50 is the trade name of this product. Potassium is a major nutrient, which is required by all crops. The main role of the potassium in the plant is as a water regulator. It is highly mobile and quickly distributed in the plant. Potassium carbonate (K50) is used by the growers to decrease disease incidence such as Powdery mildew and *Botrytis*. Potassium carbonate can be applied in a wide range of crops including fruit, flower and salad crops. Recommended dose for application in fruit is 300-500 ml/100l water. Potassium carbonate does not have a harvest interval, so application of potassium carbonate with Omex SW7 is an ideal product to use at the time the fruit is being picked (Anon., 2012h).

1.3. Rationale, Aims and Objectives

1.3.1 Rationale

There has been little research on the role of silicon as a nutrient supplement in strawberries. Also there has not been research on the ability of silicon to enhance disease resistance in strawberries. However anecdotal observations suggested that when Omex SW7, a silicon wetter, was used to apply potassium carbonate, a synergistic effect occurred whereby there was enhanced disease control in the presence of the silicon wetter. It is not clear how the silicon wetter reduced the infection. Moreover, it was not known if the application of Omex SW7 enhanced silicon levels in plant tissues. Trichomes (containing silicon), generally known as hairs serve several functions including protection against the attacks of herbivorous animals, especially insects. However, there is little information available about the role of hairs during the interaction with other organisms, especially with fungi.

1.3.2. Hypothesis

Application of silicon (in the form of Omex SW7) sprayed onto leaves or applied to roots would give enhanced silicon levels in strawberry plants, stimulate formation of leaf hairs and also reduce strawberry powdery mildew infection.

1.3.3. Aims and Objectives

1.3.3.1. Aim (overall aim)

To investigate the uptake of silicon through the leaves and roots of several varieties of strawberry and to study the effect of enhanced silicon levels on the density and length of leaf hairs on the leaves of strawberry plants and its role in reducing infection by *Podosphaera aphanis* causal agent of strawberry powdery mildew in the field.

1.3.3.2. Objectives

- To determine if there are enhanced levels of silicon in strawberry plants treated with silicon wetter (Omex SW7) when applied on leaves (foliar application) and to quantify the effect of applying different concentrations of silicon on the density and length of leaf hairs of the leaves of strawberry plants.
- To determine the enhanced levels of silicon in strawberry plants when applying different concentrations of Omex SW7 (silicon wetter) to the root zone (root application) and to assess the effect of applying different concentrations of silicon on the density and length of leaf hairs of the leaves of strawberry plants.
- To investigate the effects of silicon wetter with and without the use of potassium carbonate to limit the development of strawberry powdery mildew in the field.

Chapter-2: General Materials and Methods

The strawberry varieties used in the different experiments are described below.

2.1. Elsanta

Elsanta is a mid season June-bearing variety. The high production, firmness and long shelf life of the fruit make Elsanta extremely suitable for both growers and customers. Elsanta has a good flavour with a strong strawberry aroma. Elsanta's fruit has a strong skin which makes it more insensitive to damage due to pressure, thus very suitable for eating fresh. The juicy berries can be picked from mid-June through mid-July. The yield of Elsanta is high to very high and can be compared with Sonata. Elsanta is recommended for all types of cultivation, indoor as well as outdoor and from early to fairly late in the season. However, it is susceptible to some diseases such as wilt (*Verticillium dahliae*), crown rot (*Phytophthora cactorum*), powdery mildew (*Podosphaera aphanis)* and also susceptible to frost (Anon., 2012c)

2.2. Symphony

Symphony is a late season June-bearing variety. Symphony produces similar yields to Elsanta but with a higher proportion of marketable fruit. Symphony produces high quality bright red firm fruit with excellent flavour and good shelf-life. High yielding, vigorous Symphony is very good for disease resistance especially red core (*Phytophthora fragariae*) and also shows resistance to vine weevil (*Otiorhynchus sulcatus*). Symphony tolerates more extreme soil conditions due to its vigorous nature and disease resistance capabilities (Anon., 2012d).

2.3. Florence

Florence is an excellent late season June-bearing variety bred by HRI East Malling. Florence produces delicious sweet strawberries from the end of June to the end of July. The berries have a regular conical shape with firm skin and flesh and very good flavour. Florence is moderately resistant to powdery mildew and other fungal leaf diseases. The variety has also shown tolerance to vine weevil (Anon., 2012a; Anon., 2012e).

2.4. Rhapsody

Rhapsody was bred by SCRI (now James Hutton Institute) and released in 1990. The long conical fruits are medium to large, attractively glossy and with an excellent flavour.

Rhapsody is a mid to late season June-bearing variety. Rhapsody is resistant to red core and has moderate resistance to verticillium wilt and powdery mildew (Anon., 2012f).

2.5. Growing strawberry plants in the glasshouse

The different strawberry varieties Elsanta, Symphony, Rhapsody and Florence were used in different parts of this study. Elsanta was used in the pilot experiment (chapter 3) as well as in the main experiment (chapter 3). For the pilot experiment Elsanta was grown in the Hatfield glasshouse and for the main experiment plants were grown in the Bayfordbury glasshouses. Symphony, Rhapsody, Florence were used as additional varieties (chapter 3) and were grown in the Bayfordbury glasshouses. For growing June-bearing varieties 12-14 hours daylight are necessary. For this reason, if the plants were grown in the autumn, winter or spring (October to April) artificial light was used to create 12-14 hours of light during the day. During the summer (May to September) no artificial light was used, because in the summer days are long i.e. day light 14-16 hours/day, which was enough to grow plants of these varieties.

Figure 13: Growing strawberry plants on the capillarity bench in the glasshouse

Plants (runners of Elsanta) were provided by Harriet and Henry Duncalfe from Maltmas Farm and planted in the Hatfield glasshouse for the pilot experiment into individual 12X6 cm plastic pots filled with compost (Miracle Gro, Company) in the beginning of the

September, 2008. Pots were placed on capillary matting on bench at Hatfield glasshouse (Figure 13). Plants were watered through the capillary matting and no water was sprayed over leaves. In September, natural daylight (12-14 hours/day) was used but from October natural day light was only 8-9 hours/day. Artificial light (incandescent light) which provided additional light 4-6 hours/day was therefore used to create 12-14 hours light during the day. Temperatures were approximately 18-22^{0}C during the day and 15-18^{0}C at night. Normal plant fertilizer (organic strawberry fertilizer, Vitex Ltd) was applied to each pot after the runners were planted in the pots. Organic strawberry fertilizer 15ml was mixed with 4.5 litres of water (according to the manufacturer's instructions) and 125 ml was applied to each pot once a week and continued until the application of the treatments. In the main experiment different strawberry varieties such as Elsanta, Symphony, Rhapsody and Florence were grown in the Bayfordbury glasshouses and runners were used for growing plants which were provided by Harriet and Henry Duncalfe from Maltmas Farm. All these varieties were grown in the same way as described above.

2.6. Application of the treatments

Treatments were not applied until the plants were well established. After about 6-7 weeks when the runner plants had 10-12 leaves, treatments were applied to the leaves (foliar) or roots. Omex SW7 (details are described in chapter 1, section 1.2.8) was applied as the source of silicon. Details of foliar application and root application will be described in chapter 3 and chapter 4. Leaves were collected after completing the application of the treatments. Details of sample collection will also be described in chapter 3, section 3.2.2.2 and chapter 4, section 4.2.4. All leaves were washed after collection to remove the silicon on the surface of the leaves (foliar application). Leaves were kept in sample bags labelled with the date of collection and the treatment (rate + timing). All samples were stored in the -70^{0}C freezer. Collected leaves (number of leaves will be mentioned in chapter 3 and chapter 4) which were used for silicon extraction were oven dried at 67^{0}C for up to 36 hours and dried leaves were then ground using pestle and mortar. Some of the leaves collected (number of leaves will be mentioned in chapter 3 and chapter 4) were used to measure density and length of leaf hairs using a dissection microscope.

2.7. Silicon determination

In the present study, a modified form of Autoclave Induced Digestion (AID) method as described by Elliott and Snyder (1991) was selected for the determination of the silicon content because of its rapidity, simplicity and appropriate sensitivity. The AID method is more rapid than other methods and involves fairly low cost equipment and a minimum of hazardous chemicals (Elliott and Snyder, 1991).

2.7.1. Determination of the standard curve

In order to determine the concentrations of silicon present in strawberry leaves (treated and untreated), a standard curve had to be worked out each time the experiment was carried out. Silicon dioxide (Sigma Aldrich Company, UK) was used to make a standard curve. The concentrations of known samples were 1.562, 3.125, 6.25, 12.5, 25, 50, 100 mg/l Si. These known concentrations would give the absorbance value, when put in the spectrophotometer (Bausch and Lomb spec 21). From these concentrations and absorbance value a standard curve was drawn, shown in Figure 14. From this graph it was possible to determine the concentrations of unknown samples by drawing a line from the absorbance of the unknown sample. The other way to do this is by using a regression line. In this study, the known concentrations and absorbance value from the spectrophotometer, were put into an excel program and from this excel program a standard curve was drawn (Figure 14). By the use of the equation, Y=0.0036x (excel program) the concentrations of unknown samples were found. In this standard curve, concentrations were expressed in mg/l.

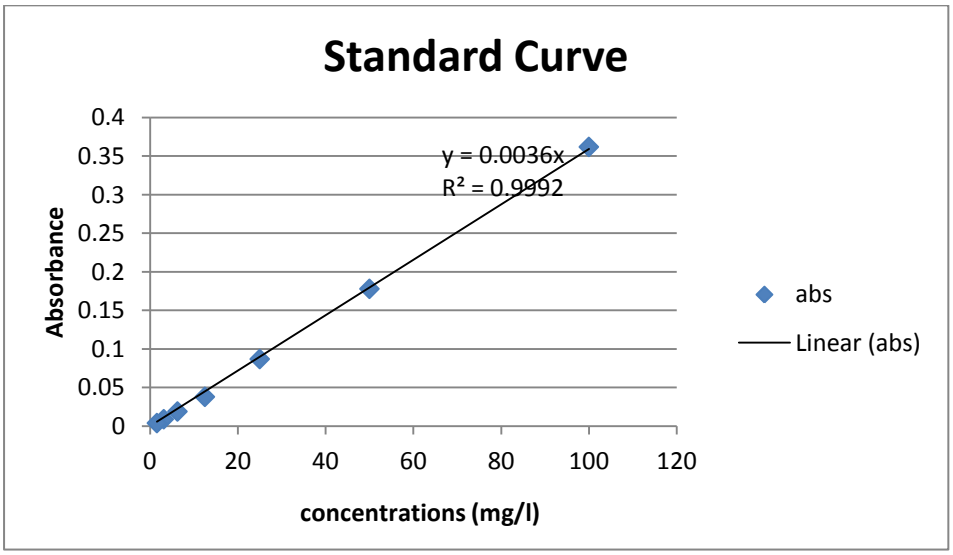


Figure 14: Example of a standard curve.

2.7.2. Methods for extraction of silicon from strawberry leaves

Silicon content was determined by a modification of the autoclave induced digestion method. The extraction procedure was similar to Elliott and Snyder (1991); however the amount of H_2O_2, NaOH and other chemicals used according to Taber *et al.*, (2002).

The following chemicals used in the AID methods were bought from the Sigma Aldrich Company, UK.

1) Hydrogen peroxide (H_2O_2)
2) Sodium Hydroxide (NaOH)
3) Boric acid (H_3BO_3)
4) Tartaric acid $(CHOH.CHOH)_2$
5) Ammonium molybdate $(NH4)6MO_7O_{24}.4H_2O$
6) Sodium sulfite (Na_2SO_3)
7) 1 amino-2 napthol-4 sulphonic acid ($NH_2C_{10}H_5(OH)SO_3H$)
8) Sodium bisulfite ($NaHSO_3$)

2.7.2.1. Preparation of solutions

Sodium Hydroxide (50%): 50 g of sodium hydroxide pellets were dissolved in 100 ml of DI (Deionized or Demineralised) water.

Boric acid (2.5%): 2.5 g of boric acid were dissolved in 100 ml of DI water.

Tartaric acid (20%): 20 g of tartaric acid were dissolved in 100 ml of DI water

Ammonium molybdate (54 g/l, pH 7): 54 g of ammonium molybdate were dissolved in 1000 ml of DI water. After mixing with DI water the pH of the solution was measured using the pH meter (Mettler Toledo). The pH of the solution was adjusted to 7 by using NaOH or HCl (Hydrochloric acid). If the pH of the solution was more than 7 then HCl was added on the other hand, when the pH of the solution was less than 7 then NaOH was added.

The reducing solution was made by combining the two solutions (solution A and B). Solution A was made by adding 2 g of Na_2SO_3 and 0.4 g of 1-amino-2-napthol-4-sulfonic acid in 25 ml DI and solution B was made by 25 g of NaHSO3 dissolved in 200 ml DI water. Solution A and B were mixed together and brought to 250 ml final volume with DI.

2.7.2.2. Extraction of silicon from ground leaves

1) First of all 100 mg samples (dried ground leaves) were added to an autoclave resistant 100 ml polyethylene bottle. Then 3.0 ml of 50% H_2O_2 were added to moisten the samples followed by the addition of 3.5 ml of 50% NaOH.

2) The bottles were gently vortexed and the bottles were individually covered with loose fitting plastic caps and autoclaved for 1 hour at 138 kPa at 120^0C. When the contents cooled, then the solutions were quantitatively transferred to 50 ml polyethylene tube through an ashless coarse filter paper.

3) The silicon content was then determined colorimetrically using the procedure described below.

4) 1 ml was taken from the digested plant tissue (mentioned above in step 2) and 3 ml of 2.5% boric acid was added and the solution mixed by inversion. The next step was the addition of 1 ml of ammonium molybdate (54 g/l, pH 7) solution. The mixture of the solution was left to stand for 5 minutes.

5) 0.5 ml of 20% tartaric acid was then added, the solution was shaken and finally 0.5 ml of the reducing solution was added.

6) After addition of all the reagents including the reducing solution (section 2.7.2.1) the final volume of the solution was made up to 10 ml with adding DI water. The mixture was allowed to stand for 30 minutes before taking the reading of absorbance at 650 nm by a spectrophotometer (Bausch and Lomb Spec 21). Glassware contains silicon; therefore to avoid contamination all equipment used was polyethylene or polycarbonate that had been rinsed with 0.1M NaOH.

7) A standard curve which had been prepared earlier (section 2.7.1) was used to determine the silicon content in the plant material. The value of x (concentration) for any given value of y (absorbance) was calculated using the following equation:

y=0.0036x (regression equation from standard curve)

Silicon content was calculated, adjusted for weight of plant material used and expressed as mg/kg silicon in dry matter using the following equation:

$$\text{Si (mg/kg)} = \frac{\text{Si in extract (mg/l)*10}}{\text{Sample size (g)}}$$

For an example, sample A (100 mg) has an absorbance value y=0.044 (at 650 nm with spectrophotometer). To calculate the value of x (concentration of Si) the equation y=0.0036x was used.

0.044 = 0.0036x

Now x= 12.22 mg/l (concentrations of Si).

To convert Si concentrations mg/l into mg/kg used the formula given above.

That is

$$\text{Si (mg/kg)} = \frac{\text{Si in extract (mg/l)}*10}{\text{Sample size (g)}}$$

$$\text{Si (mg/kg)} = \frac{12.22*10}{0.1}$$

Concentrations of Si = 1222 mg/kg

8) All above methods were done in triplicate and the results were analyzed by statistical (section 2.9) procedure.

2.7.2.3. Determination of silicon in the different treatments

Different treatments were used in this study (chapters 3, 4 and 5). Standard, high and very high concentrations of silicon were made by adding DI water to 2.5 ml, 25 ml and 50 ml Omex SW7 respectively and made the final volume 1000 ml. After that 1 ml was taken from 1000 ml for each treatment (standard, high and very high) and the amounts of silicon in the different treatments were determined colorimetrically following the steps from (4 to 6) described above (section 2.7.2.2). A standard curve which was prepared before (section 2.7.1) was used to determine the silicon content in the different treatments. The value of x (concentration) for any given value of y (absorbance) was calculated using the following equation:

y=0.0036x (regression equation from standard curve)

Using the above equation the value of x (concentrations of silicon) in the different treatments was calculated and expressed in mg/l.

2.8. Microscopic observation of density and length of leaf hairs

In order to determine the density and length of leaf hairs, 20 leaves from each treatment group were examined under the microscope. There were 4 leaflets in each leaf in the different varieties (Elsanta, Rhapsody, Florence and Symphony) of strawberry plants. To identify the effect of silicon on the density and length of leaf hairs a $1cm^2$ square was cut out of a piece of Acetate Sheet and used as a quadrat. This $1cm^2$ square was placed six times randomly on the adaxial (upper) and abaxial (lower) surface of the leaf. Number of leaf hairs on each $1cm^2$ on the upper and lower leaf surfaces were determined by observing the leaves under the dissection microscope (Hampshire Micro, Meiji EMZ-TR) using a X30 magnification. The length of leaf hairs were measured using a microscope eyepiece graticule. To measure the length of leaf hairs, a $1cm^2$ quadrat was placed on the upper and lower surface of the leaf. The lengths of 10 leaf hairs from each quadrat were measured using the eyepiece graticule in the dissection microscope (X30). This was repeated for the remaining 3 leaflets from each leaf and also repeated for the remaining leaves. Average number and length of leaf hairs were calculated from 20 leaves and results were analysed using the statistical procedure described below.

2.9. Data analysis

Data were analysed using the statistical software program, SPSS for windows, version 17. Data were analysed by one way ANOVA (analysis of variance) under the Compare Mean in the SPSS version 17. Individual means were subjected to further *post-hoc* analyses using Tukey's HSD (Honestly Significant Difference) test to determine the differences between treatments. Homogeneous subsets were used to differentiate the treatment in different groups. A value of $P<0.05$ was considered to be statistically significant. Pearson's correlation and linear regression analysis were used to find the relationship between silicon level and density and length of leaf hairs.

Chapter -3: An investigation of the effect of foliar application of silicon (Omex SW7) on strawberry plants in the glasshouse

3.1. Introduction

Even though from a scientific point of view silicon is not considered as an essential nutrient for most plants to complete their life cycle, it has been reported that silicon reduces the effects of biotic and abiotic stresses (Epstein, 2009). Silicon is the second most abundant element in mineral soils and this is perhaps the reason that silicon has been under evaluated as an important nutrient for so many years. Moreover, little research has been performed and there is limited availability of commercial silicon supplements or concentration recommendations for different crops. Recently, there has been an increased interest for sustainable crop production and silicon can contribute to that direction with its prophylactic properties and promotion of plant health. Several studies have found that silicon enhances metabolic function and plant growth rates by balancing nutrient uptake, distribution and transport and increasing chlorophyll concentration in leaves resulting in improved pollen fertility and increased yield (Epstein, 1994; Wang and Galletta, 1998; Ma and Takahashi, 2002). Lewin and Reimann (1969) in their review paper said that silicon is responsible for improved growth of a wide variety of plant species, both monocotyledons and dicotyledons. Various symptoms develop when silicon is absent or present in only small amounts.

Although the beneficial effects of silicon on plant growth and development have been observed in a wide variety of plant species, little information is available about foliar application of silicon on plant growth and development. Menzies *et al*., (1992) reported that foliar application of silicon was absorbed through the cuticle and was transported in the xylem and deposited in the epidermal cell, cell wall of the leaves and also deposited in other parts of the plants (mentioned in chapter 1). In rice, silicon accumulates in the epidermal cells of the leaves, motor cells and hulls. The deposition of silicon enhances the strength and rigidity of cell walls and thus increases the resistance of rice to diseases and pests (Epstein, 1999; Ma and Takahashi, 2002). Beneficial effects of silicon on rice have been reported by several workers; however the response of dicotyledons to silicon has received less attention. Strawberry is a dicotyledon, as is cucumber and contains less silicon than cucumber (Kanto *et al*., 2007). Wang and

Galletta (1998) found that strawberry plants fertilised with silicon produce more dry matter in roots and shoots and they also described that plants sprayed with silicon had enhanced strawberry plant growth. Epstein (1994) reported that deposition of silicon in the plant tissue decreases the transpiration rate and increases stem thickness and strength. Omex SW7 is a silicon based wetter which is routinely used by the growers on different crops including strawberry. However, it is not clear that application of Omex SW7 can enhance the silicon levels in plant tissues. The hypothesis tested in this chapter was that application of silicon (Omex SW7) to the leaves of the different varieties of strawberry would lead to enhanced levels of silicon in the plants. The enhanced levels of silicon in the plants would lead to morphological changes in the leaves with regard to the formation of a greater number and longer leaf hairs of all the varieties used.

3.1.1. Objectives

- To investigate the effect of foliar application of Omex SW7 on the accumulation of silicon in the leaves of strawberry plants of all the varieties used in this study.
- To investigate the effect of foliar application of Omex SW7 on the morphological changes that occurs in the leaves with regard to the modification of leaf hair length and density of different varieties used in this study.
- To determine the relationships between accumulation of silicon and density and length of leaf hairs of all the varieties used in this study.

3.2. Materials and Methods

3.2.1. Pilot experiment

A pilot experiment was conducted in the glasshouse at the College Lane Campus. The strawberry variety Elsanta was used in the pilot experiment and the plants of Elsanta were grown on the capillarity bench of the Hatfield glasshouse which was described in chapter 2, section 2.5. In the pilot experiment 80 plants were grown and finally 64 healthy plants were selected for the experiment. Silicon wetter (Omex SW7) was used as the source of silicon. The recommended dose of the silicon wetter is 0.1-0.25% dilution with foliar nutrients in a minimum of 200 l/ha water.

There were two different application rates (standard and high) and the product was applied at two different timings (sprayed each week for 5 weeks i.e. 5 sprays and sprayed in weeks 1 and 5 i.e. 2 sprays). There were two controls, one where the plants were sprayed with water alone and a second where plants received no foliar spray. Different treatments in the pilot experiment are shown in Table 6. The treatments were applied to 2 different blocks (Block A and Block B) which were situated in one glasshouse cubicle. Within each block each treatment occurred once and the treatments were distributed randomly (Table 7A). Each block was divided into 6 rows and each row consisted of 4 plants. The treatments which were applied in the different rows of block A are shown in Table 7B, and the treatments in block B are shown in Appendix 1. The pilot experiment helped design of the main experiment which was conducted at Bayfordbury glasshouse.

Table 6: Treatment rates and spray timings

Treatment rates and spray timings
Standard (Omex SW7at 0.25%) which contains 27.02 mg/l Si was applied each week for 5 weeks (total 5 sprays)
Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si was applied in weeks 1 and 5 (total 2 sprays)
High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si was applied each week for 5 weeks (total 5 sprays)
High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si was applied in weeks 1 and 5 (total 2 sprays)
Water (only) was applied each week for 5 weeks (total 5 sprays)
Water (only) was applied in weeks 1 and 5 (total 2 sprays)
Untreated (control)

Table 7A: Distribution of treatments in the Hatfield glasshouse (variety Elsanta)

Block	Row	Treatment
A	1	Standard-(2 sprays)
	2	Control
	3	High-(5 sprays)
	4	Water-(5 sprays)
	5	High-(2 sprays)
	6	Standard-(5 sprays)
	7	Control
	8	Water-(2 sprays)

Block	Row	Treatment
B	9	Control
	10	High-(5 sprays)
	11	Water-(2 sprays)
	12	High-(2 sprays)
	13	Standard-(2 sprays)
	14	Standard-(5 sprays)
	15	Water-(5 sprays)
	16	Control

Table 7B: Randomised distribution of the treatments, date of application of the treatments and leaf collections from different rows of block A (variety Elsanta).

Block	Row numbers and treatments	Application of the treatments date					Leaf collection
A		Week 1 14.10.08	Week 2 21.10.08	Week 3 28.10.08	Week 4 4.11.08	Week 5 11.11.08	Week 6 18.11.08
	Row-1 Standard (sprayed twice weeks 1 and 5)	Standard				Standard	Collection of leaves
	Row-2 Control	Control	Control	Control	Control	Control	Collection of leaves
	Row-3 High weekly (sprayed 5 times)	High	High	High	High	High	Collection of leaves
	Row-4 Water weekly (sprayed 5 times)	Water	Water	Water	Water	Water	Collection of leaves
	Row-5 High (sprayed twice, weeks 1 and 5)	High				High	Collection of leaves
	Row-6 Standard weekly (sprayed 5 times)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Row-7 Control	Control				Control	Collection of leaves
	Row-8 Water (sprayed twice weeks 1 and 5)	Water				Water	Collection of leaves

3.2.1.1. Application of the treatments

Treatments were not applied until the plants were established. Runner plants which were provided by Harriet and Henry Duncalfe were planted in the month of September 2008 and after about 6-7 weeks when the runner plants had 10-12 leaves treatments were applied on the leaves. For foliar application a hand held sprayer was used and 100 ml Omex SW7 was applied to each plant. The sprays were applied until run off from the upper surface of the leaves. When applying the treatments, the plants were away from the other plants, so that there was no cross contamination of treatments. Application of the treatments was started from 14 October 2008 and the treatments were applied on the leaves of the plants in the different rows of block A (Table 7B). Coloured tape was used to mark the treated leaves. After finishing each spray when the leaves were dried then $1cm^2$ tape was stuck on the underside of the treated leaves.

3.2.1.2. Collection of leaves

Samples were collected one week after finishing the last spray on 11 November 2008. At the end of the different treatments leaves were collected on the same date (18 November 2008) from all the rows of both blocks. Marked leaves were collected throughout the sample collections. Leaves collected from all the rows of block A are shown in Table 7B and leaves collected from block B are shown in Appendix 1. During sampling, 10 leaves were collected from each plant, 40 from each block for each treatment (total 80 from two blocks). Leaves were collected from all treatments. From 40 collected leaves, 20-30 leaves were kept to use for measuring leaf hair length and density. Length and density of the leaf hairs were measured by using a dissection microscope and details were described in chapter2, section 2.8. The remaining 10-12 leaves were washed after collection to remove the silicon on the surface of the leaves and then dried in the oven at 67^0C for up to 36 hours. Pestle and mortar were then used to grind the dried leaves. Ground leaves were kept in sample bags labelled with the date of collection and the treatment (rate + timing) and kept in the cupboard at room temperature, 15-20^0C and then used for measuring silicon concentrations using the AID method described in chapter 2, section 2.7.2.

3.2.2. Main experiment

The main experiment was conducted in the Bayfordbury glasshouse and Elsanta was used. In the main experiment (210-215) runners (Elsanta) were used to grow the plants and finally 192 healthy plants were selected for the experiment. In April 2009 runners were planted in the 12x6 cm pots filled with compost and pots were placed on the capillarity bench in the glasshouse. Temperatures were approximately 15-20^{0}C during the day and 12-15^{0}C at night. In April, natural daylight (8-9 hours/day) and artificial light which provided additional light 4-6 hours/day were used and from May to July natural day light (12-14 hours/day) was used and no artificial light was used. Normal plant fertilizer described in chapter 2 (section 2.5) was provided to all the pots. Plants were watered through the capillarity bench and no water was spread over the leaves. There were three different application rates and the product was applied at three different timings. Control plants received no foliar spray. Different treatments in the main experiment are shown in Table 8. The treatments were applied to 4 different blocks (Block A, B, C and D). Each block was divided into 12 rows and each treatment was applied to an individual row which consisted of 4 plants. Within each block each treatment occurred once and they were distributed randomly (Table 9A). Each treatment was applied to 16 plants (4 rows from 4 different blocks). Block A and B were situated in one glasshouse cubicle and block C and D were situated in another glasshouse cubicle. Distribution of the treatments and application of the treatments and leaf collections from different rows of block A are shown in Table 9B and from block B, C, and D are shown in Appendix 2.

3.2.2.1. Application of the treatments

The treatments were applied when the plants had 10-12 leaves, as described in the previous section 3.2.1.1. The method used for the application of the treatments was described in the previous section 3.2.1.1. and Omex SW7 (100 ml) was applied to each plant with a hand held sprayer. Application of the treatments was started from 8 June 2009. On 8 June all the treatments were applied on the leaves of the plants in the different rows of block A (Table 9B). After finishing each spray when the leaves were dried then 1cm^2 coloured tape was stuck on the underside of the treated leaves (mentioned in section 3.2.1.1.).

Table 8: Treatment rates and spray timings

Treatment rates and spray timings
Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si was applied each week for 5 weeks (total 5 sprays)
Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si was applied in weeks 1, 3 and 5 (total 3 sprays).
Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si was applied in weeks 1 and 5 (total 2 sprays)
High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si was applied each week for 5 weeks (total 5 sprays).
High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si was applied in weeks 1, 3 and 5 (total 3 sprays).
High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si was applied in weeks 1 and 5 (total 2 sprays).
Very high (Omex SW7 at 5%) which contains 540.40 mg/l Si was applied each week for 5 weeks (total 5 sprays).
Very high (Omex SW7 at 5%) which contains 540.40 mg/l Si was applied in week 1, 3 and 5 (total 3 sprays).
Very high (Omex SW7 at 5%) which contains 540.40 mg/l Si was applied in weeks 1 and 5 (total 2 sprays).
Untreated (control).

3.2.2.2. Collection of leaves

Leaves were collected on 11 July 2009 from all the rows of 4 different blocks after the application of the last treatment on 4 July. Treated and untreated (control) leaves were collected from four different blocks (A-D). Treated and untreated (control) leaves collected from different rows of block A are shown in Table 9B and leaves collected from block B, C and D are shown in Appendix 2. During the collection of samples marked leaves were collected. During sampling 40 leaves were collected from each block for each treatment (10 leaves from each plant). Collected leaves were used for measuring

hair length, density and measuring silicon concentrations as described in the previous section 3.2.1.2.

Table 9A: Distribution of treatments in the Bayfordbury glasshouse (variety Elsanta)

Block	Row	Treatment
A	1	Standard-(2 sprays)
	2	High-(3 sprays)
	3	Control
	4	Very high-(2 sprays)
	5	Very high-(3 sprays)
	6	High-(5 sprays)
	7	High-(2 sprays)
	8	Standard-(3 sprays)
	9	Control
	10	Standard-(5 sprays)
	11	Very high-(5 sprays)
	12	Control

Block	Row	Treatment
B	13	High-(3 sprays)
	14	Control
	15	Standard-(3 sprays)
	16	High-(5 sprays)
	17	Very high-(5 sprays)
	18	Control
	19	High-(2 sprays)
	20	Standard-(2 sprays)
	21	Standard-(5 sprays)
	22	Very high-(3 sprays)
	23	Very high-(2 sprays)
	24	Control

Block	Row	Treatment
C	25	Standard-(5 sprays)
	26	Control
	27	Standard-(3 sprays)
	28	High-(2 sprays)
	29	High-(3 sprays)
	30	Very high-(3 sprays)
	31	Very high-(2 sprays)
	32	Control
	33	High-(5 sprays)
	34	Standard-(2 sprays)
	35	Very high-(5 sprays)
	36	Control

Block	Row	Treatment
D	37	High-(2 sprays)
	38	Very high-(2 sprays)
	39	Control
	40	Standard-(3 sprays)
	41	Standard-(5 sprays)
	42	Control
	43	High-(5 sprays)
	44	High-(3 sprays)
	45	Standard-(2 sprays)
	46	Very high-(3 sprays)
	47	Very high-(5 sprays)
	48	Control

Table 9B: Randomised distribution of the treatments, date of application of the treatments and leaf collections from different rows of block A (variety Elsanta).

Block	Row numbers and treatments	Application of the treatments date					Leaf collection
A		Week 1 8.06.09	Week 2 15.06.09	Week 3 22.06.09	Week 4 29.06.09	Week 5 4.07.09	Week 6 11.07.09
	Row-1 Standard sprayed twice (weeks 1 and 5)	Standard				Standard	Collection of leaves
	Row-2 High sprayed 3 times (weeks 1, 3 and 5)	High		High		High	Collection of leaves
	Row-3 Control	Control	Control	Control	Control	Control	Collection of leaves
	Row-4 Very high sprayed twice (weeks 1 and 5)	Very high				Very high	Collection of leaves
	Row-5 Very high sprayed 3 times (weeks 1, 3 and 5)	Very high		Very high		Very high	Collection of leaves
	Row-6 High weekly (sprayed 5 times)	High	High	High	High	High	Collection of leaves
	Row-7 High sprayed twice (weeks 1 and 5)	High				High	Collection of leaves
	Row-8 Standard sprayed 3 times (weeks 1, 3 and 5)	Standard		Standard		Standard	Collection of leaves
	Row-9 Control	Control		Control		Control	Collection of leaves
	Row-10 Standard weekly (sprayed 5 times)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Row-11 Very high weekly (sprayed 5 times)	Very high	Very high	Very high	Very high	Very high	Collection of leaves
	Row-12 Control	Control				Control	Collection of leaves

3.2.3. Main experiment (used different varieties)

This experiment investigated the uptake of silicon in different varieties, Rhapsody, Florence, Symphony and Elsanta. This experiment was conducted in the Bayfordbury glasshouse. Runners (35-40) from each variety were used to grow the plants in the beginning of the September 2009 and finally 24 healthy plants for each variety were selected for the experiment. Runners of different varieties were grown in the glasshouse which was already described in chapter 2, section 2.5. Temperature and day light were used, as described in chapter 2, section 2.5. There were two different application rates and control. There were 3 different treatments for each variety (Table 10). The treatments were divided into 2 blocks of 4 plants each. Control plants received no foliar spray. Within each block each treatment appeared once and they were distributed randomly (Table 11A). There were 4 blocks (A-D) situated in 2 glasshouses at Bayfordbury. Block A and B were situated in one glasshouse cubicle and block C and D were situated in the other glasshouse cubicle (mentioned in section 3.2.2). Distribution of treatments and application of the treatments in block A and B are shown in Table 11B and in block C and D are shown in Appendix 3.

3.2.3.1. Application of the treatments

The method for the application of the treatments was also used here which is described in the section 3.2.1.1. Application of the treatments was started from 16 October 2009. On 16 October all the treatments were applied on the leaves of the plants in the different rows of block A and B. After that treatments were applied on 23, 30 October and then on 7 and 14 November 2009. After finishing each spray when the leaves were dried then 1cm^2 coloured tape as mentioned in section 3.2.1.1. was used to mark the treated leaves.

Table 10: Treatment rates and spray timings

Variety	Name of the treatments
Elsanta	Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si was applied each week for 5 weeks (total 5 sprays)
Elsanta	High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si was applied each week for 5 weeks (total 5 sprays)
Elsanta	Untreated (control)
Symphony	Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si was applied each week for 5 weeks (total 5 sprays)
Symphony	High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si was applied each week for 5 weeks (total 5 sprays)
Symphony	Untreated (control)
Florence	Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si was applied each week for 5 weeks (total 5 sprays)
Florence	High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si was applied each week for 5 weeks (total 5 sprays)
Florence	Untreated (control)
Rhapsody	Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si was applied each week for 5 weeks (total 5 sprays)
Rhapsody	High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si was applied each week for 5 weeks (total 5 sprays)
Rhapsody	Untreated (control)

Table 11A: Distribution of treatments in the Bayfordbury glasshouse (different varieties)

Block	Variety	Row	Treatment
A	Elsanta	1	Standard-(5 sprays)
	Elsanta	2	Control
	Elsanta	3	High-(5 sprays)
	Symphony	4	High-(5 sprays)
	Symphony	5	Standard-(5 sprays)
	Symphony	6	Control

Block	Variety	Row	Treatment
B	Florence	7	Control
	Florence	8	High-(5 sprays)
	Florence	9	Standard-(5 sprays)
	Rhapsody	10	Standard-(5 sprays)
	Rhapsody	11	High-(5 sprays)
	Rhapsody	12	Control

Block	Variety	Row	Treatment
C	Symphony	13	Standard-(5 sprays)
	Symphony	14	Control
	Symphony	15	High-(5 sprays)
	Elsanta	16	High-(5 sprays)
	Elsanta	17	Standard- (5 sprays)
	Elsanta	18	Control

Block	Variety	Row	Treatment
D	Rhapsody	19	Control
	Rhapsody	20	High-(5 sprays)
	Rhapsody	21	Standard-(5 sprays)
	Florence	22	Standard-(5 sprays)
	Florence	23	High-(5 sprays)
	Florence	24	Control

Table 11B: Randomised distribution of the treatments, date of application of the treatments and leaf collections from different rows of blocks A and B (different varieties).

Blocks	Variety	Row numbers and treatments	Applications of the treatments date					Leaf collections
A			Week 1 16.10.09	Week 2 23.10.09	Week 3 30.10.09	Week 4 7.11.09	Week 5 14.11.09	Week 6 21.11.09
	Elsanta	Row-1 Standard (5 sprays)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Elsanta	Row-2 Control	Control	Control	Control	Control	Control	Collection of leaves
	Elsanta	Row-3 High (5 sprays)	High	High	High	High	High	Collection of leaves
	Symphony	Row-4 High (5 sprays)	High	High	High	High	High	Collection of leaves
	Symphony	Row-5 Standard (5 sprays)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Symphony	Row-6 Control	Control	Control	Control	Control	Control	Collection of leaves
B	Florence	Row-7 Control	Control	Control	Control	Control	Control	Collection of leaves
	Florence	Row-8 High (5 sprays)	High	High	High	High	High	Collection of leaves
	Florence	Row-9 Standard (5 sprays)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Rhapsody	Row-10 Standard (5 sprays)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Rhapsody	Row-11 High (5 sprays)	High	High	High	High	High	Collection of leaves
	Rhapsody	Row-12 Control	Control	Control	Control	Control	Control	Collection of leaves

3.2.3.2. Collection of leaves

Leaves were collected on 21 November 2009 from all the rows of 4 blocks after the application of the final spray on 14 November 2009. During the collection of samples only marked leaves were collected. Treated (weekly) and untreated leaves from four

different varieties were collected from four different blocks (A-D). Treated and untreated (weekly) leaves from four different varieties were collected from different rows of block A and B are shown in Table 11B and leaves collected from block C and D are shown in Appendix 3. During sampling 10 leaves from each plant, 40 from each block were collected. Leaf samples were collected in a similar way from all varieties and all the treatments. Some of the leaves collected were used for measuring hair length and density, as described in the Chapter 2, section 2.8. The remaining leaves were used for measuring silicon concentrations, as described in the chapter 2, section 2.7.2.

3.2.4. Data analysis

Statistical method one way ANOVA under the Compare Mean in the SPSS version 17 was used to analyse the data of concentrations of silicon in strawberry leaves, density and length of leaf hairs after foliar application of different concentrations of Omex SW7 in different varieties (Elsanta, Rhapsody, Florence and Symphony). Tukey's HSD test and homogeneous subsets also used here to differentiate the treatment in different groups and the relationships between accumulation of silicon and density and length of leaf hairs of all the varieties used in this study were determined by using the method described in section 2.9.

3.3. Results

3.3.1. Pilot experiment in Hatfield Glasshouse

Strawberry variety Elsanta was used in the pilot experiment. Leaves collected from Elsanta after different treatments showed different levels of silicon concentrations (Figure 15). It was observed that there were significant differences in silicon concentrations in different treatments. All treated plants showed significantly higher ($P<0.05$)) silicon concentrations compared to that in leaves collected from the untreated control and water treated plants. Leaves collected from control (untreated) and water treated plants showed the background level of silicon in strawberry plants. No significant difference ($P>0.05$) in silicon concentrations was observed between the leaves collected from water treated plants and control plants (Appendix 11). Therefore

silicon concentrations from water treated plants were not shown in the results (Figure 15). Because of this when the main experiment was conducted in the Bayfordbury glasshouse the untreated control was used as a control. Standard and high concentrations of Omex SW7 were applied weekly and a total of 2000 ml (400 ml X 5 times) Omex SW7 was applied. Similarly when Omex SW7 was applied in weeks 1 and 5, a total of 800 ml (400 ml X 2 times) Omex SW7 was applied. Spectrophotometric analysis showed that a 2000 ml Omex SW7 contained 54.04 mg Si (standard), 540.40 mg Si (high) and 800 ml Omex SW7 contained 21.61 mg Si (standard) and 216.16 mg Si (high). When different concentrations of Omex SW7 were applied weekly, concentrations of silicon in the leaves were 18.22 mg/l Si (standard), 23.74 mg/l Si (high) and 11.30 mg/l Si (control). When different concentrations of silicon were applied in weeks 1 and 5 concentrations of silicon in the leaves were 14.29 mg/l Si (standard), 19.39 mg/l Si (high) and 11.03 mg/l Si (control). However, the silicon concentrations measured by the spectrophotometer were adjusted with the weight of plant material (dried leaf) used and expressed as mg/kg silicon in dried leaf tissue (Figure 15).

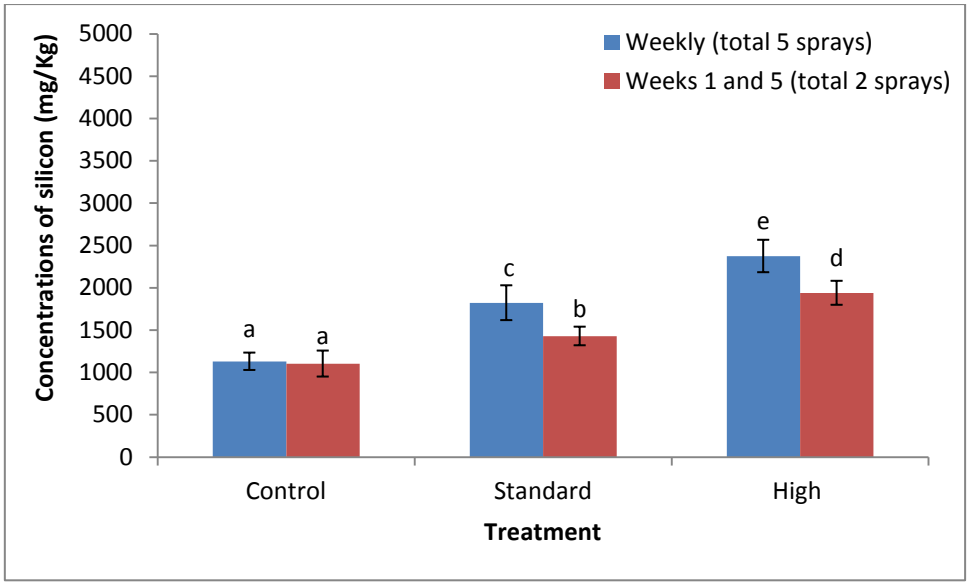


Figure 15: Concentrations of silicon in strawberry leaves (variety Elsanta) sprayed weekly (total 5 sprays) and weeks 1 and 5 (total 2 sprays) with different concentrations (standard, high) of silicon wetter and control (no application). Samples were collected one week after the final spray. Each value represents the mean of 6 replications (n=6). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

Strawberry leaves collected from standard and high concentrations of Omex SW7 treated plants showed significantly ($P<0.05$) higher silicon concentrations compared to that in leaves collected from control (untreated) plants (Figure 15). Results revealed that weekly application (total 5 sprays) of 2 different concentrations of Omex SW7 showed significantly ($P<0.05$) higher silicon concentrations compared to the application of the treatments in weeks 1 and 5 (total 2 sprays) and the highest level of silicon concentrations was observed in the leaves treated with the high concentration of Omex SW7 which was applied weekly (total 5 sprays) (Figure 15). Weekly (total 5 sprays) application of standard and high concentrations of Omex SW7 increased the silicon concentrations by up to 61.23% (standard) and 110.08% (high) compared to the control (Table 12). When standard and high concentrations of Omex SW7 were applied in weeks 1 and 5 (total 2 sprays) the silicon concentrations increased by up to 29.60% (standard) and 75.85% (high) compared to the control (Table 12).

Table 12: Increased percentage of silicon concentrations in leaves after different treatments

Treatments	Mean concentrations of silicon (mg/kg) in leaves	Increased silicon concentrations in leaves after treatment compared to control (mg/kg)	Percentage of increased silicon concentrations in leaves compared to control
Control	1130.33		
Standard weekly (sprayed 5 times)	1822.50	692.17	61.23%
High weekly (sprayed 5 times)	2374.66	1244.33	110.08%
Control	1103.00		
Standard sprayed twice (weeks 1 and 5)	1429.50	326.50	29.60%
High sprayed twice (weeks 1 and 5)	1939.66	836.66	75.85%

The highest percentage (110.08%) of silicon accumulation was observed in the leaves treated with the high concentration of Omex SW7 which was applied weekly and the

lowest percentage (29.60%) was observed in the leaves treated with the standard concentration of Omex SW7 which was applied in weeks 1 and 5 compared to the control (Table 12). The work reported here showed that concentrations of silicon in strawberry leaves were increased by the addition of the silicon wetter. Moreover, the more silicon that was applied the higher the levels of silicon absorbed by the plants (Figure 15). Weekly application of Omex SW7 gave significantly ($P<0.05$) greater accumulation of silicon compared to application of Omex SW7 in weeks 1 and 5. Results of the pilot experiment helped to design the main experiment in the Bayfordbury glasshouse.

3.3.2. Main experiment in the Bayfordbury glasshouse (effect of treatments on silicon concentrations in strawberry leaves)

In the Bayfordbury glasshouse (main experiment) four different treatments, control (untreated) and three different concentrations of silicon (Omex SW7), standard, high and very high were applied weekly (sprayed each week for 5 weeks total 5 sprays), on the leaves of strawberry plants (variety Elsanta). Extraction of silicon from strawberry leaves with the AID method and analysis of the silicon by spectrophotometric procedure are shown in different graphs. Leaves collected from strawberry plants after different treatments showed different levels of silicon concentrations (Figure 16). It was observed that there was a significant difference in silicon concentrations in different treatments. Strawberry leaves collected from standard, high, and very high concentrations of Omex SW7 treated plants showed significantly ($P<0.05$) higher silicon concentrations compared to that in leaves collected from control (untreated) plants (Figure 16).

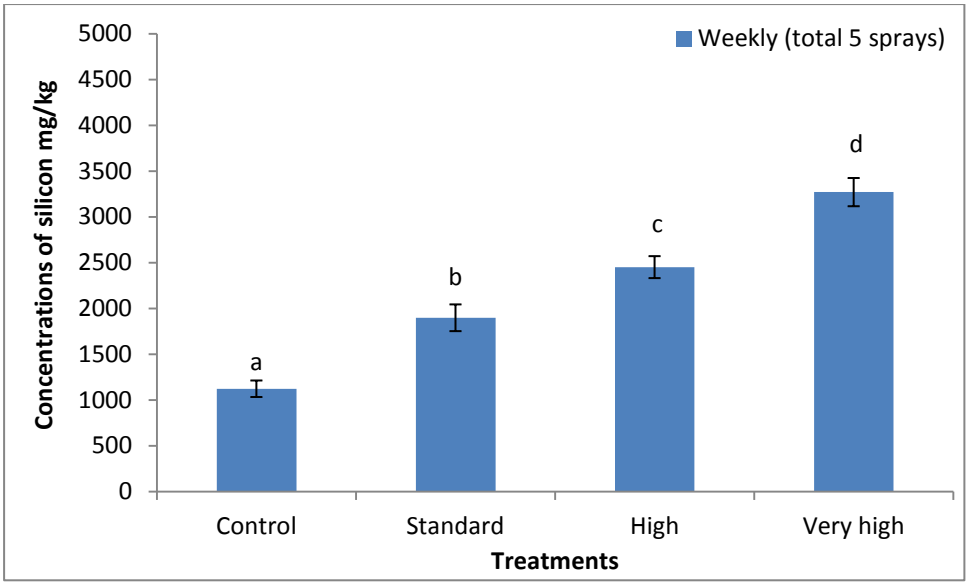


Figure 16: Concentrations of silicon in strawberry leaves (variety Elsanta) sprayed weekly (total 5 sprays) with different concentrations of silicon wetter (standard, high, and very high) and untreated control. Samples were collected one week after the final spray. Each value represents the mean of 6 replications (n=6). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

The leaves collected from the high concentration of Omex SW7 treated plants showed significantly (P<0.05) higher concentrations of silicon compared to the control and the standard Omex SW7 treated plants but significantly (P<0.05) lower compared to the very high concentration of Omex SW7 treated plants (Figure 16). Moreover, the highest levels of silicon concentrations were observed in leaves collected from the very high concentration of Omex SW7 treated leaves compared to all other treatments (Figure 16).

3.3.3. Effect of timing on silicon concentrations in strawberry leaves

Strawberry plants were treated with different concentrations of silicon wetter (Omex SW7) at three different timings. During the weekly application, a total of 2000 ml (400 ml X 5 times) of different concentrations of Omex SW7 were applied. Similarly when different concentrations of Omex SW7 were applied in weeks 1, 3 and 5, a total of 1200 ml (400 ml X 3 times) and in weeks 1 and 5, a total of 800 ml (400 ml X 2 times) was applied. Total amounts of silicon in the different treatments and concentrations of

silicon in leaves after the application of the different treatments were determined by spectrophotometer which has been shown in Table 13A. However, in this study, dried leaf samples were used to determine the concentrations of silicon. For this reason, the silicon concentrations measured by the spectrophotometer were adjusted in relation to the weight of plant material used and expressed as mg/kg silicon in dried leaf tissue (mentioned in chapter 2) which is shown in the graphs (Figures 16 and 17) and also shown in Table 13B.

Table 13A: Total amounts of silicon in the different treatments and accumulation of silicon in the leaves after different treatments.

Treatments	Total amounts of Omex SW7 applied	Total amounts of silicon in the different treatments (mg)	Mean concentrations of silicon in the leaves (mg/l)
Control	No application of Omex SW7		11.25
Standard weekly (sprayed 5 times)	2000 ml	54.04	18.98
High weekly (sprayed 5 times)	2000 ml	540.40	24.51
Very high weekly (sprayed 5 times)	2000 ml	1080.80	32.70
Control	No application of Omex SW7		11.23
Standard sprayed 3 times (weeks 1, 3 and 5)	1200 ml	32.42	15.99
High Sprayed 3 times (weeks 1, 3 and 5)	1200 ml	324.24	21.25
Very high Sprayed 3 times (weeks 1, 3 and 5)	1200 ml	648.48	28.29
Control	No application of Omex SW7		10.94
Standard sprayed twice (weeks 1 and 5)	800 ml	21.61	13.30
High sprayed twice (weeks 1 and 5)	800 ml	216.16	17.96
Very high sprayed twice (weeks 1 and 5)	800 ml	432.32	24.33

It was observed that absorption of silicon in strawberry leaves was significantly different with different timing of applications (Figure 17). Results showed that weekly

foliar application of Omex SW7 showed more silicon accumulation in leaves with 3 different silicon treatments. It was observed that the weekly application (total 5 sprays) of 3 different concentrations of Omex SW7 showed significantly ($P<0.05$) higher silicon concentrations compared to 3 applications in weeks 1, 3 and 5 or 2 applications in weeks 1 and 5 (Figure 17).

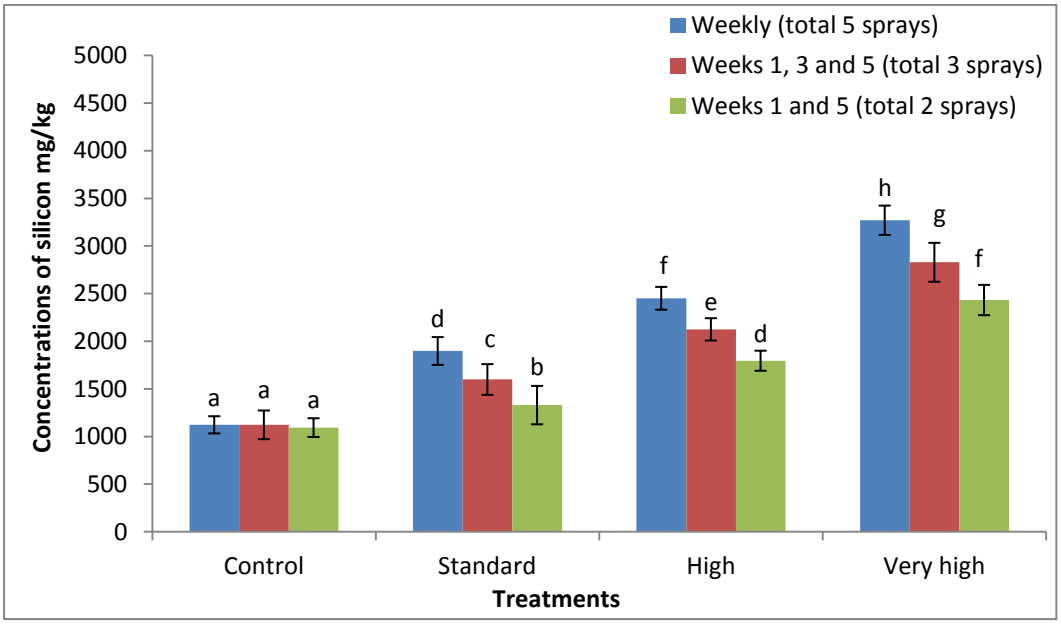


Figure 17: Concentrations of silicon in strawberry (variety Elsanta) leaves treated weekly (total 5 sprays), weeks 1, 3, 5 (total 3 sprays) and weeks 1 and 5 (total 2 sprays) with different concentrations (standard, high, and very high) of silicon wetter and control (no application). Samples were collected one week after the final spray. Each value represents the mean of 6 replications (n=6). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (Post-hoc test) in the statistical software program, SPSS for Windows version 17.

Silicon concentrations in leaf samples indicated that weekly (total 5 sprays) application of Omex SW7 increased the silicon concentrations by up to 68.98% (standard), 118.16% (high) and 191.05% (very high) compared to control (Table 13B). Similarly concentrations of silicon increased in leaves after applications of different concentrations of silicon in weeks 1, 3 and 5 (total 3 sprays) and in weeks 1 and 5 (total 2 sprays) (Table 13B). Highest levels (191.05%) of silicon were observed in the leaves treated weekly with the very high concentration of Omex SW7 and lowest levels (21.53%) of silicon were observed in the leaves treated with the standard concentration of Omex SW7 which was applied in weeks 1 and 5 (total 2 sprays) compared to the control (Table 13B).

Table 13B: Increased percentage of silicon concentrations in leaves after different treatments

Treatments	Mean concentrations of silicon (mg/kg) in leaves	Increased silicon concentrations in leaves after treatment compared to control (mg/kg)	Percentage of increased silicon concentrations in leaves compared to control
Control weekly	1123.50		
Standard weekly (sprayed 5 times)	1898.58	775.08	68.98%
High weekly (sprayed 5 times)	2451.08	1327.58	118.16%
Very high weekly (sprayed 5 times)	3270.00	2146.50	191.05%
Control	1123.41		
Standard sprayed 3 times (weeks 1, 3 and 5)	1599.33	475.92	42.36%
High Sprayed 3 times (weeks 1, 3 and 5)	2125.75	1002.34	89.22%
Very high Sprayed 3 times (weeks 1, 3 and 5)	2829.50	1706.09	151.86%
Control	1094.50		
Standard sprayed twice (weeks 1 and 5)	1330.25	235.75	21.53%
High sprayed twice (weeks 1 and 5)	1796.00	701.50	64.09%
Very high sprayed twice (weeks 1 and 5)	2433.16	1338.66	122.30%

3.3.4. Effect of silicon on the density of leaf hairs

This experiment aimed to investigate whether the silicon supplements had any effect on the number of leaf hairs. Interestingly, microscopic observations of silicon treated leaves showed that there were increased numbers of leaf hairs on both the upper and lower surfaces of leaves (Figure 18A). The numbers of leaf hairs were observed more on the lower surface compared with the upper surface of leaves. It was also found that there were significant differences in the number of leaf hairs in different treatment groups.

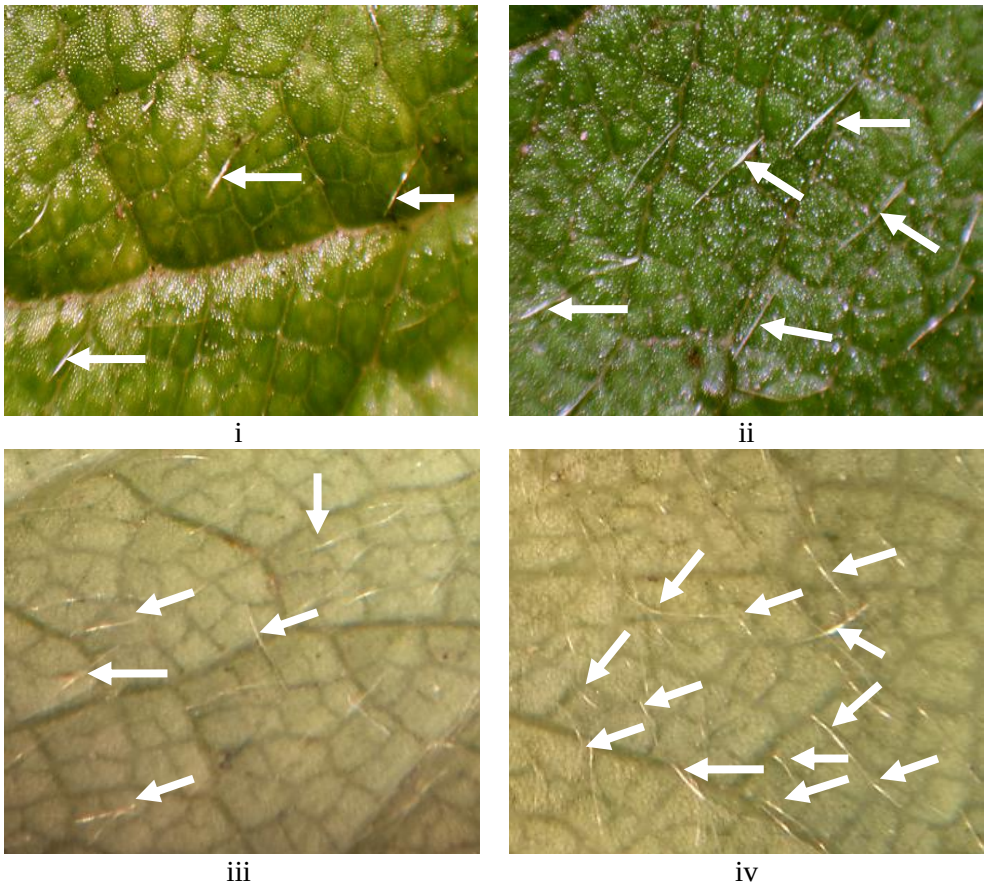


Figure 18 A: Number of leaf hairs on the i) upper surface without silicon treament ii) upper surface with silicon (standard) treatment iii) lower surface without silicon treament iv) lower surface with silicon (standard) treatment. Leaves were examined under the dissection microscope (X30). Leaf hairs are indicated by arrows.

The upper surface of leaves treated with different concentrations of Omex SW7 showed significantly ($P<0.05$) higher leaf hair numbers compared to the control (untreated) leaves (Figure 18B). Moreover, leaves treated with the high concentration of Omex SW7 showed significantly ($P<0.05$) higher leaf hair numbers compared to the standard Omex

SW7 treated leaves but significantly (P<0.05) lower compared to the very high concentration of Omex SW7 treated leaves (Figure 18B).

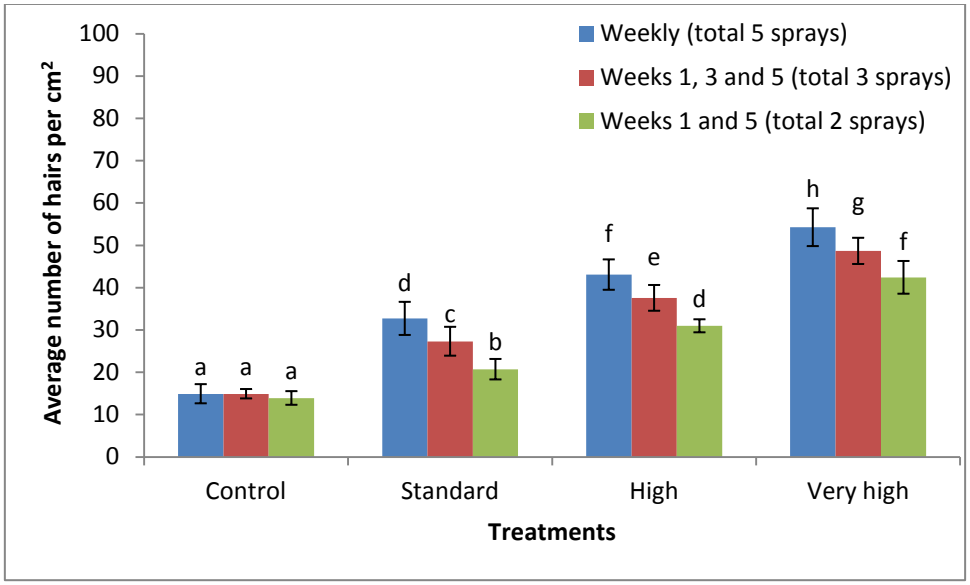


Figure 18 B: Number of leaf hairs on the upper surface of leaves in strawberry variety Elsanta treated weekly (total 5 sprays), weeks 1, 3, 5 (total 3 sprays) and weeks 1 and 5 (total 2 sprays) with different concentrations (standard, high, and very high) of silicon wetter and control (no application). Samples were collected one week after the final spray. A total of 20 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (Post-hoc test) in the statistical software program, SPSS for Windows version 17.

The number of leaf hairs on the lower surface of leaves collected after different treatments, shown in Figure 19. It was observed that the foliar application of silicon on strawberry leaves increased the leaf hair numbers on the lower surface (Figure 19). The number of leaf hairs was significantly (P<0.05) higher in the high and very high Omex SW7 treated leaves compared to the control and standard Omex SW7 treated leaves (Figure 19). Moreover, the highest levels of Omex SW7 treated leaves showed significantly higher (P<0.05) leaf hair numbers compared to all other treatment groups (Figure 19). Different timings, weekly (sprayed each week for 5 weeks, total 5 sprays), sprayed in weeks 1, 3 and 5 (total 3 sprays) and sprayed in weeks 1 and 5 (total 2 sprays) also had an effect on the density of leaf hairs on both the upper and lower surfaces of leaves.

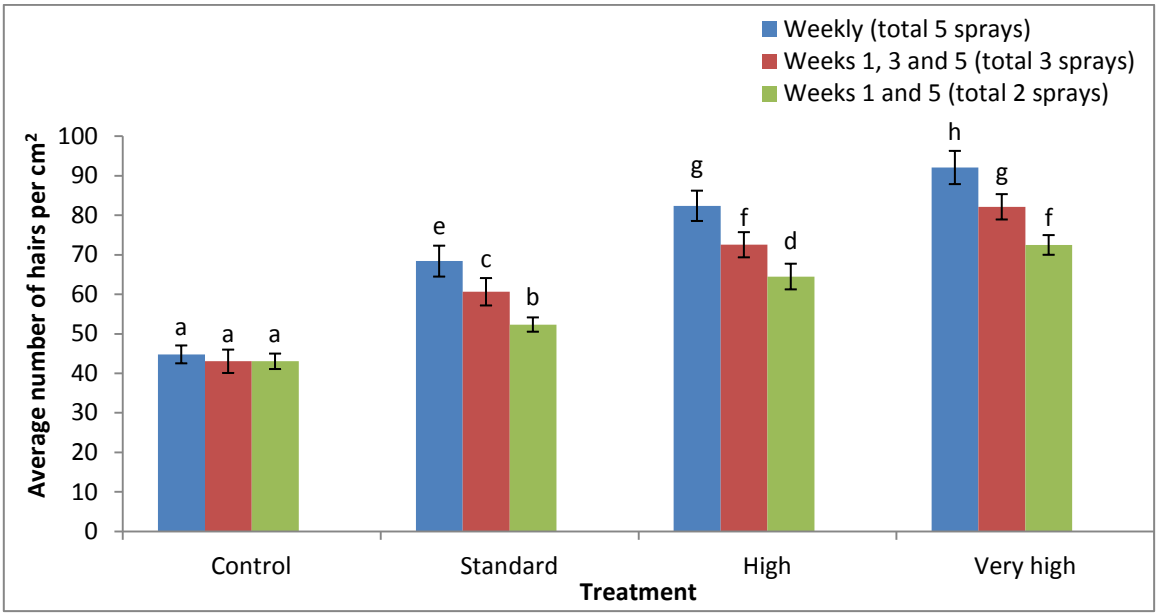


Figure 19: Number of leaf hairs on the lower surface of leaves in the strawberry variety Elsanta treated weekly (total 5 sprays), weeks 1, 3, 5 (total 3 sprays) and weeks 1 and 5 (total 2 sprays) with different concentrations (standard, high, and very high) of silicon wetter and control (no application). Samples were collected one week after the final spray. A total of 20 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 (n=20). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (Post-hoc test) in the statistical software program, SPSS for Windows version 17.

There was no significant ($P>0.05$) difference in the leaf hair numbers on the leaves collected from control plants in any of the three different intervals. On the other hand, weekly (total 5 sprays) foliar application of Omex SW7 showed more leaf hairs on both the upper and lower surfaces of leaves with three different silicon treatments. Furthermore, it was also observed that the weekly (total 5 sprays) applications of three different concentrations of Omex SW7 showed significantly ($P<0.05$) higher leaf hair numbers on both the upper (Figure 18B) and lower surfaces (Figure 19) of leaves than when the application was 3 times in weeks 1, 3 and 5 or twice in weeks 1 and 5. Moreover, when different concentrations of Omex SW7 (standard, high and very high) were applied in weeks 1, 3 and 5 (total 3 sprays) there were significantly ($P<0.05$) higher leaf hair numbers on both the upper and lower surfaces of leaves compared with application in weeks 1 and 5 (total 2 sprays) (Figures 18B and 19).

3.3.5. Relationship between the leaf hair numbers and accumulation of silicon

The results showed that foliar application of standard, high and very high concentrations of silicon increased silicon concentrations in leaves as well as increased the number of leaf hairs on both the upper and lower surfaces of leaves. The results from the leaves collected on 11 July 2009 from all the different treatments (standard, high and very high concentrations of Omex SW7 were applied at three different timings) were used to find the relationships between density and length of leaf hairs and accumulation of silicon in the variety Elsanta. Pearson's correlation as mentioned in section 2.9 was used to find a correlation. It was observed that there was a significant positive correlation ($P<0.01$) between accumulation of silicon and the number of leaf hairs on both the upper and lower surfaces of leaves (Figures 20 and 21). The correlation coefficients (r) were 0.992 and 0.977 and regression coefficients (R^2) were 0.984 and 0.954 for the upper and lower surfaces of leaves respectively.

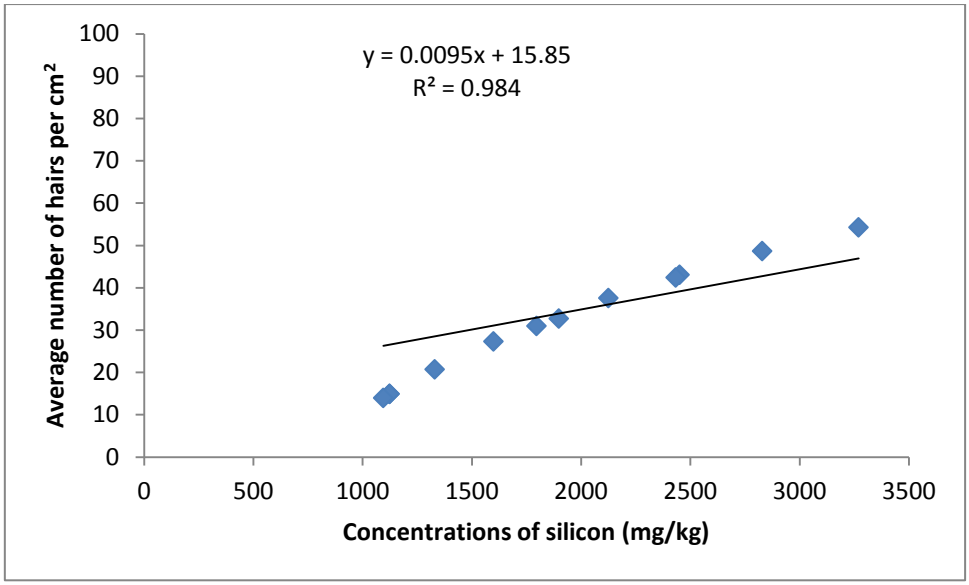


Figure 20: Relationship between concentrations of silicon and average number of leaf hairs (per cm^2) on the upper surface of leaves (variety Elsanta). The correlation coefficient (r) was 0.992 and regression coefficient (R^2) was 0.984.

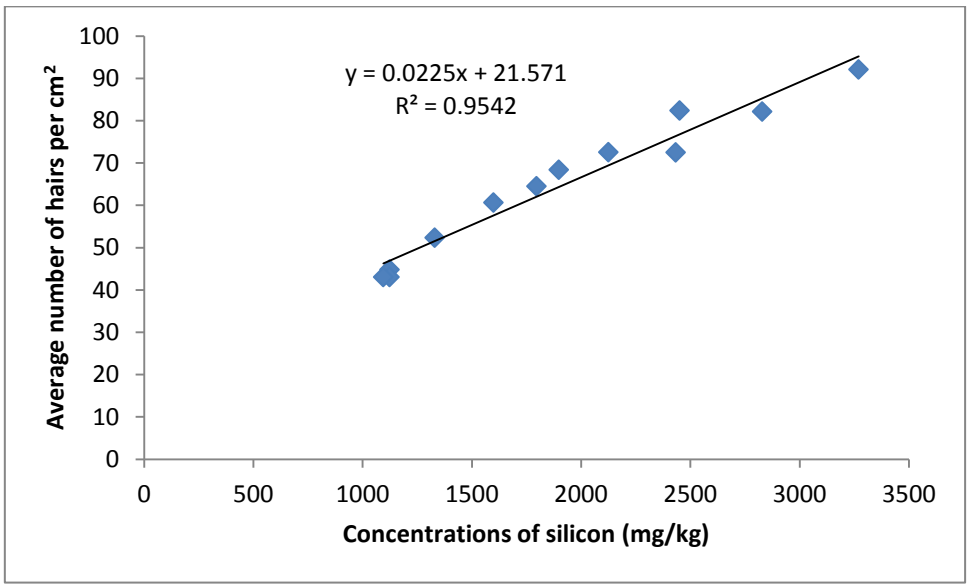


Figure 21: Relationship between concentrations of silicon and average number of leaf hairs (per cm^2) on the lower surface of leaves (variety Elsanta). The correlation coefficient (r) was 0.977 and regression coefficient (R^2) was 0.954.

3.3.6. Effect of silicon on the length of leaf hairs

This study examined whether the silicon supplement had any effect on the length of leaf hairs. It was observed that the foliar application of silicon had an effect on the length of leaf hairs on both the upper and lower surfaces of leaves. The application of three different silicon concentrations (standard, high and very high) of Omex SW7 showed significantly ($P<0.05$) longer leaf hair length compared to control leaves (Figures 22 and 23). It was observed that the length of leaf hairs in the presence of the high concentration of silicon were significantly ($P<0.05$) longer in length than those treated with the standard Omex SW7 but significantly ($P<0.05$) shorter when compared with the very high concentration of Omex SW7 treated leaves (Figures 22 and 23) on both the upper and lower surfaces of leaves. Furthermore, it was also observed that the weekly (total 5 sprays) application of three different concentrations of Omex SW7 showed significantly ($P<0.05$) longer leaf hair length on both the upper (Figure 22) and lower (Figure 23) surfaces of leaves than when the application was 3 times in weeks 1, 3 and 5 (total 3 sprays) or twice in weeks 1 and 5 (total 2 sprays).

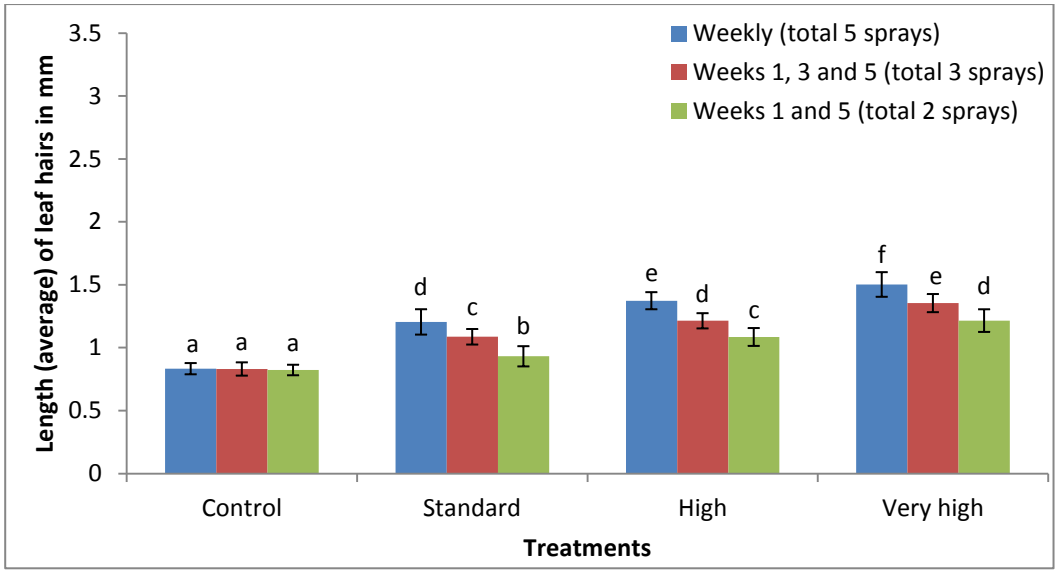


Figure 22: Length of leaf hairs on the upper surface of leaves (variety Elsanta) treated weekly (total 5 sprays), weeks 1, 3, 5 (total 3 sprays) and weeks 1 and 5 (total 2 sprays) with different concentrations (standard, high, and very high) of silicon wetter and control (no application). Samples were collected one week after the final spray. A total of 20 leaves for each treatment were used to measure the average length of leaf hairs (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (Post-hoc test) in the statistical software program, SPSS for Windows version 17.

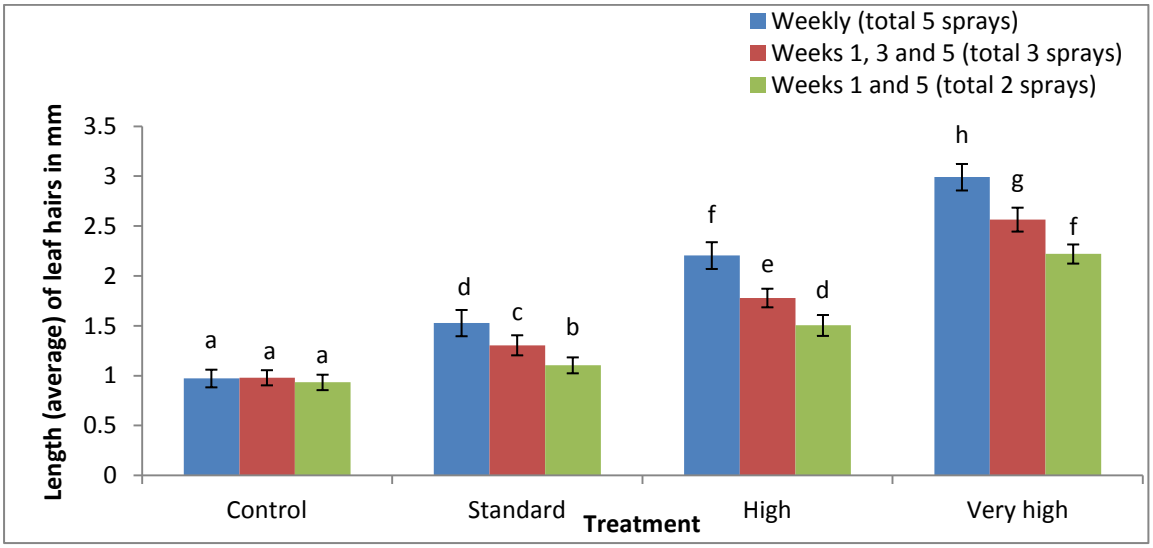


Figure 23: Length of leaf hairs on the lower surface of leaves (variety Elsanta) treated weekly (total 5 sprays), weeks 1, 3, 5 (total 3 sprays) and weeks 1 and 5 (total 2 sprays) with different concentrations (standard, high, and very high) of silicon wetter and control (no application). Samples were collected one week after the final spray. A total of 20 leaves for each treatment were used to measure the average length of leaf hairs (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (Post-hoc test) in the statistical software program, SPSS for Windows version 17.

3.3.7. Relationship between the length of leaf hair and accumulation of silicon

Results showed that foliar application of standard, high and very high concentrations of silicon wetter increased silicon concentrations in leaves as well as increased the length of leaf hairs on both the upper and lower surfaces of leaves. Therefore, the relationship between the length of leaf hairs and the silicon level was investigated. It was observed that there was a significant positive correlation ($P<0.01$) between accumulation of silicon and the length of leaf hairs on both the upper and lower surfaces of leaves (Figures 24 and 25). The correlation coefficients (r) were 0.970 and 0.996 and regression coefficients (R^2) were 0.941 and 0.991 for the upper and lower surfaces of leaves respectively. These results indicated the possibility that silicon application on the leaf surfaces increased the length of leaf hairs on both the both upper and lower surfaces of leaves.

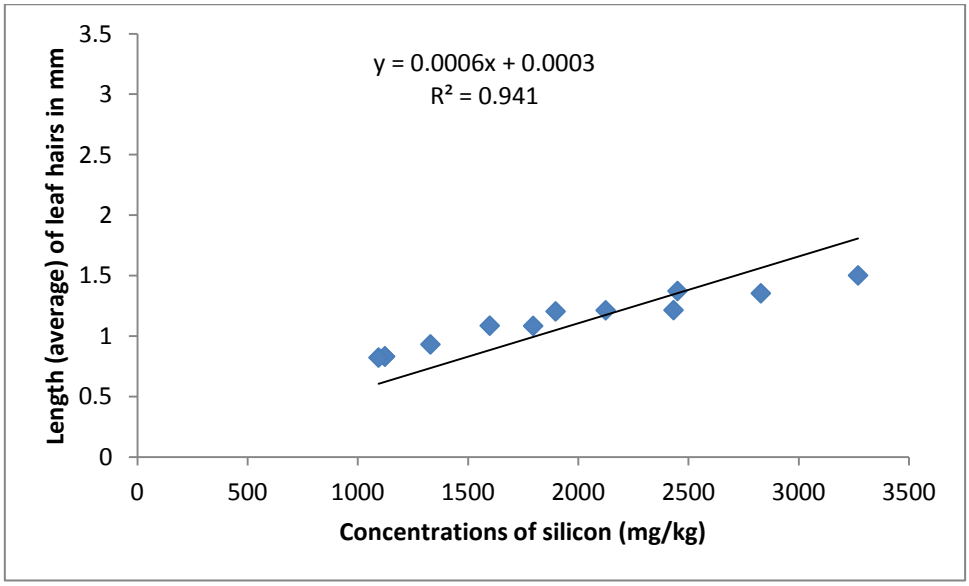


Figure 24**:** Relationship between concentrations of silicon and average length of leaf hairs on the upper surface of leaves (variety Elsanta). The correlation coefficient (r) was 0.970 and regression coefficient (R^2) was 0.941.

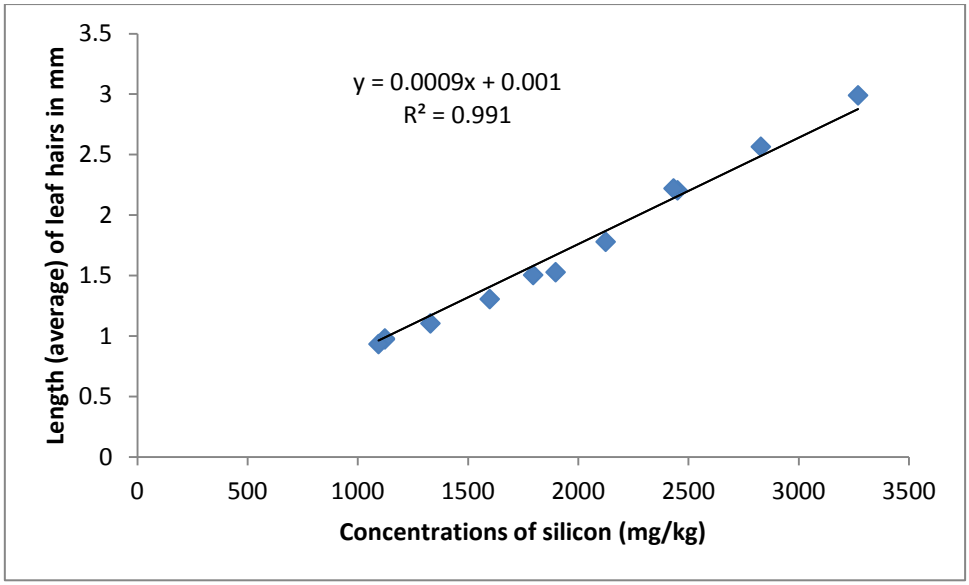


Figure 25: Relationship between concentrations of silicon and average length of leaf hairs on the lower surface of leaves (variety Elsanta). The correlation coefficient (r) was 0.996 and regression coefficient (R^2) was 0.991.

3.3.8. Effect of treatments on accumulation of silicon in different strawberry varieties

This project also investigated the uptake of silicon in different strawberry varieties Symphony and Elsanta which are susceptible to strawberry powdery mildew and Florence and Rhapsody which are moderately resistant to powdery mildew. The accumulation of silicon and the physical changes in leaves with regard to the density and length of leaf hairs of all the four varieties were assessed. Two different concentrations of Omex SW7 standard, high and a control were used to assessed the accumulation of silicon on strawberry leaves in four strawberry varieties. The very high concentration of Omex SW7 used in the main experiment was not used here. Observations in the main experiment showed that the very high concentration of Omex SW7 had a harmful effect on plant growth and development. It was observed that leaves of plants treated with the very high concentration of Omex SW7 had a white surface which may be detrimental to photosynthesis. For this reason plants treated with the very high concentration of Omex SW7 did not survive more than two weeks after finishing the treatment.

Silicon was applied weekly (sprayed each week for 5 weeks, total 5 sprays) and a total of 2000 ml (400 ml X 5 times) Omex SW7 was applied. Spectrophotometer analysis showed that 2000 ml Omex SW7 contained 54.04 mg of silicon for standard and 540.40

mg of silicon for high treatment. Concentrations of silicon in leaves after the application of the different treatments were determined by spectrophotometer and adjusted in relation to the weight of plant material (dried leaf) used and expressed as mg/kg silicon in dried leaf tissue (mentioned in section 3.3.3) (Figure 26).

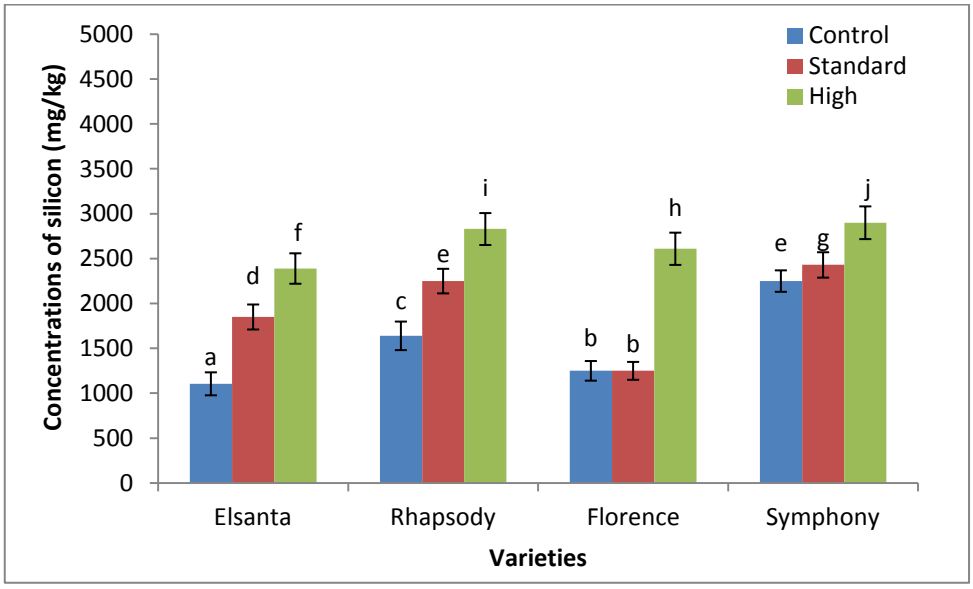


Figure 26: Concentrations of silicon in strawberry leaves in different varieties treated weekly (sprayed each week for 5 weeks, total 5 sprays) with different concentrations of silicon (standard and high) wetter and untreated control. Samples were collected one week after the final spray. Each value represents the mean of 6 replications (n=6). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (Post-hoc test) in the statistical software program, SPSS for Windows version 17.

Leaves collected from the strawberry variety Elsanta and the other three varieties Rhapsody, Florence and Symphony after different treatments showed different levels of silicon concentrations (Figure 26). All treated plants showed significantly (P<0.05) higher silicon concentrations compared to that in leaves collected from the control (untreated) plants (Figure 26). The highest levels of silicon were seen in leaves collected from the highest treatment compared to all other treatments. Results showed that in the varieties Elsanta, Rhapsody and Symphony there was a significant difference in silicon concentrations in the leaves collected from different treatments (Figure 26). However, in Florence there was no significant difference (P>0.05) in silicon concentrations in the leaves collected from the control and the standard concentration of Omex SW7 treated plants (Figure 26). Although there was a significant difference (P<0.05) in silicon concentrations between standard and high concentrations of Omex

SW7 treated leaves in Florence (Figure 26). Highest levels of silicon (116.18%) were observed in the leaves treated with the high concentration of Omex SW7 in Elsanta followed by 108.8% (Florence), 72.56% (Rhapsody) and 33.33% (Symphony) (Table 14).

Table 14: Increased percentage of silicon concentrations in leaves after different treatments

Variety	Treatments and application time	Mean concentrations of silicon (mg/kg) in leaves	Increased silicon concentrations in leaves after treatment compared to control (mg/kg)	Percentage of increased silicon concentrations in leaves compared to control
Elsanta	Control	1105.54		
Elsanta	Standard (sprayed 5 times)	1850	744.46	67.33%
Elsanta	High (sprayed 5 times)	2390	1284.46	116.18%
Rhapsody	Control	1640		
Rhapsody	Standard (sprayed 5 times)	2250	610	37.19%
Rhapsody	High (sprayed 5 times)	2830	1190	72.56%
Florence	Control	1250		
Florence	Standard (sprayed 5 times)	1250		
Florence	High (sprayed 5 times)	2610	1360	108.8%
Symphony	Control	2250		
Symphony	Standard (sprayed 5 times)	2530	280	12.45%
Symphony	High (sprayed 5 times)	3000	750	33.33%

3.3.9. Effect of silicon on the density of leaf hairs in different strawberry varieties

Microscopic observations of silicon treated leaves showed that there were increased numbers of leaf hairs on both the upper and lower surfaces of leaves in all the four

varieties when compared to the control (Figures 27 and 28). The numbers of leaf hairs were observed more on the lower surface in comparison with the upper surface of leaves. It was found that there were significant differences in the number of leaf hairs in different treatments in all the four varieties tested. Furthermore it was observed that on the upper surface of leaves two different treatments of Omex SW7 treated leaves showed significantly ($P<0.05$) higher leaf hair numbers compared to the control in the varieties Elsanta, Florence and Symphony (Figure 27). On the other hand, untreated leaves from Rhapsody had no hairs on the upper leaf surface, but a low density of hairs was observed after treatment with the high concentration of Omex SW7 (Figure 27). The number of leaf hairs on the lower surface of leaves collected after different treatments are shown in Figure 28. It was observed that the foliar application of silicon on strawberry leaves increased the lower surface leaf hair numbers (Figure 28) in all the varieties tested. However, the number of leaf hairs on the lower surface was significantly ($P<0.05$) higher on leaves treated with the high concentration of Omex SW7 compared to the control and the standard Omex SW7 treated leaves (Figure 28).

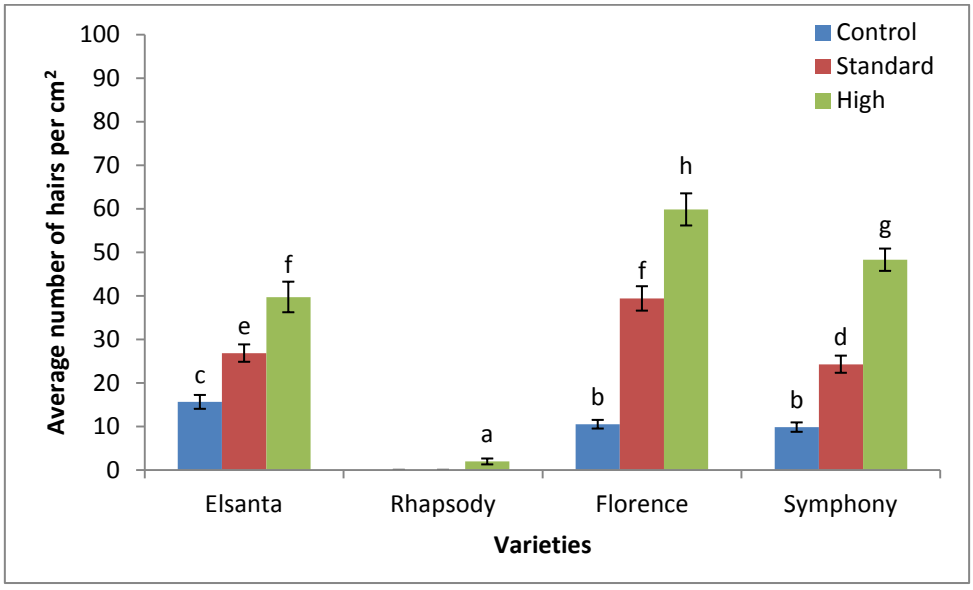


Figure 27: Number of leaf hairs on the upper surface of leaves in different strawberry varieties treated weekly (sprayed each week for 5 weeks, total 5 sprays) with different concentrations of silicon (standard and high) wetter and untreated control. Samples were collected one week after the final spray. A total of 12 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 (n=12). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (Post-hoc test) in the statistical software program, SPSS for Windows version 17.

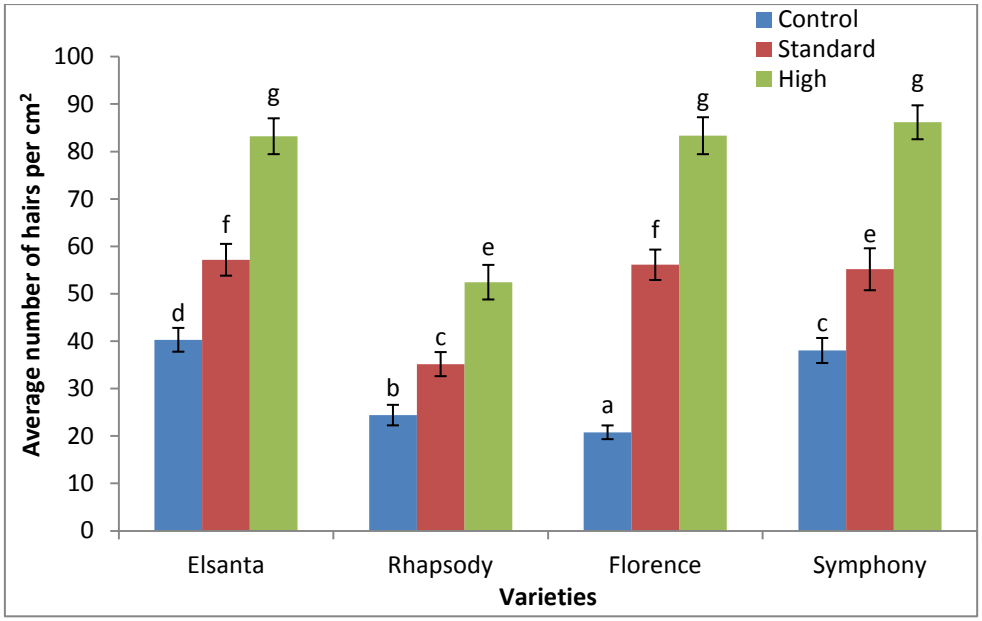


Figure 28: Number of leaf hairs on the lower surface of leaves in different strawberry varieties treated weekly (sprayed each week for 5 weeks, total 5 sprays) with different concentrations (standard and high) of silicon wetter and control (no application). Samples were collected one week after the final spray. A total of 12 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 (n=12). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (Post-hoc test) in the statistical software program, SPSS for Windows version 17.

3.3.10. Relationship between the leaf hair numbers and accumulation of silicon (different varieties)

It has been observed that different treatments enhanced both silicon levels as well as the number of leaf hairs in all different varieties. The results from the leaves collected on 21 November 2009 from all the treatments and all the varieties were used to find the relationships between density and length of leaf hairs and accumulation of silicon. The relationship between silicon accumulation in leaves and the number of leaf hairs on both the upper and lower surfaces of leaves in different varieties are shown in Table 15. In Elsanta a positive correlation was observed between silicon accumulation and the number of leaf hairs on both the upper and lower surfaces of leaves (Table 15). The correlation coefficients (r) were 0.991 and 0.977 and regression coefficients (R^2) were 0.983 and 0.955 for the upper and lower surfaces of leaves respectively. In Rhapsody there was a positive correlation between silicon accumulation and the number of leaf hairs on the lower surfaces of leaves and the correlation coefficient (r) was 0.989 and regression coefficient (R^2) was 0.978 respectively. However no relationship was

observed on the upper surface in this variety because there were no leaf hairs on the leaves collected from the control and standard treated plants (Table 15). In Florence and Symphony positive correlation between silicon accumulation and the number of leaf hairs on both the upper and lower surfaces of leaves was also observed (Table 15). However the correlation coefficient (r) in Symphony was higher on both the upper and lower surfaces of leaves in comparison with Florence.

Table 15: Relationship between concentrations of silicon and the number of leaf hairs on both the upper and lower surfaces of leaves in the different varieties

Variety	Upper surface		Lower surface	
	Correlation coefficient (r)	Regression coefficient (R^2)	Correlation coefficient (r)	Regression coefficient (R^2)
Elsanta	0.991	0.983	0.977	0.955
Rhapsody	-	-	0.989	0.978
Florence	0.813	0.660	0.826	0.683
Symphony	0.994	0.988	0.996	0.992

3.3.11. Effect of silicon on the length of leaf hairs in different strawberry varieties

Furthermore, it was also demonstrated that the accumulation of silicon affects the morphology of the strawberry leaves resulting in the formation of additional leaf hairs, which were longer than when the plants were untreated. This effect occurs on all varieties tested. It was observed that the foliar application of silicon had an effect on the length of leaf hairs on both the upper and lower surfaces of leaves (Figures 29 and 30). The application of different silicon concentrations showed significantly ($P<0.05$) longer leaf hair length compared to control (Figures 29 and 30). It was observed that the length of leaf hairs in the high silicon treated leaves showed significantly ($P<0.05$) longer length compared to that in the standard Omex SW7 treated leaves (Figures 29 and 30). On the other hand, in Rhapsody the high concentration of Omex SW7 treatment stimulated the growth of hairs which was observed on the upper surface (Figure 29). However, in Rhapsody there were no significant differences in length of leaf hairs were

(P<0.05) observed between the control and the standard concentration of Omex SW7 treated leaves on the lower surface of leaves (Figure 30).

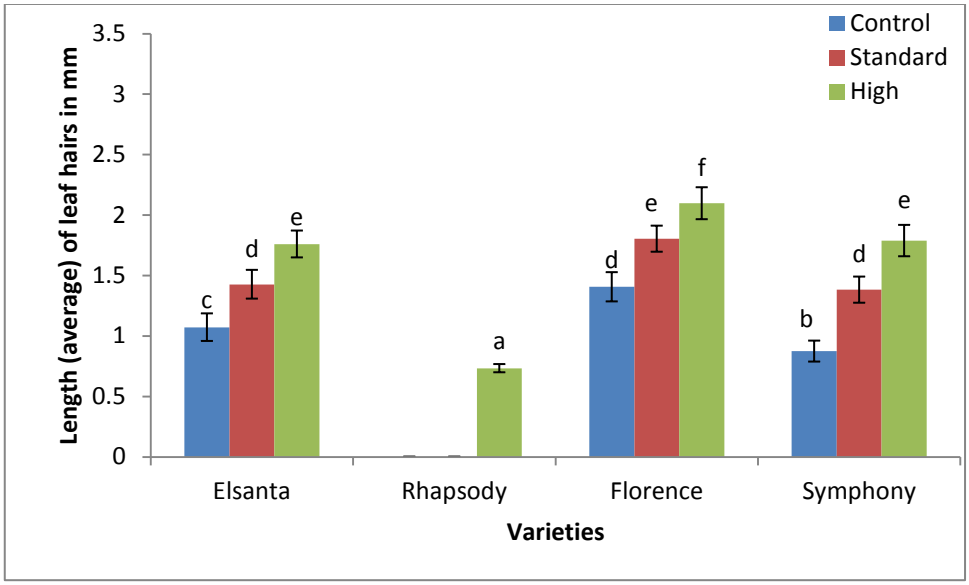


Figure 29: Length of leaf hairs on the upper surface of leaves in different strawberry varieties treated weekly (sprayed each week for 5 weeks, total 5 sprays) with different concentrations (standard and high) of silicon wetter and control (no application). Samples were collected one week after the final spray. A total of 12 leaves for each treatment were used to measure the average length of leaf hairs (n=12). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (Post-hoc test) in the statistical software program, SPSS for Windows version 17.

Moreover, on the lower surface, in Rhapsody there were significant differences of length of leaf hairs were observed between the control and the high concentration of Omex SW7 treated leaves. The high concentration of Omex SW7 treated leaves showed longer leaf hair length compared to the control and the standard treatment. In Elsanta, Florence and Symphony, significant differences were observed between control and treated leaves. Standard and high concentrations of Omex SW7 treated leaves showed significantly (P<0.05) longer leaf hair length compared to hairs on untreated leaves.

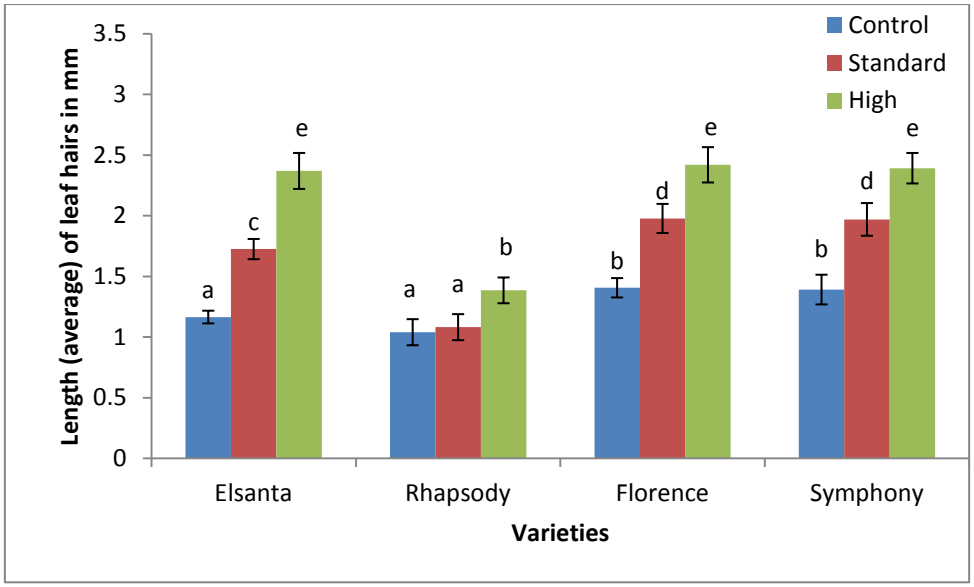


Figure 30: Length of leaf hairs on the lower surface of leaves in different strawberry varieties treated weekly (sprayed each week for 5 weeks, total 5 sprays) with different concentrations (standard and high) of silicon wetter and control (no application). Samples were collected one week after the final spray. A total of 12 leaves for each treatment were used to measure the average length of leaf hairs (n=12). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (Post-hoc test) in the statistical software program, SPSS for windows version 17.

3.3.12. Relationship between the length of leaf hairs and accumulation of silicon

The foliar application of standard and high concentrations of silicon showed increased silicon concentrations as well as increased the length of leaf hairs on both the upper and lower surfaces of leaves in all varieties used in the study. In Elsanta it was observed that there was a positive correlation between accumulation of silicon and the length of leaf hairs on both the upper and lower surfaces of leaves (Table 16). The correlation coefficients (r) were 0.997 and 0.992 and regression coefficients (R^2) were 0.993 and 0.983 for the upper and lower surfaces of leaves respectively. In Rhapsody there was a positive correlation between silicon accumulation and the length of leaf hairs on the lower surface of leaves and no relation was observed on the upper surface of the leaves in this variety due to lack of hairs (Table 16). For the lower surface of leaves the correlation coefficient (r) was 0.906 and regression coefficient (R^2) was 0.821.

Table 16: Relationship between concentrations of silicon and the length of leaf hairs on both the upper and lower surfaces of leaves in the different varieties

Variety	Upper surface		Lower surface	
	Correlation coefficient (r)	Regression coefficient (R^2)	Correlation coefficient (r)	Regression coefficient (R^2)
Elsanta	0.997	0.993	0.992	0.983
Rhapsody	-	-	0.906	0.821
Florence	0.817	0.667	0.827	0.684
Symphony	0.951	0.905	0.942	0.888

In both Florence and Symphony it was observed that there was a positive correlation between accumulation of silicon and the length of leaf hairs on both the upper and lower surfaces of leaves (Table 16).

3.4. Discussion

The work reported here showed that though strawberry is a non-accumulator of silicon (Miyake and Takahashi, 1986) spraying with the silicon wetter (Omex SW7) onto the leaf surface does result in accumulation of silicon in the leaves. There were significant differences in concentrations in different treatments and different timings. Strawberry leaves collected from standard, high and very high concentrations of Omex SW7 treated plants showed significantly ($P<0.05$) higher silicon concentrations compared to that in leaves collected from the control plants (Figure 17). This effect occurs on all varieties tested although there was a significant difference in the accumulation of silicon in the leaves collected from four varieties (Elsanta, Rhapsody, Florence and Symphony) after being treated with different concentrations of Omex SW7 (Figure 26). Leaves collected from the high concentration of Omex SW7 treated plants from all the four varieties showed significantly ($P<0.05$) higher silicon concentrations compared to that in control and standard Omex SW7 treated leaves. There was no significant difference ($P>0.05$) found on the leaves collected from the standard Omex SW7 treated leaves and the leaves collected from untreated plants in Florence (Figure 26). This might be due to the varietal difference whereby the silicon was not taken up so readily from the low concentrations of silicon in standard Omex SW7. Liang *et al*., (2009) found that high

concentrations of foliar application of silicon (Potassium metasilicate-K_2SiO_3) increased the silicon content in the leaves of cucumber plants which was also a silicon non-accumulator plant. This experiment (chapter 3) showed that, concentrations of silicon increased by the addition of silicon wetter Omex SW7 which was also supported by Wang and Galletta (1998) where they found that foliar application of silicon increased the silicon levels in the strawberry leaves.

After foliar application of Omex SW7, silicon is absorbed through the cuticle and transported in the xylem and deposited in the epidermal cell, cell wall, hairs or trichomes. There is a debate about foliar application of silicon and increased concentrations of silicon in leaves. In rice, X-ray analysis indicated that silicon accumulated in leaves when silicon was root applied and foliar applied silicon could be easily removed by rinsing (Rezende *et al*., 2009). However, Dallagnol *et al*., (2012) showed that rinsing could not remove silicon completely. They also found that foliar applied silicon treated leaves (rinsed) decreased the powdery mildew colony in melon compared to that in control. The study of Dallagnol *et al*., (2012) suggested that rinsing reduced the concentration of silicon in leaves to some extent but rinsing could not prevent silicon absorption on the leaf surface through the cuticle. The amount of silicon which is deposited on the external surface of the leaves could be removed by rinsing. Although rinsing removed the silicon from the external surface however rinsing could not remove the amount of silicon which is deposited in the epidermal cell wall and also in the other parts of the plants. Menzies *et al*., (1992) observed that silicon sprayed leaves that were washed to remove surface silicon 4 to 5 hours before inoculation had significantly fewer colonies than non sprayed leaves. As a result of this, Menzies *et al*., (1992) stated that silicon absorbed before the wash may have been sufficient to reduce the establishment of pathogen colonies by enhancing resistance. In this study (chapter 3) all leaves were washed to remove any surface silicon. Subsequently silicon was extracted from the leaves and then measured (chapter 2, section 2.7.2).

To investigate the effect of timing, strawberry plants were treated with silicon wetter (Omex SW7) at three different intervals and it was observed that absorption rate of silicon in strawberry leaves was significantly different with different timing of applications. In Elsanta, it was observed that weekly application (total 5 sprays) of 3

different concentrations of Omex SW7 showed significantly ($P<0.05$) higher silicon concentrations than when the application was 3 times in weeks 1, 3 and 5 or twice in weeks 1 and 5 (Figure 16). This was also observed in all other varieties and showed that weekly application of the high concentration of Omex SW7 increased the silicon levels in leaves. According to Datnoff *et al*., (2007) accumulation of silicon in the leaves depends on the source and continuous supply of the silicon. Bowen *et al*., (1992) observed that foliar application of extremely high concentrations of silicon increased the silicon concentrations in the leaves. After foliar application, silicon deposits on the upper surface of the leaves which may be removed by rinsing, this is perhaps the reason that foliar application of silicon needs high concentrations of silicon. Silicon has not been reported to cause toxicity in excess level (Ma *et al*., 2001) however; Kamenidou *et al*., (2008) observed that high concentration of silicon (Sodium silicate 100 mg/l weekly application) had a detrimental effect on plant growth and development. The results reported in chapter 3 showed that the very high concentration of Omex SW7 (Omex SW7 at 5% which contains 540.40 mg/l Si) had a harmful effect on plant growth and development. It was observed that leaves of plants treated with the very high concentration of Omex SW7 had a white surface which may be detrimental to photosynthesis.

In this experiment, it was examined whether the silicon supplement had any effect on the morphological changes of the strawberry leaves. Results revealed that the accumulation of silicon affects the morphology of the strawberry leaves resulting in the formation of additional leaf hairs, which were longer than when the plants were untreated. Microscopic observations of silicon treated leaves showed that there were increased density of leaf hairs on both the upper (Figure 18B) and lower surfaces (Figure 19) of leaves. It was observed that the weekly application (total 5 sprays) of three different concentrations of Omex SW7 showed significantly higher ($P<0.05$) leaf hair numbers on both the upper (Figure 18B) and lower surfaces (Figure 19) of leaves compared to application 3 times in weeks 1, 3 and 5 or twice in weeks 1 and 3. Leaf trichomes (leaf hairs) are hair like appendages that develop from epidermal cells; some epidermal cells developed into trichome whereas surrounding cells developed into regular epidermal cells (Werker, 2000). Leaf trichome's size and density vary genetically among plant species and may also vary within individual plants (Dalin *et al*.,

2008). In strawberries, the length and densities of leaf hairs varies between varieties which was observed in the different varieties tested in this study. The genetic basis of leaf hair production has been explored in detail in the model species *Arabidopsis thaliana*. Studies of the genetic basis of leaf hair formation have identified that many genes involved in leaf hair initiation, spacing and shapes. Besides this, abiotic stress, such as drought and UV-radiation, may influence leaf hair formation (Nagatta *et al*., 1999). In *Arabidopsis thaliana*, artificial damage, but also application of jasmonic acid and application of gibberellin increases leaf hair production (Traw and Bergelson, 2003). The information reported in different studies indicates that there are many factors which influence leaf hair initiation, spacing and density. The results reported here in this chapter showed that application of different concentrations of Omex SW7 increased leaf hair numbers on both the upper (Figure 27) and lower (Figure 28) surfaces of leaves. Moreover, this effect occurs on all varieties tested and in Rhapsody had the effect of actually stimulating the production of hairs where there were none on the untreated control. In Rhapsody there were no hairs on the upper surface of leaves but at a high concentration of Omex SW7 stimulated the hair growth (Figure 27). The results also showed the differences in the density of leaf hairs in the four varieties, this was a result of the rate at which each variety accumulates silicon (Figures 27 and 28).

Results also showed that hairs on the lower surface were more than the leaf hairs on the upper surface when the four varieties were compared. On the lower surface of leaves, the high concentration of Omex SW7 treated leaves showed significantly ($P<0.05$) higher leaf hair numbers compared to the control and the standard Omex SW7 treated leaves in all the four varieties (Figure 28). According to Hayward and Parry (1973) deposition of silicon differs between the upper and the lower leaf surfaces. In barley, Hayward and Parry (1973) found that total silicon content was greatest on the lower surface and on the upper surface silicon was confined to the trichomes. However, Kaufmann *et al*., (1985) found that in different grasses (C3 and C4) silicon deposition was more on the upper surface in comparison with the lower surface of leaves. The information from the above studies stated that silicon deposition differs between plant species. However, most of the studies indicated that trichomes or leaf hairs are the most important sites of silicon deposition. Non uniform deposition and distribution of silicon and the mechanism by which tissues avoid silicon deposition has not been understood.

The information reported in the literature stated that there are some significant factors which could influence silicon deposition in plants, such as, age of the plants, type and location of tissues as well as uptake through leaves or roots. Relevant soil factors would include silica, nutrient, water content, pH and soil type. However, the mechanism by which tissues avoid silicon deposition and also the mechanism by which accumulation of silicon in the trichome's base increases leaf hair numbers remain to be elucidated.

The results showed that foliar applications of standard, high and very high concentrations of silicon increased silicon concentrations in leaves as well as increased the number of leaf hairs on both the upper and lower surfaces of leaves. This suggests that there is a relationship between density of leaf hairs and the availability of silicon. It was observed that there was a significant positive correlation ($P<0.01$) between accumulation of silicon and the number of leaf hairs on both the upper and lower surfaces of leaves in the variety Elsanta (Figures 20 and 21). Positive correlation was also observed in the other three varieties used in the study; however the correlation coefficients (r) and regression coefficients (R^2) were different for the different varieties, as shown in Table 15. These results suggested that there was a correlation between silicon levels and leaf hair numbers in all varieties studied, though there were varietal differences.

This study also revealed the difference in length of leaf hairs in the four varieties as a result of different rates of silicon accumulation. Length of leaf hairs were increased when the four varieties of strawberry leaves were treated at the high rate of Omex compared to control and the standard rate of Omex SW7 treated leaves. This was observed on both the upper (Figure 29) and lower surfaces (Figure 30) of leaves in all the four varieties. These findings showed that weekly application of the high rate of Omex SW7 resulted in changes in morphological structure with respect to density and length of leaf hairs. These two findings regarding the morphological changes of strawberry leaves such as increased leaf hair numbers as well as lengths have been supported by several investigators where they mentioned that application of silicon induced physiological and physical changes in the plants to increase resistance against pathogen (Kanto *et al*., 2007). The distribution of silicon in the leaf epidermis of cucumber was examined using scanning electron microscope and x-ray analysis and

silicon was found primarily in cells surrounding the bases of the trichomes (Samuels *et al*., 1991). According to Adatia and Besford (1986) silicon was translocated in the xylem but may then be taken up by certain cells, e.g. the trichomes. According to the above investigation it can be postulated that the application of silicon accumulated in the trichome (leaf hairs) bases and this may have increased the number and length of leaf hairs on the upper and lower surfaces of the leaves in this study. The relationship between the length of leaf hairs and the uptake of silicon was investigated. It was observed that there was a significant positive correlation ($P<0.01$) between accumulation of silicon and the length of leaf hairs on both the upper and lower surfaces of leaves in Elsanta (Figures 24 and 25). Besides this, positive correlations were observed in all the varieties (Table 16); however the correlation coefficients (r) and regression coefficients (R^2) were different for the different varieties. These might be due to the varietal efficiency which is mentioned above. These relationships indicated the possibility that silicon application on leaf surface increased the length of leaf hairs on both the upper and lower surfaces of leaves.

The present study revealed that silicon uptake can be enhanced by the application of Omex SW7. Results also revealed that the enhanced levels of silicon affect the morphology of the strawberry leaves resulting in the formation of additional leaf hairs, which were longer than when the plants were untreated. This effect observed on all the varieties used in the study. Wang and Galletta (1998) showed that the application of silicon stimulated the growth and production of strawberry plants. This study showed accumulation of silicon and a correlation between silicon levels and leaf hair density and length. This could contribute to disease resistance (Kortekamp and Zyprian, 1999). Silicon had been shown to increase resistance to the powdery mildew fungus in a number of plant species such as cucumber, melon, rose (Menzies *et al*., 1991; Dallagnol *et al*., 2012; Shetty *et al*., 2012). Therefore the application of the silicon based wetter may help to strawberry plant to absorb silicon and improve resistance against powdery mildew infections. However, further work is required to establish evidence for this protective effect in the field. This effect was further examined in a field trial in the 2011 season.

Chapter 4: An investigation of the effect of root application of silicon (Omex SW7) on strawberry plants in the glasshouse

4.1. Introduction

Silicon accumulation differs greatly between plant species. Differences in silicon content between plant species are related to differences in silicon uptake by the roots. The beneficial effects of silicon are mainly associated with its high deposition in plant tissues, enhancing their strength and rigidity. However, until the beginning of the 20th century the beneficial role of silicon in plant growth and development was overlooked (Epstein, 1999; Ma and Takahashi, 2002). The abundance of the silicon element in nature and fact that the silicon deficiency symptoms are not visible in all plant species might be the reason for the ignorance of silicon by plant physiologists. However, most soils contain significant quantities of silicon, but continuous cropping particularly with crops that accumulate significant quantities of silicon can reduce plant available silicon. Repeated rice cropping can reduce silicon levels in many rice growing soils which initially contained significant quantities of silicon. When silicon is depleted silicon fertilization becomes beneficial for growth and disease resistance in rice (Datnoff *et al*., 1997). Therefore silicon fertilization is required, particularly in soil which contains low amounts of plant available silicon and for known silicon accumulating plants such as rice and sugarcane. Factors that can contribute to low silicon concentrations in the soil solution are the low solubility of silicon minerals in the parent materials (Jones and Handreck, 1967).

Plants take up silicon by the roots from solutions below pH 9 in the form of silicic acid (Jones and Handreck, 1965; Raven, 2003). Following uptake by the roots, silicon is translocated to the shoot via the xylem through transpiration. The form in which silicon is ultimately deposited is mainly amorphous silica $SiO_2.\ nH_2O$ (Epstein, 1994). Many studies have investigated silicon in the above ground plant organs, but there is still a great lack of knowledge with regard to silicon in roots. In rice and wheat silica deposition was found in the root endodermis (Parry and Soni, 1972; Bennett, 1982). On the other hand no silica deposition was observed in maize roots (Bennett and Sangster, 1982). In roots, the amounts of silicic acid are low compared to shoots. In general, the

major proportion of silicon which is taken up by the roots is translocated to the shoots (Ma and Yamaji, 2006). In strawberry silicon is translocated from the roots to the shoots and leaves (Miyake and Takahashi, 1986). Miyake and Takahashi (1986) reported that after the application of 50 ppm SiO_2 in strawberry plants, silicon was translocated to all the organs of the strawberry plants. Hairs or trichomes are another important site of silicon deposition (Kaufmann *et al*., 1981). The hydrated amorphous silica is immobile and not redistributed. For this reason, after polymerization, silicic acid is no longer available as a source of silicon for any other parts of the plant. According to Jones and Handreck (1965) only 1% of the total silicon is present in the form of silicic acid.

Nowadays, silicon is still not recognised as an essential element for plant growth and development but the beneficial effects of this element on the growth, development, yield and disease resistance have been observed in a wide variety of plant species (Epstein, 1999; Ma, 2004). Okuda and Takahashi (1965) showed that silicon influenced the growth of rice plant kept in plastic container. Both roots and shoots were longer in the presence of silicon and the grain yield was greater. At harvest, the dry weight of ears was half or two-thirds in control plants (without silicon) compared with silicon treated plants. Beneficial effects of silicon on rice have been reported by several workers; however the response of dicotyledons to silicon has received less attention. Cucumber is a dicotyledon and contains less silicon than rice. Adatia and Besford (1986) showed that although cucumber is a dicotyledon and contains less silicon, addition of silicon could be beneficial to cucumbers grown in a medium which contains less silicon, e.g. peat. Adatia and Besford (1986) stated that addition of silicon in the growing media increased the silicon content of cucumber leaves and found that deposition of silicon induced physical changes in the leaves. The lower leaves on the plants treated with high levels of silicon were darker green and remained well presented to intercept light efficiently, while the leaves on the plants treated with low levels of silicon were more prone to wilting. An experiment conducted by Miyaki and Takahashi (1986) stated that silicon increased the total amount of strawberry fruit and total yield of useful fruit (fruit weight of 6g or above) in a hydroponic culture. This is thought to be due to increased chlorophyll content (Wang and Galletta, 1998) and enhanced pollen fertility (Miyaki and Takahashi, 1986). Fruit quality traits influenced by silicate treatments are increased citric acid, malic acid and membrane bound glycol and phospholipids and decreased fructose,

glucose and myo-inositol content (Wang and Galletta, 1998). In this study, Omex SW7, a silicon based wetter was applied to the roots of strawberry plants and silicon accumulation and morphological changes in leaves were assessed. The hypothesis tested in this chapter was that application of silicon (Omex SW7) to the roots of the strawberry plants would lead to enhanced levels of silicon in the plants. The enhanced levels of silicon in the plants would lead to morphological changes in the leaves with regard to the density and length of leaf hairs.

4.1.1. Objectives

- To investigate the effect of root application of Omex SW7 on the accumulation of silicon in the leaves of strawberry plants.
- To investigate the effect of root application of Omex SW7 on the morphological changes that occurs in the leaves with regard to the modification of leaf hair length and density.
- To determine the relationships between accumulation of silicon and length and density of leaf hairs after root application of Omex SW7.
- To compare the effect of foliar and root applications of Omex SW7 on the accumulation of silicon in the leaves including modification of leaf hair length and density.

4.2. Materials and Methods

4.2.1. Plant material and growth

The strawberry variety Elsanta was used in this experiment and the plants of Elsanta were grown on the capillarity bench of the Hatfield glasshouse which was described in chapter 2, section 2.5. In this experiment (160-165) runners (Elsanta) were used to grow the plants and finally 144 healthy plants were selected for the experiment. In April 2009 runners were planted in the 12x6 cm pots filled with compost (Miracle Gro, Company) and pots were placed on the capillarity bench in the glasshouse. As plants were grown in April, artificial light was used to create 12-14 hours light during the day

(mentioned before in chapter 2, section 2.5). In April, natural day light (8-9 hours/day) and incandescent light which provided additional light 4-6 hours/day was used and from May to July natural day light was used and no artificial light was used. Temperatures were approximately 15-20^{0}C during the day and 12-15^{0}C at night. Normal plant fertilizer (described in chapter 2, section 2.5) was provided to all the pots. Plants were watered through the capillarity bench and no water was spread over the leaves (mentioned before in chapter 2, section 2.5).

4.2.2. Experimental design of the silicon treatments

Silicon wetter Omex SW7 was used as the source of silicon. Silicon was applied to the roots of the plants. There were two application rates (standard and high) and the product was applied at three different timings. Control plants received no treatment. Treatment rates and timings are shown in Table 17.

Table 17: Treatment rates and timings

Treatment rates and timings
Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si was applied each week for 5 weeks (total 5 applications)
Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si was applied in weeks 1, 3 and 5 (total 3 applications)
Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si was applied in weeks 1 and 5 (total 2 applications)
High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si was applied each week for 5 weeks (total 5 applications)
High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si was applied in weeks 1, 3 and 5 (total 3 applications)
High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si was applied in weeks 1 and 5 (total 2 applications)
Untreated (control)

The treatments were applied to 4 different blocks (Block A, B, C and D). Each block was divided into 9 rows and each treatment was applied to an individual row which

consisted of 4 plants. Within each block each treatment appeared once and they were distributed randomly (Table 18A). Each treatment was applied to 16 plants (4 rows from 4 different blocks). Date of application of the treatments and leaf collections from different rows of block A are shown in Table 18B and from block B, C and D are shown in Appendix 4.

4.2.3. Application of the treatments

Treatments were applied to the roots when the plants had 10-12 leaves. Omex SW7 (100 ml) was applied to the roots of each pot containing a plant. For applying Omex SW7, a small hole was made in the soil near the roots of the plant in each pot. A funnel was placed inside the hole and 100 ml Omex SW7 was poured in the funnel. After finishing the pouring of Omex SW7, the funnel was removed from the soil and placed in the hole by the roots in the soil of another pot. To avoid cross contamination different funnels for different treatments were used during application of the treatments. Application of the treatments was started from 7 June 2010. On 7 June all the treatments were applied to the roots of the plants in the different rows of block A (Table 18B). After that, treatments were applied on 14, 21, 28 June and 5 July 2010.

Table 18A: Distribution of treatments in the Hatfield glasshouse (variety Elsanta)

Block	Row	Treatment
A	1	Standard-(2 applications)
	2	High-(3 applications)
	3	Control
	4	High- (5 applications)
	5	High-(2 applications)
	6	Standard-(3 applications)
	7	Control
	8	Standard-(5 applications)
	9	Control

Block	Row	Treatment
B	10	High-(3 applications)
	11	Control
	12	Standard-(3 applications)
	13	High-(5 applications)
	14	Control
	15	High-(2 applications)
	16	Standard-(2 applications)
	17	Standard-(5 applications)
	18	Control

Block	Row	Treatment
C	19	Standard- (5 applications)
	20	Control
	21	Standard-(3 applications)
	22	High-(2 applications)
	23	High-(3 applications)
	24	Control
	25	High-(5 applications)
	26	Standard-(2 applications)
	27	Control

Block	Row	Treatment
D	28	High-(2 applications)
	29	Control
	30	Standard-(3 applications)
	31	Standard- (5 applications)
	32	Control
	33	High-(5 applications)
	34	High-(3 applications)
	35	Standard-(2 applications)
	36	Control

Table 18B: Randomised distribution of treatments, date of application of the treatments and leaf collections from different rows of block A (variety Elsanta).

Block	Row numbers and treatments	Application of the treatments date					Leaf collections
		Week 1 7.06.10	Week 2 14.06.10	Week 3 21.06.10	Week 4 28.06.10	Week 5 5.07.10	Week 6 12.07.10
A	Row-1 Standard applied twice (weeks 1 and 5)	Standard				Standard	Collection of leaves
	Row-2 High applied 3 times (weeks 1, 3 and 5)	High		High		High	Collection of leaves
	Row-3 Control	Control	Control	Control	Control	Control	Collection of leaves
	Row-4 High weekly (applied 5 times)	High	High	High	High	High	Collection of leaves
	Row-5 High applied twice (weeks 1 and 5)	High				High	Collection of leaves
	Row-6 Standard applied 3 times (weeks 1, 3 and 5)	Standard		Standard		Standard	Collection of leaves
	Row-7 Control	Control		Control		Control	Collection of leaves
	Row-8 Standard weekly (applied 5 times)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Row-9 Control	Control				Control	Collection of leaves

4.2.4. Collection of leaves

Leaves were collected on 12 July 2010 from all the rows of 4 different blocks after the application of the last treatment on 5 July. Treated and untreated (control) leaves were collected from four different blocks (A-D). Treated and untreated (control) leaves were collected from different rows of block A, shown in Table 18B, and leaves collected from block B, C and D, shown in Appendix 4. Leaves were kept in sample bags labelled with the date of collection and the treatment (rate + timing). All samples were stored in the -70^{0}C freezer (mentioned in chapter 2, section 2.6). During sampling 40 leaves were collected from each block for each treatment (10 leaves from each plant) and from 40 leaves, 20-30 leaves were used for measuring hair length and density as described in chapter 2, section 2.8. The remaining leaves were oven dried according to the method described in chapter 2, section 2.6 and dried leaves were then used for measuring silicon concentrations using the AID method described in chapter 2, section 2.7.2.1.

4.2.5. Data analysis

Data from studies of concentrations of silicon in strawberry leaves, density and length of leaf hairs after root application of different concentrations of Omex SW7 in the variety Elsanta were analysed by one way ANOVA (analysis of variance) under the Compare Mean in the SPSS version 17. Comparison between foliar and root treatments were analysed by a Univariate model under the general linear model in the statistical software program SPSS version 17. For further *post-hoc* analysis Tukey's HSD test was used and to differentiate the treatments in different groups homogeneous subsets test was used. The relationships between silicon level and density and length of leaf hairs were analysed by the method described in section 2.9.

4.3. Results

4.3.1. Effect of treatments on silicon concentrations in strawberry leaves (silicon wetter Omex SW7 was applied to roots)

Leaves collected from strawberry plants (variety Elsanta) after treatment with different concentrations of Omex SW7, which were applied to roots showed different levels of silicon absorption (Figure 31). It was observed that there was a significant difference in silicon absorption in different treatments. Strawberry leaves collected from standard and high concentrations of Omex SW7 (to roots) treated plants showed significantly ($P<0.05$) higher silicon accumulation compared to that in leaves collected from control (untreated) plants (Figure 31). Leaves collected from control plants showed the background level of silicon in strawberry plants. Highest levels of silicon accumulation were detected in leaves collected from the high concentration of Omex treated plants compared to all other treatments (Figure 31).

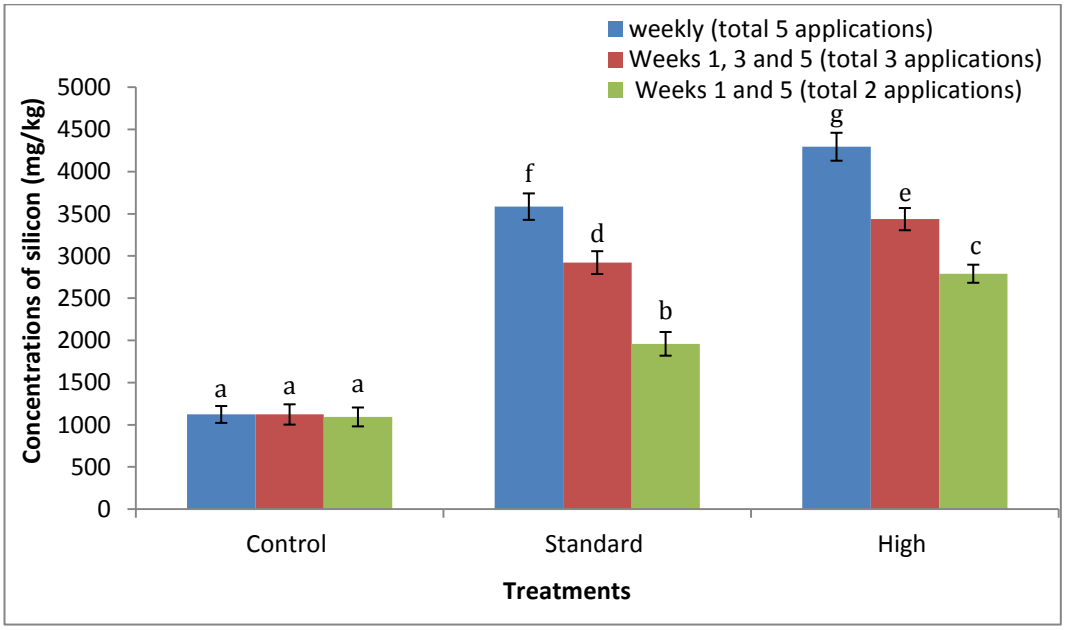


Figure 31: Concentrations of silicon in strawberry (variety Elsanta) leaves treated weekly (total 5 applications), weeks 1, 3 and 5 (total 3 applications) and weeks 1 and 5 (total 2 applications) with different concentrations (standard, high) of silicon wetter, which was applied to roots and control (no application). Samples were collected one week after the final application. Each value represents the mean of 6 replications (n=6). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

4.3.2. Effect of timing on silicon accumulation in strawberry leaves

Silicon wetter Omex SW7 was applied to roots of strawberry plants at three different timings, weekly (applied each week for 5 weeks, total 5 applications), applied in weeks 1, 3 and 5 (total 3 applications) and applied in weeks 1 and 5 (total 2 applications). Results showed that when standard and high concentrations of Omex SW7 were applied weekly a total of 2000 ml (400 ml X 5 times) Omex SW7 was applied. Similarly when standard and high concentrations of Omex SW7 were applied in weeks 1, 3 and 5, a total of 1200 ml (400 ml X 3 times) and in weeks 1 and 5, a total of 800 ml (400 ml X 2 times) Omex SW7 was applied. Total amounts of silicon in 2000 ml, 1200 ml and 800 ml of Omex SW7 (standard and high) and concentrations of silicon in the leaves after the application of the different treatments were determined by spectrophotometer which has been shown in Table 19A.

Table 19A: Total amounts of silicon in the different treatments and accumulation of silicon in the leaves after different treatments.

Treatments	Total amounts of Omex SW7 applied	Total amounts of silicon in the treatments (mg)	Mean concentrations of silicon in the leaves (mg/l)
Control	No application of Omex SW7		11.23
Standard weekly (applied 5 times)	2000 ml	54.04	35.86
High weekly (applied 5 times)	2000 ml	540.40	42.96
Control	No application of Omex SW7		11.23
Standard applied 3 times (weeks 1, 3 and 5)	1200 ml	32.42	29.23
High applied 3 times (weeks 1, 3 and 5)	1200 ml	324.24	34.38
Control	No application of Omex SW7		10.94
Standard applied twice (weeks 1 and 5)	800 ml	21.61	19.60
High applied twice (weeks 1 and 5)	800 ml	216.16	27.91

Dried leaf samples were used in this study to determine the concentrations of silicon. The silicon concentrations measured by the spectrophotometer were therefore adjusted in relation to the weight of plant material used and expressed as mg/kg silicon in dried

leaf tissue. This is shown in Figure 31 and in Table 19B. Concentrations of silicon in the leaves indicated that weekly (total 5 times) application of Omex SW7 increased the silicon concentrations by up to 219.21% (standard) and 282.43% (high) compared to control (Table 19B). Similarly after applications of different concentrations of silicon in weeks 1, 3 and 5 (total 3 applications) and in weeks 1 and 5 (total 2 applications) concentrations of silicon increased in leaves, as shown in Table 19B.

Table 19B: Increased percentage of silicon concentrations in the leaves after different treatments

Treatments	Mean concentrations of silicon (mg/kg) in leaves	Increased silicon concentrations in leaves after treatment compared to control (mg/kg)	Percentage of increased silicon concentrations in leaves compared to control
Control	1123.50		
Standard weekly (applied 5 times)	3586.33	2462.83	219.21%
High weekly (applied 5 times)	4296.67	3173.17	282.43%
Control	1123.42		
Standard applied 3 times in (weeks 1, 3 and 5)	2923.33	1799.83	160.20%
High applied 3 times in (weeks 1, 3 and 5)	3438.00	2314.5	206.02%
Control	1094.50		
Standard applied twice in (weeks 1 and 5)	1960.83	866.33	79.15%
High applied twice in (weeks 1 and 5)	2791.67	1668.17	152.41%

Highest levels (282.43%) of silicon were observed in the leaves treated weekly with the high concentration of Omex SW7 and lowest levels (79.15%) of silicon were observed in

the leaves treated with the standard concentration of Omex SW7 which was applied in weeks 1 and 5 (total 2 applications) compared to the control (Table 19B). It was observed that the absorption rate of silicon in strawberry leaves was significantly different with different timing of applications (Figure 31). Results revealed that the weekly application (total 5 times) of 2 different concentrations (standard and high) of Omex SW7 showed significantly ($P<0.05$) higher silicon accumulation compared to 3 applications in weeks 1, 3, and 5, or 2 applications in weeks 1 and 5 (Figure 31). Moreover, standard and high concentrations of Omex SW7 when applied in weeks 1, 3 and 5 (total 3 applications) showed significantly ($P<0.05$) more silicon accumulation in leaves compared to treatments applied in weeks 1 and 5 (total 2 applications) (Figure 31).

4.3.3. Effect of silicon on the density of leaf hairs

The effect of root application of two different concentrations of Omex SW7 on the density of leaf hairs of strawberry leaves was examined using a dissection microscope. Microscopic observation of silicon treated leaves showed that there were increased number of leaf hairs on both the upper and lower surfaces of leaves. The number of leaf hairs observed was more on the lower surface in comparison with the upper surface of leaves. Results also indicated that there was a significant difference in number of leaf hairs in different treatment groups. On the upper surface, leaves collected from the plants after being treated with the two different concentrations of Omex SW7 which were applied to roots showed significantly ($P<0.05$) higher leaf hair numbers compared to the leaves collected from the control plants (Figure 32). Moreover, leaves collected from the plants treated with the high concentration of Omex SW7 showed significantly ($P<0.05$) higher leaf hair numbers compared to the leaves collected from the standard and control (untreated) plants. The number of leaf hairs on the lower surface of leaves collected after different treatments are shown in Figure 33. It was observed that the number of leaf hairs was significantly ($P<0.05$) higher in the leaves collected from the plants treated with the high concentration of Omex SW7 compared to the leaves collected from the control and the standard Omex SW7 treated plants.

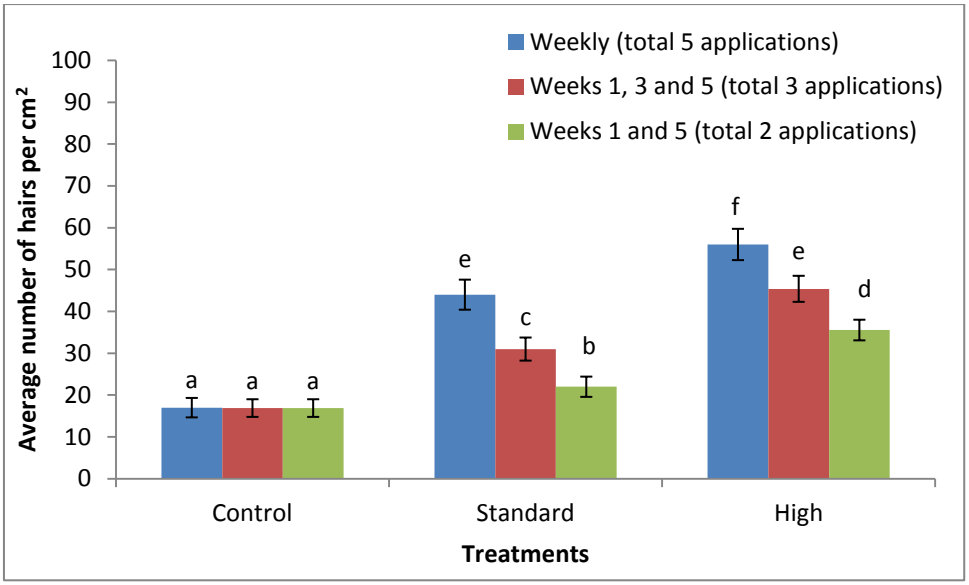


Figure 32: Number of leaf hairs on the upper surface of leaves in strawberry variety Elsanta treated weekly (total 5 applications), weeks 1, 3 and 5 (total 3 applications) and weeks 1 and 5 (total 2 applications) with different concentrations (standard, high) of silicon wetter, which was applied to roots and control (no application). Samples were collected one week after the final application. A total of 20 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 (n=20). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

Timing also had an effect on the density of leaf hairs on both the upper (Figure 32) and lower surfaces (Figure 33) of treated leaves. It was observed that the weekly (5 applications) application of two different concentrations of Omex SW7 showed significantly ($P<0.05$) higher leaf hair numbers on both the upper (Figure 32) and lower surfaces (Figure 33) of leaves compared to 3 applications in weeks, 1, 3 and 5 or 2 applications in weeks 1 and 5. Moreover, standard and high concentrations of Omex SW7 when applied in weeks 1, 3 and 5 (total 3 applications) showed significantly ($P<0.05$) higher leaf hair numbers compared to those applied in weeks 1 and 5 (total 2 applications) on both the upper and lower surfaces of leaves (Figures 32 and 33).

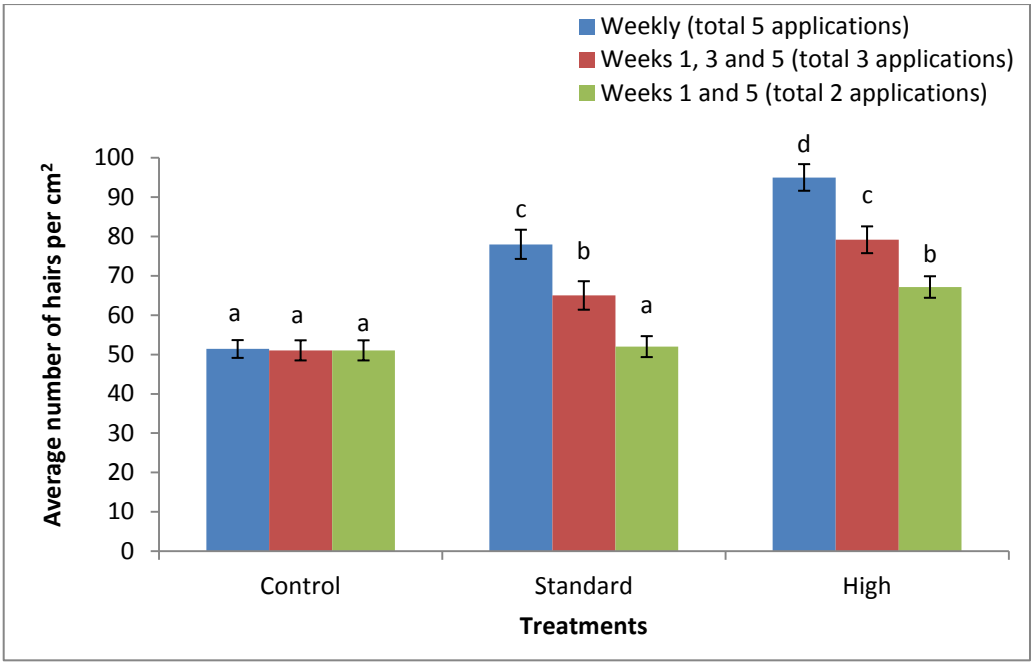


Figure 33: Number of leaf hairs on the lower surface of leaves in strawberry variety Elsanta treated weekly (total 5 applications), weeks 1, 3 and 5 (total 3 applications) and weeks 1 and 5 (total 2 applications) with different concentrations (standard, high) of silicon wetter, which was applied to roots and control (no application). Samples were collected one week after the final application. A total of 20 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 (n=20). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

4.3.4. Relationship between the leaf hair numbers and accumulation of silicon

The results showed that root applications of standard and high concentrations of silicon increased silicon concentrations in leaves as well as increased the number of leaf hairs on both the upper and lower surfaces of leaves. The results from the leaves collected on 12 July 2010 were used to find the relationship. It was observed that there was a significant positive correlation ($P<0.01$) between accumulation of silicon and the number of leaf hairs on both the upper and lower surfaces of leaves (Figures 34 and 35). The correlation coefficients (r) were 0.980 and 0.958 and regression coefficients (R^2) were 0.960 and 0.918 for the upper and lower surfaces of leaves respectively.

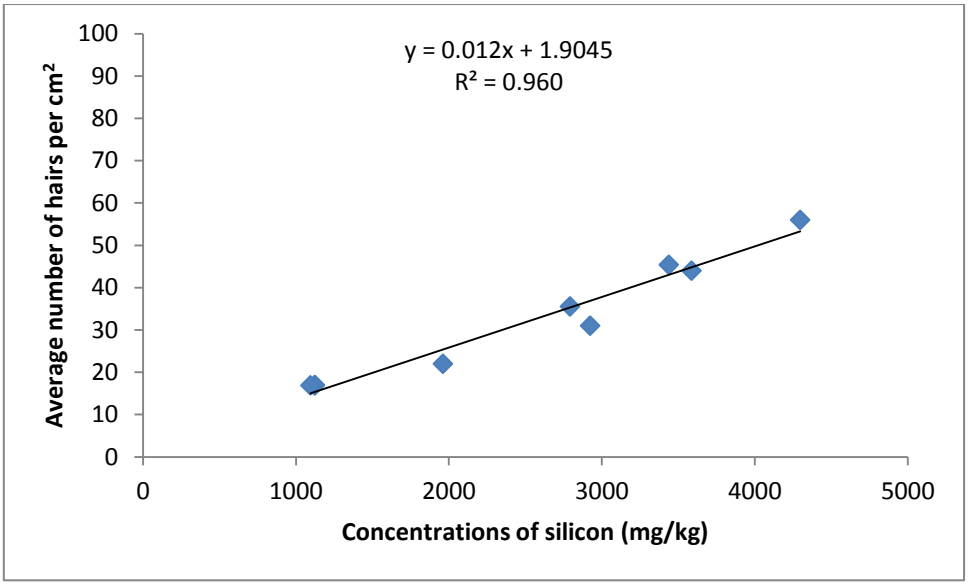


Figure 34**:** Relationship between concentrations of silicon and average number of leaf hairs (per cm^2) on the upper surface of leaves (variety Elsanta). The correlation coefficient (r) was 0.980 and regression coefficient (R^2) was 0.960.

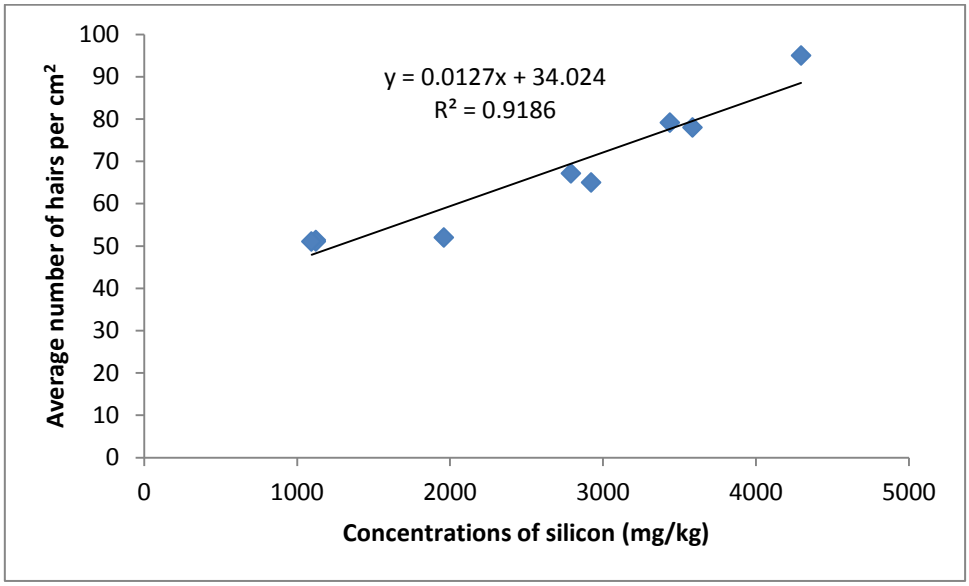


Figure 35: Relationship between concentrations of silicon and average number of leaf hairs (per cm^2) on the lower surface of leaves (variety Elsanta). The correlation coefficient (r) was 0.958 and regression coefficient (R^2) was 0.918

4.3.5. Effect of silicon on the length of leaf hairs

This study examined whether the silicon supplement had any effect on the length of leaf hairs. It was observed that the root application of silicon wetter Omex SW7 had an effect

on the length of leaf hairs on both the upper and lower surfaces of leaves. The application of two different silicon concentrations (standard and high) of Omex SW7 showed significantly ($P<0.05$) longer leaf hair length compared to control leaves (Figures 36 and 37). It was observed that the length of leaf hairs in the high concentration of silicon treated leaves showed significantly ($P<0.05$) longer length compared to that in the standard Omex SW7 treated leaves and also the leaves collected from the control plants (Figures 36 and 37) on both the upper and lower surfaces of leaves. Furthermore, it was also observed that the weekly (total 5 applications) application of two different concentrations of Omex SW7 showed significantly ($P<0.05$) longer leaf hair length on both the upper (Figure 36) and lower (Figure 37) surfaces of leaves compared to 3 applications in weeks 1, 3 and 5 or 2 applications in weeks 1 and 5.

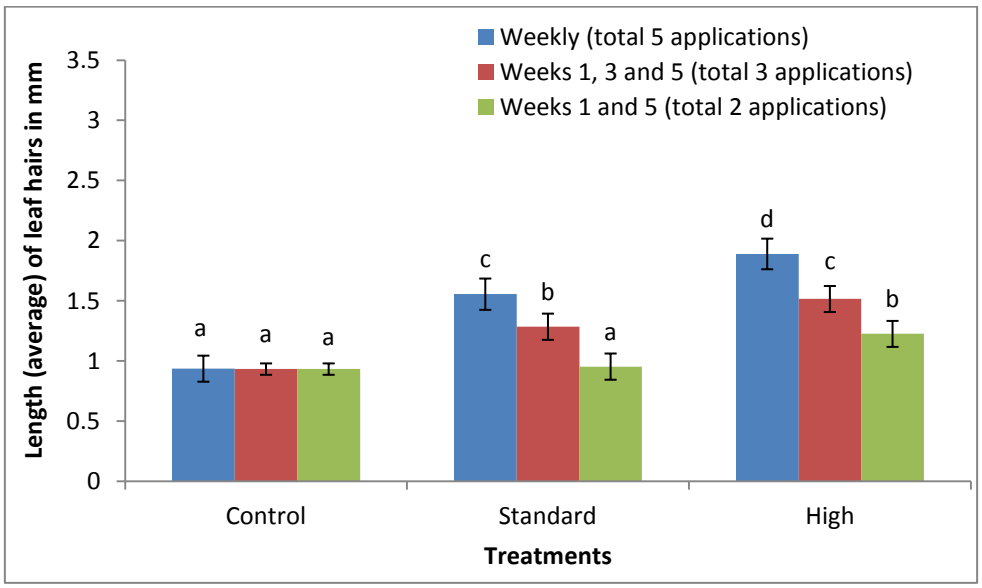


Figure 36: Length of leaf hairs on the upper surface of leaves in strawberry variety Elsanta treated weekly (total 5 applications), weeks 1, 3 and 5 (total 3 applications) and weeks 1 and 5 (total 2 applications) with different concentrations (standard, high) of silicon wetter, which was applied to roots and control (no application). Samples were collected one week after the final application. A total of 20 leaves for each treatment were used to measure the average length of leaf hairs (n=20). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

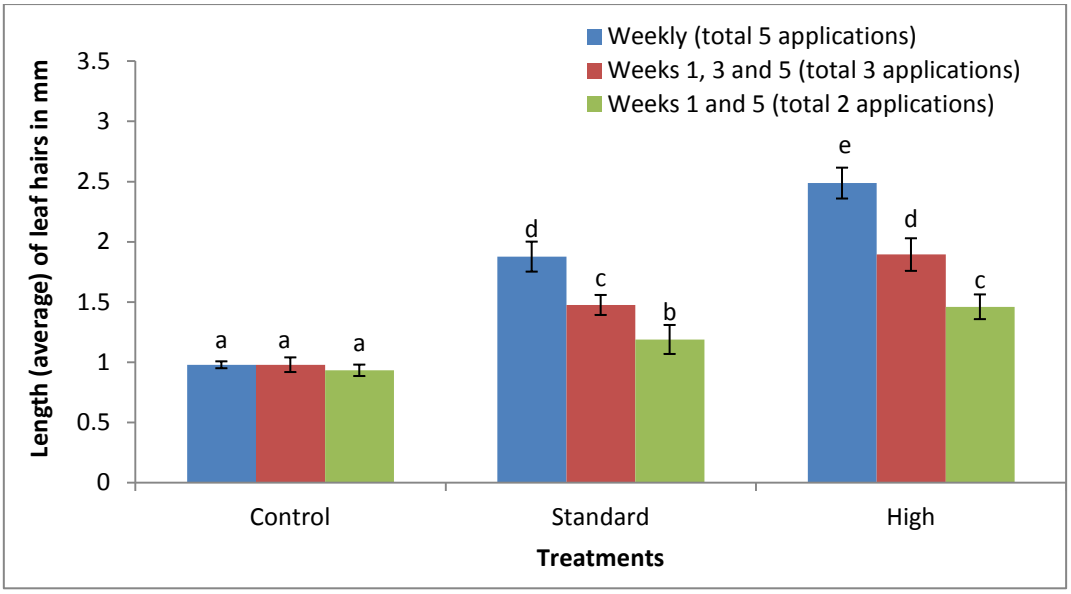


Figure 37: Length of leaf hairs on the lower surface of leaves in strawberry variety Elsanta treated weekly (total 5 applications), weeks 1, 3 and 5 (total 3 applications) and weeks 1 and 5 (total 2 applications) with different concentrations (standard, high) of silicon wetter, which was applied to roots and control (no application). Samples were collected one week after the final application. A total of 20 leaves for each treatment were used to measure the average length of leaf hairs (n=20). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

4.3.6. Relationship between the length of leaf hairs and accumulation of silicon

Root applications of standard and high concentrations of silicon showed increased silicon concentrations in leaves as well as increased the length of leaf hairs on both the upper and lower surfaces of leaves. Therefore, the relationship between the length of leaf hairs and the silicon level was investigated. It was observed that there was a significant positive correlation ($P<0.01$) between accumulation of silicon and the length of leaf hairs on both the upper and lower surfaces of leaves (Figures 38 and 39). The correlation coefficients (r) were 0.961 and 0.968 and regression coefficients (R^2) were 0.923 and 0.937 for the upper and lower surfaces of leaves respectively. These results indicated the possibility that silicon application on the leaf surfaces increased the length of leaf hairs on both the upper and lower surfaces of leaves.

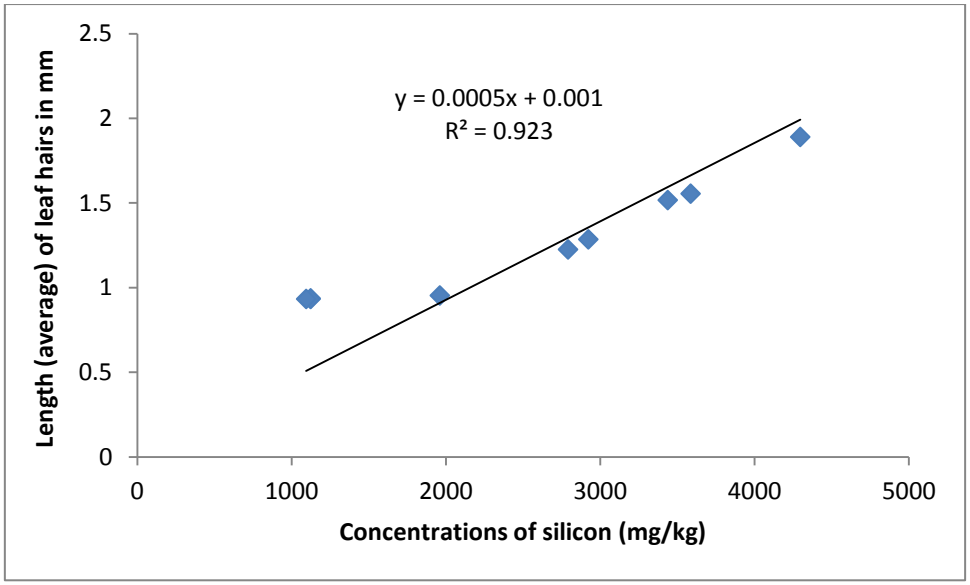


Figure 38**:** Relationship between concentrations of silicon and average length of leaf hairs on the upper surface of leaves (variety Elsanta). The correlation coefficient (r) was 0.961 and regression coefficient (R^2) was 0.923.

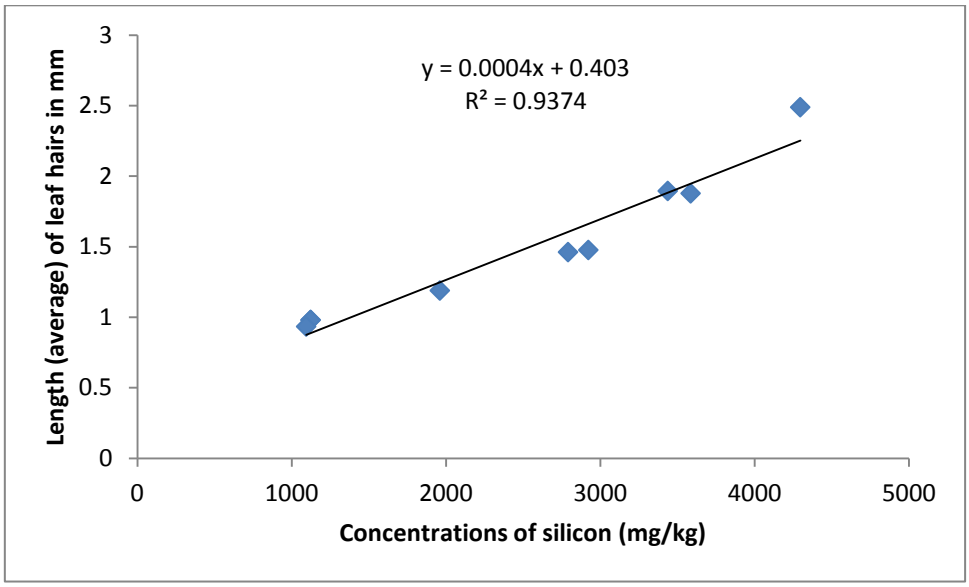
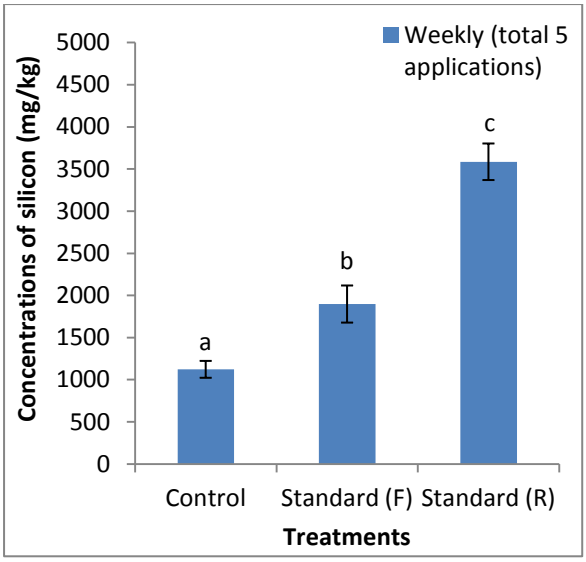


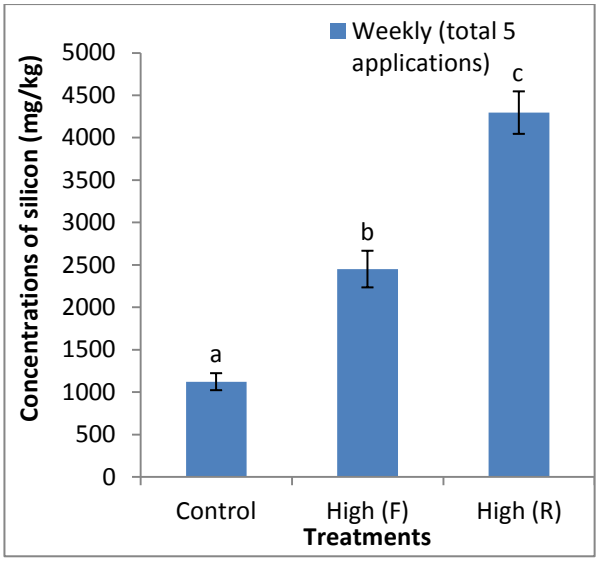
Figure 39: Relationship between concentrations of silicon and average length of leaf hairs on the lower surface of leaves (variety Elsanta). The correlation coefficient (r) was 0.968 and regression coefficient (R^2) was 0.937.

4.3.7. Comparison between foliar and root applications of Omex SW7 on strawberry plants

Foliar and root applications of different concentrations of Omex SW7 were evaluated for their effects on strawberry plants. Three different concentrations of Omex SW7 (standard, high and very high) were applied to strawberry plants at three different timings. Results of silicon accumulation and physical changes on leaves were shown in different graphs in chapter 3. In chapter 4 two different concentrations (standard and high) of Omex SW7 were applied to the root zone at three different timings. The very high concentration of Omex SW7 was not applied to roots. Reasons for not using the very high concentration of Omex SW7 has already been described in chapter 3 (section 3.3.8). Leaves collected from strawberry plants (variety Elsanta) after being treated weekly with foliar and root applications of different concentrations (standard and high) of Omex SW7 showed different levels of silicon absorption. Standard (F) represents standard foliar application i.e. the standard concentration of Omex SW7 applied on leaves and Standard (R) represents standard root application i.e. the standard concentration of Omex SW7 applied to roots. High (F) represents high foliar application i.e. the high concentration of Omex SW7 applied on leaves and High (R) represents high root application i.e. the high concentration of Omex SW7 applied to roots. Results showed that there was a significant difference in silicon absorption when different concentrations of silicon wetter were applied to strawberry plants at three different timings. Leaves collected from control plants showed significantly ($P<0.05$) lower silicon accumulation compared to the leaves collected from standard foliar and standard root applications. There was a significant difference in silicon accumulation between standard foliar and standard root treatment when the standard concentration of Omex SW7 was applied weekly (total 5 applications) (Figure 40A).

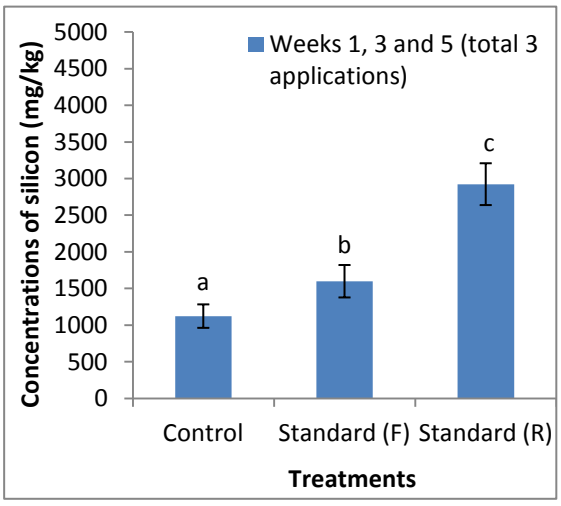


A

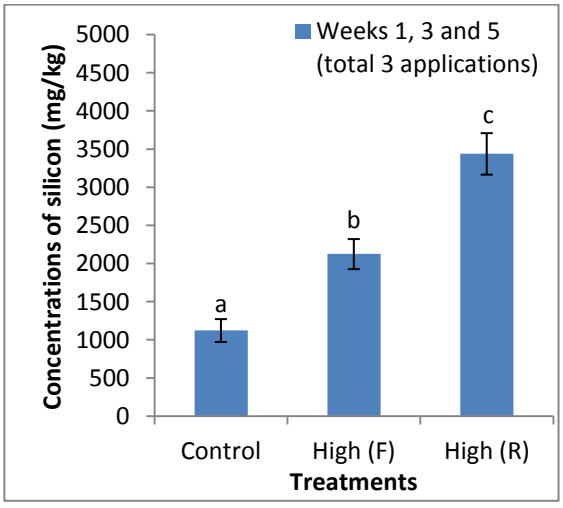


B

Figure 40(A-B): Comparison of silicon concentrations in strawberry leaves (variety Elsanta) after being treated weekly with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). Each value represents the mean of 6 replications (n=6). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

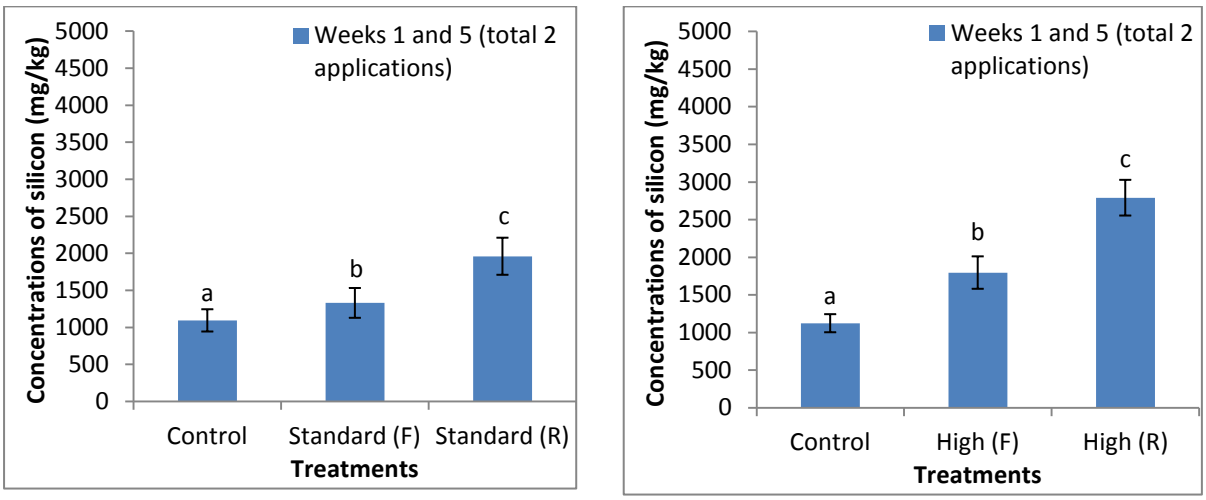


A



B

Figure 41(A-B): Comparison of silicon concentrations in strawberry leaves (variety Elsanta) after being treated in weeks 1, 3 and 5 with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). Each value represents the mean of 6 replications (n=6). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.



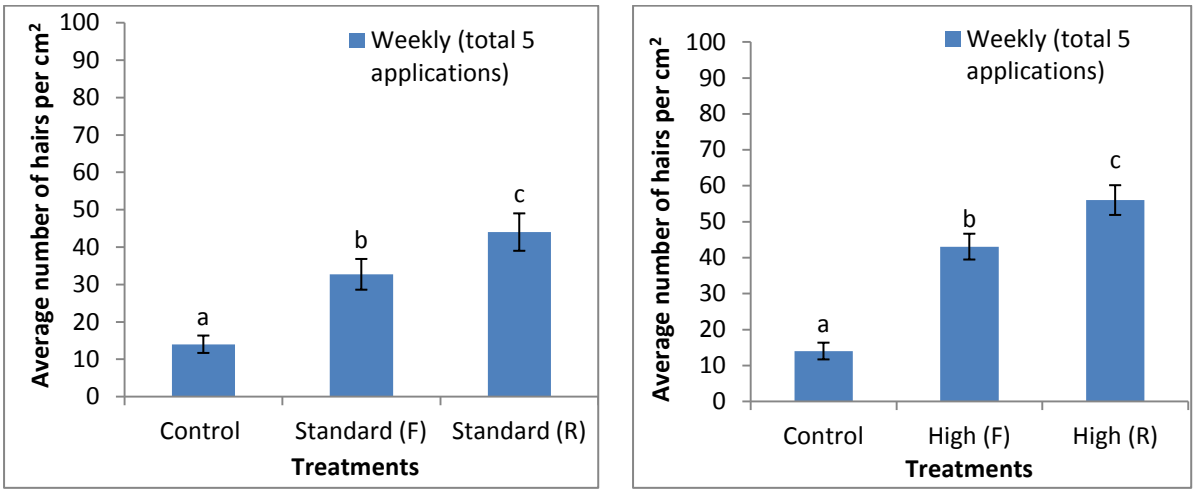
A B

Figure 42(A-B): Comparison of silicon concentrations in strawberry leaves (variety Elsanta) after being treated in weeks 1 and 5 with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). Each value represents the mean of 6 replications (n=6). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

Significant difference was also observed in silicon accumulation between high foliar and high root treatment when the high concentration of Omex SW7 was applied weekly (Figure 40B). Similar results were also found in silicon accumulation between foliar and root treatment when standard and high concentrations of Omex SW7 were applied in weeks 1, 3 and 5 (total 3 applications) and also applied in weeks 1 and 5 (total 2 applications) (Figures 41A and B and Figures 42A and B). Results revealed that root application significantly ($P<0.05$) increased silicon accumulation compared to the foliar application. Observation also indicated that when standard and high concentrations of Omex SW7 were applied to roots at three different timings significantly ($P<0.05$) more silicon accumulated in the leaves compared to the foliar treatments.

4.3.8. Comparison and effect of foliar and root applications of silicon on the density of leaf hairs (upper surface)

Results showed that standard and high concentrations of Omex SW7 when applied to leaves (foliar) and roots had an effect on the density of leaf hairs. There was a significant difference in the density of leaf hairs on both the upper and lower surfaces of leaves between foliar and root application.

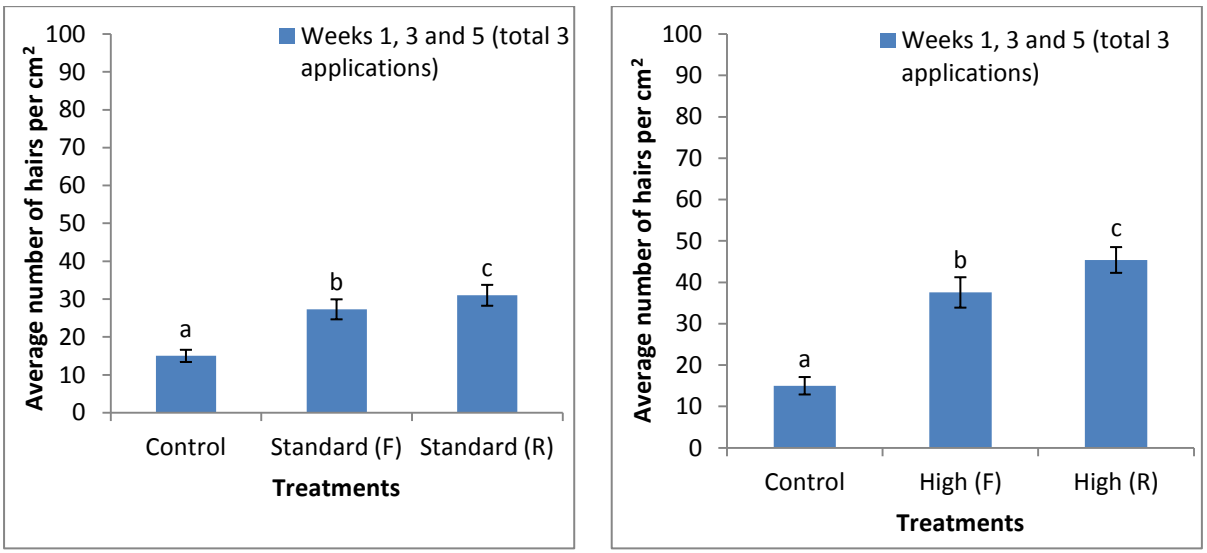


A B

Figure 43(A-B): Comparison of leaf hair numbers on the upper surface of strawberry leaves (variety Elsanta) after being treated weekly with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). A total of 20 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 (n=20). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

On the upper surface of leaves, the standard concentration of Omex SW7 when applied weekly (total 5 applications) showed significantly higher ($P<0.05$) leaf hair numbers on the leaves collected from the root treated plants compared to the leaves collected from standard concentration of foliar treatment (Figure 43A). Furthermore when the high concentration of Omex SW7 was applied weekly (total 5 applications) significant difference between high foliar and high root treatment was also observed (Figure 43B). On the upper surface of leaves significant ($P<0.05$) difference in leaf hair numbers was

observed between standard foliar and standard root treatment, when the standard concentration of Omex SW7 was applied in weeks 1, 3 and 5 (total 3 applications) (Figure 44A). Leaves collected from standard root treatment showed significantly ($P<0.05$) higher leaf hair numbers compared to the leaves collected from standard foliar treatment (Figure 44A). Similar results were also found between high foliar and high root treatment when the high concentration of Omex SW7 was applied in weeks 1, 3 and 5 (total 3 applications) (Figure 44B).

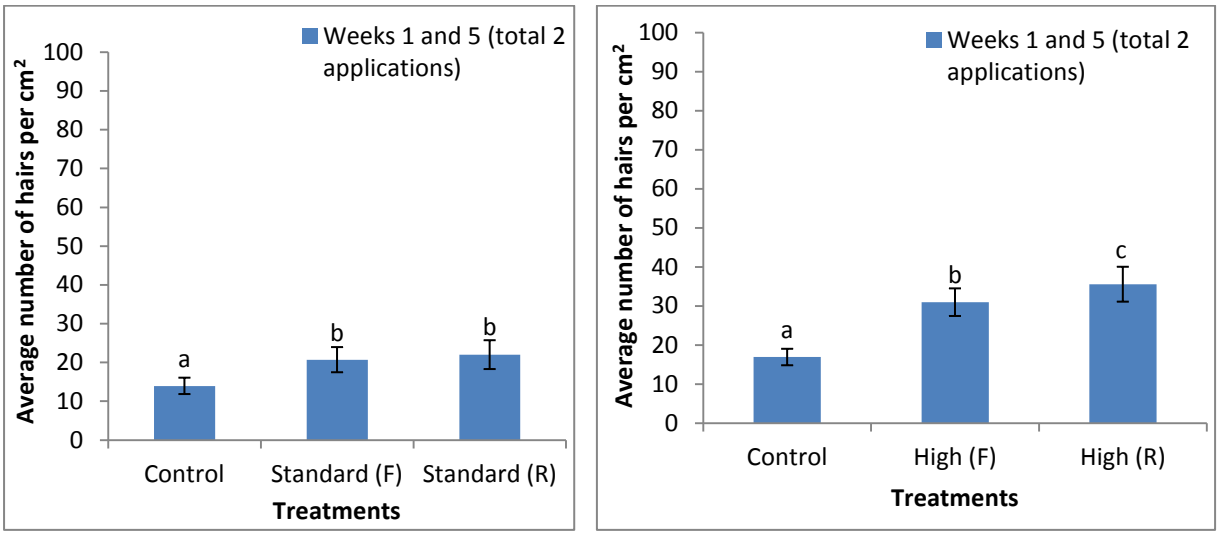


A B

Figure 44(A-B): Comparison of leaf hair numbers on the upper surface of strawberry leaves (variety Elsanta) after being treated in weeks 1, 3 and 5 with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). A total of 20 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 ($n=20$). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

On the upper surface of leaves standard and high concentrations of foliar and root treatment when applied in weeks 1 and 5 (total 2 applications) also had an effect on the density of leaf hairs. No significant difference ($P>0.05$) in leaf hair numbers was observed between standard foliar and standard root treatment when the standard concentration of Omex was applied in weeks 1 and 5 (total 2 applications) (Figure 45A). On the other hand a significant difference was found between high foliar and high root treatment when the high concentration of Omex was applied in weeks 1 and 5 (total 2 applications) (Figure 45B). Application of the high concentration of Omex SW7 to roots

in weeks 1 and 5 (total 2 applications) showed significantly (P<0.05) higher leaf hair numbers compared to the foliar treatment (Figure 45B).



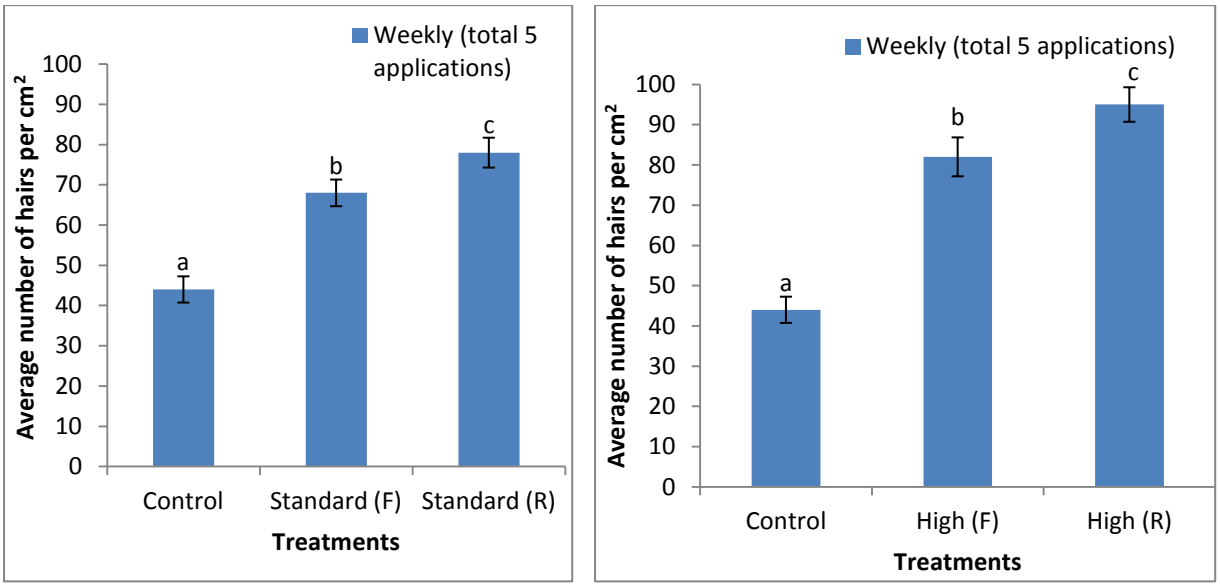
A B

Figure 45(A-B): Comparison of leaf hair numbers on the upper surface of strawberry leaves (variety Elsanta) after being treated in weeks 1 and 5 with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). A total of 20 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

4.3.9. Comparison and effect of foliar and root applications of silicon on the density of leaf hairs (lower surface)

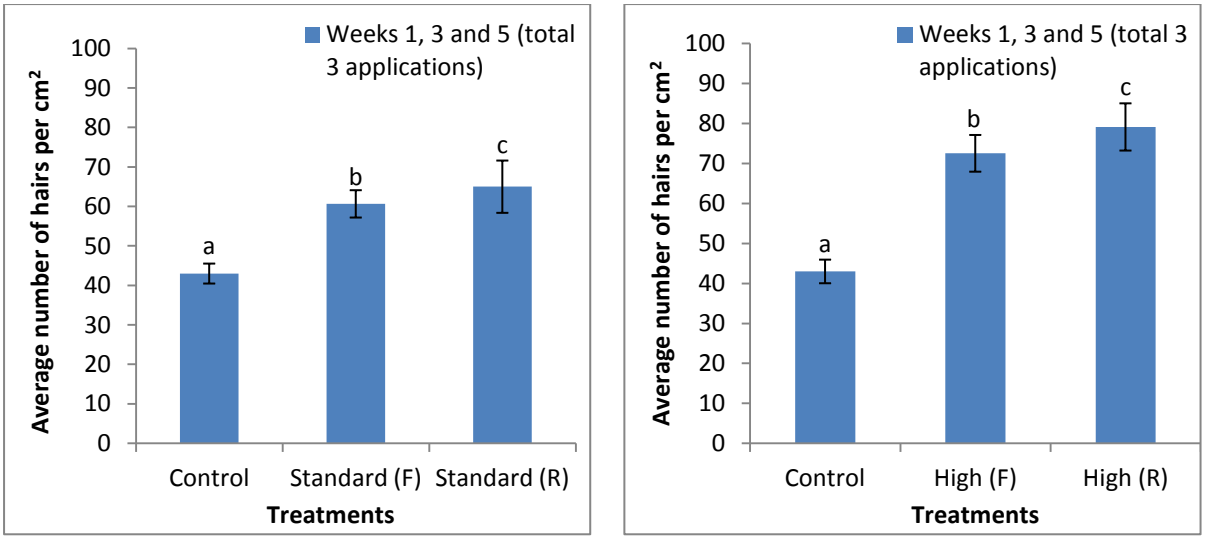
On the lower surface of leaves, significant difference in leaf hair numbers was observed between standard foliar and standard root treatment when the standard concentration of Omex SW7 was applied weekly (total 5 applications) (Figure 46A). Moreover, weekly (total 5 applications) application of the high concentration of Omex SW7 to roots showed significantly (P<0.05) higher leaf hair numbers compared to the leaves collected from high foliar treatment and the leaves collected from control plants (Figure 46B). When the standard concentration of Omex SW7 was applied in weeks 1, 3 and 5 (total 3 applications) a significant difference (P<0.05) between standard foliar and standard root treatment was observed (Figure 47A). Similar results were also found

between high foliar and high root treatment when the high concentration of Omex SW7 was applied in weeks 1, 3 and 5 (total 3 applications) (Figure 47B). However, no significant difference (P>0.05) was observed between foliar and root treatment when standard and high concentrations of Omex SW7 were applied in weeks 1 and 5 (total 2 applications) (Figures 48A and B).



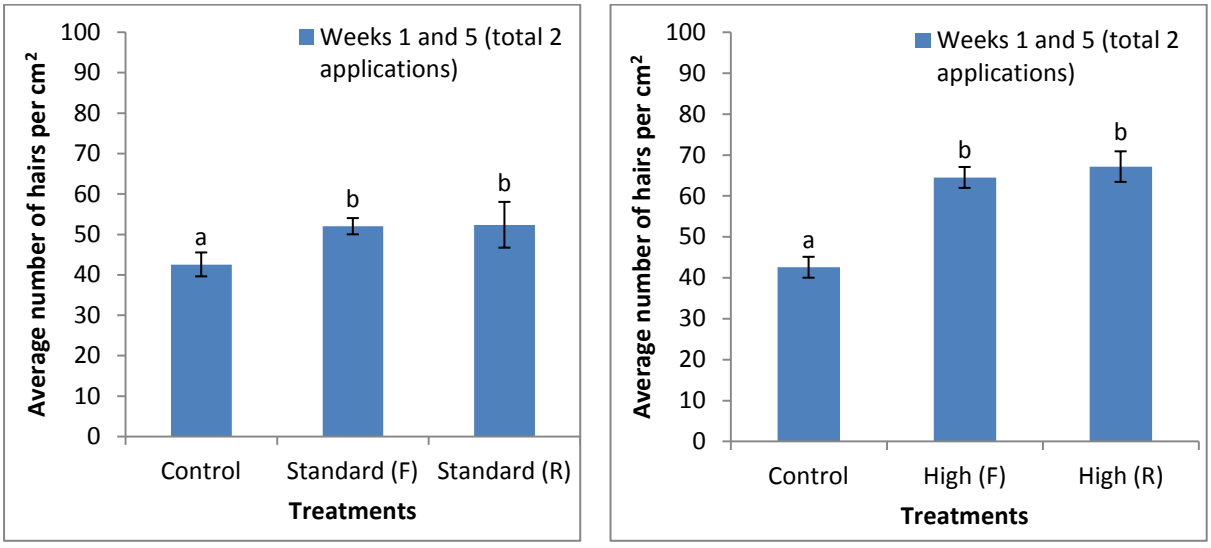
A B

Figure 46(A-B): Comparison of leaf hair numbers on the lower surface of strawberry leaves (variety Elsanta) after being treated weekly with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). A total of 20 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.



A B

Figure 47(A-B): Comparison of leaf hair numbers on the lower surface of strawberry leaves (variety Elsanta) after being treated in weeks 1, 3 and 5 with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). A total of 20 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

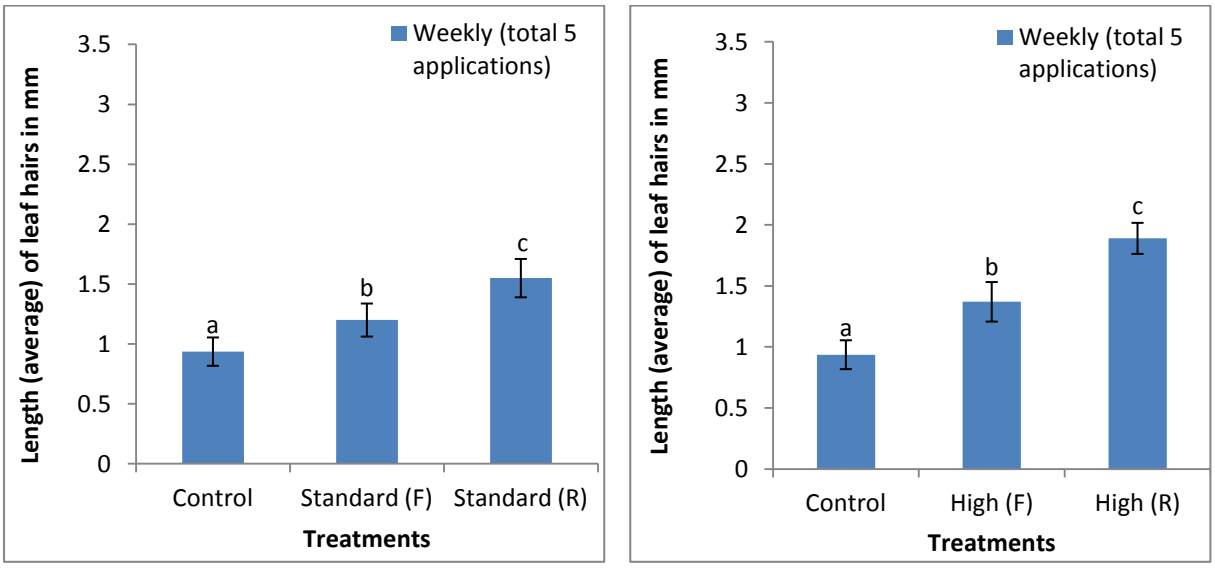


A B

Figure 48(A-B): Comparison of leaf hair numbers on the lower surface of strawberry leaves (variety Elsanta) after being treated in weeks 1 and 5 with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). A total of 20 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

4.3.10. Comparison and effect of foliar and root applications of silicon on the length of leaf hairs (upper surface)

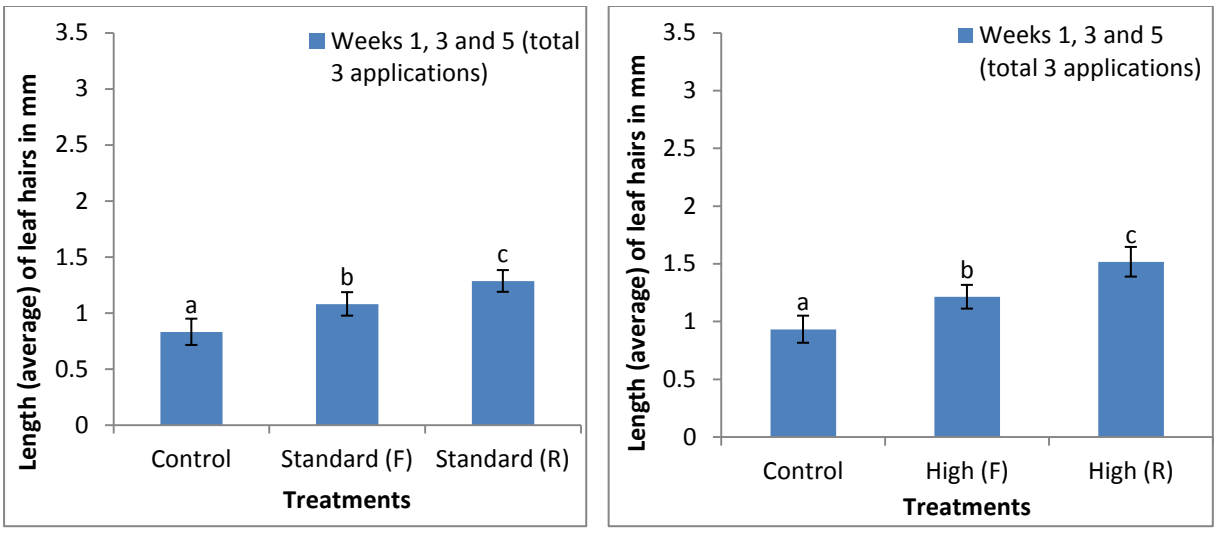
Results showed that standard and high concentrations of Omex SW7 had an effect on the length of leaf hairs on both the upper and lower surfaces of leaves when applied to leaves and roots. Timing also had an effect on the length of leaf hairs on both the upper and lower surfaces of leaves. The standard concentration of Omex SW7 when applied to roots weekly (total 5 applications) showed significantly ($P<0.05$) longer leaf hair length compared to the standard foliar treament and control (Figure 49A). The high concentration of Omex SW7 when applied to roots weekly (total 5 applications) also showed longer leaf hair length compared to foliar treatment (Figure 49B).



A B

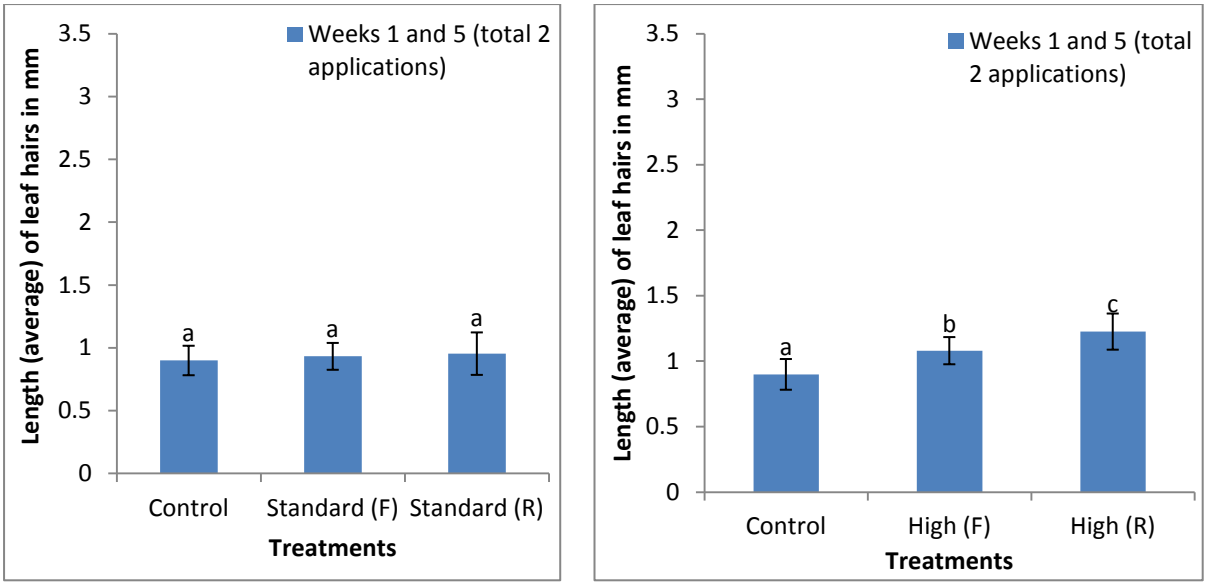
Figure 49(A-B): Comparison of length of leaf hairs on the upper surface of strawberry leaves (variety Elsanta) after being treated weekly with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). A total of 20 leaves for each treatment were used to measure the average length of leaf hairs (n=20). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

The standard concentration of Omex SW7 when applied to roots in weeks 1, 3 and 5 (total 3 applications) showed significantly longer (P<0.05) leaf hairs compared to the leaves collected from foliar treatment (Figure 50A). Similar results were also found between foliar and root treatment when the high concentration of Omex SW7 was applied in weeks 1, 3 and 5 (total 3 applications) (Figure 50B). However no significant difference (P>0.05) in length of leaf hairs was observed between foliar and root treatment when the standard concentration of Omex SW7 was applied in weeks 1 and 5 (total 2 applications) (Figure 51A). Results also indicated that there was a significant difference (P<0.05) between high foliar and high root treatment when the high concentration of Omex SW7 was applied to roots and leaves in weeks 1 and 5 (total 2 applications) (Figure 51B). The high concentration of Omex SW7 applied to roots showed significantly (P<0.05) longer leaf hair length compared to the foliar treatment (Figure 51B).



A B

Figure 50(A-B): Comparison of length of leaf hairs on the upper surface of strawberry leaves (variety Elsanta) after being treated in weeks 1, 3 and 5 with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). A total of 20 leaves for each treatment were used to measure the average length of leaf hairs (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

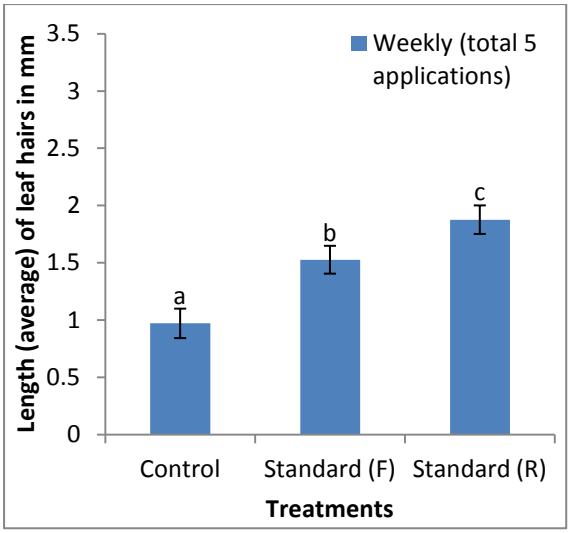


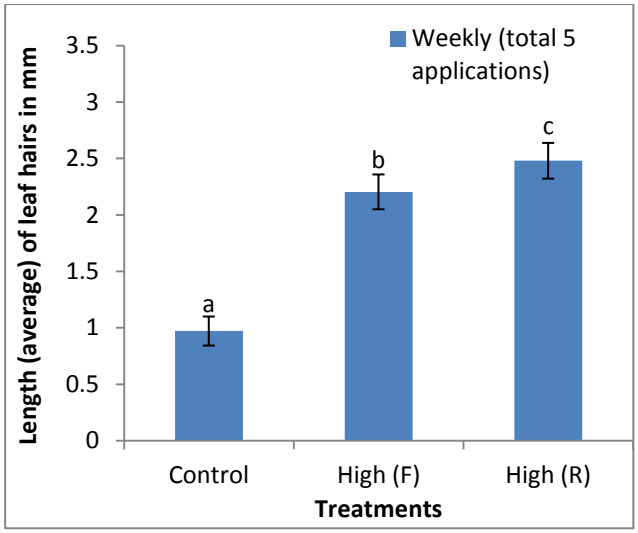
A B

Figure 51(A-B): Comparison of length of leaf hairs on the upper surface of strawberry leaves (variety Elsanta) after being treated in weeks 1 and 5 with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). A total of 20 leaves for each treatment were used to measure the average length of leaf hairs (n=20). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

4.3.11. Comparison and effect of foliar and root applications of silicon on the length of leaf hairs (lower surface)

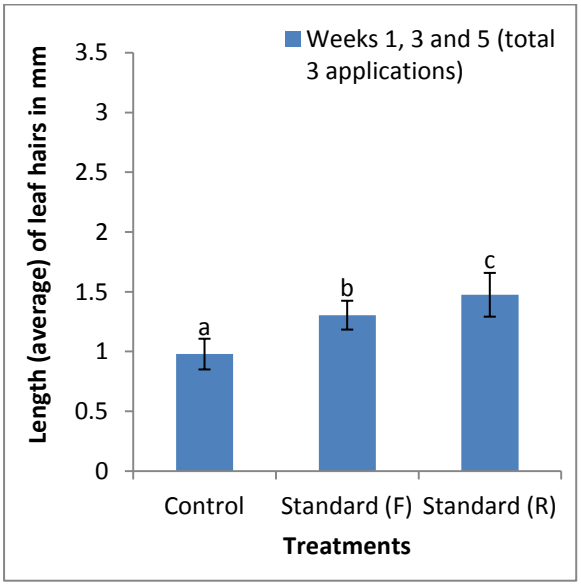
Results showed that on the lower surface of leaves, length of leaf hairs in the root treated leaves was significantly ($P<0.05$) longer compared to the foliar treated leaves and control (untreated) leaves. Significant difference ($P<0.05$) in length of leaf hairs was observed between foliar and root treatment when standard and high concentrations of Omex SW7 were applied weekly (total 5 applications) and also when applied in weeks 1, 3 and 5 (total 3 applications) to roots and leaves (Figures 52A and B and Figures 53A and B). On the other hand no significant difference ($P>0.05$) was observed between foliar and root treatment when standard and high concentrations of Omex SW7 were applied in weeks 1 and 5 (total 2 applications) (Figures 54A and B).

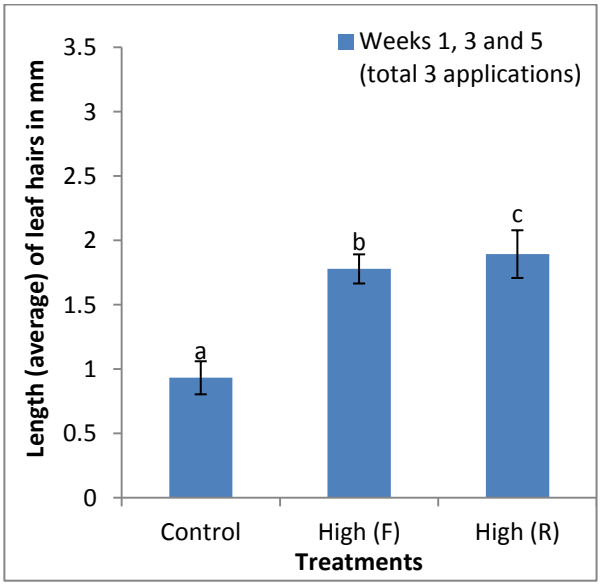




A B

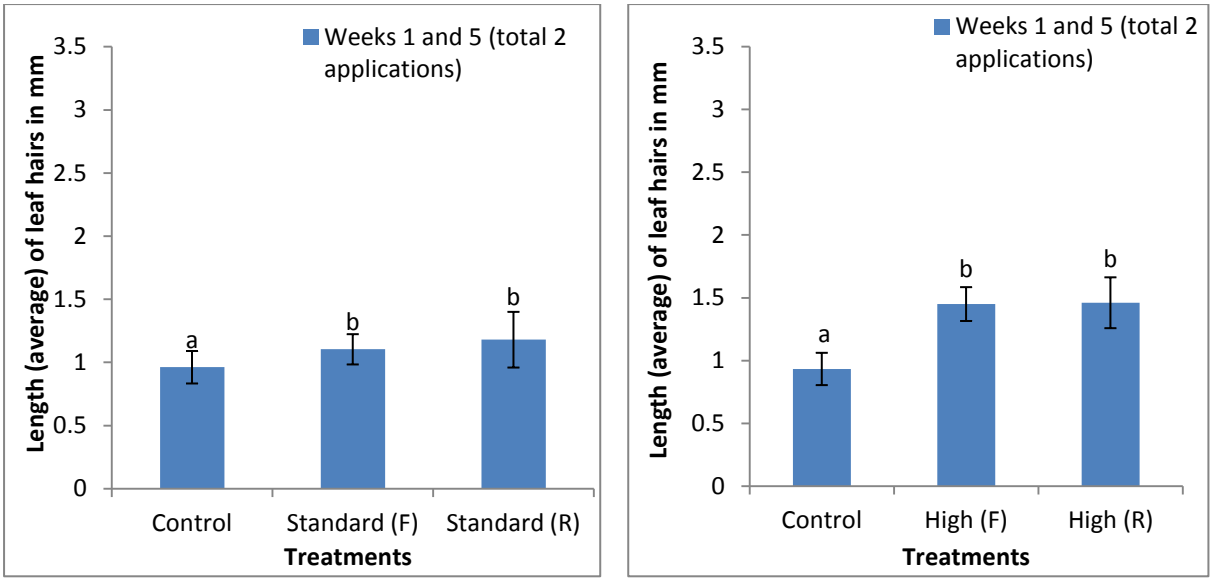
Figure 52(A-B): Comparison of length of leaf hairs on the lower surface of strawberry leaves (variety Elsanta) after being treated weekly with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). A total of 20 leaves for each treatment were used to measure the average length of leaf hairs (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.





A B

Figure 53(A-B): Comparison of length of leaf hairs on the lower surface of strawberry leaves (variety Elsanta) after being treated in weeks 1, 3 and 5 with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). A total of 20 leaves for each treatment were used to measure the average length of leaf hairs (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.



A B

Figure 54(A-B): Comparison of length of leaf hairs on the lower surface of strawberry leaves (variety Elsanta) after being treated in weeks 1 and 5 with (A) standard concentration of Omex SW7 to leaves (Standard F) and roots (Standard R) and (B) high concentration of Omex SW7 to leaves (High F) and roots (High R). A total of 20 leaves for each treatment were used to measure the average length of leaf hairs (n=20). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for Windows version 17.

4.4. Discussion

Leaves collected from strawberry plants after treatment with different concentrations of Omex SW7, which was applied to roots at different timings, showed different levels of silicon absorption. There were significant differences in concentrations in different treatments and different timings. Strawberry leaves collected from standard and high concentrations of Omex SW7 treated plants showed significantly ($P<0.05$) higher silicon concentrations compared to that in leaves collected from control plants (Figure 31). Highest levels of silicon accumulation were detected in leaves collected from the high concentration of Omex SW7 treated plants compared to all other treatments (Figure 31). Results also showed that the high concentration of Omex SW7 when applied weekly (total 5 applications) to roots increased the silicon concentrations by up to 282.43% compared to control. Shetty *et al.*, (2012) observed that application of silicon (Potassium silicate) to roots increased the silicon levels in the leaves of the rose plants. The observations reported above showed that root application of silicon increased the concentrations of silicon in both strawberries (above) and roses (Shetty *et al.*, 2012).

Accumulation of silicon in plant tissues varies greatly among plant species and the difference in silicon accumulation has been attributed to the ability of the roots to take up silicon (Takahashi *et al*., 1990). According to Takahashi *et al*., (1990) there are three different modes of silicon uptake that is active, passive and rejective uptake. Plants species, which take up silicon faster than water have an active mechanism of silicon uptake and are classified as silicon accumulators (mentioned in chapter 1). According to the above classification plants belonging to the group of silicon excluders or silicon non-accumulators have a rejective mode of silicon uptake and consequently take up silicon to a lower degree than water. Takahashi and Miyake (1976a and 1976b) made an extensive study covering 147 plant species grown under similar soil conditions and measured silicon concentrations from the leaves. Takahashi and Miyake (1976a and 1976b) observed that there is a characteristic distribution of silicon accumulation in the plant kingdom. They observed that silicon accumulators belong to the plant families Gramineae and Cyperaceae and show high silicon accumulation. Intermediate plants (mentioned in chapter 1) are found within the orders Cucurbitales and Urticales and show intermediate silicon accumulation. Whereas species of all other plant families were silicon excluders and show low silicon accumulation. Based on this, strawberry has been classified as a silicon excluder type plant (Takahashi and Miyake, 1976a and 1976b; Miyake and Takahashi, 1986). Although strawberry is considered as a silicon excluder plant, addition of silicon in the hydroponic solution showed (Kanto *et al*., 2006) increased concentrations of silicon in the strawberry plants. The results from chapter 4 and the results from Shetty *et al*., (2012) showed that although strawberry and rose are not classified as silicon accumulator plants application of silicon to roots increased the silicon concentrations in leaves of strawberry and rose plants. Shetty *et al*., (2012) also found that accumulation of silicon was different in different varieties used in their study. They (Shetty *et al*., 2012) found that accumulation of silicon was 365.3% in a powdery mildew resistant variety (genotype 99/9496-19) of rose and 198.9% in a powdery mildew susceptible variety (genotype 95/5166-1) of rose compared to the control. The difference of accumulation of silicon between strawberry and rose and the discrepancy between the two varieties (resistant and susceptible variety of rose) indicated that accumulation of silicon depends on plant species and their ability to take up silicon. Therefore according to the results of the study (chapter 4) and the results from Shetty *et al*., (2012) it can be postulated that strawberry plants

can accumulate silicon. The classification of Takahashi and Miyake (1976a and 1976b) were merely based on silicon contents in the plant dry tissue whereas detailed studies involving the measurements of silicon uptake rates, soil, climatic factors and transpiration are missing. Such a detailed study could be specially interesting for a more precise characterization of silicon excluder plants.

Silicon wetter Omex SW7 was applied to roots of strawberry plants (variety Elsanta) at three different timings and it was observed that the absorption rate of silicon in strawberry leaves was significantly different with different timing of applications. Results revealed that weekly (total 5 applications) application of 2 different concentrations (standard and high) of Omex SW7 showed significantly ($P<0.05$) higher silicon concentrations compared to 3 applications in weeks 1, 3 and 5 or 2 applications in weeks 1 and 5 (Figure 31). In order to guarantee a continuous disease control, silicon must be constantly available for root uptake because once it is deposited in the plant tissue it is not retranslocated (Datnoff *et al*., 2007). This might be the reason that weekly application of standard and high concentrations of Omex SW7 increased the concentrations of silicon in the plants compared to 3 applications in weeks 1, 3 and 5 or 2 applications in weeks 1 and 5. Besides this, Ma and Yamaji (2006) stated that the amount of silicon in soil or media is also important for plant growth and development. This is applicable in this study which showed that application of the high concentration of Omex SW7 increased the concentrations of silicon in the strawberry plants, because of high amount of silicon in the high Omex SW7 treatment compared to the standard treatment.

The effect of root application of Omex SW7 on the density and length of leaf hairs was investigated using a dissection microscope. It was observed that the root application of Omex SW7 had an effect on the density and length of leaf hairs on both the upper and lower surfaces of leaves. Furthermore, it was also revealed that the weekly (total 5 times) applications of two different concentrations (standard and high) of Omex SW7 showed significantly higher leaf hair numbers and longer leaf hair length on both the upper and lower surfaces of leaves compared to applications 3 times in weeks 1, 3 and 5 or twice in weeks 1 and 5. Plants take up silicon by the roots. Following uptake by the roots, silicon is translocated to the shoot through transpiration. Preferential sites of silica deposition are the cell wall, cell lumen, intercellular space of epidermal tissue.

Hairs or trichomes are another important site of silica deposition. Samuels *et al*., (1993) stated that trichomes are the most common site where the silica is found. Application of standard and high concentrations of Omex SW7 to the roots of strawberry plants showed increased density and increased length of leaf hairs in leaves. According to the above discussion it can be explained that after application of Omex SW7, silicon is taken up by the roots of strawberry plants and then silicon is deposited in the base of the leaf hair which stimulated the number and growth of leaf hairs. The results of this chapter also showed that there was a significant positive correlation ($P<0.01$) between accumulation of silicon and the number of leaf hairs on both the upper and lower surfaces of leaves. Significant positive correlation ($P<0.01$) was also observed between accumulation of silicon and the length of leaf hairs on both the upper and lower surfaces of leaves. These results emphasize the possibility that application of silicon to the roots increased the number and length of leaf hairs on both the upper and lower surfaces of leaves.

Foliar and root applications of different concentrations of Omex SW7 were evaluated for their effects on strawberry plants. The purpose of the study was to compare the effect of foliar and root applications of Omex SW7 on silicon accumulation and morphological changes in leaves of strawberry plants. Results showed that there was a significant difference in silicon absorption at different concentrations of silicon wetter application to strawberry plants. Results showed that standard and high concentrations of Omex SW7 when applied weekly to roots showed significantly higher accumulation of silicon in the leaves compared to the leaves collected from foliar application (Figures 40A and B). The difference in silicon accumulation has been attributed to the differences in the silicon uptake mechanism although the mechanism responsible for these different uptake systems is poorly understood. In a study Ma *et al*., (2004) reported that a gene Lsi1 is mainly expressed in the root which is responsible for active silicon uptake, suggesting that root absorption is optimal. On the other hand, after foliar application silicon is not totally absorbed through the cuticle and this might be the reason why foliar application of silicon leads to less silicon accumulation compared to the root application. In the literature there are varying reports about levels of accumulation of silicon in different plant species. Guevel *et al*., (2007) reported that in wheat silicon deposition was highest when different silicon based products were applied to roots as opposed to leaves. Bowen *et al.*, (1992) observed that both foliar and root applications

of silicon increased silicon concentrations in the leaves of grapes. Wang and Galletta (1998) observed that foliar application of silicon increased silicon accumulation and also increased growth in strawberry plants. Foliar application of silicon has been shown to increase concentrations of chlorophyll per unit area of leaf tissue (Wang and Galletta, 1998). Although there is a debate about foliar and root application, to date many studies have confirmed the beneficial role of the application of silicon on plant growth and development either through leaves or roots. The present study showed that standard and high concentrations of Omex SW7 had an effect on the density and length of leaf hairs when applied to leaves (foliar) and roots. There was a significant difference in the density and length of leaf hairs between foliar application and root application on both the upper and lower surfaces of leaves. Results revealed that standard and high concentrations of silicon when applied weekly (total 5 applications) and also when applied in weeks 1, 3 and 5 (total 3 applications) showed significantly more leaf hairs and significantly longer leaf hair length compared to the foliar treatment.

The above comparison revealed that the impact of silicon was best achieved when this element was taken up by the roots. This was supported by Shetty *et al.,* (2012) who found that application of silicon to the roots significantly increased the silicon content in the rose plant which is considered as a silicon non-accumulator plant. Another investigator Dallagnol *et al*., (2012) found that in melon which is not a silicon accumulator plant, concentration of silicon increased by the application of silicon. They stated that best results could be achieved by root application compared to foliar application. Both authors also found that root application of silicon was effective in the reduction of powdery mildew in melon and in rose (Dallagnol *et al*., 2012; Shetty *et al.,* 2012). Although silicon is not considered as an essential element, its role in plant resistance to both biotic and abiotic stresses has received increasing attention (Ma and Yamaji, 2006). To date most studies showed that disease control by silicon had involved root absorption of silicon, either from soils or application of silicon to the root zone (Guevel *et al*., 2007; Rezende *et al*., 2009). The objectives of this chapter were achieved and thus revealed that accumulation of silicon could be increased by the application of Omex SW7 and best result could be achieved by root application compared to foliar application. This study also indicated that accumulation of silicon stimulated the increase in density and length of leaf hairs which might be a positive tool in the decrease of powdery mildew infection in strawberry.

Chapter 5: A study of the role of a silicon wetter (Omex SW7) in the control of strawberry powdery mildew (*Podosphaera aphanis*) in a field trial

5.1. Introduction

Growers are under pressure from the retailers to reduce the amount of fungicides that they use to control *P. aphanis*. A prediction system has been developed especially for *P. aphanis* that has the potential to reduce the number of fungicide applications applied by the growers and still give good disease control. The prediction system identified when strawberry plants would be at greatest risk of infection by *P. aphanis*. This helps growers to apply the fungicides when their crop is at greatest risk of infection by *P. aphanis*. The high risk day predicted by the prediction system corresponds to the onset of the epidemic often at the start of the exponential phase (Dodgson, 2008). An initial set of parameters for the prediction system was developed from the literature review. Temperature and relative humidity that favours development of *P. aphanis* infection were then confirmed from field observation data (Dodgson, 2008). The prediction system identifies when there have been 144 hours of temperature and humidity which are in the optimum range for disease development. The development time for a fungal infection by *P. aphanis* is 144 (6+138) hours of suitable conditions from spore germination to visible symptoms. Germination of a spore requires a total of 6 hours and further growth of the germinated spore requires a further 138 hours where the optimum temperature is 15-25^{O}C and relative humidity 97-100% (Dodgson, 2008). In this study the temperature and relative humidity data that were used in the prediction system were measured on the grower's own weather station situated on the farm for their use. The data from the weather station was input into the prediction system. In the field trial reported here, the prediction system was not used to predict high risk days for fungicide applications but was used in the results section to demonstrate that the environmental conditions during the trial were not conducive to disease development.

A field trial experiment applies scientific method to examine intervention and innovative ideas which come from farmers and researchers in the naturally occurring environment. In the laboratory, researchers develop scientific method. Field trial experiments test this scientific method in the real world and then transfer the new

invention to the farmers. There are many designs which can be used in the field trial experiment. Each design has its own advantages and disadvantages. Basically, four types of design are used in plant pathological field trials: demonstration strip design, randomised complete block design, split plot design and factorial design. Among these designs randomised complete block design is generally used in the field trials. Randomization and replication (i. e. repetition) are the two major components of the randomized complete block design. Repetition and randomization help the researcher to determine the difference in results between treatments being real or simply due to chance. According to Goddard (2010) each treatment needs to be replicated at least three times. In each block of replications, each treatment is included once. Palmer (2007) used randomised block design in a field trial experiment and found that potassium silicate had an effect on strawberry powdery mildew. Randomised complete block design was used in the field trial experiment by Dallagnol *et al*., (2012) and showed the effect of silicon supplied in the form of potassium silicate to control the powdery mildew infection in melon.

It has been reported that silicon reduces several diseases caused by biotrophic pathogens in both monocotyledons and dicotyledons (Datnoff *et al*., 2007). Buck *et al.,* (2008) reported that foliar application of potassium silicate at rates ranging from 1 to 16 g/l reduce blast incidence in rice leaves. In grape, Bowen *et al.,* (1992) found that feeding plants 1.7 mM silicon had no effect on the reduction of disease severity caused by *Uncinula necator* whereas 17mM silicon spraying on leaves one day before inoculation substantially reduced the development of diseases. Scanning electron micrographs and x-ray analysis revealed that silicon deposition on the leaf surface of grapes prevented the fungal hyphae development. Conidia of *U. necator* were cultured on an agar medium and 0-17mM silicon was added to the agar medium to test whether silicon was an inhibitor of conidia germination or germ tube development. Results showed that the presence of silica weakly promoted conidia germination and germ tube development. The above study showed that foliar application of silicon reduced the fungal infection. Silicon has been shown to control a number of diseases and it is believed that silicon creates a physical barrier which can restrict fungal hyphae penetration (Epstein, 1994). However, the mechanism of suppression against powdery mildews in strawberry is still not clear. Anecdotal observations suggested that a silicon

based wetter (Omex SW7) had a synergistic effect with potassium carbonate (described in chapter 1) in controlling *Podosphaera aphanis* on strawberries. The work reported here demonstrates this effect in the field in a commercial setting. Therefore, it has been hypothesized that supplying silicon as a wetter would help the strawberry plants to absorb silicon and improve resistance against the pathogen and help to find out how silicon reduces disease development.

5.1.1. Objectives

- To investigate the effect of foliar applications of silicon wetter (Omex SW7) and potassium carbonate to limit *P. aphanis* infection in a field trial under polythene tunnel at Wisbech.
- To determine the uptake of silicon from silicon based wetter into strawberry plants and the effect of absorbed silicon on the relationships between leaf hair length and density and reduction in the severity of powdery mildew.
- To determine the relationships between density of leaf hairs with germinating ascospores and colonies.
- To determine the relationships between length of leaf hairs with germinating ascospores and colonies.
- To determine the relationships between silicon level and germinating ascospores and also colonies.

5.2. Materials and Methods

5.2.1. Sonata

Sonata is a mid season June-bearing strawberry variety. Sonata was used in the field trial experiment. The variety Sonata was developed from a cross between Elsanta and Polka (Meulenbroek, 2005). Elsanta was used in the glasshouse experiment and the work reported in chapter 3 showed that spraying with the silicon wetter (Omex SW7) onto the leaf surface does result in accumulation of silicon in the leaves. Accumulation of silicon affects the morphology of the strawberry leaves resulting in the formation of additional leaf hairs, which were longer than when the plants were untreated. This effect occurred on all varieties tested (Rhapsody, Florence and Symphony) after being treated with different concentrations of Omex SW7. It could be postulated that the effect

of spraying silicon would be observed in the variety Sonata which would be used in the field trial study. Sonata was used in this field trial study because it is now a widely grown variety by the growers. Sonata produces beautiful conically shaped strawberries and the amount of misshaped berries is minimal. On average Sonata is more productive than Elsanta with a more uniform fruit size and shape throughout the picking season with significantly higher levels of class I fruit. The fruits are bright red and taste a little sweeter and juicer than Elsanta. Sonata is more winter hardy and less prone to winter frost. Sonata is less susceptible to powdery mildew but is susceptible to wilt (*Verticillium*), crown rot (*Phytophthora cactorum*) and root rot (*Rhizoctonia fragariae*) (Meulenbroek, 2005).

5.2.2. Experimental design of the field trial

The effect of foliar applications of silicon wetter and K50 (Potassium carbonate) to limit strawberry powdery mildew infection was examined in a field trial in a polythene tunnel at Wisbech on a commercial crop. Silicon was applied to strawberry plants with a hand held spray boom. Silicon wetter (Omex SW7-Omex Agrifluids Ltd) was used as the source of silicon. K50 (Potassium carbonate-Omex Agrifluids Ltd) is used by the growers to reduce disease incidence (Anon, 2012h). Treatments were arranged in a randomised block design of 3 replicates (Figure 56). Each plot was 3 metres (m) long and separated by 1 metre guard row. The trial plot was 25 metres long and 36 metres wide. The whole polythene tunnel was 172 metres long (Figure 55). There were a total of 18 plots. There were five rows and treatments were applied to the alternate rows, no treatment was applied to the guard rows. The untreated plots received no treatments and no water was sprayed as free water alone inhibits the germination of powdery mildews (Jhooty and McKeen, 1965; Schnathorst, 1965; Amsalem *et al*., 2006). All treatments were applied to the strawberry variety Sonata. There were six treatments and each treatment was applied weekly. The plot was sprayed weekly starting from 7 April until 11 May 2011. The spray dates, disease assessment date and leaf collection date are shown in Table 20. In this trial 500 ml was sprayed on each plot. Product and dilution rates used in this trial are shown in Table 21. Treatments used in this trial are shown in Table 22.

Table 20: Spray and date of assessment

Spray date	Assessment date in field	Leaf collection for assessment for disease and silicon level
1st spray- 7.04.11	7.04.11	7.04.11-before spray (morning)
	11.04.11	
2nd spray-13.04.11	14.04.11	
	18.04.11	
3rd spray-20.04.11	21.04.11	
4th spray-27.04.11	28.04.11	28.04.11
5th spray-4.05.11	5.05.11	
Last spray-11.05.11	12.05.11	
	19.05.11	19.05.11
	26.05.11	
	10.06.11	10.06.11

Table 21: Product and dilution rates

Name of the chemicals	Dilution rate
1) Omex SW7 (0.25%)	2.5 ml/l
2) Omex SW7 (2.5%)	25 ml/l
3) K50 (Potassium carbonate)	10 gm/l

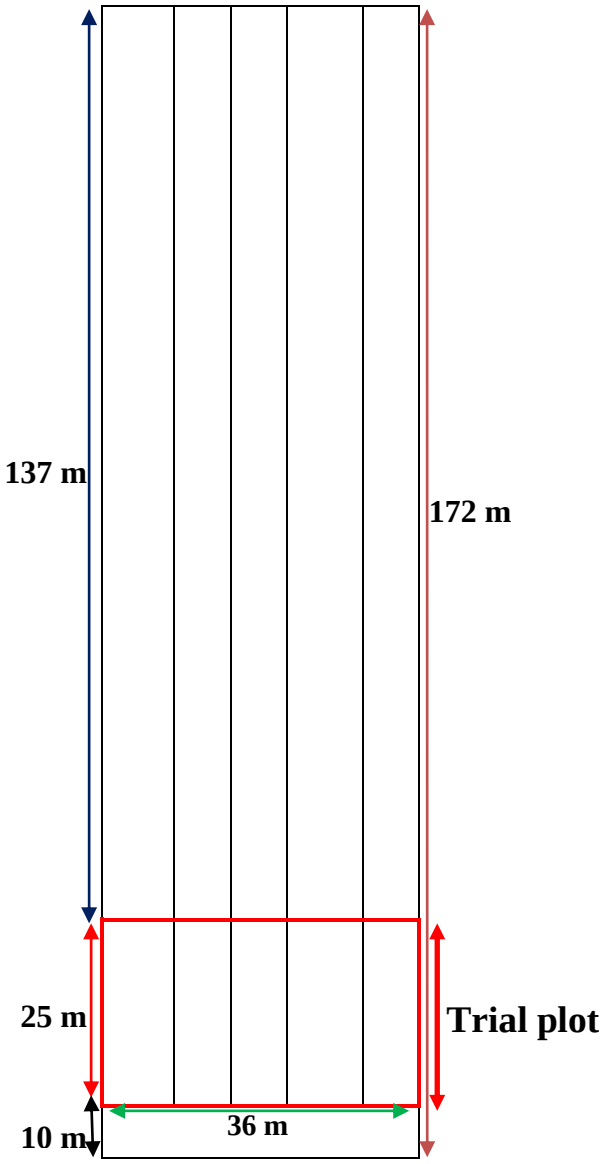


Figure 55: Plan of the trial under polythene tunnel at Wisbech field site. The trial plot was 25 m long and 36 m wide. The whole polythene tunnel was 172 m long.

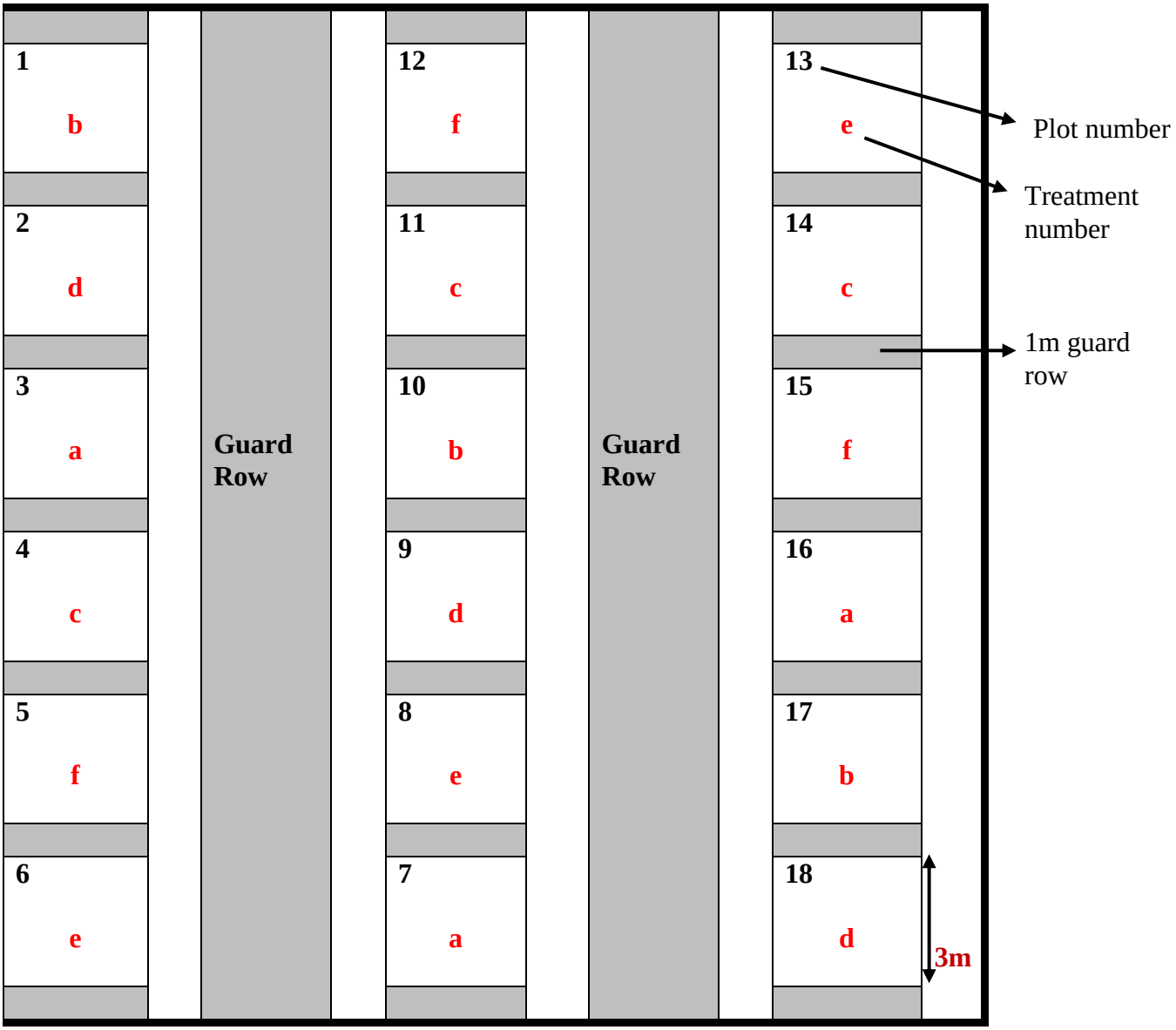


Figure 56: Trial design. Number shows the plot number and lower case letter indicates the treatment number. Each trial plot was 3 m long and each plot was separated by 1 m guard row. In this trial there were 5 rows. Spray was applied to 3 rows and spray was not applied to the guard row.

Table 22: List of different treatments

Treatment no	Name of the treatment
a	Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si
b	High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si
c	Standard + K50
d	High + K50
e	K50 (Potassium carbonate) used at field rate
f	Untreated (Unt)

5.2.3. Microscopic assessment of *P. aphanis* from trial

Strawberry leaves were collected from the variety Sonata from the trial at Wisbech. To identify *P. aphanis* symptoms 20 leaves were collected from each trial plot at each sample date. Each set was assessed for visible *P. aphanis* symptoms on each assessment date. There were 4 to 5 leaflets in each leaf in the variety Sonata. Each leaflet was then placed in a Petri dish and submerged in 0.1% trypan blue stain (trypan blue in lactic acid) (Waller *et al*., 2002). Leaflets were left to stain for 24 hours at room temperature, after which they were washed in water and cut into 4 strips (parallel to the mid rib). Of the 4 strips, 2 were placed on microscope slides upper surface up and 2 were placed lower surface up (Figure 57). The slide was placed on the microscope stage and viewed at X100 magnification (Nikon, model YS100). One transect of the leaflet strip was viewed. The number of germinating ascospores (size, length 10-50 µm and width 8-30 µm) and the number of distinct *P. aphanis* colonies (mycelium was assessed as a colony) were recorded. This was repeated for the remaining 3 strips from each leaflet. The number of germinating ascospores and number of colonies were recorded from all the 4 or 5 leaflets from each leaf. This was repeated for the remaining leaves. Average number of germinating ascospores and average number of colonies were calculated from 20 leaves.

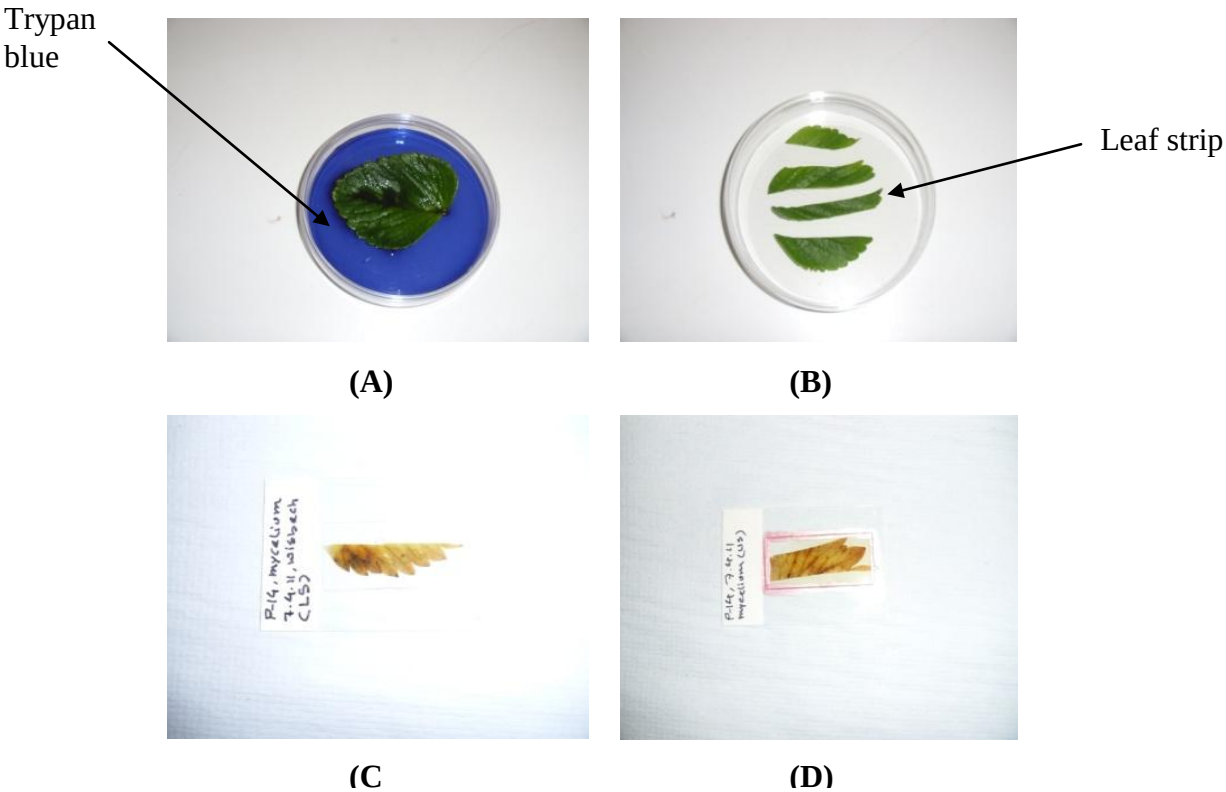


Figure 57: (A) Leaflet submerged in trypan blue stain for 24 hours. (B) After 24 hours staining at room temperature, leaflet was washed in water and cut into 4 strips (parallel to the mid rib). (C) Leaf strip was placed on microscope slide and covered with cover slip. (D) Cover slip was sealed with nail polish.

5.2.4. Microscopic observation of leaf hair length and density

In order to determine the density and length of leaf hairs, 20 leaves from each plot were examined under the microscope. Length and density of the leaf hairs were measured by using a dissection microscope and details were described in chapter 2, section 2.8.

5.2.5. Silicon determination

Details of Silicon determination are described in chapter 2, section 2.7.

Collection of leaves for silicon extraction:

When sampling, 10 leaves were taken from each plot, 180 leaves from 18 plots. Samples were collected four times during the trial; the first sample was collected on 7 April 2011

before spraying started in order to measure the background level of silicon in untreated plants throughout the trial. The second sample was collected on 28 April 2011 to measure the effect of spray, third sample was collected on 19 May 2011 after completing the five weeks of spray treatments to measure the effect of spray and to analyse the level of silicon after the final spray. The last sample was collected on 10 June 2011 to measure the level of silicon one month after the final spray. Leaves were kept in a cold room at 4^0C in sample bags labelled with the date of collection and the treatments (rate, timing). Drying of the leaves, grinding of the leaves and extraction method of silicon are described in chapter 2.

5.2.6. Data analysis

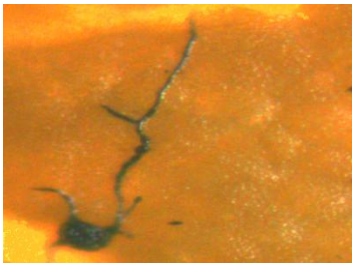
Statistical method one way ANOVA (analysis of variance) under the Compare Mean in the SPSS version 17 was used to analyse the data, number of germinating ascospores, and colonies, concentrations of silicon in leaves, density and length of leaf hairs after foliar application of different concentrations of Omex SW7 and potassium carbonate. Tukey's HSD test was used for further *post-hoc* analysis and homogeneous subsets also used here to differentiate the treatment in different groups (mentioned in section 2.9). Pearson's correlation and linear regression analysis were used to find the relationships between silicon level and density and length of leaf hairs and also the relationships between density and length of leaf hairs and disease incidence. The relationship between silicon level and disease incidence was analysed using the correlation and regression analysis.

5.3. Results

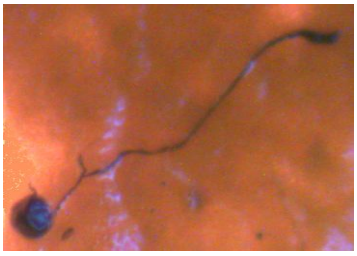
5.3.1. Assessment of *P. aphanis* in field trial

Leaves collected from different trial plots on 7 April 2011 before the trial was sprayed showed that there were germinating ascospores and colonies on all the treated and untreated plots. While no symptoms of powdery mildew infection were observed with the naked eye, microscopic observation showed that there were germinating ascospores and colonies present throughout the trial (Figure 58). When the trial was carried out the

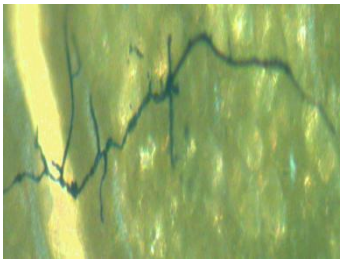
temperature and relative humidity were not conducive for growth and development of *P. aphanis* infection, so the symptoms were not visible in the trial, but there was still evidence that germinating ascospores and colonies were present in 18 trial plots (Figures 59 and 60).



A. Germinating ascospore (X400)



B. Germinating ascospore (X400)



C. Mycelium (X400)

Figure 58. (A) Germinating ascospore seen under the microscope (X400). (B) Another germinating ascospore seen under the microscope (X400). (C) Mycelium seen under the microscope (X400).

Results showed that there were more germinating ascospores and colonies in the leaves collected from all trial plots on 7 April before the trial was sprayed. Though there were no colonies visible to the naked eye, ascospores and colonies were found throughout the trial (Figures 59 and 60). On the untreated plots there were germinating ascospores and colonies throughout the trial. On K50 (Potassium carbonate) treated plots there were germinating ascospores and colonies throughout the trial. However, some reduction in germinating ascospores was found in K50 (Potassium carbonate) treated plots when compared with untreated plots on 28 April, 19 May and 10 June. On these four silicon and K50 (Standard, High, Standard+K50, High+K50) treated plots there were no germinating ascospores after 28 April 2011. However colonies were present throughout the trial on the untreated plots and there was a reduction in colony numbers in both the treated and untreated plots over time. However the reduction in treated plots was greater than in the untreated plots. Thereafter the number of colonies was greatly reduced on 19 May in all plots treated with Omex SW7 with or without K50. Whilst K50 (Potassium carbonate) alone gave some reduction in the number of colonies, all treatments with silicon gave a significant ($P<0.05$) reduction in colonies on 28 April, 19 May and 10 June when compared with the untreated control. Effect of silicon wetter and

K50 in the reduction of the number of germinating ascospores and colonies was shown in the decreasing order high+K50>high>standard+K50>standard>K50>untreated.

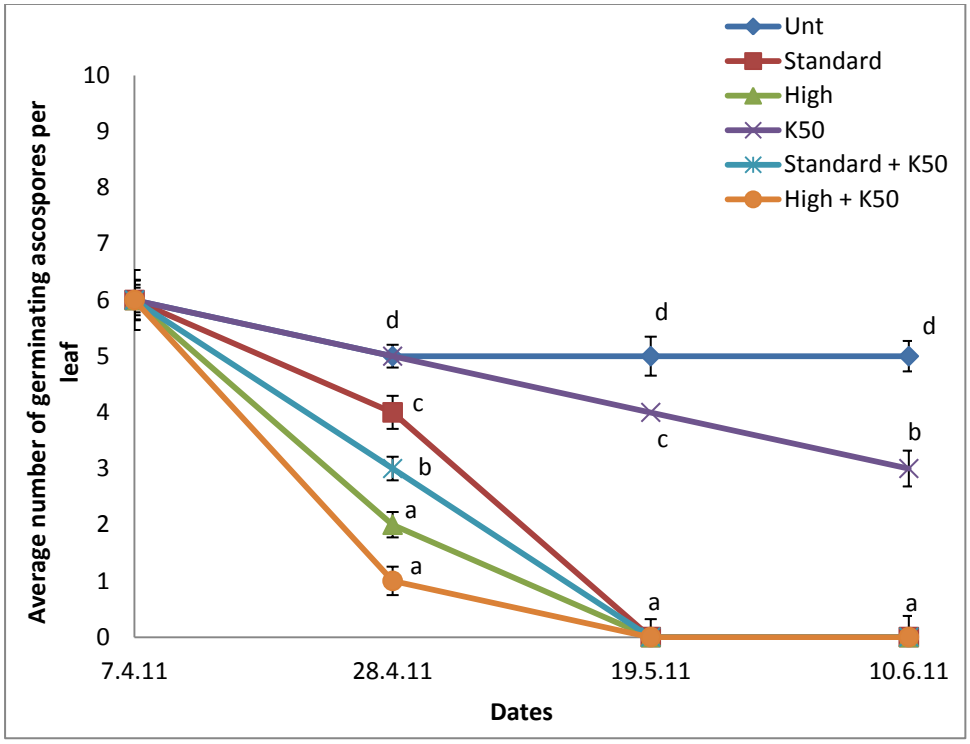


Figure 59: Number of germinating ascospores on strawberry leaves (variety Sonata) from 18 trial plots. The disease was assessed on 7 April before beginning of treatment/spray and also on 28 April, 19 May and 10 June after being treated with different concentrations (standard and high) of silicon wetter (Omex SW7) with and without the use of K50 (Potassium carbonate) and control (untreated). A total of 20 leaves for each treatment were used to calculate the average number of germinating ascospores (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for windows version 17.

Colonies were present on all treatments throughout the trial. There was a reduction in the number of colonies on the untreated plot on 28 April, 19 May and 10 June. The reduction in the number of colonies was probably due to the weather conditions (Figure 61). There was no epidemic build up in the control plots which were unexpected. The lack of an epidemic in the period of the trial was due to the low disease pressure caused by the particular weather conditions in 2011 as shown by the print out from the prediction system for that year (Figure 61).

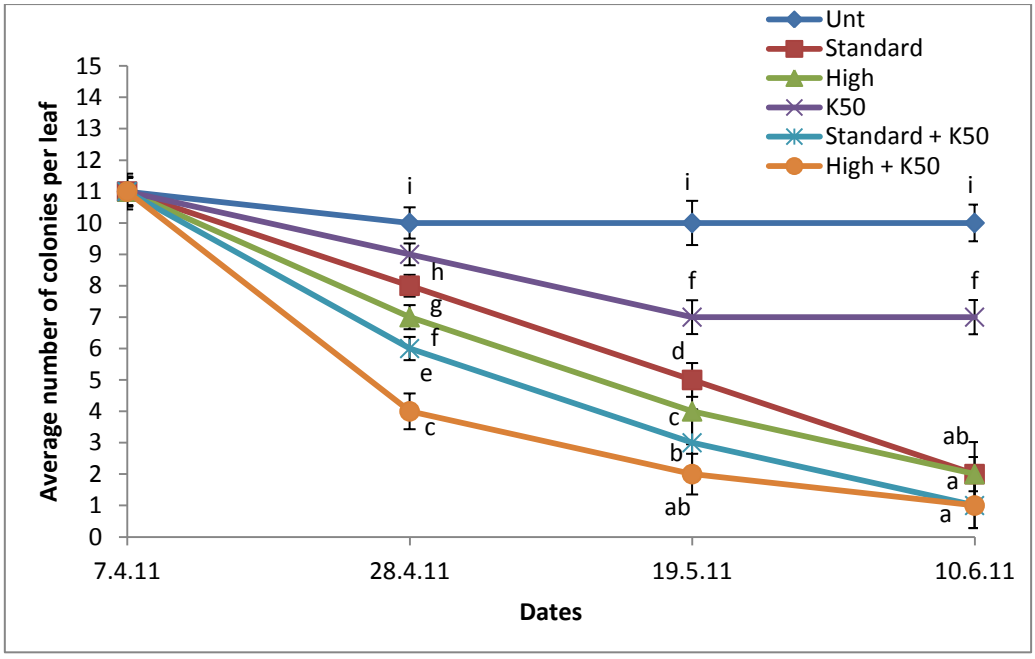


Figure 60: Number of colonies on strawberry leaves (variety Sonata) from different trial plots. The disease was assessed on 7 April before beginning of treatment/spray and also on 28 April, 19 May and 10 June after being treated with different concentrations (standard and high) of silicon wetter (Omex SW7) with and without the use of K50 (Potassium carbonate) and control (untreated). A total of 20 leaves for each treatment were used to calculate the average number of colonies (n=20). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for windows version 17.

The prediction system was used here not to predict high risk days for fungicide application but to show that environmental conditions during the trial were not conducive to disease development. Temperature and relative humidity affect ascospore germination and colony development. If conditions are not suitable for germination and colony development there will be no epidemic build up. However, if conditions are not suitable for germination or growth of *P. aphanis* this does not reduce the level of development the fungus has already achieved. Further fungal growth starts again from the point it had reached previously when conditions are suitable. There was very low disease pressure from 7 April to 1 June (trial period) where the prediction system (denoted by green to red lines) shows that it took 54 days to accumulate 144 hours of disease conducive conditions (temperature 15-25^{0}C and relative humidity 97-100%) (Figure 61). However, from 1 June to 16 June where the prediction system shows that it took only 16 days to accumulate 144 hours of disease conducive conditions.

Temperature and relative humidity data showed that the environmental conditions were not conducive to *P. aphanis* development during the first 5 weeks of the trial so no visible epidemic occurred in the untreated plots. However the trial does demonstrate the effect of silicon in reducing both ascospore development and colony growth.

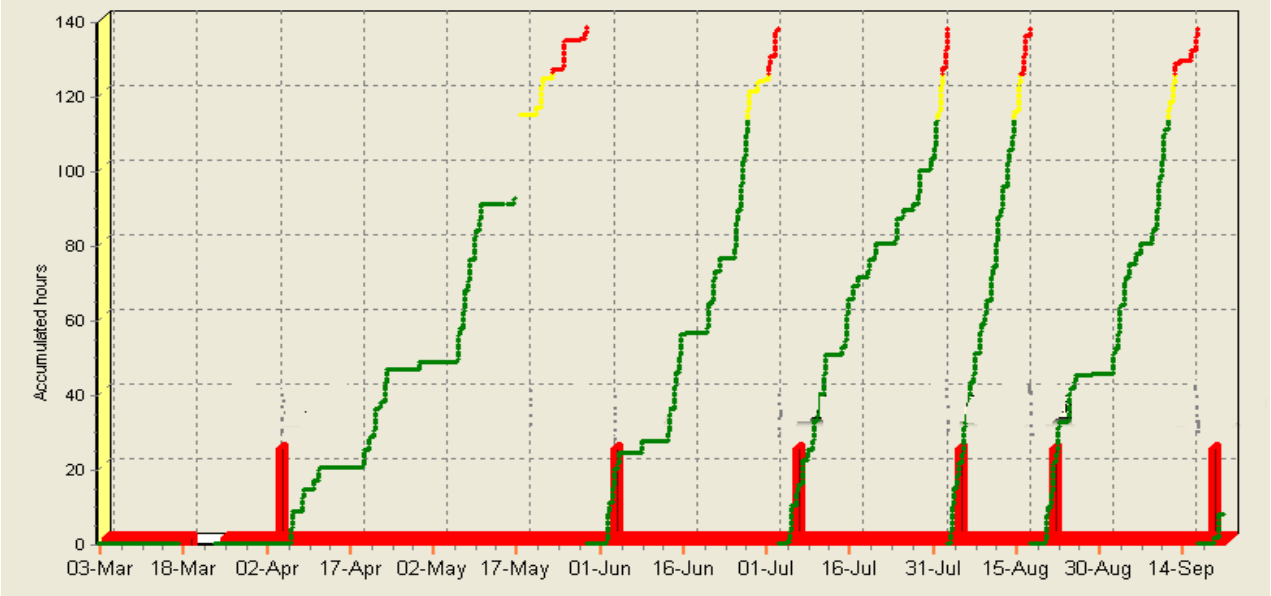


Figure 61: Disease development predictions for Wisbech, 2011. The green lines are accumulated hours with both above 20^0C and 95% relative humidity. The yellow/red is the warning period (action threshold) for the growers, showing the need to spray. As soon as the crop is sprayed, the prediction system is reset to zero and the accumulated hours calculation is restarted.

5.3.2. Effect of silicon on the density of leaf hairs

This experiment aimed to investigate whether the silicon supplements had any effect on the number of leaf hairs. Interestingly, microscopic observations of leaves which were collected on 28 April 2011 after being treated with silicon wetter and K50 (Potassium carbonate) showed that there were increased number of leaf hairs on both the upper and lower surfaces of leaves (Figure 62). It was also found that there was a significant difference in number of leaf hairs in different treatment groups. On the upper surface of leaves there was no significant ($P>0.05$) difference in leaf hair numbers between control and K50 (Potassium carbonate) treated leaves. However, Omex SW7 treated leaves showed significantly ($P<0.05$) higher leaf hair numbers compared to control and K50 (Potassium carbonate) treated leaves (Figure 62). Moreover, high Omex SW7 treated

leaves showed significantly (P<0.05) higher leaf hair numbers compared to standard Omex SW7 treated leaves (Figure 62).

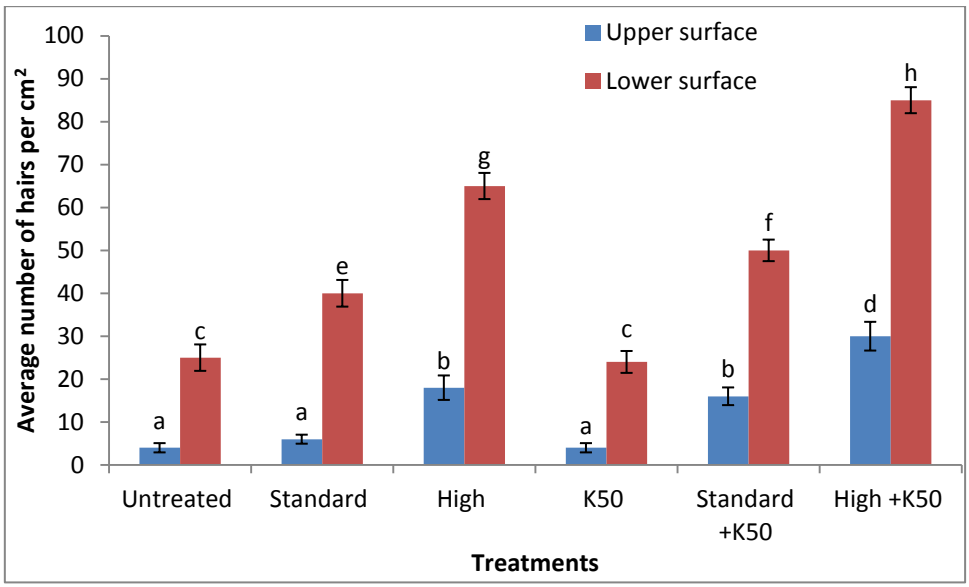
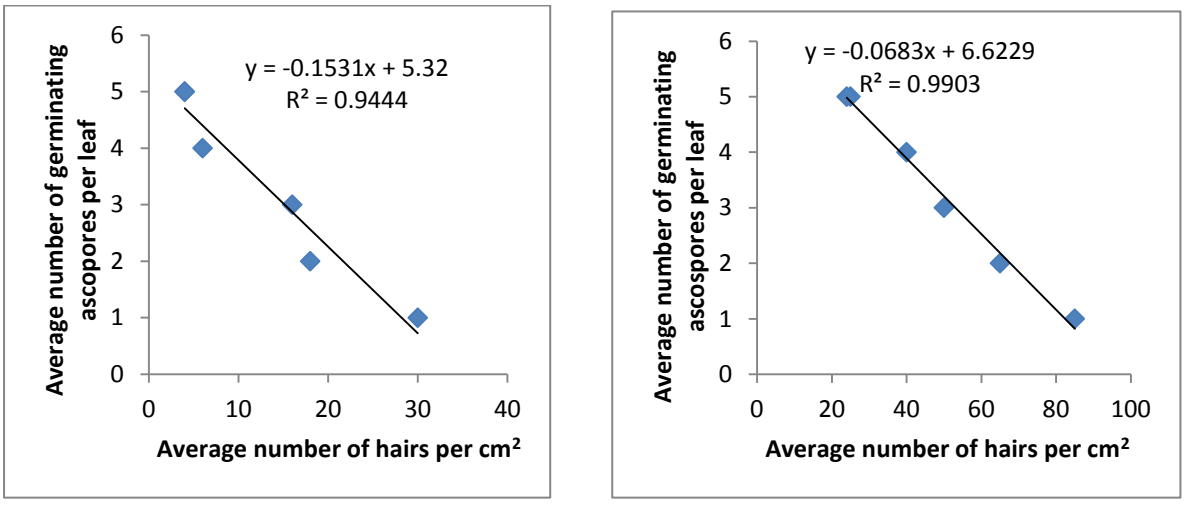


Figure 62: Number of leaf hairs on both the upper and lower surfaces of the strawberry leaves (variety Sonata) collected from different trial plots. Leaves were collected on 28 April 2011, after being treated with different concentrations (standard and high) of silicon wetter (Omex SW7) with and without the use of K50 (Potassium carbonate) and control (untreated). A total of 20 leaves for each treatment were used to measure the average number of leaf hairs per cm^2 (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for windows version 17.

It was observed that the foliar application of silicon on strawberry leaves increased the number of leaf hairs on the lower surface (Figure 62). There was no significant (P>0.05) difference in leaf hair numbers between control and K50 (Potassium carbonate) treated leaves (Figure 62). However, the number of leaf hairs on the lower surface was significantly different in standard and high Omex SW7 treated leaves compared to control and K50 (Potassium carbonate) treated leaves (Figure 62). Moreover, the highest level of Omex SW7 treated leaves showed significantly higher (P<0.05) leaf hair numbers compared to all other treatment groups (Figure 62). Results also showed that the leaf hair numbers were not increased in K50 (Potassium carbonate) treated leaves but when K50 (Potassium carbonate) was mixed with Omex SW7 an increase in the number of leaf hairs on both the surfaces was observed.

5.3.3. Relationship between the leaf hair numbers and the number of germinating ascospores

The above results showed that application of standard and high concentrations of Omex SW7 and standard and high concentrations of Omex SW7 mixed with K50 increased the number of leaf hairs on both the upper and lower surfaces of leaves (Figure 62). Leaves collected on 28 April 2011 showed that standard and high concentrations of Omex SW7 and standard and high concentrations of Omex SW7 mixed with K50 decreased the number of germinating ascospores (Figure 59). The results from the leaves collected on 28 April 2011 were used to find a relationship between the number of leaf hairs and the number of germinating ascospores. The results from the leaves collected on 19 May and 10 June were not used to find the relationship because no ascospores were observed on the leaves collected on 19 May and 10 June 2011 (Figure 59). The Pearson's correlation was used to find a correlation. It was observed that there was a significant ($P<0.01$) negative correlation between increased number of leaf hairs on both the upper and lower surfaces of leaves and decreased number of germinating acospores. The correlation coefficients (r) were -0.972 and -0.995 for the upper and lower surfaces of leaves respectively. The negative r value means that there was a negative relationship between the number of leaf hairs and the number of germinating ascospores.



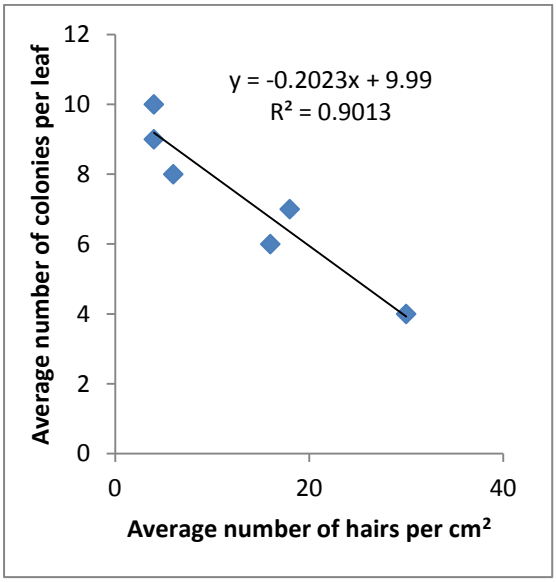
A. Upper surface B. Lower surface

Figure 63(A-B): Relationship between the number of leaf hairs and the number of germinating ascospores on both the upper (A) and lower (B) surfaces of leaves (variety Sonata). Leaves were collected on 28 April 2011, after being treated with different concentrations (standard and high) of Omex SW7 with and without the use of K50 (Potassium carbonate) and untreated (control).

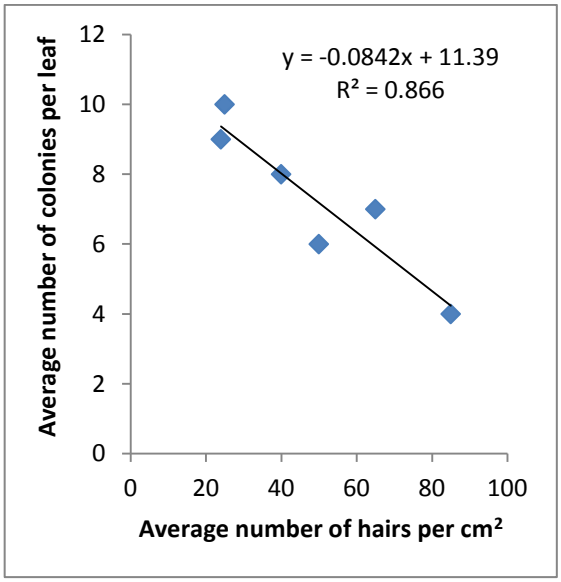
This means that the increased number of leaf hairs is correlated with the decreased number of germinating ascospores on both the upper and lower surfaces of leaves. Regression analysis also showed a negative relationship between the number of leaf hairs and the number of germinating ascospores on both the upper and lower surfaces of leaves (Figures 63A and B).

5.3.4. Relationship between the leaf hair numbers and the number of colonies

The results from the leaves collected on 28 April 2011 were used to find a relationship between the number of leaf hairs and the number of colonies. The results from the leaves collected on 19 May and 10 June were not used to find the relationship because very few colonies were observed on the leaves collected on those days (Figure 60). A significant ($P<0.01$) negative correlation was observed between increased number of leaf hairs on both the upper and lower surfaces of leaves and decreased number of colonies. The correlation coefficients (r) were -0.950 and -0.927 for the upper and lower surfaces of leaves respectively. The negative r value means that there was a negative relationship between the number of leaf hairs and the number of colonies.



A. Upper surface



B. Lower surface

Figure 64(A-B): Relationship between the number of leaf hairs and the number of colonies on both the upper (A) and lower (B) surfaces of leaves (variety Sonata). Leaves were collected on 28 April 2011, after being treated with different concentrations (standard and high) of Omex SW7 with and without the use of K50 (Potassium carbonate) and untreated (control).

Significant negative correlation indicated the possibility that the increased number of leaf hairs is correlated with the decreased number of colonies on both the upper and lower surfaces of leaves. Regression analysis also showed a negative relationship between the number of leaf hairs and the number of colonies on both the upper and lower surfaces of leaves (Figures 64A and B).

5.3.5. Effect of silicon on the length of leaf hairs

This study examined whether the silicon supplement had any effect on the length of leaf hairs. It was observed that the foliar application of silicon (Omex SW7) had an effect on the length of leaf hairs on both the upper and lower surface of leaves (Figure 65). On the upper surface, application of different silicon concentrations showed significantly ($P<0.05$) longer leaf hair length compared to control and K50 (Potassium carbonate) treated leaves, whereas there was no significant ($P>0.05$) difference in length of leaf hairs between control and K50 (Potassium carbonate) treatment (Figure 65). The length of leaf hairs in high silicon (Omex SW7) treated leaves was significantly ($P<0.05$) longer compared to that in standard Omex SW7 treated leaves (Figure 65).

It was observed that the foliar application of silicon (Omex SW7) on strawberry leaves increased leaf hair length on the lower surface (Figure 65). There was no significant ($P>0.05$) difference in leaf hair length between control and K50 (Potassium carbonate) treated leaves (Figure 65). However, the length of leaf hairs on the lower surface was significantly different in standard and high Omex SW7 treated leaves compared to control and K50 (Potassium carbonate) treated leaves (Figure 65). Moreover, the highest level of Omex SW7 treated leaves showed significantly ($P<0.05$) longer leaf hair length compared to all other treatment groups (Figure 65). Results also showed that leaf hair length was not increased in K50 (Potassium carbonate) treated leaves but when K50 (Potassium carbonate) was mixed with Omex SW7 an increase in the length of leaf hairs on both the surfaces was observed.

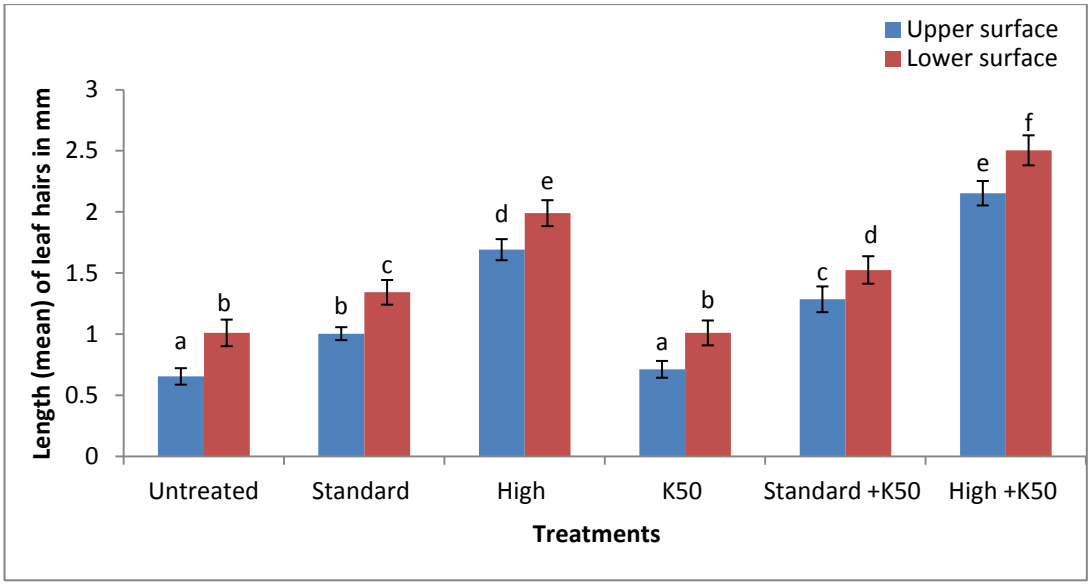
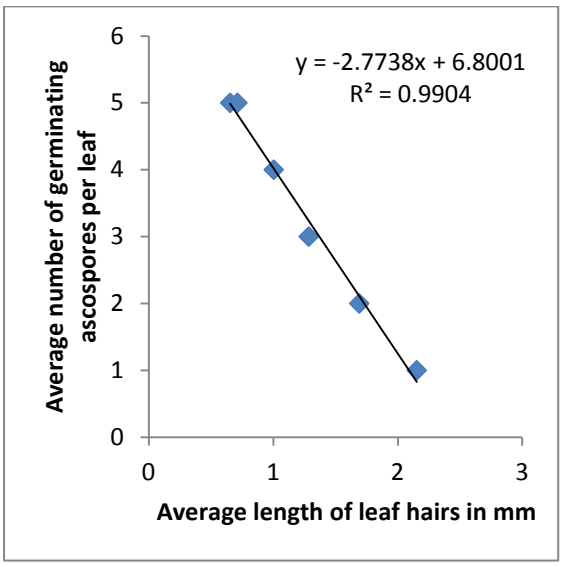
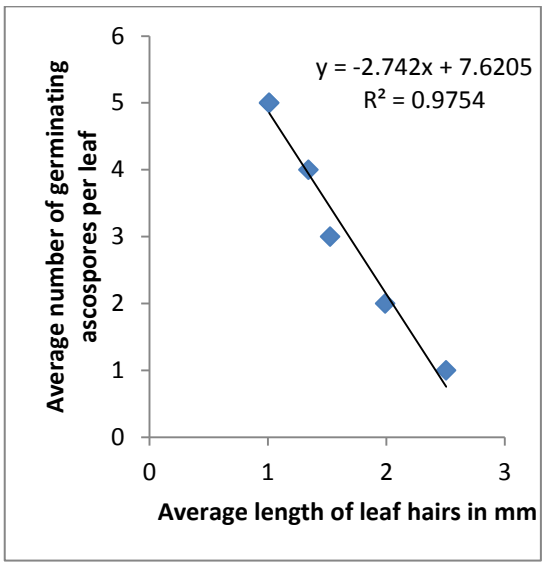


Figure 65: Length of leaf hairs on both the upper and lower surfaces of the strawberry leaves (variety Sonata). Leaves were collected on 28 April 2011, after being treated with different concentrations (standard and high) of silicon wetter (Omex SW7) with and without the use of K50 (Potassium carbonate) and control (untreated). A total of 20 leaves for each treatment were used to measure the average length of leaf hairs (n=20). Means (± standard error of means) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for windows version 17.

5.3.6. Relationship between the length of leaf hairs and the number of germinating ascospores

The results from the leaves collected on 28 April 2011 were used to find a relationship between the length of leaf hairs and the number of germinating ascospores. The results from the leaves collected on 19 May and 10 June were not used to find the relationship which was mentioned in section 5.3.3. Correlation analysis showed that there was a relationship between the length of leaf hairs and the number of germinating ascospores on both the upper and lower surfaces of leaves. A significant (P<0.01) negative correlation was observed between the length of leaf hairs and the number of germinating ascospores. The negative correlation coefficients (r) were -0.995 and -0.988 for both the upper and lower surfaces of leaves which indicated the possibility that the increased length of leaf hairs is correlated with the decreased number of germinating ascospores. Regression analysis also showed a negative relationship between the length of leaf hairs and the number of germinating ascospores on both the upper and lower surfaces of leaves (Figures 66A and B).



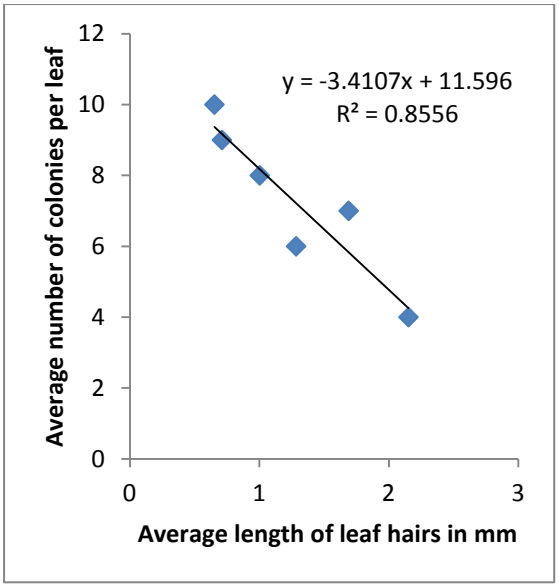


A. Upper surface B. Lower surface

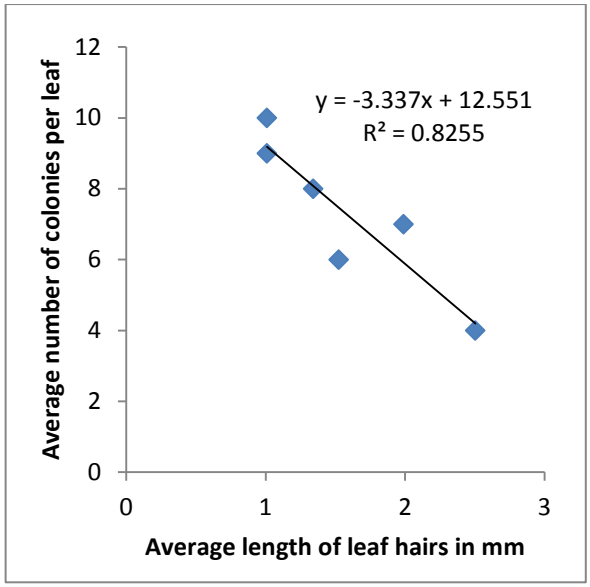
Figure 66(A-B): Relationship between the length of leaf hairs and the number of germinating ascospores on both the upper (A) and lower (B) surfaces of leaves (variety Sonata). Leaves were collected on 28 April 2011, after being treated with different concentrations (standard and high) of Omex SW7 with and without the use of K50 (Potassium carbonate) and untreated (control).

5.3.7. Relationship between the length of leaf hairs and the number of colonies

To find a relationship, Pearson's correlation was used and results from the leaves collected on 28 April 2011 were also used. Correlation analysis showed that there was a relationship between the length of leaf hairs and the number of colonies on both the upper and lower surfaces of leaves. The negative correlation coefficient values (r) were -0.925 and -0.909 for both the upper and lower surfaces of leaves which indicated the possibility that the increased length of leaf hairs is correlated with the decreased number of colonies. A significant ($P<0.01$) negative correlation was observed between colonies and length of leaf hairs for the upper surface of leaves. For the lower surface of leaves significant ($P<0.05$) negative correlation between colonies and length of leaf hairs was observed at the 5% level. Regression analysis also showed a negative relationship between the length of leaf hairs and the number of colonies on both the upper and lower surfaces of leaves (Figures 67A and B)



A. Upper surface



B. Lower surface

Figure 67(A-B): Relationship between the length of leaf hairs and the number of colonies on both the upper (A) and lower (B) surfaces of leaves (variety Sonata). Leaves were collected on 28 April 2011, after being treated with different concentrations (standard and high) of Omex SW7 with and without the use of K50 (Potassium carbonate) and untreated (control).

5.3.8. Concentrations of silicon in leaves collected from trial plot

Leaves collected on 7 April before the first spray and also on 28 April, 19 May and 10 June from strawberry plants showed different levels of silicon concentrations (Figure 68). Results showed that silicon concentrations in the leaves collected on 7 April (before spray) were the same in all the plots. Concentrations of silicon in the leaves collected on 7 April showed the background level of silicon in all trial (18) plots. It was observed that there was a significant difference in silicon concentrations between different treatments. Strawberry leaves collected from standard, high, standard+K50, high+K50 treated plants showed significantly ($P<0.05$) higher silicon concentrations compared to that in leaves collected from control and K50 (Potassium carbonate) treated plants. However, there was no significant difference ($P>0.05$) of silicon concentrations in leaves collected from control and K50 (Potassium carbonate) treated plants. The silicon concentration in leaves collected from the high Omex SW7 treated plants showed significantly higher ($P<0.05$) concentrations compared to control, K50 (Potassium carbonate) treated and the standard Omex SW7 treated plants (Figure 68).

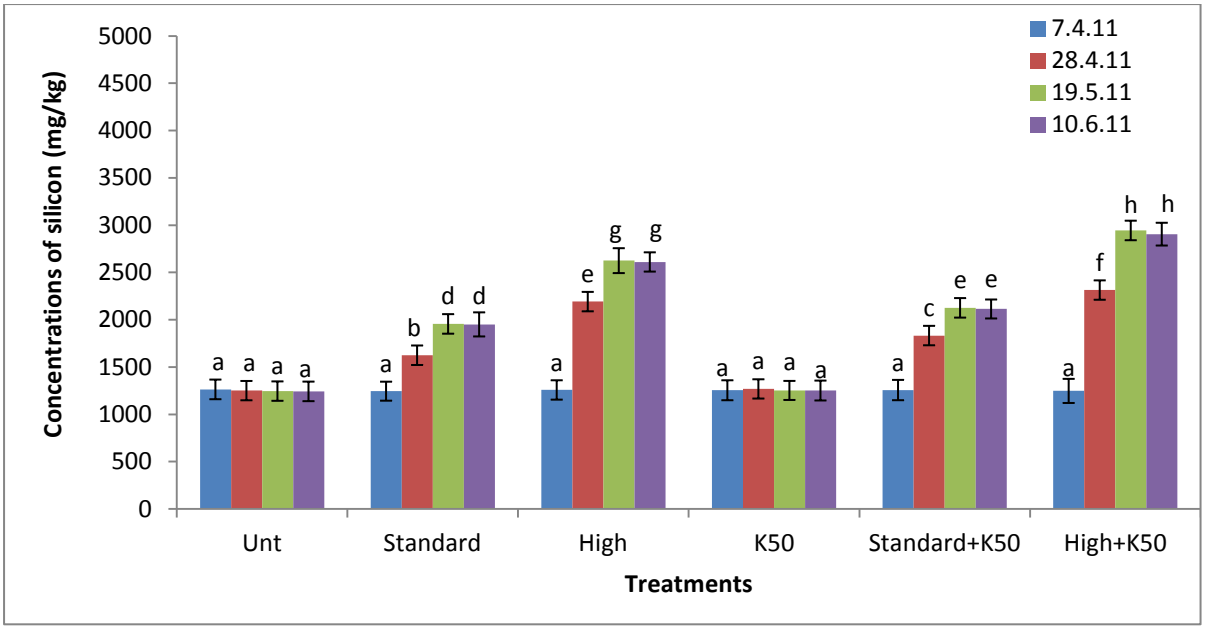


Figure 68: Concentrations of silicon in the strawberry leaves (variety Sonata). Strawberry plants were treated with different concentrations (standard and high) of silicon wetter (Omex SW7) with and without the use of K50 (Potassium carbonate) and control (untreated). Leaves were collected on 7 April before beginning of treatment/spray and also on 28 April, 19 May and 10 June. Each value represents the mean of 6 replications (n=6). Means (± standard error of means) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by Tukey's HSD (*Post-hoc* test) in the statistical software program, SPSS for windows version 17.

However concentrations of silicon were significantly different on the leaves collected on 28 April in different treated plots after the weekly application of different concentrations of silicon and K50 (Potassium carbonate). There was no significant difference found in silicon concentrations in the leaves collected from untreated and K50 (Potassium carbonate) treated plots. On 28 April there were significant differences observed in standard, high, standard+K50, high+K50 treated plots. Weekly spraying ceased on 11 May and the silicon level was maintained at the same level until 10 June (Figure 68). This demonstrates that the silicon is absorbed into plant cell structures and not lost from the plant in the four weeks since the last application of silicon to the leaves. This result indicated that after foliar application of Omex SW7, silicon is absorbed through the cuticle and is available for plant development for at least one month.

5.3.9. Relationship between concentrations of silicon and the number of germinating ascospores

The above results showed that application of standard and high concentrations of Omex SW7 and standard and high concentrations of Omex SW7 mixed with K50 increased the concentrations of silicon in the strawberry leaves. The results also showed that standard and high concentrations of Omex SW7 and standard and high concentrations of Omex SW7 mixed with K50 decreased the number of germinating ascospores. The results from the leaves collected on 28 April were used to find the relationship and were also used to find the other relationship described in this chapter. It was observed that there was a significant ($P<0.01$) negative correlation between increased levels of silicon and decreased number of germinating ascospores.

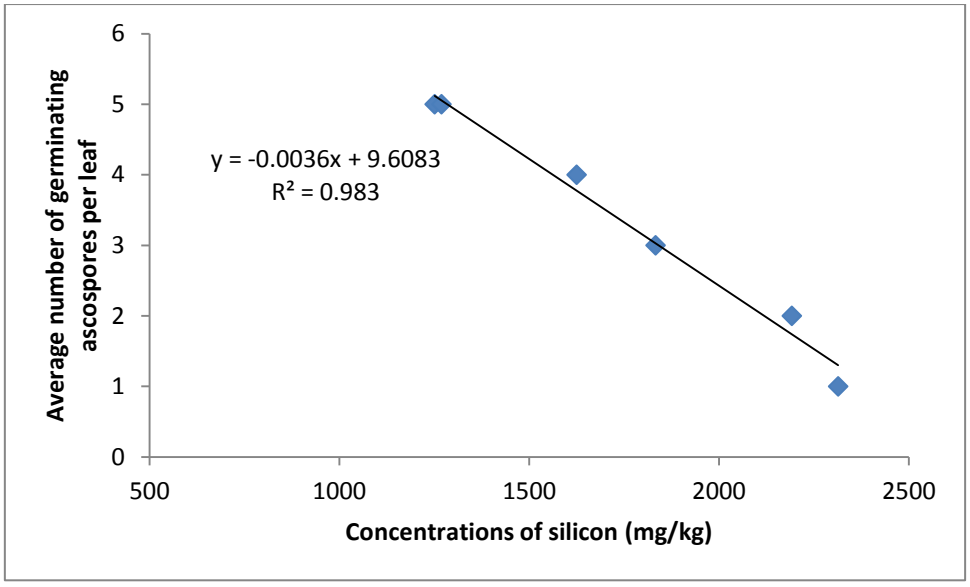


Figure 69: Relationship between concentrations of silicon and the number of germinating ascospores. Leaves were collected on 28 April 2011, after being treated with different concentrations (standard and high) of Omex SW7 with and without the use of K50 (Potassium carbonate) and untreated (control).

The correlation coefficient (r) was -0.991. The negative r value means that the increased levels of silicon is correlated with the decreased number of germinating ascospores. Regression analysis also showed a negative relationship between increased levels of silicon and decreased number of germinating ascospores (Figure 69).

5.3.10. Relationship between concentrations of silicon and the number of colonies

Correlation analysis showed that there was a significant ($P<0.05$) negative correlation between increased levels of silicon and decreased number of colonies. The correlation coefficient (r) was -0.894. The negative r value means that the increased levels of silicon is correlated with the decreased number of colonies. Regression analysis also showed a negative relationship between increased levels of silicon and decreased number of colonies (Figure 70).

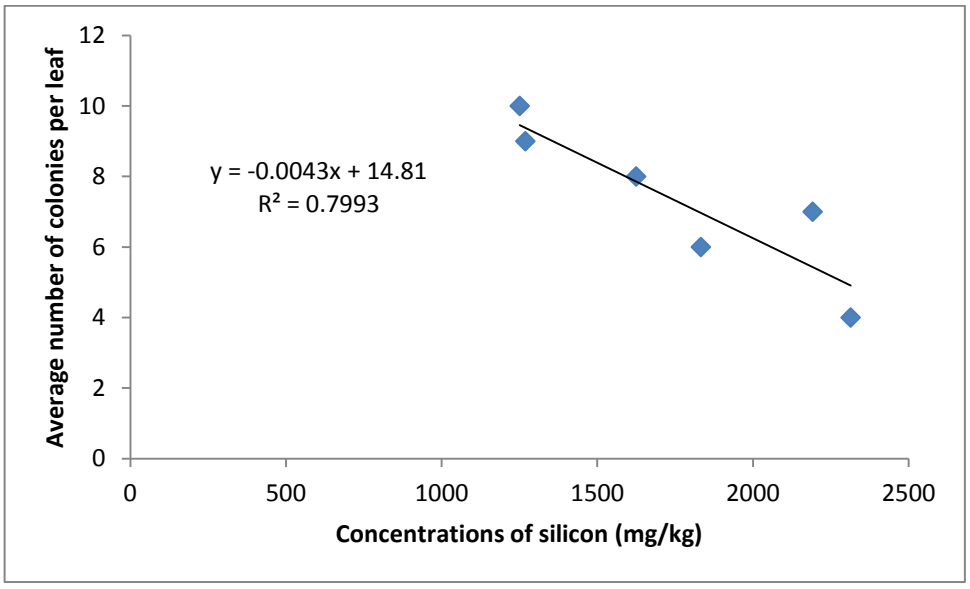
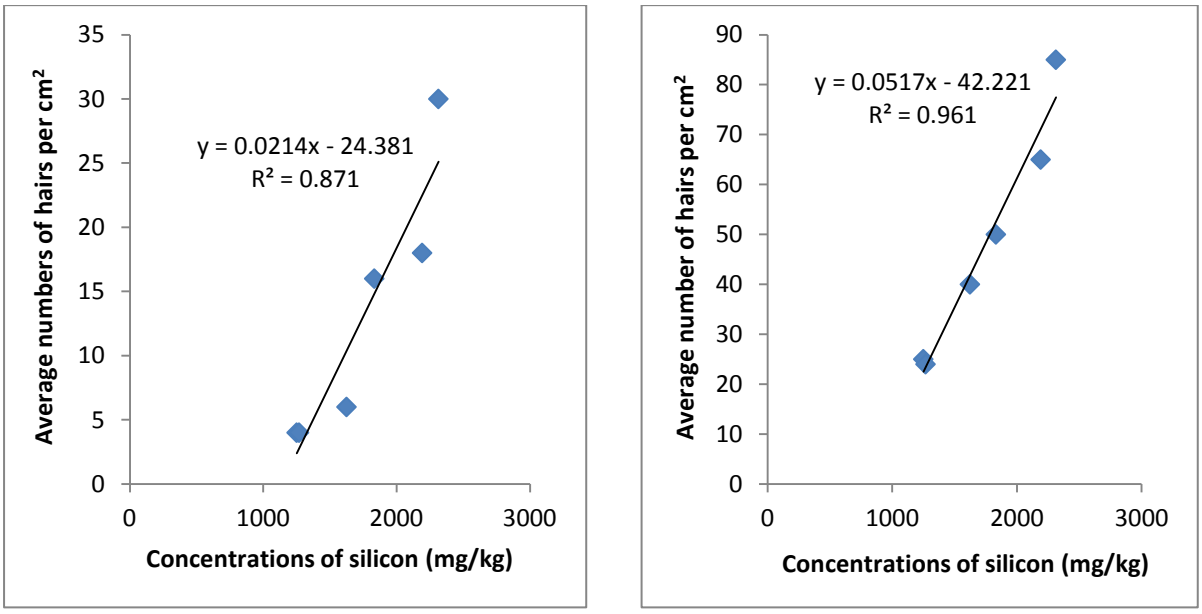


Figure 70: Relationship between concentrations of silicon and the number of colonies. Leaves were collected on 28 April 2011, after being treated with different concentrations (standard and high) of Omex SW7 with and without the use of K50 (Potassium carbonate) and untreated (control).

5.3.11. Relationship between number of leaf hairs and concentrations of silicon

The above results showed that application of standard and high concentrations of Omex SW7 and standard and high concentrations of Omex SW7 mixed with K50 increased concentrations as well as increased the number of leaf hairs on both the upper and lower surfaces of leaves. A significant ($P<0.01$) positive correlation was observed

between increased levels of silicon and increased number of leaf hairs on both the upper and lower surfaces of leaves. The correlation coefficient values (r) were 0.933 and 0.980 for the upper and lower surfaces of leaves respectively. Regression analysis also showed a positive relationship between increased levels of silicon and increased number of leaf hairs on both the upper and lower surfaces of leaves (Figures 71A and B).

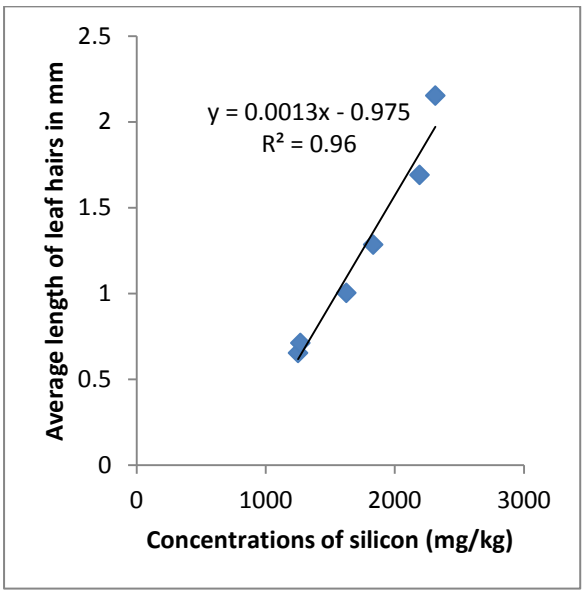


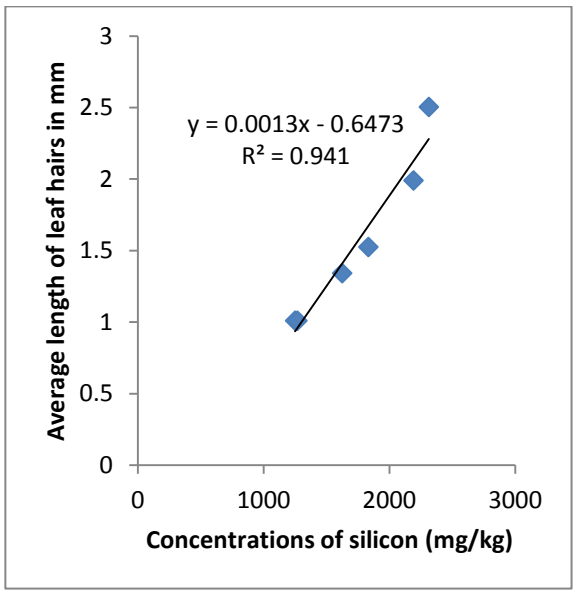
A. Upper surface B. Lower surface

Figure 71(A-B): Relationship between concentrations of silicon and number of leaf hairs on both the upper (A) and lower (B) surfaces of leaves (variety Sonata). Leaves were collected on 28 April 2011, after being treated with different concentrations (standard and high) of Omex SW7 with and without the use of K50 (Potassium carbonate) and untreated (control).

5.3.12. Relationship between length of leaf hairs and concentrations of silicon

Correlation analysis showed that there was a significant positive ($P<0.01$) relationship between increased levels of silicon and increased length of leaf hairs on both the upper and lower surfaces of leaves. The correlation coefficient values (r) were 0.980 and 0.970 for the upper and lower surfaces of leaves respectively. Regression analysis also showed a positive relationship between increased levels of silicon and increased length of leaf hairs on both the upper and lower surfaces of leaves (Figures 72A and B).





A. Upper surface B. Lower surface

Figure 72(A-B): Relationship between concentrations of silicon and length of leaf hairs on both the upper (A) and lower (B) surfaces of leaves (variety Sonata). Leaves were collected on 28 April 2011, after being treated with different concentrations (standard and high) of Omex SW7 with and without the use of K50 (Potassium carbonate) and untreated (control).

5.4. Discussion

The results showed that standard and high concentrations of silicon (Omex SW7) significantly ($P<0.05$) reduced the number of germinating ascospores and colonies compared with the untreated. Moreover, standard and high concentrations of silicon (Omex SW7) mixed with K50 gave the greatest reduction in disease compared with the untreated. On 7 April before the start of the spray there were germinating ascospores and colonies on all the trial plots. No germinating ascospores were observed after 28 April on silicon (Omex SW7) and potassium carbonate (standard, high, standard+K50, high+K50) treated plots whilst colonies were present throughout the trial. There was a significant reduction in the number of colonies observed after 28 April on silicon (Omex SW7) and potassium carbonate (standard, high, standard+K50, high+K50) treated plots compared with the untreated plots. There are different hypotheses regarding the role of silicon in inhibition of fungal pathogens. According to the mechanical barrier theory,

application of silicon accumulates in the epidermal cell wall (Epstein, 1994). The silicon deposition in the cell wall acts as a barrier against fungal germ tube penetration in the epidermis (Epstein, 1994). Bowen *et al*., (1992) reported that the thick potassium silicate deposits that coated a significant portion of the grape leaf cuticle prevented the penetration by germinating ascospores of *Uncinula necator*. Fungal development was more extensive in the areas of the leaf surface that had not been coated. Bowen *et al*., (1992) suggested that foliar application of silicon gave a satisfactory disease control probably through a physical barrier of silicon deposited on leaf surfaces. Menzies *et al*., (1992) showed that foliar applications of silicon (Potassium silicate) to cucumber, muskmelon and zucchini squash reduced the number of colonies of *Podosphaera xanthii* and as a consequence severity of powdery mildew decreased. From this explanation it might be stated that the reduction in the number of germinating ascospores and colonies by silicon (Omex SW7) sprays might be partly due to a physical barrier to hyphal penetration and the deposition of silicon within the leaf at fungal penetration sites.

The results showed that there were germinating ascospores and colonies on the leaves collected from all the trial plots on 7 April before spraying. Although there were no colonies visible to the naked eye, microscopic observation showed that germinating ascospores and colonies were present throughout the trial. This means that ascospores and colonies were present in the tunnels before the trial started. Ascospores germinate in the spring after conducive conditions developed in the tunnel and whilst the plants were covered with fleece and mulch. After germination, mycelium developed. The mycelium of the fungus then grew in a thin layer on the surface of the leaf and formed a colony. The above explanation showed that colonies developed from ascospores. However the results indicated that there were more colonies than ascospores. According to Glawe (2008) ascospores germinate and initiate an epidemic in the spring following the growing season during which they were formed. The trial was started on 7 April 2011 and the field used in the trial was a second year crop. Chasmothecia which contained ascospores had overwintered in the tunnel and according to Glawe (2008) ascospores germinate in the spring (March) which was before the trial started. The use of fleece before the trial had allowed a high temperature and humidity to develop and so acospores were released and ascospores germinated. In this trial, ascospores would

have been released and started to germinate whilst the plants were covered with fleece. For this reason more colonies than germinating ascospores were observed in the trial.

When the trial was conducted the temperature and relative humidity were not conducive for disease development therefore no epidemic built up on the untreated plots where no treatments and no water were sprayed at all (mentioned in section 5.2.2). Water was not used as a control; as several studies have reported that although high moisture levels favour strawberry powdery mildew development, free water (moisture) is inhibitory to powdery mildews (Jhooty and McKeen, 1965; Schnathorst, 1965; Amsalem *et al*., 2006). Results showed that there was a reduction in the number of germinating ascospores and colonies on all the treated and untreated plots after 7 April. The reduction in the number of germinating ascospores and colonies on the treated plots was greater than the untreated plots. The reduction in the number of germinating ascospores and colonies observed on the untreated plots on 28 April was unexpected. The reduction in the number of germinating ascospores and colonies on the untreated plots on 28 April was very small compared to 7 April. However there was no further reduction of germinating ascospores and colonies on 19 May and 10 June on the untreated plots. No significant reduction in the number of germinating ascospores and colonies was observed on the untreated plots on 28 April, 19 May and 10 June. The number of germinating ascospores and colonies gradually decreased on the treated plots on 28 April, 19 May and 10 June and no germinating ascospores were observed after 28 April on silicon (Omex SW7) and K50 treated plots. Therefore it might be stated that the reduction in the number of germinating ascospores and colonies on the untreated plots was probably due to the weather conditions (Figure 61). In addition due to the weather conditions there was no real increase in disease during the experimental period in all the treated and untreated plots, because the colonies formed did not mature to produce the conidiospores which give epidemic build up. The study of Glawe (2008) demonstrated that temperature and relative humidity affect ascospore germination and colony development. During the experimental period weather conditions (temperature and relative humidity) were not conducive for disease development. Therefore no epidemic was observed and the small number of ascospore germinated and the colonies formed were observed by dissection microscope.

Microscopic observations of silicon treated leaves showed that there was increased density of leaf hairs on both the upper and lower surfaces (Figure 62) and increased length of leaf hairs on both the upper and lower surfaces compared with the leaves collected from the untreated plots (Figure 65). However increased density and length of leaf hairs were not found in the leaves collected from the plots treated with K50 (Potassium carbonate). Moreover it was also found that leaves collected from silicon (Omex SW7) and K50 (Potassium carbonate) treated plots had an effect on density and length of leaf hairs. In this study it was found that application of silicon increased the number and length of leaf hairs. Results also indicated that the number of germinating ascospores and colonies also decreased when silicon (Omex SW7) was applied to the different trial plots. These findings support the hypothesis that the silicon from the silicon wetter is absorbed by the plant and changes the physical properties of the leaves, including number and length of leaf hairs and in this way helps the plants to reduce fungal infection. This is supported by the work of Kanto *et al*., (2004) and showed that silicon was absorbed by strawberry plants and improved the physical properties of the strawberry leaves, such as increased the hardness of the strawberry leaves. In another study, Kanto *et al*., (2007) showed that silicon was absorbed by strawberry plants which led to physiological changes in the strawberry leaves. As a result of these physiological changes, Kanto *et al*., (2007) reported that germination and appressoria formation of powdery mildew conidia were inhibited. Samuels *et al*., (1991) revealed that when a silicon supplement (Potassium silicate) was added to hydroponic solution used for growing cucumber, high silicon concentrations were found at the basal cells surrounding the trichomes. They (Samuels *et al*., 1991) described that fungal colonies were smaller in silicate treated leaves in comparison with the control. The findings of Samuels *et al*., (1991) indicated that silicon supplement was absorbed by the plants, changed the physical properties of the leaves, including the trichomes and in this way helped the cucumber plants to reduce fungal infection. The discrepancy between the studies of Samuels *et al*., (1991) and Kanto *et al*., (2007) and the work reported in chapter 5 is that in this study silicon wetter (Omex SW7) was applied on leaves, whereas in the above studies (Samuels *et al*., 1991; Kanto *et al*., 2007) silicon (Potassium silicate) was applied in the hydroponic solution. However, the studies referred to above (Samuels *et al*., 1991; Kanto *et al*., 2007) and the results in this chapter (5) showed that silicon had an influence on changing the physical properties of

the leaves which play an important role in plant resistance against pathogens. The results in chapter 5 do not demonstrate the direct inhibitory effects of increased leaf hair numbers and length against the strawberry powdery mildew infection which will be resolved by further investigations.

Trichomes and bristles, generally known as hairs play an important role against the pathogen attack to prevent mycelia penetration and infection (Bonos *et al.,* 2004). Bonos *et al*., (2004) found that a resistant variety of creeping bentgrass had 42 to 64% larger trichomes than a susceptible variety. For this reason they stated that large trichomes work as a physical hindrance to the pathogen infecting the host. Shaik (1985) explained that larger trichomes work as a structural defence in bean rust. He also described that the presence of long, straight trichomes on the adaxial surfaces of bean leaves helps to reduce the intensity of disease. The results described in chapter 5 indicated that application of silicon (Omex SW7) increased the number and length of leaf hairs. Strawberry powdery mildew symptoms first appeared on the lower surface of leaves and in general the number of leaf hairs is more on the lower surface in comparison with upper surface of leaves which had already been observed in different strawberry varieties. In strawberries, density and length of leaf hairs varies between varieties. However the natural high density and length of leaf hairs on the lower surface is not sufficient to limit the strawberry powdery mildew. Treatment with silicon (Omex SW7) stimulated the production of leaf hairs and increases their density and length which does result in disease reduction. Significant negative correlation was observed between the number of leaf hairs on the lower surface of leaves and the number of germinating ascospores (Figure 63B). Significant negative correlation was also observed between the number of leaf hairs on the lower surface of leaves and the number of colonies (Figure 64B). These relationships emphasize the possibility that an increased number of leaf hairs on the lower surface helps to reduce the number of germinating ascospores and colonies which leads to disease reduction. Kortecamp and Zyprian (1999) observed that the high density of hairs on the leaves of grapes (variety, *Vitis labrusca*) inhibit colonization of the leaf cuticle by *Plasmopara viticola*. Kortecamp and Zyprian (1999) mentioned that the leaf hairs of *Vitis labrusca* were arranged very closely and completely covered the leaf surface. Kortecamp and Zyprian (1999) showed that leaf hairs prevent a successful penetration of the host by the fungal germ tube. The

study of Kortecamp and Zyprian (1999) and the study of Shaik (1985) showed that although rust and downy mildew pathogen enters the plant through stomata, leaf hairs inhibit fungal disease by physically inhibiting fungal germ tube penetration into the stomata. However, *P. aphanis* enters the plant by direct penetration and not through stomata. In addition, Shaik (1985) stated that leaf hairs could limit infection in the following manner. Leaf hairs may distract a germ tube and in some instances the germ tube twined from one hair to another like wire without touching the leaf surface. This could be a mechanism by which the stimulation in leaf hair density and length causes the reduction in disease development in *P. aphanis*. From the above explanation, it can be stated that leaf surface characteristics, such as leaf hair numbers and length also have an influence on pathogen infection.

The results showed that concentrations of silicon were significantly higher in the leaves collected from standard and high concentrations of Omex SW7 treated plots than the untreated plots (Figure 68). Leaves collected on 28 April and 19 May showed that weekly application of standard and high concentrations of Omex SW7 significantly increased the silicon concentrations in the strawberry leaves. Though strawberry is a silicon non-accumulator plant, the results in chapter 3 showed that foliar application of Omex SW7 increased silicon concentrations in the strawberry leaves in the variety Elsanta, and the other three varieties which were observed in the glasshouse experiment. The results in chapter 5 showed that foliar applications of Omex SW7 increased silicon concentrations in the leaves of strawberry variety Sonata which was observed in the field trial experiment. Results in chapter 5 also stated that weekly spraying ceased on 11 May and that concentrations of silicon were the same in the leaves collected on 19 May and also on 10 June. This result indicated that after foliar application, silicon is absorbed through the cuticle and is deposited in the epidermal cell and available for plant development for at least one month. The information in the literature indicated that after foliar application silicon accumulates in leaves and it accumulates especially below and above the cuticle of leaves (Epstein, 1994). However, silicon uptake and accumulation depends on plant species. Monocotyledon plants take up more silicon than dicotyledon plants. However, the exact mechanism of uptake and accumulation and how long the accumulated silicon is available for plant growth and development is not clear and remains to be determined.

Correlation analysis showed that there was a negative relationship between concentrations of silicon and the number of germinating ascospores (Figure 69). A negative relationship was also observed between concentrations of silicon and the number of colonies (Figure 70). These relationships showed that increased concentrations of silicon are correlated with the decreased number of germinating ascospores and colonies. Suppressive effects of silicon against plant pathogens especially for blast and brown spot of rice have been known for a long time (Datnoff and Rodrigues, 2005). Belanger *et al*., (2003) reported a suppressive effect of silicon and its mode of action against powdery mildew on wheat (a silicon accumulator). Miyake and Takahashi (1983) examined the suppressive effect of silicon against Fusarium wilt of cucumber (a silicon non-accumulator) and reported that both potassium silicate and calcium silicate alone suppressed Fusarium wilt of cucumber for 3 years. However there was little relationship between the amount of applied silicon and the suppressive effect against cucumber wilt. They showed that potassium silicate was more effective than calcium silicate against Fusarium wilt of cucumber. Kanto *et al*., (2006) examined the suppressive effect of solid potassium silicate and calcium silicate against strawberry powdery mildew. They (Kanto *et al*., 2006) observed that solid potassium silicate and calcium silicate was not effective against strawberry powdery mildew. Therefore, they stated that solid potassium silicate cannot be absorbed by strawberry to any great extent. However, in another study Kanto *et al*., (2004) used liquid potassium silicate and examined the suppressive effect of liquid potassium silicate against strawberry powdery mildew. They (Kanto *et al*., 2004) observed that little mildew developed in the plots treated with low concentrations of silicon and no powdery mildew developed in the plots treated with high concentrations of silicon. They also observed that suppressive effect lasted for about 4 months and even longer on leaves. They also observed a relationship between increased silicon content and decreased plant diseases. The results in the chapter 5 showed that a silicon based wetter Omex SW7 significantly reduced the number of germinating ascospores and colonies. Correlation analysis showed that increased silicon concentrations are correlated with decreased plant diseases which were also observed by Kanto *et al.,* (2004). Kanto *et al.,* (2004) used liquid potassium silicate and showed the suppressive effect against powdery mildew of strawberries; Menzies *et al*., (1992) used potassium silicate and showed the

suppressive effect against cucumber powdery mildew. The above studies (Menzies *et al*., 1992; Kanto *et al.,* 2004) used potassium silicate as a silicon source, however in this study (chapter 5) Omex SW7 (a liquid form of silicon) was used as a silicon source and the results confirmed for the first time that silicon wetter (Omex SW7) had an effect on the reduction of disease incidence.

The objectives of this study (Chapter 5) have been achieved and show that though strawberry is classified as a silicon non-accumulator plant (Miyake and Takahashi, 1986) application of silicon based wetter Omex SW7 onto the leaf surface does result in accumulation of silicon in the leaves. The application of Omex SW7 has stimulated an increase in the number and length of leaf hairs in strawberry plants. Furthermore, the applications of the silicon wetter lead to a reduction in the germination of ascospores and a reduction in the number of colonies of *P. aphanis* on the leaf surface. This reduction in germination and colony number after the use of Omex SW7 was greater than if potassium carbonate as K50 was used alone. The greatest reduction was in the plots treated with high rate of Omex SW7 mixed with K50. Significant negative correlations were observed between density and length of leaf hairs and number of germinating ascospores and number of colonies. Significant negative correlations were also observed between increased silicon level and decreased number of germinating ascospores and colonies. These relationships emphasize the possibility of the use of a silicon wetter in the reduction of strawberry powdery mildew. Therefore the protection offered by the application of silicon based wetter Omex SW7 mixed with K50 might have important practical implications in commercial strawberry production. However, further work is required to establish evidence for this protective effect under high disease pressure.

Chapter-6: General discussion and conclusions

6.1. Discussion and conclusions

The work reported here showed that though strawberry is a dicotyledon, and a silicon non-accumulator plant, the application of the silicon based wetter Omex SW7 enhanced the silicon levels in the leaves of strawberry plants. Enhanced levels of silicon were observed through foliar application (Omex SW7 applied to leaves) and root application (Omex SW7 applied to the root zone). The silicon based wetter Omex SW7 was sprayed at different concentrations and different time intervals on the strawberry leaves. It was observed that both the concentration and timing of applications had an effect in increasing the silicon concentrations in the strawberry leaves. According to Menzies *et al.,* (1992) foliar application of silicon was absorbed through the cuticle and deposited in the epidermal cell and cell wall. Silicon concentration was significantly higher ($P<0.05$) in leaves treated with standard, high and very high concentrations of Omex SW7 compared to that in leaves collected from control plants (Figure 17). These findings are supported by the work of Liang *et al*., (2005) where they demonstrated that in plants such as cucumber, which is also known as a silicon non-accumulator plant, foliar application of silicon increased the silicon content in the plants. However there are differences between studies using different silicon based products as a source of silicon. In this study (chapters 3, 4 and 5) the silicon wetter Omex SW7 (Silicon dioxide) was used as a silicon source whereas other studies used potassium silicate or metasilicate as a source of silicon (Kanto *et al*., 2006; Dallagnol *et al*., 2012). In Europe, potassium silicate or metasilicate is available commercially and is marketed for the greenhouse industry as a plant nutrient (Belanger *et al*., 1995). While it has been impossible to learn the exact proportion of growers using potassium silicate, companies estimate that more than 60% of cucumber growers and over 30% of rose growers use it on a regular basis (Belanger *et al*., 1995). However, the silicon based product Omex SW7 is routinely used by the strawberry growers as a wetter. Omex SW7 is used by the growers for the application of a wide range of nutrients and is also used in the application of fungicides (mentioned in chapter 1). The silicon wetter (Omex SW7) helps the absorption of nutrients by spreading the nutrients very quickly thereby covering a large surface area. The results in this thesis demonstrate that the silicon in

the wetter is taken up into the plants through the leaves. In addition when Omex SW7 is used at higher than the recommended field rate, then more silicon is absorbed by the plants. The results reported in this study (chapter 3) proved that the highest levels of silicon accumulated in the leaves that were treated by the very high concentration of silicon wetter Omex SW7. Moreover, the more silicon that was applied the higher the levels of silicon absorbed by the plants. This effect was observed not only in the variety Elsanta but also in the other varieties Rhapsody, Florence and Symphony used in the glasshouse experiment (chapter 3).

The results in chapter 4 indicated that root application of silicon (Omex SW7) gave more accumulation of silicon in the leaves of strawberry plants compared to the foliar treatment. The difference in silicon content has been attributed to the ability of the roots to take up silicon (Takahashi *et al*., 1990). Although strawberry is considered as a silicon non-accumulator plant, addition of silicon in the hydroponic solution showed (Kanto *et al*., 2007) the increased concentrations of silicon in the strawberry plants. This finding supported the results that although strawberry plant is a silicon non-accumulator but application of standard and high concentrations of Omex SW7 to roots significantly ($P<0.05$) increased the silicon concentrations in leaves of strawberry plants. However, the mechanisms involved in the different uptake modes are not fully understood and scientists are still trying to find the exact mechanism involved in the different uptake system. Mitani and Ma (2005) conducted a study to examine the uptake system of silicon through the roots in rice, cucumber and tomato which accumulate high, medium and low levels of silicon, respectively. The uptake system of silicon involves at least two processes: radial transport of silicon from the external solution to the root cortical cells (outer layer of the root) and the release of silicon from the cortical cells to the xylem (xylem uptake) in rice, cucumber and tomato. Evidence has shown that the uptake of silicon is an energy-dependent process (Mitani and Ma, 2005). In rice, cucumber and tomato, it has been demonstrated that radial transport of silicon is mediated by a transporter (SITI) and passive diffusion. However, the density of the transporter differs among plant species following the order of rice>cucumber>tomato. The next step of the process that is xylem uptake is mediated by a transporter (SIT2) in rice. In cucumber and tomato xylem uptake is mediated by diffusion. Mitani and Ma (2005) observed that the silicon concentration of xylem sap in rice was 20 and 100 fold

higher than that in cucumber and tomato, respectively. The much lower accumulation of silicon in cucumber and tomato might be explained by a lower density of transporter (SITI) to transport silicon from the external solution to the cortical cells and a defective transporter or the absence of a transporter (SIT2) to transport silicon from cortical cells to xylem (Mitani and Ma, 2005). Rice is a typical silicon accumulating plant which can accumulate 10% (dry weight) silicon in the shoots. High levels of silicon in rice tissues are attributed to the ability of the roots to take up this element. According to Ma and Takahashi (2002) rice takes up silicon actively and the uptake is much faster than that of water. Recently, a gene (*Lsi1*) that is responsible for active silicon uptake has been identified from rice (Ma *et al*., 2004). Lsi1 is mainly expressed in the roots and the expression of Lsi1 is regulated by silicic acid availability and supplying constant silicic acid results in decreased expression. The identification of a gene in rice has been proposed that governs a specific uptake system in rice that facilitates the uptake of silicon. However, molecular mechanisms underlying silicon uptake in dicotyledonous plants including cucumber, tomato and strawberry are still unknown and hence, silicon uptake genes in dicotyledonous plants need to be isolated and characterized. The beneficial effects of silicon are mainly associated with its high deposition in plant tissues, enhancing their strength and rigidity. However, many plants especially dicotyledonous plants are not able to accumulate a large amount of silicon in the shoot from the soil (Mitani and Ma, 2005). Therefore, genetically manipulating the silicon uptake system of the roots might help dicotyledonous plants to accumulate more silicon, thereby enhancing the resistance of plants to multiple stresses.

The results reported in the present study (chapters 3, 4 and 5) showed that foliar and root applications of silicon (Omex SW7) stimulated both the number and length of leaf hairs on the leaves of strawberry plants. Moreover, it was also observed that the weekly applications (foliar and root) of different concentrations of Omex SW7 showed significantly ($P<0.05$) increased leaf hair numbers on both the upper and lower surfaces of leaves compared to the other two intervals of applications. Silicon is rapidly bound in leaf tissue and once it is deposited in the leaf tissue, it is not retranslocated (Datnoff *et al*., 2007). Therefore continuous supply of silicon is very important in order to guarantee a continuous beneficial effect of silicon on plants. This is perhaps the reason that weekly application (foliar and root) of silicon wetter increased silicon levels in the

strawberry plants, which stimulated an increase in the number and length of hairs on the leaves of strawberry plants.

Following uptake by the roots, silicon is translocated to the shoot via the xylem through transpiration but may then be taken up actively by certain cells e.g. trichomes. Samuels *et al*., (1993) stated that trichomes are the most common site where the silica is found. Another investigator Adaita and Besford (1986) observed that leaves containing high levels of silicon in the cucumber plants were rougher and the leaf hairs were also more noticeable. These findings suggest that silicon has an effect on the leaves especially in trichomes. The present study (chapter 3) also revealed that spraying the silicon wetter (Omex SW7) onto the leaf surface affects the morphology of the strawberry leaves resulting in the formation of additional leaf hairs which were longer than when the plants were untreated. This effect was observed not only in the variety Elsanta. It was observed in the varieties Rhapsody, Florence and Symphony. In the variety Rhapsody although there were no hairs on the upper surface (Figure 27) of control leaves a high concentration of Omex SW7 stimulated the growth of hairs on the upper surface. A significant positive correlation was observed between silicon level and number of leaf hairs on both the upper and lower surfaces of leaves in the variety Elsanta (Figures 20 and 21) and also observed in other varieties used in the study. Correlation analysis also showed a significant positive relationship between silicon level and length of leaf hairs on both the upper and lower surfaces of leaves (Figures 24 and 25) in the variety Elsanta. Significant positive relationship between silicon level and length of leaf hairs was also observed in other varieties used in the study. These relationships emphasize that the silicon wetter has an influence in increasing the number and length of leaf hairs. The results in chapter 4 showed that root application at the same concentrations of silicon as the foliar application increased more silicon accumulation in leaves as well as increased numbers and length of leaf hairs compared to that in the foliar application. This can also be explained by the relationship mentioned above. The above positive significant relationship showed that increased silicon levels are correlated with increased leaf hair numbers and increased length of leaf hairs.

This is the first report demonstrating that absorbed silicon stimulated the number and length of leaf hairs. Previous studies showed that silicon might be accumulated at the

base of the trichome but those studies did not show the effect of silicon on the physiological change of the trichomes. The present study showed the effect of silicon on trichomes. This result is supported by another study of Samuels *et al*., (1993) where they found that addition of silicon to the hydroponic nutrient medium has been shown to change the fruit trichome morphology. They (Samuels *et al*., 1993) found that trichomes from silicon treated fruit had a coarse appearance compared to the untreated fruit where the trichomes were smooth. These findings showed that deposition of silicon in the trichome base changed the surface characteristics. The amount of silicon accumulated in the trichomes depends on the species as well as the concentration of the silicate in the growing medium or soil solution (Sangster and Hodson, 1986). Besides this, there are other factors which could influence the accumulation of silicon on trichomes but not in the surrounding epidermis. The cuticular membrane of the trichomes may have a different ability to absorb silicon than the cuticle of the surrounding epidermis. The high transpiration rate in this region may lead to super saturation of the silicate solution and subsequent polymerisation. In addition Samuels *et al*., (1993) stated that trichomes are the common site where the epicuticular wax was observed. The component of the wax may have the ability of binding and concentrating silicate, because the hydroxyl group of the primary alcohol of the wax bind the silicate (Samuels *et al*., 1993).

The results reported in chapter 5 showed that at the beginning of the experiment on 7 April 2011 there were germinating ascospores and colonies on all the trial plots. The four different silicon and K50 (standard, high, standard+K50, high +K50) treated plots showed no germinating ascospores after 28 April (after 21 days of treatment). A reduction in the number of colonies was also observed on these four silicon and K50 treated plots on 28 April 2011. The effect of silicon and K50 in reducing the number of germinating ascospores and colonies can be shown in the decreasing order (high+K50>standard+K50>high>standard>K50). This finding showed that silicon played a role in the reduction of germinating ascospores and number of colonies. In cucumber Liang *et al*., (2005) found that foliar application of silicon (Potassium metasilicate) was effective in the reduction of powdery mildew diseases. They (Liang *et al*., 2005) suggested that foliar applied silicon in their experiments probably played a role as a physical barrier in preventing the penetration of fungal hyphae into host tissue.

The mode of action of foliar applied Omex SW7 in reducing germinating ascospores and reducing the number of colonies was not determined in chapter 5. However, it appears that silicon is the active ingredient in the foliar spray (Omex SW7) which might help to reduce the number of germinating ascospores and colonies.

The mechanisms as to how silicon mediates disease resistance against pathogens are still under debate among scientists. Kunoh and Ishizaki (1975) generalized that in contrast to monocotyledons; in dicotyledons the deposition of silicon at the penetration site of the pathogens does not confirm resistance to pathogens. This view is however challenged by other workers. The reduction in cucumber powdery mildew with addition of silicon has since been shown to be coincident with an accumulation of silicon in the leaves (Menzies *et al*., 1992). Scanning electron microscope and X-ray analysis showed that accumulation of silicon in rice leaves was closely associated with enhanced resistance to rice blast (Kim *et al*., 2002). However, the concept that the accumulation of silicon in cell walls is related to the inhibition of pathogens has become widely accepted, but little emphasis has been placed on the possibility that silicon plays a role in activating host defences. It appears that amendment of plants with silicon may result in enhancement of defence responses. Therefore, in order to study the mechanism by which silicon reduces disease severity, it is necessary to study if defence responses in the host are enhanced, i.e. whether induced resistance is involved. For cucumber, Menzies *et al*., (1991) reported that silicon might induce host resistance by triggering a cascade of defence mechanisms of plants, leading to the accumulation of antifungal compounds such as phytoalexins and pathogenesis-related proteins. Strawberry is a dicotyledon, as is cucumber, and contains less silicon than cucumber. The results in chapter 5 showed that application of silicon wetter Omex SW7 significantly reduced the disease incidence in comparison with the control. Kanto *et al*., (2007) mentioned that silicon had a role in the reduction of powdery mildew in strawberry plants. Although both studies showed the suppressive effect of silicon against powdery mildew in strawberry plants, the difference between the two studies is that Kanto *et al*., (2007) showed that silicon was effective in the reduction of the germination of conidia. On the other hand the results reported in chapter 5 showed that germination of ascospores were reduced after the application of silicon wetter Omex SW7.

Correlation analysis showed a negative correlation between silicon content in leaves and the disease severity (chapter 5). A similar relationship was also observed by Kanto *et al*., (2004). In another study Kanto *et al*., (2006) observed that powdery mildew in strawberry plants was strongly suppressed by the application of silicon (as potassium silicate) when applied before mildew appeared. Kunoh and Ishizaki (1975) observed that silicon accumulated at the penetration sites in powdery mildew pathogens; however accumulation of silicon was not correlated with increased resistance to fungal penetration. For this reason, Kunoh and Ishizaki (1975) stated that silicon accumulation might not constitute a physical barrier to block mildew penetration. Kanto *et al*., (2006) also observed that although leaf hardness seemed to be greater than in control leaves, leaf hardness did not differ significantly between control and silicon treated leaves. Therefore, they (Kanto *et al*., 2006) stated that the main suppressive effect of silicon against mildew is not only due to physical changes, but also it was absorbed into strawberry plants and subsequently induced physiological changes in the strawberry plants. Cherif *et al*., (1994) observed that incorporation of silicon into a hydroponics system stimulated the activities of peroxidises and chitinases which can enhance defence resistance in response to infection by the pathogen *Pythium* spp. in cucumber. For this reason, Kanto *et al*., (2006) stated that silicon plays a role not only as a physical barrier but also as a resistance inducer in plants.

The field trial (chapter 5) revealed that application of Omex SW7 significantly reduced the number of germinating ascospores and colonies. This study (chapter 5) also revealed that foliar application of Omex SW7 (standard and high) significantly ($P<0.05$) increased the number and length of leaf hairs on both the upper and lower surfaces of leaves (Figures 62 and 65). These increased number and length of leaf hairs might work as a physical barrier and reduce the number of germinating ascospores and colonies. These two findings regarding the morphological changes of strawberry leaf structure such as increased number and length of leaf hairs could act as a physical barrier against penetration of fungal hyphae that might help to prevent germination rate of powdery mildew (Lewin and Reimann, 1969). Significant negative correlation was observed between number of leaf hairs and disease severity (chapter 5). Correlation analysis also showed a significant negative correlation between length of leaf hairs and disease severity (chapter 5). This relationship further establishes the important role silicon

plays in disease resistance in strawberry. In addition, correlation analysis demonstrates that number and length of leaf hairs might play a significant role in the reduction of disease severity. Plant leaf hairs play important physiological roles. It has long been known that glandular hairs protect the plants against the attacks of herbivorous animals and especially of insects (Dalin *et al*., 2008). According to Brewer and Smith (1997) leaf hairs can decrease pathogen germination rates by decreasing leaf wettability. Kortekamp and Zyprian (1999) stated that leaf hairs work as a basic protective barrier against downy mildew of grapes. Kortekamp and Zyprian (1999) observed that the high density of leaf hairs on the leaves of grapes inhibit colonization of the leaf cuticle by *Plasmopora viticola*, the downy mildew fungus of grape. According to the above findings it might be stated that foliar application of Omex SW7 which increases the number and length of leaf hairs might work as a protective barrier and decrease the number of germinating ascospores and colonies in this trial. However this study (results of this thesis) does not exclude the possibility that silicon may enhance host resistance by triggering a systemic defence mechanism in strawberry plants leading to the accumulation of antifungal compounds such as phytoalexin and pathogenesis-related proteins in plants. In a study Wang and Galletta (1998) reported that spraying silicon (Potassium silicate) increased unsaturated fatty acids (linoleic, linolenic acids) in strawberry leaves. Linolenic acid is an unsaturated fatty acid related to the production of jasmonate, a key substance in induced systemic resistance (Creelman and Mullet, 1997). According to the report of the different studies it can be stated that the mechanism of silicon mediated resistance to fungal attacks in plants appears to be extremely complicated, depending on the fungus involved and many other unknown factors. In conclusion it can be said that silicon wetter Omex SW7 did not directly act against infection by powdery mildew, but it was absorbed into strawberry plants and subsequently induced physiological changes in the strawberry plants. According to Wang and Galletta (1998) as a result of these physiological changes, after the application of Omex SW7 on the strawberry leaves might be increased the unsaturated fatty acids which could play an important role in the reduction of powdery mildew infections. Thus the reduction in the number of germinating ascospores and colonies after the application of silicon wetter Omex SW7, could indicate that silicon wetter (Omex SW7) plays a role not only as a physical barrier, but also as a resistance inducer in plants. However further study is required to investigate the presence and role of

induced compounds in silicon wetter Omex SW7 treated strawberry plants infected by powdery mildew. It would be interesting to clarify whether silicon wetter treated strawberry plants infected with powdery mildew exhibit the presence of phenol-like material associated with degraded powdery mildew haustoria.

The results of this thesis support the hypothesis that silicon levels can be enhanced by the application of Omex SW7 through leaves or roots and the application of silicon has been shown to increase the number and length of leaf hairs in strawberry plants. The field trial study also revealed that the application of the silicon based wetter significantly ($P<0.05$) reduced the number of germinating ascospores and colonies. It has also been observed that K50 (Potassium carbonate) alone can give some reduction in the number of germinating ascospores and colonies. Potassium carbonate (K50) is a contact type fungicide and direct contact with the fungus is necessary for control. In order to achieve a good spread of K50, Omex SW7 is used as a wetter in the application of potassium carbonate to reduce the infection of strawberry powdery mildew. Therefore the result of the study suggests that foliar application of standard and high concentrations of Omex SW7 mixed with K50 might limit the powdery mildew infection in the field. The use of same mode of action of fungicides has resulted in the development of fungicide resistance in *P. aphanis*. There are limited ranges of products available for strawberry growers to control *P. aphanis* infection. According to Dodgson (2008) effective control of *P. aphanis* infection could be achieved by using Systhane (Myclobutanil) a systemic, protectant and curative fungicide. The use of natural and low-toxicity substances such as sodium or potassium carbonate can reduce the number of applications of fungicides that need to be used. For example, sodium bicarbonate has been shown to control powdery mildew of rose (Horst *et al*., 1992). Potassium carbonate however only works by contact with fungus and it has no lasting effect so can require more frequent applications than fungicides. Potassium carbonate also could not provide the same level of disease control which is achievable by using traditional fungicides. For this reason potassium carbonate can be used in the integrated control programme to complement fungicides rather than replace them completely. Potassium carbonate does not have a harvest interval. Therefore, it is suggested that growers could use standard and high concentrations of Omex SW7 and potassium carbonate routinely in the immediate pre-harvest period to control the disease without using conventional

fungicides thus reducing the risk of occurrence of residues in the fruit and also reducing conventional fungicide use.

The reduction of *P. aphanis* infection after the use of silicon wetter Omex SW7 was greater than when K50 was used alone (chapter 5). Significant reduction was observed in the plots treated with the standard and high concentrations of Omex SW7. The reduction of disease severity as a result of foliar application of Omex SW7 was similar in magnitude to that observed in melon plants infected with powdery mildew and supplied with silicon (Dallagnol *et al*., 2012). Moreover, reductions of disease severity were greater than those observed in cucumber and strawberry plants. In cucumber and strawberry, foliar application of silicon did not show significant disease control (Liang *et al*., 2005; Palmer, 2007). Therefore the result of the study suggests that weekly application of standard and high concentrations of foliar application of Omex SW7 might be effective in reducing the powdery mildew severity in the field. In UK, strawberry growers who are closely involved with this work used less than 14 applications of fungicide in each season; other growers sometimes applied fungicide more than 14 times. In the Netherlands, a study carried out by Van Drooge *et al*., (2001) showed that in strawberry more than 50 applications of pesticides were used in a season, of which just less than half were fungicides. This number of applications greatly increases the possibility of finding residues in the fruit as well as posing a health risk to the operator. The use of the same mode of action of fungicides has resulted in the development of fungicide resistance in *P. aphanis*. Some investigators have observed that regular use of other fungicides and regular use of Systhane had adverse effects on strawberry crops (Palmer, 2007; Dodgson, 2008). A reduction in fungicide applications could be achieved in part by using alternate products where appropriate. According to Ma *et al*., (2001) silicon is the only element that is not harmful for plants when accumulated in excess levels. For this reason, the standard concentration of Omex SW7 could be used in the interval after use of conventional fungicides. Moreover, standard concentration of silicon wetter could be used frequently when there is high disease pressure. This would help growers to reduce the amount of fungicides they used to control powdery mildew infection in strawberry production. Therefore, use of silicon based wetter Omex SW7 as a supplement to fungicide in disease control has a strong potential in integrated pest management and might help to ensure that the production of strawberry is more sustainable.

Silicon concentrations in the leaves indicated that root application of Omex SW7 accumulates more silicon compared to the foliar application. The same results were observed in rice, wheat and melon (Rezende *et al*., 2009; Guevel *et al.,* 2007; Dallagnol *et al*., 2012). All the above investigator findings supported the findings that concentrations of silicon were higher when silicon was applied to roots compared to foliar application. The results reported in chapter 5 showed that weekly (foliar) application of standard and high concentrations of Omex SW7 significantly reduced the powdery mildew infection. Application of silicon proved to be an effective means to increase the resistance of plants for many combinations of plants and pathogens (Ma, 2004; Dallagnol *et al*., 2012). Some investigators have found that root application of silicon (Potassium silicate) was effective in the reduction of powdery mildew in strawberry, melon and rose (Kanto *et al*., 2006; Shetty *et al*., 2012; Dallagnol *et al*., 2012). Therefore, the results of this study suggest that the incorporation of Omex SW7 through the irrigation water might be more effective in controlling powdery mildew disease than the foliar treatment. Growers have complained that other silicon based products especially potassium silicate can create a blockage when used in the irrigation pipes (Personal communication). The advantage of using Omex SW7 is that when silicon wetter is applied using the irrigation pipes it does not block the pipes. Growers usually supply water to the strawberry plants through the irrigation pipes after planting up to harvesting. Therefore, the standard concentration of Omex SW7 might be applied weekly using the irrigation pipes after planting and continue up to harvesting. The application of Omex SW7 in the irrigation pipes might help strawberry plants to absorb more silicon and increase resistance against strawberry powdery mildew. This treatment strategy might facilitate effective management of powdery mildew in strawberries and increase productivity and marketability of strawberry crops. Thus the incorporation of Omex SW7 in an integrated management programme might bring positive results both in terms of reducing disease severity and consequently reducing the amount of fungicides used.

6.2. Future Work

From the results described in this study and the information reported in the literature, it becomes clear that application of silicon can contribute to disease control. Previous reports from the literature clearly suggest that even though foliar application of silicon can decrease the intensity of disease, the level of control achieved was not as great when the silicon was applied to the roots. The present study revealed that concentrations of silicon in the leaves were more when Omex SW7 was applied to the roots compared to the foliar treatment. However, the molecular mechanism responsible for the uptake of silicon is unknown and genes responsible for silicon uptake have not been identified so far in the strawberry plants. Therefore, silicon uptake genes need to be isolated and characterized. The gene responsible for silicon uptake in strawberry plants could be isolated by using both expressed sequence tag based (EST) PCR and microsatellite markers (Ma *et al*., 2004). Besides this, the ability of silicon applied as Omex SW7 to the roots was not evaluated to control the powdery mildew disease in the field. Therefore, further work is needed to investigate the effect of root applied Omex SW7 in controlling strawberry powdery mildew disease in the field. Foliar application of Omex SW7 significantly reduced the number of germinating ascospores and colonies which was observed in the field trial. Therefore, further field trial study is needed to identify whether the incorporation of Omex SW7 through the irrigation water (root treatment) would be more effective in controlling powdery mildew disease compared to the foliar treatment. The progress of the strawberry powdery mildew epidemic could be monitored in each plot allowing comparison of treated and untreated plots. The area under the disease progress curve (AUDPC) could be used to quantify the epidemics in each treatment and used to compare epidemics by ANOVA to identify differences between different treatments.

Many investigators have shown that silicon is deposited under the cell wall and forms a silica-cuticle double layer (Kim *et al*., 2002; Raven, 2003). The deposition of silicon enhances the strength and rigidity of the cell walls and thus increases the resistance of plants to diseases. According to Kim *et al*., (2002) silicon accumulation on leaf surface and under the cell wall might limit fungal penetration and invasion by acting as a physical barrier. Therefore, further study needs to be carried out to determine whether

root applied Omex SW7 can enhance resistance due to accumulation and polymerization of silicic acid under the cell wall of strawberry leaves. It would also be interesting to know whether there is a relationship between cuticle thickness of leaves and resistance against strawberry powdery mildew. This could be examined in a field trial under high disease pressure. Location of silicon accumulation in strawberry leaves and its possible association with resistance to strawberry powdery mildew could be investigated by electron microscope and X-ray microanalysis. Localization of silicon deposition in the strawberry leaves could be identified by using transmission electron microscope coupled with energy-dispersive x-ray microanalysis. Transmission electron microscope could also be used to measure the cuticle thickness. Silicon accumulation and cuticle thickness measurement would identify whether root applied silicon wetter could enhance cuticle thickness due to accumulation of silicon. To identify the relationship between cuticle thickness and resistance against powdery mildew correlation and regression analysis could be used. These relationships would determine whether cuticle thickness has any effect against powdery mildew infections.

There is evidence that leaf hairs play an important role by protecting plants against pathogen attack either by secreting chemicals or by acting as a physical barrier against pathogen attack (Kumar *et al*., 2004; Dalin *et al*., 2008). In particular, trichome formation in *Arabidopsis thaliana* has been used as a model system and many of the genes which are involved in trichome initiation, spacing and shape have been identified (Marks, 1997). Recent work on *Arabidopsis* indicates that artificial damage, and application of jasmonic acid increases trichome production. The number of trichomes produced and trichome density vary genetically within plant species. The results in the present study (chapters 3, 4 and 5) showed that foliar and root applications of different concentrations of Omex SW7 increased leaf hair density and length on both the upper and lower surfaces of leaves. The results in chapter 5 showed that foliar application of silicon wetter increased leaf hair numbers and length and decreased powdery mildew infections. Therefore, further study needs to be carried out to examine the role of leaf hairs during the infection of *Podosphaera aphanis*. In future, it could be investigated whether high density of hairs on the leaf surface inhibits germ tube penetration and thus inhibits colonization. Different strawberry varieties could be used. After the application of different concentrations of silicon wetter to the roots, the youngest leaves

of strawberry plants could be dusted with conidia on the lower surface. After inoculation, leaf samples could be collected and examined using the scanning electron microscope. Use of a scanning electron microscope could help to identify whether different density of leaf hairs from different strawberry varieties treated with silicon wetter prevent penetration of *P. aphanis* fungus in the strawberry leaves.

There is strong evidence from the literature that silicon activates plants defence mechanisms. In a study Liang *et al*., (2005) observed that in cucumber, root applied silicon significantly suppressed powdery mildew and enhanced the activity of pathogenesis-related proteins (peroxidase, polyphenoloxidase and chitinase) which enhanced defence resistance in response to infection by the pathogen. However, it is still unknown whether root applied silicon wetter can enhance or induce resistance in strawberry plants infected with strawberry powdery mildew. In order to investigate whether root applied silicon wetter could enhance the activity of pathogenesis-related proteins would be identified by the method used by Liang *et al*., (2005). An alternative approach is to determine the defence related genes expression due to silicon treatment, which could be investigated by the use of molecular biology techniques such as subtractive cDNA libraries or microarrays. These techniques were carried out by Fauteux *et al*., (2006) to determine the defence related genes expressed in *Arabidopsis* after treatment with silicon.

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Appendix-1: Randomised distribution of the treatments, date of application of the treatments and leaf collections from different rows of block B (variety Elsanta) (Pilot experiment) (Chapter3)

Block	Row numbers and treatments	Application of the treatments date					Leaf collection
B		Week 1 14.10.08	Week 2 21.10.08	Week 3 28.10.08	Week 4 4.11.08	Week 5 11.11.08	Week 6 18.11.08
	Row-9 Control	Control	Control	Control	Control	Control	Collection of leaves
	Row-10 High weekly (sprayed 5 times)	High	High	High	High	High	Collection of leaves
	Row-11 Water sprayed 2 times (weeks 1 and 5)	Water				Water	Collection of leaves
	Row-12 High sprayed 2 times (weeks 1 and 5)	High				High	Collection of leaves
	Row-13 Standard sprayed 2 times (weeks 1 and 5)	Standard				Standard	Collection of leaves
	Row-14 Standard weekly (sprayed 5 times)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Row-15 Water weekly (sprayed 5 times)	Water	Water	Water	Water	Water	Collection of leaves
	Row-16 Control	Control				Control	Collection of leaves

Appendix-2: Randomised distribution of the treatments, date of application of the treatments and leaf collections from different rows of block B, C and D (variety Elsanta) (Main experiment) (Chapter3)

Block	Row numbers and treatments	Application of the treatments date					Leaf collection
B		Week 1 08.06.09	Week 2 15.06.09	Week 3 22.06.09	Week 4 29.06.09	Week 5 04.07.09	Week 6 11.07.09
	Row-13 High sprayed 3 times (weeks 1, 3 and 5)	High		High		High	Collection of leaves
	Row-14 Control	Control	Control	Control	Control	Control	Collection of leaves
	Row-15 Standard sprayed 3 times (weeks 1, 3 and 5)	Standard		Standard		Standard	Collection of leaves
	Row-16 High weekly (sprayed 5 times)	High	High	High	High	High	Collection of leaves
	Row-17 Very high weekly (sprayed 5 times)	Very high	Very high	Very high	Very high	Very high	Collection of leaves
	Row-18 Control	Control		Control		Control	Collection of leaves
	Row-19 High sprayed 2 times (weeks 1 and 5)	High				High	Collection of leaves
	Row-20 Standard 2 times (weeks 1 and 5)	Standard				Standard	Collection of leaves
	Row-21 Standard weekly (sprayed 5 times)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Row-22 Very high sprayed 3 times (weeks 1, 3 and 5)	Very high		Very high		Very high	Collection of leaves
	Row-23 Very high sprayed 2 times (weeks 1 and 5)	Very high				Very high	Collection of leaves
	Row-24 Control	Control				Control	Collection of leaves
	Row-25 Standard weekly	Standard	Standard	Standard	Standard	Standard	Collection of leaves

	(sprayed 5 times)						
	Row-26 Control	Control	Control	Control	Control	Control	Collection of leaves
	Row-27 Standard sprayed 3 times (weeks 1, 3 and 5)	Standard		Standard		Standard	Collection of leaves
	Row-28 High sprayed 2 times (weeks 1 and 5)	High				High	Collection of leaves
	Row-29 High sprayed 3 times (weeks 1, 3 and 5)	High		High		High	Collection of leaves
C	Row-30 Very high sprayed 3 times (weeks 1, 3 and 5)	Very high		Very high		Very high	Collection of leaves
	Row-31 Very high sprayed 2 times (weeks 1 and 5)	Very high				Very high	Collection of leaves
	Row-32 Control	Control		Control		Control	Collection of leaves
	Row-33 High weekly (sprayed 5 times)	High	High	High	High	High	Collection of leaves
	Row-34 Standard sprayed 2 times (weeks 1 and 5)	Standard				Standard	Collection of leaves
	Row-35 Very high weekly (sprayed 5 times)	Very high	Very high	Very high	Very high	Very high	Collection of leaves
	Row-36 Control	Control				Control	Collection of leaves
D	Row-37 High sprayed 2 times (weeks 1 and 5)	High				High	Collection of leaves
	Row-38 Very high sprayed 2 times (weeks 1 and 5)	Very high				Very high	Collection of leaves
	Row-39 Control	Control				Control	Collection of leaves

D							
	Row-40 Standard sprayed 3 times (weeks 1, 3 and 5)	Standard		Standard		Standard	Collection of leaves
	Row-41 Standard weekly (sprayed 5 times)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Row-42 Control	Control	Control	Control	Control	Control	Collection of leaves
	Row-43 High weekly (sprayed 5 times)	High	High	High	High	High	Collection of leaves
	Row-44 High sprayed 2 times (weeks 1 and 5)	High				High	Collection of leaves
	Row-45 Standard sprayed 2 times (weeks 1 and 5)	Standard				Standard	Collection of leaves
	Row-46 Very high sprayed 3 times (weeks 1, 3 and 5)	Very high		Very high		Very high	Collection of leaves
	Row-47 Very high weekly (sprayed 5 times)	Very high	Very high	Very high	Very high	Very high	Collection of leaves
	Row-48 Control	Control		Control		Control	Collection of leaves

Appendix-3: Randomised distribution of the treatments, date of application of the treatments and leaf collections from different rows of block C and D (different varieties) (Chapter3)

Block	Variety	Row numbers and treatments	Application of the treatments date					Leaf collection
			Week 1 16.10.09	Week 2 23.10.09	Week 3 30.10.09	Week 4 7.11.09	Week 5 14.11.09	Week 6 21.11.09
C	Symphony	Row-13 Standard (5 sprays)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Symphony	Row-14 Control	Control	Control	Control	Control	Control	Collection of leaves
	Symphony	Row-15 High (5 sprays)	High	High	High	High	High	Collection of leaves
	Elsanta	Row-16 High (5 sprays)	High	High	High	High	High	Collection of leaves
	Elsanta	Row-17 Standard (5 sprays)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Elsanta	Row-18 Control	Control	Control	Control	Control	Control	Collection of leaves
D	Rhapsody	Row-19 Control	Control	Control	Control	Control	Control	Collection of leaves
	Rhapsody	Row-20 High (5 sprays)	High	High	High	High	High	Collection of leaves
	Rhapsody	Row-21 Standard (5 sprays)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Florence	Row-22 Standard (5 sprays)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Florence	Row-23 High (5 sprays)	High	High	High	High	High	Collection of leaves
	Florence	Row-24 Control	Control	Control	Control	Control	Control	Collection of leaves

Appendix-4: Randomised distribution of the treatments, date of application of the treatments and leaf collections from different rows of block B, C and D(variety Elsanta)(Chapter 4)

Block	Row numbers and treatments	Application of the treatments date					Leaf collection
		Week 1 7.06.10	Week 2 14.06.10	Week 3 21.06.10	Week 4 28.06.10	Week 5 5.07.10	Week 6 12.07.10
B	Row-10 High applied 3 times (weeks 1, 3 and 5)	High		High		High	Collection of leaves
	Row-11 Control	Control	Control	Control	Control	Control	Collection of leaves
	Row-12 Standard applied 3 times (weeks 1, 3 and 5)	Standard		Standard		Standard	Collection of leaves
	Row-13 High weekly (applied 5 times)	High	High	High	High	High	Collection of leaves
	Row-14 Control	Control		Control		Control	Collection of leaves
	Row-15 High applied 2 times (weeks 1 and 5)	High				High	Collection of leaves
	Row-16 Standard applied 2 times (weeks 1 and 5)	Standard				Standard	Collection of leaves
	Row-17 Standard weekly (applied 5 times)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Row-18 Control	Control				Control	Collection of leaves
C	Row-19 Standard weekly (applied 5 times)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Row-20 Control	Control	Control	Control	Control	Control	Collection of leaves
	Row-21 Standard applied 3 times (weeks 1,	Standard		Standard		Standard	Collection of leaves

	3 and 5)						
	Row-22 High applied 2 times (weeks 1 and 5)	High				High	Collection of leaves
	Row-23 High applied 3 times (weeks 1, 3 and 5)	High		High		High	Collection of leaves
	Row-24 Control	Control		Control		Control	Collection of leaves
	Row-25 High weekly (applied 5 times)	High	High	High	High	High	Collection of leaves
	Row-26 Standard applied 2 times (weeks 1 and 5)	Standard				Standard	Collection of leaves
	Row-27 Control	Control				Control	Collection of leaves
D	Row-28 High applied 2 times (weeks 1 and 5)	High				High	Collection of leaves
	Row-29 Control	Control				Control	Collection of leaves
	Row-30 Standard applied 3 times (weeks 1, 3 and 5)	Standard		Standard		Standard	Collection of leaves
	Row-31 Standard weekly (applied 5 times)	Standard	Standard	Standard	Standard	Standard	Collection of leaves
	Row-32 Control	Control	Control	Control	Control	Control	Collection of leaves
	Row-33 High weekly (applied 5 times)	High	High	High	High	High	Collection of leaves
	Row-34 High applied 2 times (weeks 1 and 5)	High				High	Collection of leaves
	Row-35 Standard applied 2 times (weeks 1 and 5)	Standard				Standard	Collection of leaves
	Row-36 Control	Control		Control		Control	Collection of leaves

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Appendix-5: Published paper

Study the role of silicon in strawberries and its possible role in control of strawberry powdery mildew

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Summary

Silicon has been shown to control a number of diseases. The physical effect of silicon on strawberry plants is unknown. Omex SW7 is a silicon based wetter, which is used in the application of potassium bicarbonate for the control of strawberry powdery mildew and previous studies suggested that this combination has given enhanced control of strawberry powdery mildew. There is evidence that leaf hairs involved in plant disease resistance. This project investigated the uptake of silicon from the silicon based wetter into strawberry plants. The physical effect of the silicon on leaf hair length and density was also investigated. Three concentrations of silicon were used and three different timings were used. Whilst the main study has used the variety Elsanta, other varieties have also been used. The plants were assessed on a) silicon uptake over time, b) the density of leaf hairs on upper and lower leaf surfaces, c) the length of leaf hairs on the upper and lower surfaces, and d) accumulation of silicon in fruit. The principal findings include enhanced levels of silicon in leaves and an additional level where there were multiple application of silicon. On leaves with higher silicon levels there was an increased density of leaf hairs and the leaf hairs were longer. Six months after treatment with silicon there were no enhanced levels of silicon in the treated plants when compared with untreated controls. Silicon accumulation was not found in harvested strawberry fruit after the application of different concentrations of silicon. The different varieties of strawberry used showed anatomical differences. Untreated leaves from Rhapsody had no hairs on the upper leaf surface, but a low density of hairs was observed after treatment with silicon.

Key words: Strawberries, silicon, varietal differences, disease control, powdery mildew, Omex

Introduction

Strawberry production in the UK is one of the success stories of intensification and efficiency, as since the introduction of the use of polythene tunnels in the early 1990s strawberry production has doubled whilst the hectarage has been halved. This is coupled with very efficient production systems involving the delivery of water and fertilizer direct to the growing plants with no evaporation. The use of polythene tunnels, however, tends to create the environmental conditions (15°C to 30°C and 95% humidity, Dodgson *et al.*, 2008a)

which are conducive to epidemics of powdery mildew (*Podospheara aphanis*). This has had a major impact on strawberry production, requiring extensive use of fungicides to control these epidemics. There is also pressure for the growers to produce the 'perfect' strawberries without the use of pesticides. In order to achieve control of *P. aphanis* with reduced pesticide use growers use some sprays of (food grade) potassium bicarbonate in the integrated control programme (Dodgson *et al.*, 2008). This is generally applied using a wetter to ensure a good spread of the potassium bicarbonate. Anecdotal observations suggested that a silicon based wetter had a synergistic effect with potassium bicarbonate in controlling *P. aphanis* on strawberries. The work reported here aimed to explore possible explanations for this effect. Silicon is absorbed into plants which are described as either silicon accumulators or non accumulators. Strawberries are non accumulators of silicon (Epstein 1994). Silicon has been shown to control a number of plant diseases and it is believed that silicon creates a physical barrier which can restrict penetration of fungal hyphae and there is evidence that leaf hairs are involved in plant disease resistance.(Kortekamp & Zyprian, 1999). Trichomes and bristles, generally known as hairs, play an important role in protecting plants against fungal attack (Kortekamp & Zyprian, 1999, Bonos *et al.*, 2004). This project investigated the uptake of silicon into strawberry plants and the effect of the absorbed silicon on leaf hair length and density.

Material and Methods

A silicon wetter (Omex SW7) used to spray strawberries was used as the source of silicon and the dose rate was based on multiples of the label recommendations. Experiments were carried out in glasshouses and the treatments and timing of sprays are shown in Table 1.

Table 1 Treatment rates and spray timings

Treatment		Timing
1	0.25% Omex SW7 (label rate) 27. 02mg/l si	Weekly
		Every 2 weeks
		Every 4 weeks
2	2.5% Omex SW7 282.63 mg/l si	Weekly
		Every 2 weeks
		Every 4 weeks
3	5% Omex SW7 538.60 mg/l si	Weekly
		Every 2 weeks
		Every 4 weeks
4	Water only	Weekly
		Every 2 weeks
		Every 4 weeks
5	Untreated	Weekly
		Every 2 weeks
		Every 4 weeks

In this study, the Autoclave Induced Digestion (AID) method described by Elliott and Snyder (1991) was selected for the determination of the silicon content of the leaves because of its rapidity, simplicity and appropriate sensitivity. To quantify the effect of silicon on the density and length of leaf hairs a 1cm² quadrat was used on the leaves and placed randomly six times on the upper and lower surface of each leaf with a replicate of 20 leaves from each treatment. The number of leaf hairs in each quadrat on the upper (adaxial) and lower (abaxial) leaf surfaces was determined using a dissecting microscope. The lengths of 10 leaf hairs in each quadrat were measured at x3 using a graticule. Statistical differences between treatments were analysed by using Anova and differences between treatments were analysed by using Tukey test in SPSS for windows (16 SPSS Inc).

Results

Leaves collected from strawberry variety Elsanta after different treatments showed different levels of silicon concentrations (fig. 1). It was observed that there were significant differences of silicon concentrations in different treatments. All treated plants showed significantly higher ($P < 0.05$) silicon concentrations compared to that in leaves collected from the untreated control and water-treated plants (fig. 1). Leaves collected from these control plants show the background level of silicon in untreated strawberry plants and there were no significant differences ($P > 0.05$) between the controls. The highest levels of silicon were seen in leaves collected from the highest treatment (538.6 mg/l si) compared to all other treatments.

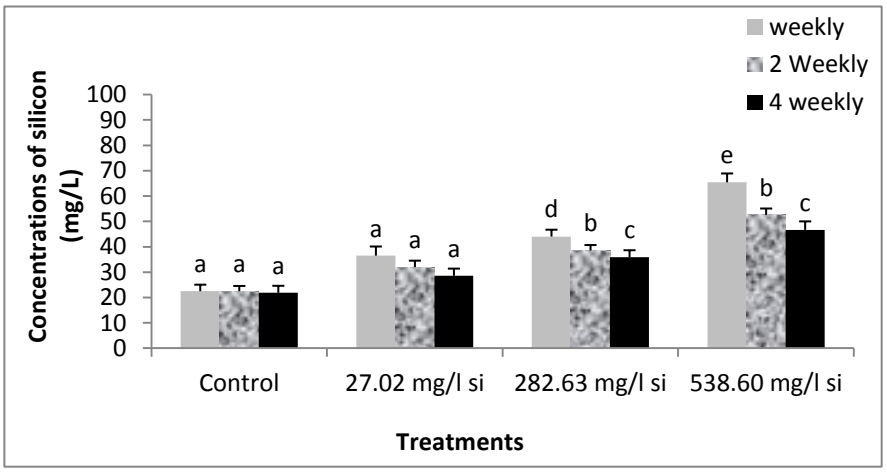

Fig. 1 Concentrations of silicon in strawberry leaves (variety Elsanta) treated by silicon wetter. Bars with different letters indicate significant differences ($P < 0.05$).

Timing influenced the silicon uptake by leaves (Fig. 1).The background level of silicon is shown in the two controls. Results showed that plants treated more frequently resulted in greater accumulations of silicon (fig. 1) with weekly application of all dose rates showing significantly higher levels ($P < 0.05$) of silicon compared to 2-week and 4-week intervals of applications. Figures 2 and 3 show the effect of silicon on leaf hair density. Observations showed that there were increased numbers of leaf hairs on both upper and lower surfaces of treated leaves. It was also found that there was a significant difference in number of leaf hairs in different treatment groups. The observations (Figures 4 and 5) showed that silicon also had an effect on the length of the leaf hairs on both upper and lower leaf surfaces. It was observed that the length of leaf hair in 282.63 mg/l si treated leaves showed significantly ($P < 0.05$)

longer hairs compared with 27.02mg/l si treated leaves but significantly shorter ($P < 0.05$) hairs than in 538.6 mg/l si treated leaves. Elsanta strawberries were grown on after silicon treatment, through flowering to fruit production and then fruit and leaves were analysed for silicon accumulation in the ripe fruits. Table 2 shows that the levels of silicon in the fruits were not as high as in the sprayed leaves, though slightly above the control.

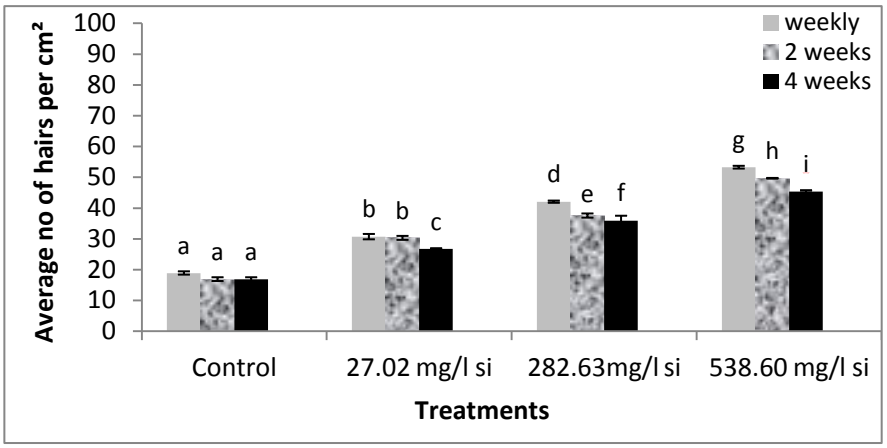

Fig. 2 Density of leaf hairs per 1cm² quadrat on upper surface of leaves in strawberry variety Elsanta treated with silicon. Bars with different letters indicate significant differences ($P < 0.05$).

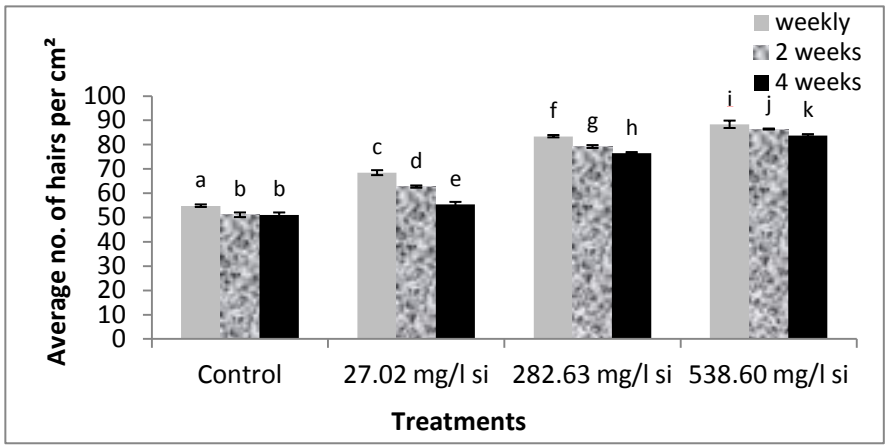

Fig. 3 Density of leaf hairs per 1cm² quadrat on lower surface of leaves in strawberry variety Elsanta treated with silicon. Bars with different letters indicate significant differences ($P < 0.05$).

Figures 7, 8, 9 and 10 show that silicon accumulated in the leaves of all strawberry varieties tested and that the silicon enhanced the number and density of leaf hairs in all four varieties. In the variety Rhapsody, which normally has no leaf hairs on the upper surface, the silicon sprays appeared to stimulate leaf hair formation.

Discussion

The work reported here shows that though strawberry is a non-accumulator of silicon (Takahashi *et al*. 1990) spraying with the silicon wetter (Omex SW7) onto the leaf surface does result in accumulation of silicon in the leaves. Futhermore it also demonstrates that the accumulation of silicon affects the physiology of the strawberry leaves resulting in the

formation of additional leaf hairs, which are longer than when the plants are untreated. Furthermore this effect occurs on all varieties tested and on Rhapsody has the effect of actually stimulating the production of hairs where there were none on the untreated control. As reported by Kortekamp & Zyprian (1999) and Bonos *et al.* (2004), leaf hairs play an important role in protecting plants against attack from fungal pathogens. The work reported here shows that silicon applied as Omex SW7 stimulated leaf hair development and that this may be a contributing factor in the observed synergistic effect between potassium bicarbonate and the silicon wetter OMEX SW. This will be further examined in a field trial in the 2011 season.

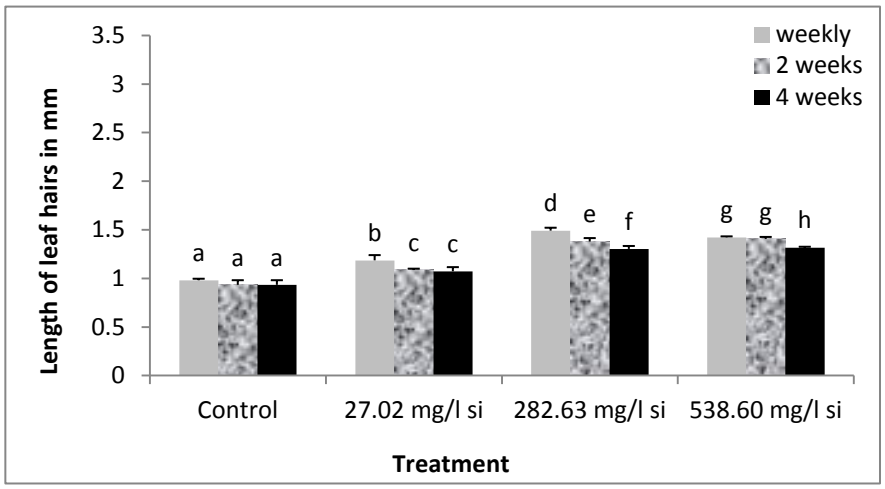

Fig. 4 Length of leaf hairs on upper surface in strawberry variety Elsanta in silicon treated leaves. Bars with different letters indicate significant differences ($P < 0.05$).

Three different varieties of strawberry (Elsanta, Rhapsody and Symphony) were used to compare the effect of silicon on different varieties. Accumulation of silicon in the leaves was measured as were leaf hair density and leaf hair length (methods as above). The treatments were an untreated control, and two different concentrations of silicon 27.02 mg/l si and 282.63 mg/l si applied weekly.

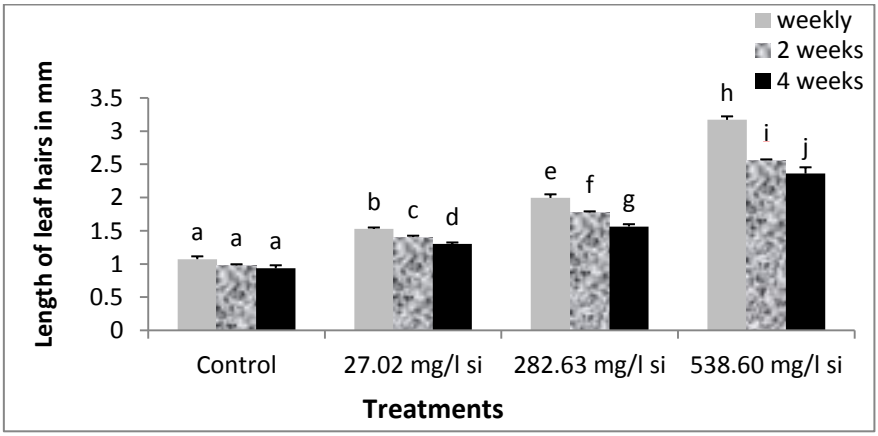

Fig. 5 Length of leaf hairs on lower surface in strawberry variety Elsanta in silicon treated leaves. Bars with different letters indicate significant differences ($P < 0.05$).

Table 2. Comparison of silicon levels in leaves and fruits after treatments with silicon wetter

Treatment	Concentrations of Si in leaves (mg/l)	Concentrations of Si in fruit (mg/l)
Control	7.5	0.625
27.02 mg/l si weekly	17.5	1.25
27.02 mg/l si every 2 weeks	10	0.625
27.02 mg/l si every 4 weeks	8.125	0.625
282.63 mg/l si weekly	26.25	1.25
282.63mg/l si every 2 weeks	20	0.625
282.63 mg/l si every 4 weeks	16.25	0.625
538.60 mg/l si weekly	33.75	1.875
538.60 mg/l si every 2 weeks	25	1.25
538.60 mg/l si every 4 weeks	18.75	1.25

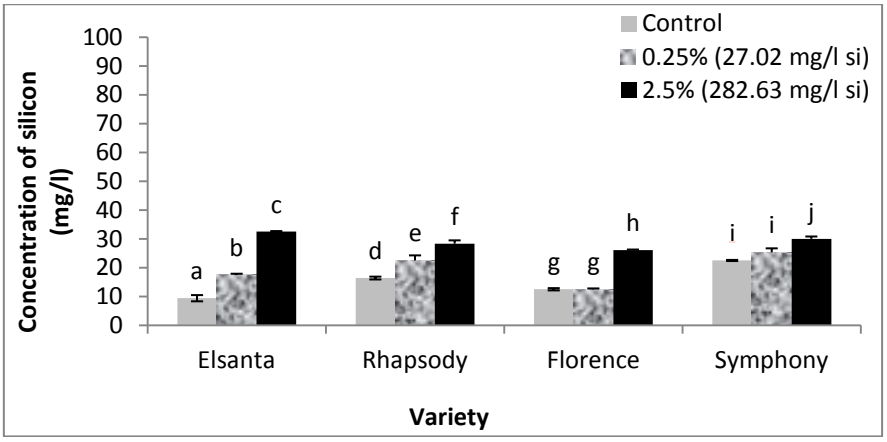

Fig. 6 Concentrations of silicon in strawberry leaves in different varieties treated by silicon wetter. Bars with different letters indicate significant differences ($P < 0.05$).

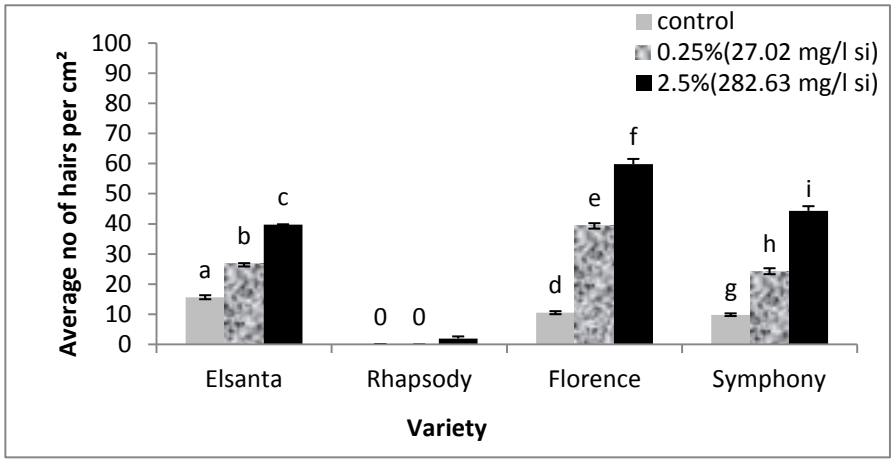

Fig. 7 Number of leaf hairs on upper surface of leaves treated by silicon wetter. Bars with different letters indicate significant differences ($P < 0.05$).

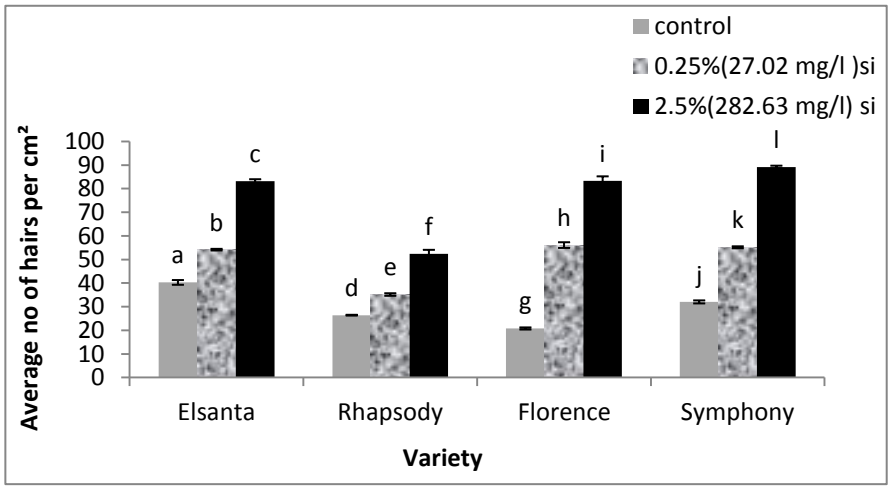

Fig. 8 Number of leaf hairs on lower surface of leaves treated by silicon wetter Bars with different letters indicate significant differences ($P < 0.05$).

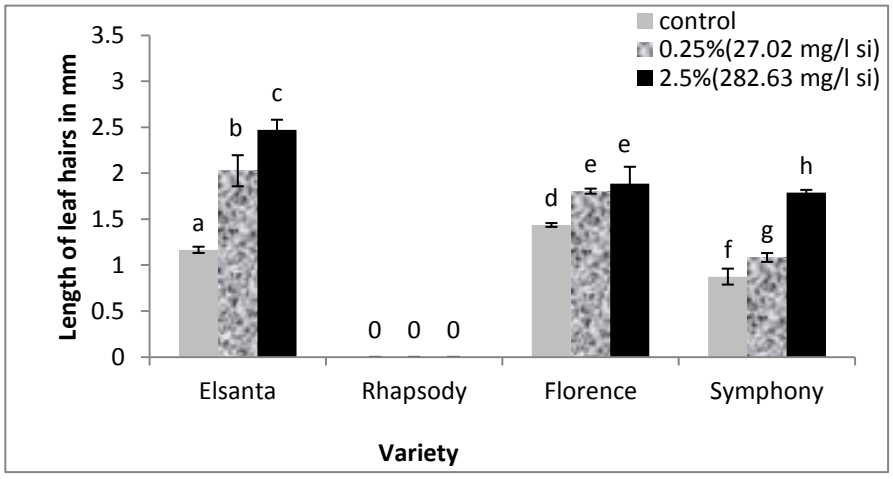

Fig. 9 Length of leaf hairs on upper surface in different strawberry varieties in silicon treated leaves. Bars with different letters indicate significant differences ($P < 0.05$).

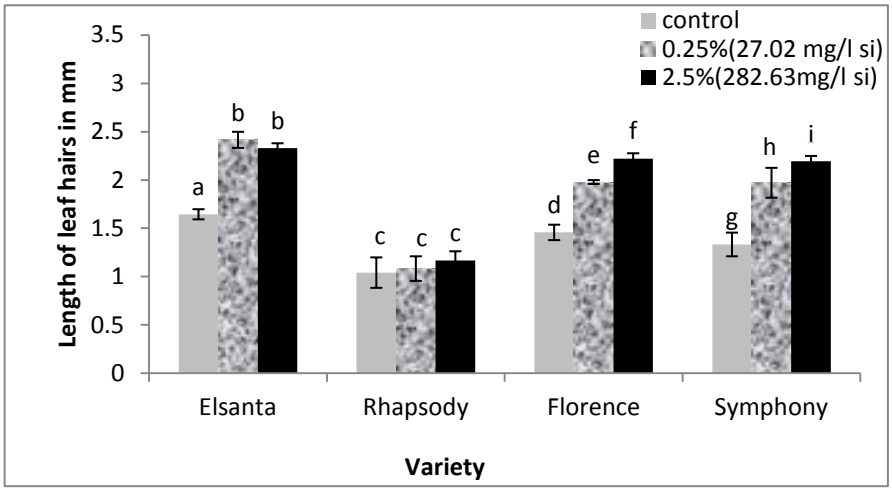

Fig. 10 Length of leaf hairs on lower surface in different strawberry varieties in silicon treated leaves. Bars with different letters indicate significant differences ($P < 0.05$).

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Appendix-6: Published paper

A study of the role of silicon in the control of strawberry powdery mildew *(Podosphaera aphanis)* in a field trial

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Summary

Silicon has been shown to reduce the severity of a number of plant diseases. Omex SW7 is a silicon based wetter, which is routinely used in the application of potassium carbonate (K50) for the control of strawberry powdery mildew. The effect of foliar applications of silicon and potassium carbonate to limit *P. aphanis* infection was examined in a field trial under polythene tunnel at Wisbech.There were six treatments, Standard (contains 27.02 mg/l Si), High (contains 270.20 mg/l Si), Potassium carbonate (K50), Standard + K50, High + K50 and Control (Untreated) plants receiving no foliar spray. Each treatment was applied weekly for 6weeks with a hand held spray boom. Results showed that germinating ascospores and colonies were present in all plots before the trial was sprayed. Treatments with Standard and High Omex SW7 significantly ($P<0.05$) reduced the number of germinating ascospores and colonies in this trial. However, Potassium carbonate alone gave some reduction in the number of colonies and number of germinating ascospore. Moreover, potassium carbonate mixed with silicon based wetter Omex SW7 significantly ($P<0.05$) reduced the number of germinating ascospores and number of colonies. Analysis showed that there were enhanced levels of silicon in plants treated with silicon wetter. On leaves with higher silicon levels there was an increased density of leaf hairs and the leaf hairs were longer.

Key words: Silicon, disease control, powdery mildew, Omex SW7, strawberry plant

Introduction

Podosphaera aphanis which causes powdery mildew of strawberry is of economic importance in strawberry production in United Kingdom as it affects yield and fruit quality (Dodgson, 2008). The main economic loss caused by powdery mildew is the amount of affected fruit discarded, followed by the expense of pesticides (Maas, 1998). Several alternative control agents, i.e. soluble silicon, oils, salts, plant extract, mineral oil alone or in combination, have been tested against powdery mildews on different crops (Pertot *et al*. 2007). Silicon has been shown to control a number of diseases and it is believed that silicon creates a physical barrier which can restrict fungal hyphae penetration (Epstein, 1994). Trichomes and bristles, generally known as hairs, play an important role in protecting plants against fungal attack. This project investigated the uptake of silicon from a silicon based wetter into strawberry plants and the effect of absorbed silicon on leaf hair length and density and reduction in the severity of powdery mildew. Silicon products have been used to control powdery mildew in cucumber, melon, rose (Dallagnol *et al*. 2012; Shetty *et al*. 2012). The mechanism of suppression by silicon of powdery mildews in strawberry is not clear. Therefore, it has been hypothesized that supplying silicon as a wetter would result in

strawberry plants absorbing silicon and improved resistance against *Podosphaera aphanis*. A field trial was designed to evaluate the effect of a silicon wetter on the severity of *P. aphanis.*

Materials and Methods

Experimental design of the field trial

The effect of foliar applications of silicon and potassium carbonate to limit Strawberry powdery mildew infection was examined in a field trial in Polythene tunnel at Wisbech. Silicon wetter (Omex SW7-Omex Agrifluids Ltd) was used as the source of silicon. K50 (Potassium carbonate- Omex Agrifluids Ltd) used by the growers to decrease of disease incidence (Anon, 2012). Potassium carbonate does not have a harvest interval, so application of potassium carbonate with Omex SW7 is an ideal product to use at the time of fruit is being picked. Treatments were arranged in a randomised block design of 3 replicates. All treatments were applied to the strawberry variety Sonata. There were six treatments see table 1. The plot was sprayed weekly started 7th April until 11th May 2011. The first spray was applied on 7th April, 2nd spray was on 13th April and also on 20th April, 27th April, 4th May and 11th May. Leaves were collected for disease assessment on 7th April before spray and also on 28th April, 19th May and 10th June. In this trial 500ml was sprayed on each plot.

Table 1**.** *List of different treatments*

Treatment no	Name of the treatment
1	Untreated (Unt)
2	Standard (Omex SW7 at 0.25%) which contains 27.02 mg/l Si
3	High (Omex SW7 at 2.5%) which contains 270.20 mg/l Si
4	K50 (Potassium Carbonate) used at field rate
5	Standard + K50
6	High + K50

Microscopic assessment of *P. aphanis* from trial

To identify the *P. aphanis* symptoms 20 leaves were collected from each trial plot at each sample date and assessed for visible *P. aphanis* symptom. Each leaf was then placed in Petri dishes and submerged in 0.1% trypan blue stain (trypan blue in lactic acid) (Waller *et al*. 2002). Leaflets were left to stain for 24 hours at room temperature, after which they were washed in water and cut into 4 strips (parallel to the mid rib). Of the 4 strips 2 were placed on microscope slides upper surface up and 2 were placed lower surface up. The slide was placed on the microscope stage and viewed at x100 magnification (Nikon, model YS100). One transect of the leaflet strip was viewed. The number of germinating ascospore and the number of distinct *P. aphanis* colonies were recorded. This was repeated for the remaining 3 strips from each leaf.

Extraction of silicon and determination of leaf hair length and density

In this study, the Autoclave Induced Digestion (AID) method described by Elliott and Snyder (1991) was selected for the determination of the silicon content of the leaves because of its rapidity, simplicity and appropriate sensitivity. To quantify the effect of silicon on the density

and length of leaf hairs a 1cm² quadrat was used on the leaves and placed randomly six times on the upper and lower surface of each leaf with a replicate of 20 leaves from each treatment. The number of leaf hairs in each quadrat on the upper (adaxial) and lower (abaxial) leaf surfaces was determined using a X30 magnification of dissecting microscope. The lengths of 10 leaf hairs in each quadrat were measured using an eyepiece graticule in the dissecting microscope (X30). Data were analysed by linear model under the Mixed model in the statistical software programme, SPSS for Windows, version 17 and subsequently, differences between treatments were determined using LSD (*Post-hoc test)* in SPSS for windows version 17.

Results

Leaves were collected from all trial plots on 7th April (2011) before the trial was sprayed this showed that there were germinating ascospores and colonies on all plots (Fig. 1 & 2). There were no visible symptoms of the powdery mildew infections but microscopic observation showed that there were germinating ascospore and colonies present throughout the trial. The environmental conditions (temperature and relative humidity in April and May 2011) were not conducive to *P. aphanis* development (results not shown) but there was evidence that germinating ascospore and colonies was present in all plots (Fig. 1 & 2). Treatments with High plus K50 and Standard plus K50 gave the greatest reduction in disease.

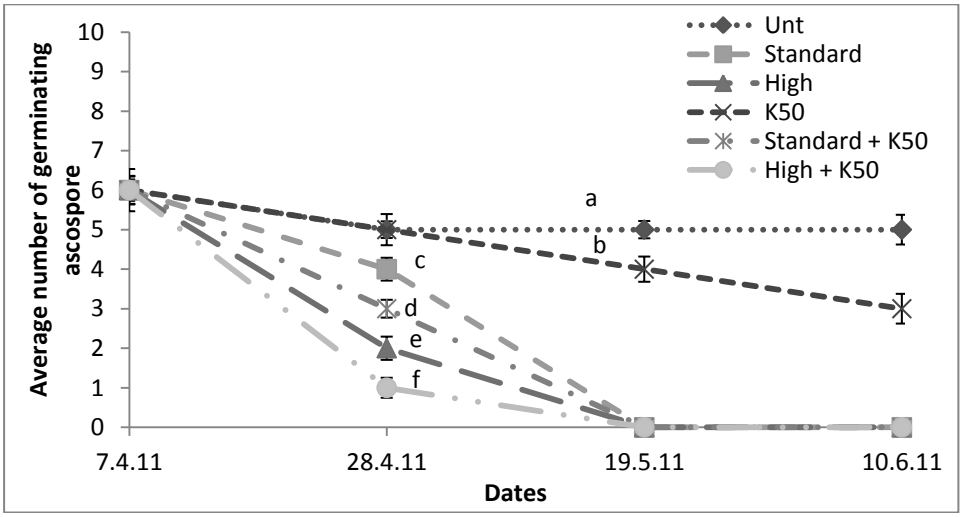

Figure 1**:** Number of germinating ascospores on strawberry leaves (variety Sonata) from different trial plots. The disease was assessed on 7th April before beginning of treatment/spray and also on 28th April, 19th May and 10th June. Lower case letters indicate significant differences (P<0.05) between treatments as indicated by LSD (*Post-hoc test)* in SPSS for Windows, version 17.

Whilst potassium carbonate alone gave some reduction in the number of colonies, all treatments with silicon gave a significant reduction (P<0.05) in colonies on 28th April, 19th May and 10th June when compared with the untreated control. The trial was carried out early in a year of very low disease pressure. There was very low disease pressure from 7th April to 1st June when the prediction system showed that it took 54 days to accumulate 144 hours of disease conducive conditions. This explains the lack of a visible epidemic.
Leaves collected on 7th April before first spray and also on 28th April, 19th May and 10th June from strawberry plants showed different levels of silicon concentrations (Table 2). Results also showed that the silicon concentration in the leaves collected on 7th April (pre spray) was

the same in all treated and untreated plots. Concentration of silicon on the leaves collected on 7th April showed the background level of silicon in all trial plots. However concentrations of silicon were significantly different in the leaves collected on 28th April in different treated plots after the weekly application of different concentration of silicon and potassium carbonate.

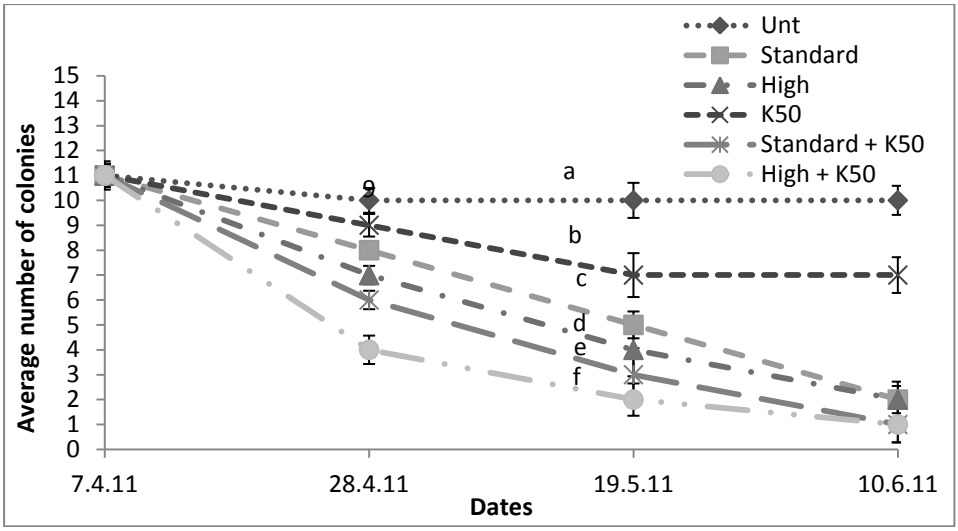

Figure 2**:** Number of colonies on strawberry leaves (variety Sonata) from different trial plots. The disease was assessed on 7th April before beginning of treatment/spray and also on 28th April, 19th May and 10th June. Lower case letters indicate significant differences (P<0.05) between treatments as indicated by LSD (*Post-hoc test)* in the statistical software programme, SPSS for Windows, version 17.

There was no significant difference (P>0.05) found in silicon concentration in the leaves collected from untreated and potassium carbonate treated plots. On 28th April significant differences (P<0.05) were observed in different treated plots such as Standard, High, Standard+K50, High+K50 treated plots. Moreover the results also indicated that in the leaves collected on 10th June, concentrations of silicon were same as in the leaves collected on 19th May. This result shows that the concentration of silicon did not decrease one month after the final spray.

Table 2. *Concentrations of silicon in strawberry leaves (variety Sonata) treated with different concentrations of Omex SW7 and K50.*

Treatment	7.4.11 (mg/kg)	28.4.11 (mg/kg)	19.5.11 (mg/kg)	10.6.11 (mg/kg)
Untreated	1361 ± 155.4^{a}	1356 ± 158.9^{a}	1353 ± 155.4^{a}	1350 ± 157.9^{a}
Standard	1333 ± 150.4^{a}	1625 ± 203.9^{b}	1956 ± 258.3^{c}	1950 ± 318.3^{c}
High	1366 ± 152.3^{a}	2291 ± 257.4^{d}	2625 ± 263.3^{e}	2611 ± 254.4^{e}
K50	1336 ± 158.1^{a}	1333 ± 203.7^{a}	1335 ± 201.3^{a}	1333 ± 262.4^{a}
Standard+K50	1356 ± 155.6^{a}	1833 ± 256.6^{c}	2125 ± 206.6^{d}	2111 ± 250.8^{d}
High K50	1357 ± 155.6^{a}	2513 ± 256.2^{e}	2944 ± 257.8^{f}	2900 ± 300.7^{f}

Leaves were collected on 7th April before beginning of treatment/spray and also on 28th April, 19th May and 10th June. Means ± SE (n=6) followed by different letters indicate significant differences (P<0.05) between treatments as indicated by LSD (*post-hoc*) analysis in the statistical software programme, SPSS for windows version 17.

Leaves from untreated plots showed that the variety Sonata has more leaf hairs on the lower than the upper surface (Table 3). All treatments with Omex SW7 showed significantly ($P<0.05$) enhanced number of leaf hairs (Table 3). Observations showed that all treatments with Omex SW7 also increased the length of leaf hairs (Table 4).

Table 3. *Number of leaf hairs on upper surface (US) and lower surface (LS) of leaves (Strawberry variety Sonata) collected from different trial plots after application of different concentration of Omex SW7 and K50.*

Treatments	Surface	Hair number	Treatments	Surface	Hair number
Untreated	US	4.08 ± 0.19^{a}	Untreated	LS	25.05 ± 0.27^{c}
Standard	US	6.25 ± 0.20^{a}	Standard	LS	40.25 ± 0.72^{e}
High	US	18.47 ± 0.21^{b}	High	LS	65.00 ± 0.43^{g}
K50	US	4.04 ± 0.17^{a}	K50	LS	24.15 ± 0.25^{c}
Standard+K50	US	16.00 ± 0.31^{b}	Standard+K50	LS	50.08 ± 0.84^{f}
High+K50	US	30 ± 0.43^{d}	High+K50	LS	85.00 ± 0.81^{h}

(Means±SE, n=20) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by LSD (*post-hoc*) analysis in the statistical software programme, SPSS for windows version 17.

Table 4: *Length of leaf hairs on upper surface (US) and lower surface (LS) of leaves (Strawberry variety Sonata) collected from different trial plots after application of different concentration of Omex SW7 and K50.*

Treatments	Surface	Hair Length (mm)	Treatments	Surface	Hair Length (mm)
Untreated	US	0.654 ± 0.167^{a}	Untreated	LS	1.01 ± 0.178^{b}
Standard	US	1.004 ± 0.122^{b}	Standard	LS	1.342 ± 0.291^{c}
High	US	1.691 ± 0.136^{d}	High	LS	1.99 ± 0.286^{e}
K50	US	0.712 ± 0.128^{a}	K50	LS	1.01 ± 0.291^{b}
Standard+K50	US	1.285 ± 0.115^{c}	Standard+K50	LS	1.525 ± 0.252^{d}
High+K50	US	2.153 ± 0.190^{e}	High+K50	LS	2.504 ± 0.232^{f}

(Means±SE, n=20) followed by different letters indicate significant differences ($P<0.05$) between treatments as indicated by LSD (*post-hoc*) analysis in the statistical software programme, SPSS for windows version 17.

Discussion

The work reported here showed that though strawberry is classified as a silicon non accumulator plant (Miyake and Takahashi, 1986) application of silicon based wetter Omex SW7 onto the leaf surface does result in accumulation of silicon in the leaves. The application of Omex SW7 has stimulated an increase the number of leaf hairs and length of leaf hairs in strawberry plants (Tables 3 & 4). It was also observed that Omex SW7 mixed with K50 significantly ($P<0.05$) reduced the number of germinating ascospore and number of colonies (Fig.1 & 2). The results showed that there were germinating ascospores and colonies in the leaves collected from all trial plots on 7th April before spraying. Although there were no colonies visible to the naked eye, microscopic observation showed that ascospores and colonies were present throughout the trial. When the trial was done the temperature and relative humidity was not conducive for growth and development of *P. aphanis* infection, so

no visible epidemic developed in the trial. The results indicated that application of Standard and High concentrations of Omex SW7 on to the strawberry plants significantly ($P<0.05$) reduced the number of germinating ascospores and the number of colonies. The above result also indicated that potassium carbonate alone can give some reduction in the number of germinating ascospores and number of colonies. Potassium carbonate mixed with a silicon based wetter Omex SW7 can significantly ($P<0.05$) reduce the number of germinating ascospores and colonies. These findings are supported by Kanto *et al*. (2007) who showed that applying silicon (Potassium silicate) to strawberry plants results in physiological changes of the strawberry leaves. As a result of these physiological changes, germination and appressoria formation of mildew conidia was inhibited. The present study revealed that silicon level can be enhanced by the application of Omex SW7, change the physical properties of the leaves, such as increased number of leaf hairs and increased length of leaf hairs and in this way help the plants to resist fungal infection. However, further work is required to establish evidence for this protective effect under high disease pressure.

Acknowledgements

The authors would like to thank Harriet and Henry Duncafe (Maltmas Farm) for providing field trial facilities, Gidon Bahiri (King's Lynn, Norfolk) to supply Omex SW7 for this experiment and Andy and Helen Barker for carrying out the spraying.

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Appendix-7: Poster for BSPP Conference 2009

Investigating the effect of foliar applications of soluble silicon and its possible role in control of strawberry powdery mildew

Kaneez Fatema, Avice Hall, David Naseby
University of Hertfordshire, College Lane, Hatfield, AL10 9AB, UK

Introduction

- Soluble silicon induces both physiological and physical changes, thereby increasing plant resistant against pathogen.[1,2]
- In strawberry, the mechanism of suppression against powdery mildew remains uncertain.
- Omex SW7 is used as a wetter in the application of potassium bicarbonate and it has been reported by growers that this has given enhanced control of strawberry powdery mildew.
- We hypothesised that supplying soluble silicon would help strawberry plants to absorb silicon and improve resistance against the pathogen.

Objectives

- To determine if there are enhanced levels of silicon in strawberry plants treated with silicon wetter (OMEX SW7).
- To identify whether the silicon supplement has any effect on the anatomy of strawberry leaves.

Materials and methods

- The silicon based wetter was sprayed at different concentrations to the strawberry plants.
- Each treatment was applied in different timings.
- Enhanced level of silicon were quantified in washed leaves by the Autoclave Induced Digestion (AID) method.[3,4]
- Numbers of leaf hairs and length of leaf hairs were measured using a dissecting microscope.

Symptoms of strawberry powdery mildew disease

Healthy plant | Mycelium on leaves | Red blotch | Mycelium on fruit

Silicon (OMEX SW7) treated leaves

0.25% | 2.5% | 5%

Results

- Concentrations of silicon in strawberry leaves were increased by the addition of silicon wetter (Figure 1).
- There was significant difference in concentrations in different treatments and in different timings. There were higher levels of silicon in plants that were treated more frequently shown in figure 1.

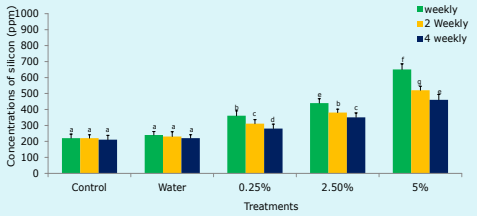

Figure-1: Concentrations of silicon in strawberry leaves treated by silicon wetter. Bars with different letters indicate significant differences(P<0.05).

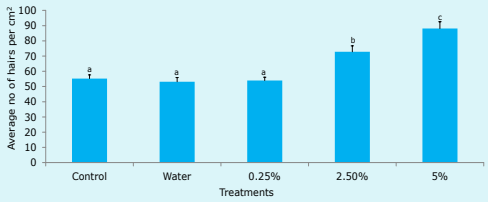

Figure-2: Number of leaf hairs on lower surface of strawberry leaves treated by silicon wetter. Bars with different letters indicate significant differences(P<0.05).

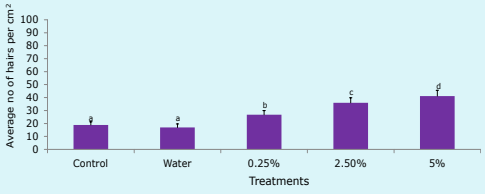

Figure-3: Number of leaf hairs on upper surface of leaves treated by silicon wetter. Bars with different letters indicate significant differences (P<0.05).

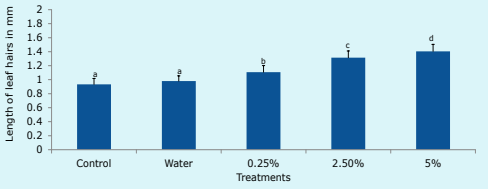

Figure-4: Length of leaf hairs on lower surface of strawberry leaves. Bars with different letters indicate significant differences (P<0.05).

- Foliar applications of OMEX SW7 at a concentration of 5% in each week gave the highest result in in terms of Si absorption.
- Microscopic observations of silicon treated leaves showed that there was increased density of leaf hairs in upper (Figure 2) and lower surfaces (Figure 3) and increased length of leaf hairs (Figure 4) on lower surface of leaves.
- The numbers of leaf hairs was more in 2.5% and 5% OMEX SW7 treated leaf compared to other treatment groups.
- Enhanced levels of silicon was not observed in silicon treated leaves after six months.

Conclusions

- There were enhanced levels of silicon in plants treated with silicon wetter.
- The more silicon that is applied, the higher the levels of silicon absorbed by the plant.
- Increased number of leaf hairs and increased length of leaf hairs were found in 2.5% and 5% treated leaves.
- There were increased numbers of hairs found on upper and lower surfaces of treated leaves.

References

1.Kanto T, Miyoshi A, Ogawa T, Maekawa K, Aino M (2004). Gen Plant Pathol, 70:207-211.
2.Epstein E (1999). Annu Rev Plant Physiol, 50 :641-64.
3.Henry G.T, Diane Shogren, Gang L (2002). Commun. Soil Sci. Plant Anal, 33:1661-70.
4.Elliott C.L, Snyder G.H (1991). J Agric Food Chem, 39:118-19.

Acknowledgement: The author would like to thank Harriet and Henry Duncafe (Maltmas Farm) for providing strawberry plant and Gidon Bahiri (King's Lynn, Norfolk) to supply OMEX SW7 for this experiment.

Appendix -8

Study of the role of silicon in strawberries and its possible role in control of strawberry powdery mildew

Kaneez Fatema, David Naseby and Avice Hall
University of Hertfordshire, School of Life Science, Hatfield, AL10 9AB

Introduction

*Soluble silicon induces both physiological and physical changes, thereby increasing plant resistant against pathogen. (Kanto et al. 2004; Epstein, 1990).
*Omex SW7 is a silicon based wetter, which is used in the application of potassium bicarbonate for the control of strawberry powdery mildew.
*Anecdotal observations suggested that a silicon based wetter had a synergistic effect with potassium bicarbonate in controlling *P. aphanis* on strawberries.
*The work reported here aimed to explore possible explanations for this effect.

Objectives

*To investigate the uptake of silicon in strawberry plants treated with silicon wetter Omex SW7.
*To determine the effect of absorbed silicon on leaf hair length and density.
*An assessment of the silicon accumulation and residues in harvested strawberry fruits.

Materials and Methods

*The silicon based wetter was sprayed at different concentrations to the strawberry plants.
*Each treatment was applied at different timings.
*Enhanced levels of silicon were quantified in washed leaves by the Autoclave Induced Digestion (AID) method.
*Numbers of leaf hairs and length of leaf hairs were measured using a dissecting microscope.

Results

*Concentrations of silicon in strawberry leaves were increased by the addition of silicon wetter..
*There was significant difference in concentration in different treatment and in different timings.
There were higher levels of silicon in plants that were treated more frequently shown in Fig.1.

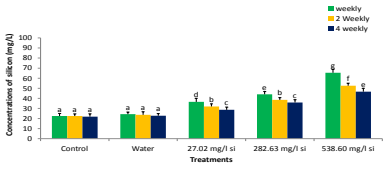

Figure-1: Concentrations of silicon in strawberry leaves (variety Elsanta) treated by silicon wetter. Bars with different letters indicate significant differences (P<0.05).

*The highest levels of silicon were seen in leaves collected from the highest treatment (538.6 mg/l si) compared to all other treatments.
*Observations showed that there were increased numbers of leaf hairs on both upper and lower surfaces of treated leaves.
*It was also found that there was a significant difference in number of leaf hairs in different treatment groups (Fig.2 & 3).

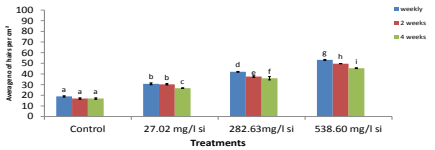

Figure-2: Density of leaf hairs on upper surface of leaves in strawberry variety Elsanta treated with silicon. Bars with different letters indicate significant differences.

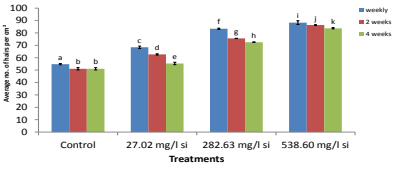

Figure-3: Density of leaf hairs on lower surface of leaves in strawberry variety Elsanta treated with silicon. Bars with different letters indicate significant differences (P<0.05).

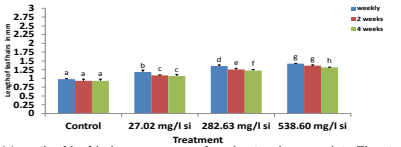

Figure-4 Length of leaf hairs on upper surface in strawberry variety Elsanta in silicon treated leaves. Bars with different letters indicate significant difference (P<0.05).

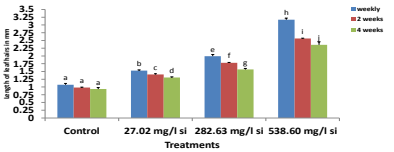

Figure-5: Length of leaf hairs on lower surface in strawberry variety Elsanta in silicon treated leaves. Bars with different letters indicate significant differences (P<0.05).

Treatment	Concentrations of Si in leaves (mg/l)	Concentrations of Si in fruit (mg/l)
Control	7.5	0.625
0.25%(27.02 mg/l si)(EW)	17.5	1.25
0.25%(27.02 mg/l si)(2W)	10	0.625
0.25%(27.02 mg/l si)(4W)	8.125	0.625
2.5%(282.63 mg/l si)(EW)	26.25	1.25
2.5%(282.63 mg/l si)(2W)	20	0.625
2.5%(282.63 mg/l si)(4W)	16.25	0.625
5%(538.60 mg/l si)(EW)	33.75	1.875
5%(538.60 mg/l si)2W)	25	1.25
5%(538.60 mg/l si)4W)	18.75	1.25

Table1: Comparison of silicon levels in leaves and fruits after treatments with silicon wetter.

*Silicon also had an effect on the length of the leaf hairs on both upper and lower leaf surfaces shown in figure 4 &5.
* It was observed that the length of leaf hair in 282.63 mg/l si treated leaves showed significantly (P<0.05) longer hairs compared with 27.02 mg/l si treated leaves but significantly shorter (P<0.05) hairs than in 538.6 mg/l si treated leaves.
*Elsanta strawberries were grown on after silicon treatment, through flowering to fruit production and then fruit and leaves were analysed for silicon accumulation in the ripe fruit.
*Levels of silicon in the fruits were not as high as in the sprayed leaves, though slightly above the control (Table-1).

Conclusions

•The principal findings include enhanced levels of silicon in leaves and an additional level where there were multiple application of silicon.
*On leaves with higher silicon levels there was an increased density of leaf hairs and the leaf hairs were longer.
•Silicon accumulation was not found in harvested strawberry fruit after the application of different concentrations of silicon.

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1.Kanto T, Miyoshi A, Ogawa T, Maekawa K, Aino M (2004). Gen Plant Pathol, 70:207-211.
2.Epstein E (1999). Annu Rev Plant Physiol, 50 :641-64.
3.Henry G.T, Diane Shogren, Gang L (2002). Commun. Soil Sci. Plant Anal, 33:1661-70.
4.Elliott C.L, Snyder G.H (1991). J Agric Food Chem, 39:118-19.

Acknowledgement: The author would like to thank Harriet and Henry Duncafe (Maltmas Farm) for providing strawberry plant and Gidon Bahiri (King's Lynn, Norfolk) to supply OMEX SW7 for this experiment.

University of Hertfordshire

Appendix-9 (Poster for BSPP Conference 2011)

Study of the role of silicon in control of strawberry powdery mildew in strawberries

Kaneez Fatema and Avice Hall
University of Hertfordshire, School of Life Science, Hatfield, AL10 9AB

Introduction

*Soluble silicon induces both physiological and physical changes, thereby increasing plant resistance against pathogens (Kanto et al. 2004; Epstein, 1990).
*Omex SW7 is a silicon based wetter, which is used in the application of potassium bicarbonate for the control of strawberry powdery mildew caused by *Podosphaera aphanis.*
*Anecdotal observations suggested that the silicon based wetter had a synergistic effect with potassium bicarbonate in controlling *Podosphaera aphanis* on strawberries.

Objectives

*To quantify the effect of silicon and potassium bicarbonate to limit the development of strawberry powdery mildew.
*Identify the source of fungal inoculum responsible for initiating primary outbreak of disease.

Materials and Methods

*The effect of foliar applications of silicon (Si) and Potassium Bicarbonate (PB) was examined in a field trial under polythene tunnels.
*Treatments were arranged in a randomised block design of 3 replicates. All treatments were applied to the strawberry variety Sonata.
*There were six treatments which consist of Untreated (Unt), Standard (recommended field rate, Contains 27.02 mg/l si), High (contains 270.20 mg/l si), Potassium Bicarbonate (PB) contains 20gm/l, Standard + PB, High + PB, Control plants receiving no foliar spray.
*The length, density of leaf hairs and number of germinating ascospore as well as number of colonies assessed by the dissection microscope.
*The field trial was sprayed weekly from 7th April until 11th May, 2011.
*Silicon levels were measured from 8 leaves per plot using the AID method (Elliott and Snyder, 1991).

Results

*Results showed that germinating ascospores and colonies were present throughout the trial on 7th April before the first spray. Though there were no colonies visible to the naked eye, ascospores and colonies were found throughout the trial at this time(Fig.1 & 2).

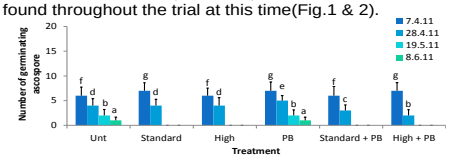

Figure-1: Number of germinating ascospore on leaves collected from different trial plots. Disease was assessed on 7th April before the first spray and also on 28th April, 19th May and 8th June. Lower case letters indicate significant differences (P<0.05) as indicated by Mixed model ANOVAs using the general linear model.

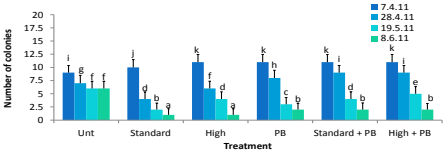

Figure-2. Number of colonies on leaves collected from different trial plots. Disease was assessed on 7th April before the first spray and also on 28th April, 19th May and 8th June. Lower case letters indicate significant differences (P<0.05) as indicated by Mixed model ANOVAs using the general linear model.

*The untreated plots had germinating ascospores and colonies throughout the trial. The levels of number of germinating ascospores in plots treated with potassium bicarbonate were similar to the controls throughout the trial. Treatments with Si and Si and PB had no germinating ascospores from 28th April onwards. Colonies visible under the microscope was present throughout the trial, on these treatments but number of colonies decreased throughout the trial (Fig. 2).

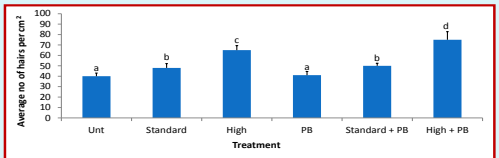

Figure-3: Density of leaf hairs on lower surface of leaves in strawberry variety Sonata collected from different trial plots. Lower case letters indicate significant differences (P<0.05) as indicated by statistical method ANOVAs.

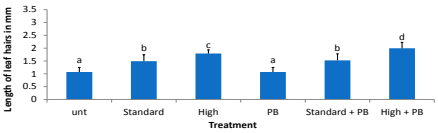

Figure-4: Length of leaf hairs on lower surface of leaves in strawberry variety Sonata collected from different trial plots. Lower case letters indicate significant differences (P<0.05) as indicated by statistical method ANOVAs.

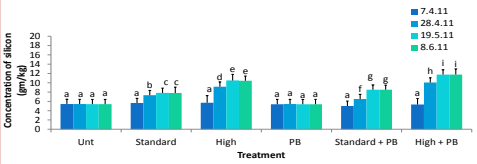

Figure-5: Concentrations of silicon on strawberry leaves (variety Sonata) collected from different trial plots.. Disease was assessed on 7th April before the first spray and also on 28th April, 19th May and 8th June. Lower case letters indicate significant differences (P<0.05) as indicated by Mixed model ANOVAs using the general linear model.

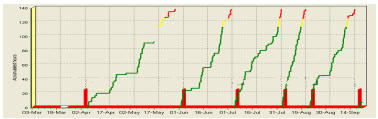

Figure-6: Disease development prediction for Wisbech

*There was very low disease pressure from 7th April to 1st June where the prediction system (Fig.6) shows that it took 54 days of accumulate of 144 hours of disease conducive conditions. This explains the lack of a visible epidemic.
*Observations showed that silicon treated leaves had greater density and length of leaf hairs in upper and lower surfaces shown in figure 3& 4.
*Concentrations of silicon in strawberry leaves were increased by the use of silicon wetter (Fig.5).
*There was significant difference in silicon levels between treatments .

Conclusions

*Potassium bicarbonate alone cannot reduce the number of germinating ascospore and colonies whereas when it mixed with silicon based wetter Omex SW7 it significantly reduce the number of germinating ascospore and colonies.

References

1.Kanto T, Miyoshi A, Ogawa T, Maekawa K, Aino M (2004). Gen Plant Pathol, 70:207-211.
2.Epstein E (1999). Annu Rev Plant Physiol, 50 :641-64.
3.Elliott C.L., Snyder G.H. (1991).J.Agric Food Chem. 39:118-119.

Acknowledgement

The authors would like to thank Harriet and Henry Duncafe (Maltmas Farm) for providing field trial facilities, Gidon Bahiri (King's Lynn, Norfolk) to supply OMEX SW7 for this experiment and Andy and Helen Barker for carrying out the spraying.

University of Hertfordshire