

Project title: The epidemiology and control of strawberry powdery mildew under protection

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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## ***GROWER SUMMARY***

### **Headline**

- A better understanding of when powdery mildew attacks strawberries has been developed and progress is being made to develop a predictive model and improved control strategies.

### **Background and expected deliverables**

Strawberry powdery mildew is a significant threat to the economic sustainability of crops grown under protection. The industry is dependent on a few cultivars, which are mostly very susceptible to the disease. Good control of powdery mildew can be achieved using fungicides, but production protocols are placing increasingly stringent limits on the products used, harvest intervals and allowable chemical residues. In addition, growers rely on a relatively limited armoury of fungicide active ingredients, placing enormous selection pressure on the pathogen population.

This is expected to improve the understanding of strawberry powdery mildew and use this to develop control strategies, which will integrate cultural and chemical control methods to reduce disease to tolerable levels.

The work is expected to:

- Assess the efficacy of fungicides currently approved on strawberry under protection, for controlling powdery mildew.
- Design chemical control schedules that are both effective and reduce selection pressure for fungicide insensitivity in the pathogen population.
- Identify agronomic management practices that can reduce disease pressure.
- Identify environmental conditions that favour the spread and development of disease, which might be used as the basis for risk warnings.

## **Summary of the project and main conclusions**

### ***Preliminary Review of the Literature***

Powdery mildew on strawberries was reported at the start of the last century. The causal pathogen has been identified as *Sphaerotheca humuli* (DC.) Burr, the cause of hop powdery mildew, and *Sphaerotheca macularis*. Some authors have suggested that the two species might be the same. However, recent taxonomic studies have shown that the correct name of the fungus causing strawberry powdery mildew is *Podosphaera aphanis*, which is not the cause of hop powdery mildew.

Despite the taxonomic confusion about the identity of the pathogen, details of its life-cycle can be derived from previous work. Of particular interest are optimum growth conditions and the upper and lower environmental boundaries in which the pathogen can survive. Laboratory experiments have been used to estimate the time for completion of important life-cycle phases. These estimates provide a useful basis for planning the investigation of disease progress in the field experiments within the current project.

### ***Inoculum and primary disease spread***

In order to establish a new infection and develop visible symptoms, the pathogen requires 144 hours of suitable environmental conditions (*i.e.*, temperature and humidity). In most situations it is likely that infected, but visually asymptomatic plants are present when tunnels are covered at the start of each cropping season. These plants act as the primary inoculum source for infection of the crop.

In the experimental work, disease developed throughout two plots of newly planted strawberry plants, which were examined frequently following establishment. Disease symptoms were not clustered at the ends of the plots, but were distributed randomly throughout the plots. This result supported work undertaken in the previous season, which suggested that infection of newly planted crops is introduced via the planting stocks. Compared with established crops, disease symptoms took longer to develop within newly planted crops. It may be that the pathogen over-winters as either cleistothecia or conidia in new plantings, whereas in established crops infection may also over-winter as mycelium, possibly within leaf buds. New inoculum can be generated more rapidly from the later source of primary infection.

### ***Cultivar resistance to strawberry powdery mildew***

Seven cultivars, untreated by fungicides, were compared for the development of strawberry powdery mildew. All of them developed symptoms of the disease, however Everest and Florence had less than 5% of their leaf surface covered with red blotches. The dose of fungicide necessary to control an epidemic is a function of the amount of disease that would develop if the epidemic was left untreated (*cf* disease pressure). Therefore reduction in disease pressure from using more resistant cultivars offer opportunities to reduce the amount of fungicide applied, especially when the environment is suboptimal for disease development.

### ***Chemical control***

Regular applications of potassium bicarbonate can reduce powdery mildew levels substantially. The magnitude of control is approximately equivalent to a single application of Systhane (myclobutanil) at the maximum permitted dose. Systhane is currently an effective mainstay in programmes targeted to control powdery mildew in strawberries. Potassium bicarbonate could therefore provide a useful tool for growers aiming to widen the range of modes of action used to combat disease – reducing selection pressure for fungicide insensitivity. It might also be deployed near harvest to decrease the likelihood of fungicide residues on the fruit. A formulation of plant nutrients with potassium bicarbonate did not provide any additional control above that achieved by the straight product.

Excellent disease control was provided by Fortress (quinoxyfen), which has not previously been used by UK growers to control strawberry powdery mildew. This protectant fungicide reduced the severity of powdery mildew below the levels achieved with the benchmark products Systhane and Corbel (fenpropimorph). Apart from the requirement to quantify the protectant efficacy of the product more completely, this should include adherence to anti-resistance strategies to protect the long-term performance of the product.

### ***Plant Stimulants***

Weekly treatments with 'plant stimulants' did not provide any reduction in the severity of strawberry powdery mildew. On the basis of these experiments, growers should not use these products as an alternative to fungicides with known activity against the pathogen. Benefits to general plant vigour from using the products were not measured by these experiments.

### ***Dipping plants to control initial disease development***

Infection may already be present in plants used to establish new crops. Due to the canopy structure of strawberry crops, it is difficult to ensure complete coverage of the plants when fungicidal products are applied. As a consequence the applied dose may be much greater than the effective dose, *i.e.* the amount reaching the intended targets. Experiments showed that, in a high-pressure mildew environment, it was possible to delay the onset of strawberry powdery mildew symptoms by at least 7 days when the plants were dipped in a chemical control product before planting. This delay was evident for plants dipped in Systhane (myclobutanil) compared to those that were not dipped or that were dipped in water or bicarbonate. However, there are currently no products approved by The Pesticide Safety Directorate (PSD) for dipping of strawberry plants to control powdery mildew.

### ***Inoculum levels linked to cupping and red blotches***

Infection by strawberry powdery mildew causes a progression of symptoms (leaf cupping, presence of mycelium on leaves, red blotching of leaves and finally mycelium on the fruit). Presence of mycelium is the only symptom that can be linked, with any certainty by visual assessment, to powdery mildew. This work has shown that compared with apparently healthy leaves (*i.e.* uncupped and green), there were significantly more mycelial colonies present on leaves that were cupped or with red blotching. In addition, significantly more mycelium was found on the lower leaf surface than on the upper leaf surface. This suggests that treatment of powdery mildew should be initiated when leaf-cupping is observed and that further treatment may remain necessary even when red blotching is the only evident symptom. Since most of the inoculum is generated on the lower leaf surfaces, it is important to achieve good spray coverage of this portion of the canopy when applying fungicides.

### ***Prediction of high risk periods***

The prediction system developed by this work and outlined in the previous annual report (SF 62, 2005) has been further refined, so that it can better predict high risk periods. When data describing disease development (collected as part of the epidemiological studies) was compared with outputs by the prediction system, the high risk periods identified coincided with the onset of the measured epidemics. The prediction system requires some additional testing against experimental data and subsequently must be field-tested rigorously, in partnership with crop managers.

## Financial benefits

### *In the short-term:*

- The work will lead to the design of improved fungicide programmes that will improve control of powdery mildew and increase the picked yields.
- The use of the commodity substance potassium bicarbonate can provide similar levels of powdery mildew to conventional fungicides. Reliance on this will help to reduce the risk of pesticide residues occurring, thus increasing customer confidence and subsequent fruit sales.

### *In the medium-term:*

- More responsible use of fungicides as a result of this work will reduce the development of fungicide resistance to commercially available fungicides, hence maintaining viable control options.
- The work will improve the targeting of fungicides and improve control.

## Action points for growers

- Growers should avoid repeated applications of fungicides with the same Mode of Action. Consecutive and frequent applications of products from the same MOA group increase the likelihood that the pathogen will develop fungicide insensitivity. Information about The mode of actions of fungicides approved for use on strawberries are available in a table in the HDC Factsheet 17/08 Control of strawberry powdery mildew under protection.
- Growers should consider using applications of potassium bicarbonate within 3 days of covering tunnels (or removing fleece) to suppress disease spread. An early application of Fortress (Quinoxifen) applied after the bicarbonate will provide further protection.
- New plantings may have low incidence of infection without any visible symptoms. Early application of potassium bicarbonate might provide cost effective management of this potential inoculum source.
- Inoculum is associated with the cupping and red blotch symptoms. Growers should aim to treat powdery mildew when these symptoms are present even if mycelium is not visible.
- Treatment should start when leaf-cupping is observed. Further treatment may be necessary, even when red blotching is the only evident symptom.



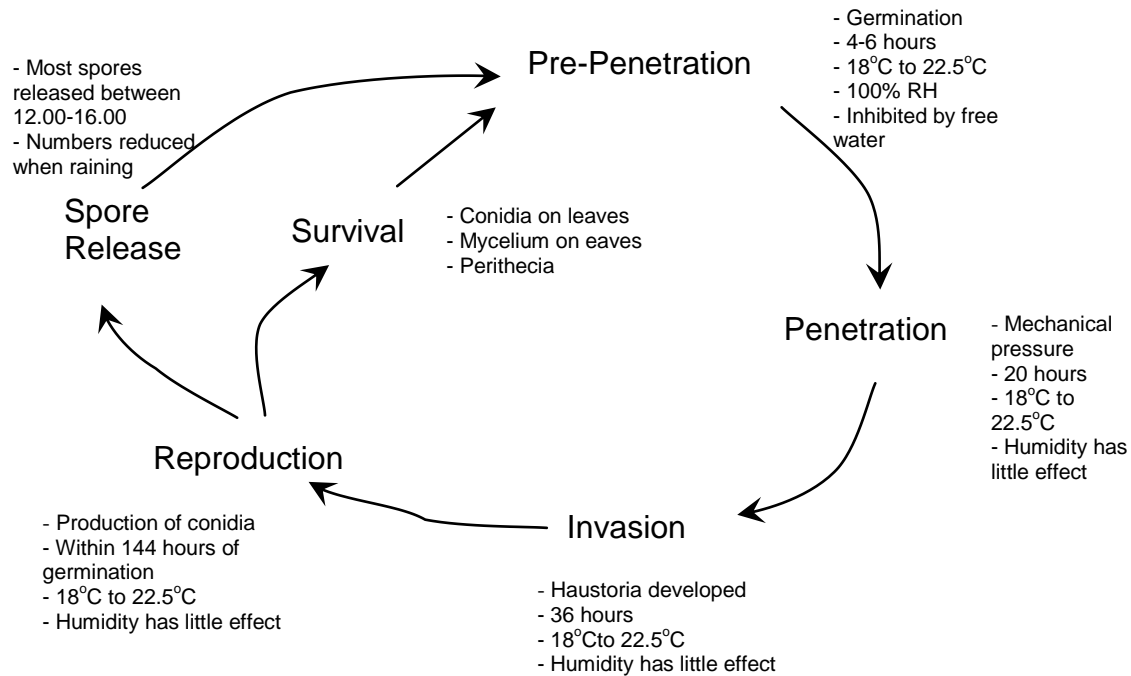
- Aim to achieve good spray coverage of the lower leaf surfaces.
- Where economically viable (and acceptable to retailers), growers should consider planting moderately resistant cultivars as part of an integrated disease management programme.

## **SCIENCE SECTION**

### **Introduction**

A powdery mildew on strawberries was reported at the start of the last century (Salmon, 1900). The causal pathogen has variously been identified as *Sphaerotheca humuli* (DC.) Burr (Peries, 1961, Rashid Khan, 1960), the cause of hop powdery mildew, and *Sphaerotheca macularis* (Peries, 1962b, Peries, 1962a, Miller *et al.*, 2003, Jhooty and McKeen, 1965, Jhooty and McKeen, 1964a, Freeman and Pepin, 1969, Jhooty and McKeen, 1964b). Some authors have suggested that the two species might be the same (Horn *et al.*, 1972, Smith *et al.*, 1988). However, *S. humuli* can be distinguished from *S. macularis* by the structure of the cleistocarp appendages (Liyanage, 1973) and is highly specialized to hop (Liyanage & Royle, 1976). So there is little doubt that powdery mildew on hops and strawberries are caused by different fungal species. Recent taxonomic studies have shown that the correct nomenclature for the fungus causing strawberry powdery mildew is *Podosphaera aphanis* (Braun 1982; Braun, 2002). These studies provide further confirmation that the fungi causing strawberry and hop powdery mildew are different.

Despite taxonomic confusion about the identity of the pathogen, details of its life-cycle can be derived from previous work (Fig. 1). Of particular interest are optimum growth conditions and the upper and lower environmental boundaries that the pathogen can survive.



**Fig. 1.** Life cycle of strawberry powdery mildew *Podosphaera aphanis* (syn. *Sphaerotheca macularis*).

Further details of fungal development are shown in Table 1. These estimates of the time for completion of life-cycle phases were obtained from laboratory experiments. However, they provide a useful basis for the investigation of disease progress in the field experiments undertaken within the current project.

**Table 1.** Time for development of major stages in fungal infection. Compiled from work by (Peries, 1962b).

Life Cycle Stage	Time since inoculation (hours)
Conidia germinate	4-6
Appressorium formed	12
Host penetration	20
Haustoria developed	36
Conidiophore start to form	96
Conidiophores fully developed	120 (5 days)
Lesion visible to naked eye	144 (6 days)

The optimum temperature for germination of the conidia was given in the range of 18°C to 22.5°C by Peries (1962a). Subsequent authors found 20°C to be the optimum temperature for conidial germination (Jhooty and McKeen, 1965, Miller *et al.*, 2003). Miller *et al.*, (2003) found that 8% of spores germinated at 4°C and, at greatly reduced frequency, could also occur at 36°C. This is supported by Jhooty and McKeen (1965), who found that the minimum and maximum temperatures for spore germination were 3°C and 38°C respectively. Peries (1962a) found that less than one percent of spores germinated at 2°C and that they did not infect the plant unless the temperature was at least 5°C. While some conidia will germinate at 10°C and 30°C these temperatures are not conducive for disease development. The amount of infection at 15°C is consistently greater than at 25°C (Jhooty and McKeen, 1965).

Relative humidity (RH) is also a major influence on the germination and development of the pathogen. Spore germination occurs best at 100% RH (Peries, 1962a, Jhooty and McKeen, 1965, Jhooty and McKeen, 1964b, Jhooty and McKeen, 1964a) and reduces greatly when RH falls below 95%. Peries (1962a) found that humidity does not affect the development of the fungus after germination had taken place.

Whilst conidia need a high RH to germinate, exposure to free water can have a detrimental effect on disease progress (Peries, 1962a). Even short periods of immersion in water inhibited germination of the majority of conidia (conditions are summarised in Table 2).

Conidia can remain viable even when conditions are not favourable for germination. For example, conidia stored for 96 hours had a 46 % germination rate (Peries, 1962a). However, conidia that remain attached to the conidiophores are more likely to germinate. For example, at 0°C conidia that were attached to conidiophores showed only a small reduction in germination frequency after 40 days storage.

Using spore traps, Peries (1962a) found that the majority of conidia are produced between 12.00 and 16.00 hours and the least between 20.00 and 08.00 hours. He also showed that rain reduces the number of air-borne conidia greatly and that it takes about 3 days for the levels to reach the pre-rain levels (Peries, 1962a). The majority of air-borne conidia were detected within a horizontal radius of 5 feet ( $\approx$ 1.5m) from their source and vertically from within 3 feet ( $\approx$ 1.0m, Peries, 1962a). Relationships between environmental conditions, incidence of strawberry

powdery mildew, and concentrations of *P. aphanis* (syn. *S. macularis*) conidia in the air, have been described recently for US conditions (Blanco *et al.*, 2004).

**Table 2.** Summary of conditions that effect the life cycle of strawberry powdery mildew (data obtained from laboratory observations).

Variable		Germination	Infection	Sporulation
<b>Temperature (°C)</b>	Minimum	3 <sup>3</sup> , 2 <sup>5</sup>	5 <sup>3,4,5</sup>	13 <sup>5</sup>
	Optimum	15-25 <sup>3</sup> , 18-25 <sup>4</sup> (15*)18-22.5 <sup>5</sup>	18-30 <sup>5</sup>	20 <sup>3</sup>
	Maximum	38 <sup>3</sup> , 30-35 <sup>5</sup>	30 <sup>4,5</sup>	35 <sup>3</sup>
<b>Relative humidity (%)</b>	Minimum	8 <sup>1</sup> , 12 <sup>5</sup>	No effect <sup>4,5</sup>	No effect <sup>4,5</sup>
	Optimum	100 <sup>2,4</sup> , 97 <sup>5</sup>	No effect <sup>4,5</sup>	No effect <sup>4,5</sup>
	Maximum	100 <sup>1,2,5</sup>	No effect <sup>4,5</sup>	No effect <sup>4,5</sup>
<b>Presence of free water (immersion time hours)</b>	Minimum	NA	No effect <sup>4,5</sup>	No effect <sup>4,5</sup>
	Optimum	0 <sup>5</sup>	No effect <sup>4,5</sup>	No effect <sup>4,5</sup>
	Maximum	24 <sup>5</sup>	No effect <sup>4,5</sup>	No effect <sup>4,5</sup>
<b>Time of day (hours)</b>	Minimum	No effect <sup>5</sup>	No effect <sup>5</sup>	20.00-8.00 <sup>5</sup>
	Maximum	No effect <sup>5</sup>	No effect <sup>5</sup>	12.00-16.00 <sup>1,5</sup>

<sup>1</sup> Blanco *et al.* (2004), <sup>2</sup> Jhooty and Mckeen (1964a), <sup>3</sup> Jhooty and Mckeen (1965), <sup>4</sup> Miller *et al.* (2003) and <sup>5</sup> Peries (1962a)

\* Radial growth is slow at 15°C but maturity is reached in the same time as at 18°C.

Peries (1962b) tested the germination and growth of *P. aphanis* (syn. *S. macularis*) on several different varieties of strawberry. He found that some varieties were more susceptible than others, but none of them were completely resistant. He found that the least susceptible varieties had greater levels of cutin acids and suggests that these are potentially fungitoxic. Cuticle penetration is achieved by mechanical pressure (Peries, 1962b). This probably explains why plants with a thick cuticles appear to be less susceptible than those with a thinner cuticles (Jhooty and McKeen, 1965).

Perithecia may provide a route for inoculum survival across strawberry production seasons and between old and new plantings. They have been observed in the field on strawberry plants (identified as *S. humuli*; Peries, 1962b, Rashid Khan, 1960, Salmon, 1900). During the experiments done by (Peries, 1962a) perithecia were only witnessed under one set of conditions. These were in green houses in specially built chambers covered with muslin (75-90% reduction in light intensity). Natural dehiscence of the perithecia was not observed.

Strawberry powdery mildew can also survive as mycelium on over wintering strawberry leaves (Smith *et al.*, 1988).

Many attempts have been made to model disease epidemics and thus provide the grower with information on the best time to apply control products. Sall (1980) developed a mathematical model of grape powdery mildew based on Vanderplank's compound interest equation for disease development. The basic infection rate ( $r$ ) varied as a function of ambient temperature and moisture conditions. The plant growth was also simulated to allow for changes in the susceptible tissue during the growth season. A spreadsheet based model of grape powdery mildew has also been developed (Chellemi and Marois, 1991). This model did not simulate the growth of the plants, but instead is based only on weather conditions. Models have also been developed that forecast disease development at a much larger scale. For example, Asher and Williams (1991) attempted to develop a system for forecasting the national incidence of sugar-beet powdery mildew from weather data in Britain. To date, however, there appear to be no models or prediction systems for strawberry powdery mildew reported in the literature.

## **Materials & Methods**

### ***Field experiments***

#### *Essex*

A field experiment was established on a commercial holding near Colchester, Essex (Grid reference: TM 068 305). The experiment consisted of a Spanish tunnel (30m × 7m, covered with normal plastic sheeting) and contained second season plants of cv Elsanta grown in peat filled troughs. The plants were grown in a glasshouse in their first season and, in the autumn of 2005, were transferred (in their troughs) to the Spanish tunnels for a second season. The plants were managed as a commercial crop whilst in the glasshouse. The original planting stock were bare root waiting bed plants supplied by Peter Wensak (Dutch plants).

The Spanish tunnel consisted of 7 rows of table tops. Each trough was 0.5m long, 0.17m wide and contained 6 plants in two off set rows. Plants were separated by 16cm within rows and the distance between rows was 8cm. Within troughs, plants were off-set by 8cm. Rows of troughs were separated by 1m. The tunnel was covered on 12 April 2006 and was vented and irrigated according to normal farm practices.

## *Cambridgeshire*

Three field experiments were established on a commercial holding near Wisbech, Cambridgeshire (Grid reference: TF 459 037). The first experiment consisted of a Spanish tunnel (150m x 6m, covered with normal plastic sheeting) and contained third season plants of cv Elsanta grown in the soil. The site was established in 2004, with plants supplied by Stefan Kraege. Prior to the experiment the crop had been managed commercially. The tunnel contained 4 beds that were 2 rows wide. Plants were separated by 30cm within rows and the distance between rows within each bed was 30cm. Within beds, plants were offset by 15cm across rows. Beds were separated by 115cm. The tunnel was fleeced in mid-March 2006 and removed from the tunnel on the 02 May 2006. The tunnel were vented and irrigated according to commercial practice.

The second experiment site consisted of two Spanish tunnels (each 10.5m x 7.5m, covered with normal plastic sheeting). The site contained one tunnel of first season Elsanta and one of first season everbearers in the ground. The everbearer tunnel was planted in the 1<sup>st</sup> week of March 2006 and the Elsanta was planted May 2006 with plants supplied by Stefan Kraege. Each tunnel contained 5 beds that were 2 rows wide. Plants were separated by 30cm within rows and the distance between rows within each bed was 30cm. Within beds, plants were offset by 15cm across rows. Beds were separated by 115cm. The everbearer tunnel was fleeced during the first week of March 2006 and the fleece was removed during the third week of April 2006. Both tunnels were covered on the 3 July 2006 and were vented and irrigated according to commercial practice.

The third experiment consisted of part of a Spanish tunnel (43m x 4.5m, covered with normal plastic sheeting). The plants were cv Everest, supplied by Edward Vinson Limited, and grown in peat filled troughs. The site had been managed commercially in the previous season. The tunnel contained 4 double rows of troughs. Each trough was 0.5m long, 0.17m wide and contained 3 plants in one row. The tunnel was fleeced in mid-March 2006 and the fleece was removed mid-June 2006. The tunnel was covered during the third week of July 2006 and was vented and irrigated according to commercial practice.

The field sites were used for the experiments described below.

### ***Inoculum and primary disease spread***

All the plants at the second Wisbech experiment were scored for the presence or absence of powdery mildew symptoms: leaf cupping, mycelium and red blotching. Both tunnels were scored 5 times between 04 July and 25 July, 2006.

### ***Analysis of disease patterns***

Disease patterns were mapped using ArcGis (ESRI Corporation, Redland California, USA), which is a geostatistical software system. The spatial patterns were analysed using SADIE (spatial analysis by distance indices) developed and supplied by J. N. Perry (Rothamsted Experimental Station). This software analyses the degree of clustering in the data, evident in the form of patches and gaps. The software produces maps with random disease patterns that have the same incidence of healthy and diseased plants as the observed map. This allows a likelihood test of whether the observed pattern is random or exhibits spatial structure due to uniformity or aggregation.

### ***Dipping plants to control initial disease development***

Four dipping treatments were compared (Table 3). Plants of cv Elsanta were removed from cold store and allowed to defrost and were then dipped for 1 minute. Following dipping, plants were allowed to drain before being placed in plastic bags. The next day 7 June 2006, they were planted at the Colchester experimental site into 3 rows of raised troughs, at the south side of the tunnel. Each treatment was replicated in 3 plots each of which contained 84 plants (42 x 2). The plots were arranged randomly. Plants were first scored for presence or absence of leaf cupping, mycelium and red blotching on the 16 June and then weekly from the 21 June until the 12 July 2006. No other powdery mildew control products were applied in this experiment.

**Table 3. Products and dilution rates for dipping trial.**

<b>Product</b>	<b>Active Ingredient</b>	<b>Dilution</b>
Untreated	Not applicable	Not applicable
Water	Not applicable	Not applicable
Bicarbonate (K50) +SW7	Potassium hydrogen carbonate	10ml/l 0.6ml/l
Systhane	Plant nutrient Myclobutanil*	0.9ml/l

\*Myclobutanil does not have approval to be used as a dip at this time. This treatment was used to prove the principle.



### *Quantification of disease progress curves*

Disease progress in each treatment was quantified by using Area Under the Disease Progress Curve (AUDPC), which was calculated, by the trapezoidal method:

$$Area = \sum_i^{n-1} \frac{1}{2} [(S_i + S_{i+1})(t_{i+1} - t_i)]$$

Where  $S_i$  is the severity of symptoms at date  $i$ ,  $t_i$  is the number of days between observations and  $n$  is the number of observations.

### ***Inoculum levels linked to cupping and red blotches***

Leaves with no mycelium visible to the naked eye, but exhibiting different powdery mildew symptoms (apparently healthy ['flat'], cupped or red blotched) were collected from 2 fields at the Wisbech site. Mycelium was visible on some leaves in field A, but was not visible in field B. Leaves from field A were collected on the 08, 22 and 30 August 2006, and from field B on the 30 August and the 5 September 2006. At all but the first sample date (8 August), when no red blotching was found, leaves were collected for all three symptom types. Leaves were frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ .

The leaves were removed from storage and allowed to defrost before being placed in a 0.1% trypan blue stain (diluted in lactic acid) for 24 hours (Waller *et al.* 2002). After this time they were washed to remove excess stain and each leaf was cut into 4 strips length ways. A transect of each strip was scored for the number of colonies and the area of each colony, under a high-powered microscope (magnification x100). Two strips from each leaf had the upper surface scored and 2 had the lower surface scored.

### ***Prediction of high risk periods***

The prediction system, reported in SF 62 annual report 2005 recommended two treatments less than were actually applied in an everbearer crop (over five months the grower applied 10 treatments and the system recommended 8 treatments). During the period covered by this report, the prediction system was developed and refined using the conditions identified in SF 62 2005 as a starting point.

Disease development data, collected over the last 3 seasons, has been used to refine the parameters. The disease development data identified when individual plants started to show symptoms of strawberry powdery mildew at the start of the season. For each disease development data set, there was also a data set of the environmental conditions from within the tunnel. The environmental conditions were run through the prediction system and the parameters were altered until the high-risk periods predicted by the system corresponded to the dates that the first signs of infection developed.

### ***Summary of other experimental work***

Other experiments were established, but did not produce any results due to the low disease levels experienced during the 2006-growing season. During the early and mid parts of the season the weather was unusually hot and hence not conducive to disease development. The growers who farm on the Wisbech site reported very low disease levels across the entire farm until the start of September. Apart from the occurrence weather unfavourable to powdery mildew, this might also be due in part to their implementing the disease management recommendations made in the first and second year reports produced by project SF 62.

In all the experiments reported here there were no differences between the treated plots and the untreated controls. Methods are summarised here for information.

### ***Disease control products***

Ten products were compared for efficacy against powdery mildew. These included a control, chemical control products, bio-pesticides and plant nutrients. The products were applied every two weeks at the recommended label rates. Leaves were tagged on selected plants from each plot, which were scored for disease symptoms weekly. The experiment was run twice on the first Wisbech experiment and once on the Colchester site.

### ***Development of control program***

Everbearers in the third Wisbech experiment were treated over the course of the season for powdery mildew. Four treatments were compared; untreated, chemical control product applied every two weeks, bio-control product applied every two weeks or alternating chemical and bio-control with one product applied every two weeks. The first products were applied on the 8 June 2006 and the last products were applied on the 23 August 2006. At the last visit to the site on the 5 October 2006 no symptoms of powdery mildew were visible. Leaves were collected on

5 September and were stored and measured as described under the method section for 'Inoculum levels linked to Cupping and red blotches'. No mycelium was present.

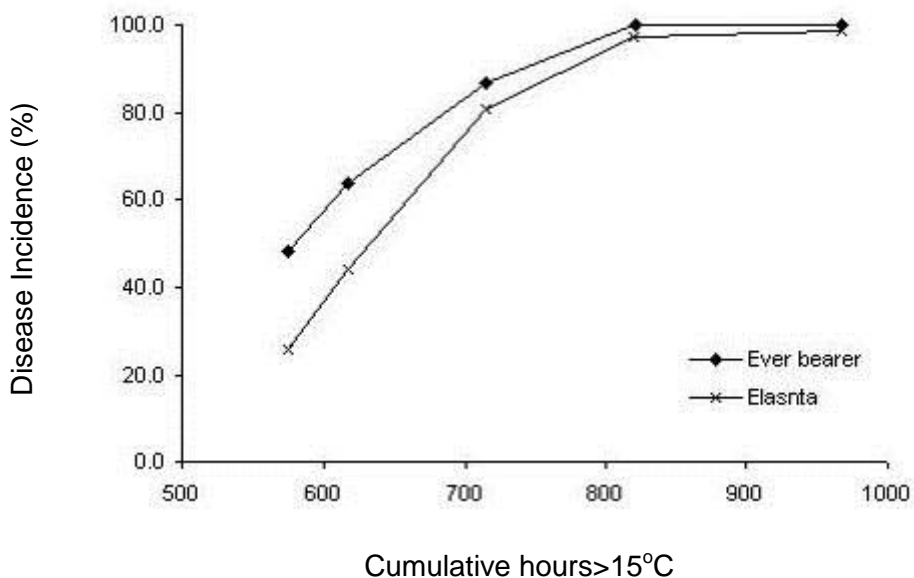
### ***Control of over wintering infection***

Various control products that are currently marketed were tested during December 2005 and April 2006 (just before the tunnel was fleeced) using part of the first Wisbech experiment. Some plots just had the control products applied during December 2005, others had the control products applied during April 2006 and some had products applied in both December 2005 and April 2006. The plots were scored 9 times at weekly intervals for symptoms of strawberry powdery mildew after the fleece was removed, beginning on 2 May 2006.

## **Results**

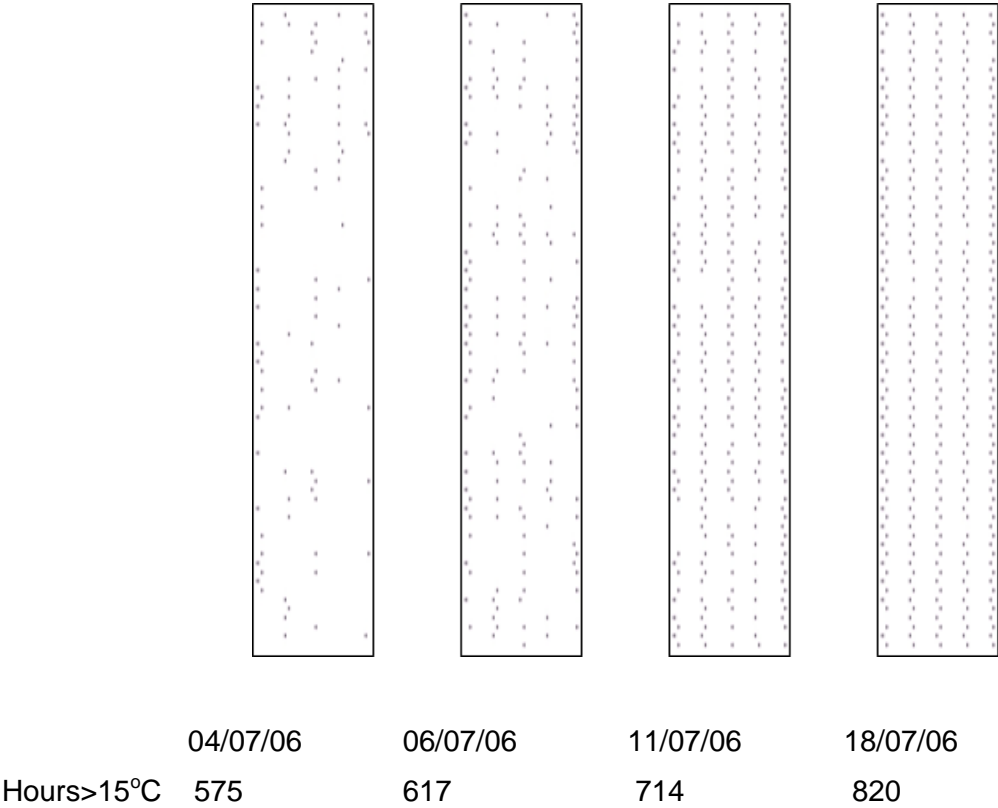
### ***Inoculum and primary disease spread***

When the tunnels were covered, 91 plants (26%) in the Elsanta and 163 plants (48%) in the everbearer had symptoms of powdery mildew. Within 617 hours >15°C, 44% of the Elsanta and 64% of the everbearer had symptoms. In both tunnels, incidence grew until all the plants were diseased (Figure 2).

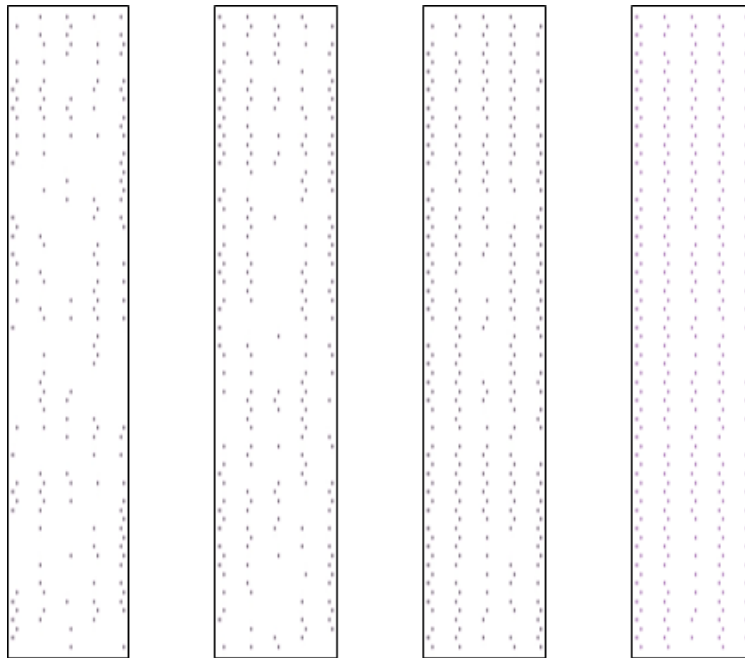


**Figure 2.** Growth in disease incidence (percent plants with symptoms), after tunnels were covered on an established site at Wisbech, Cambridgeshire.

Diseased plants in both the cv Elsanta and everbearer crops were distributed throughout the tunnels, at the first assessment (Figures 3 and 4). As the number of plants showing disease symptoms increased, the distribution remained random. By the final assessment virtually all the plants were diseased, so the disease pattern was uniform.



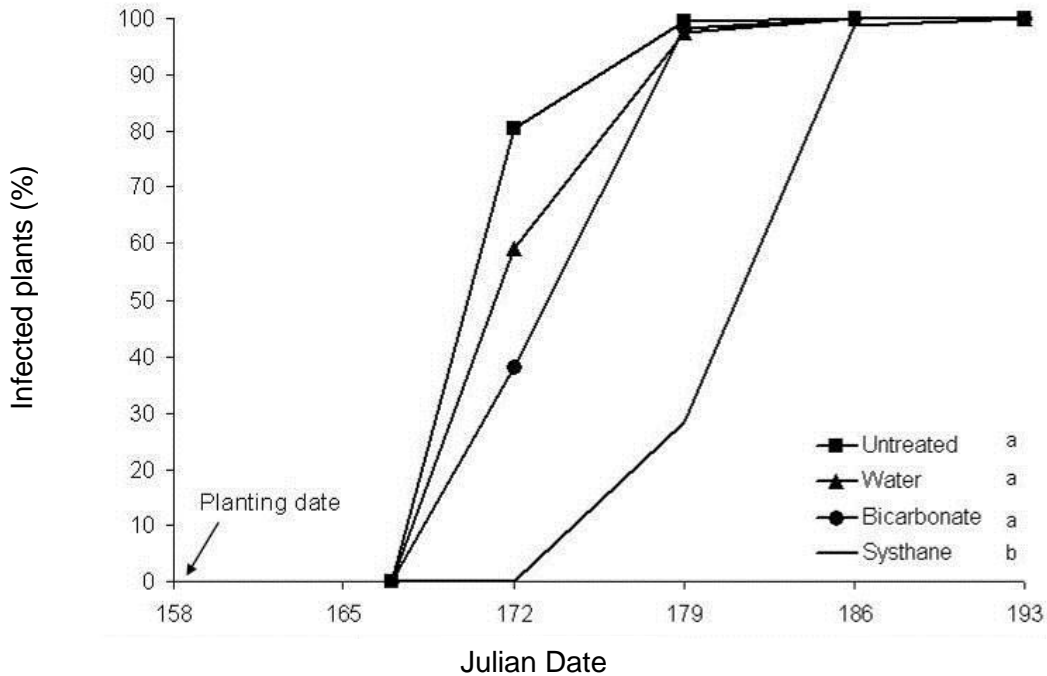
**Figure 3.** Pattern of increase for Elsanta plants with powdery mildew symptoms.



	04/07/06	06/07/06	11/07/06	18/07/06
Hours>15°C	575	617	714	820

**Figure 4.** Pattern of increase for everbearer plants with powdery mildew.  
Dipping plants to control initial disease development

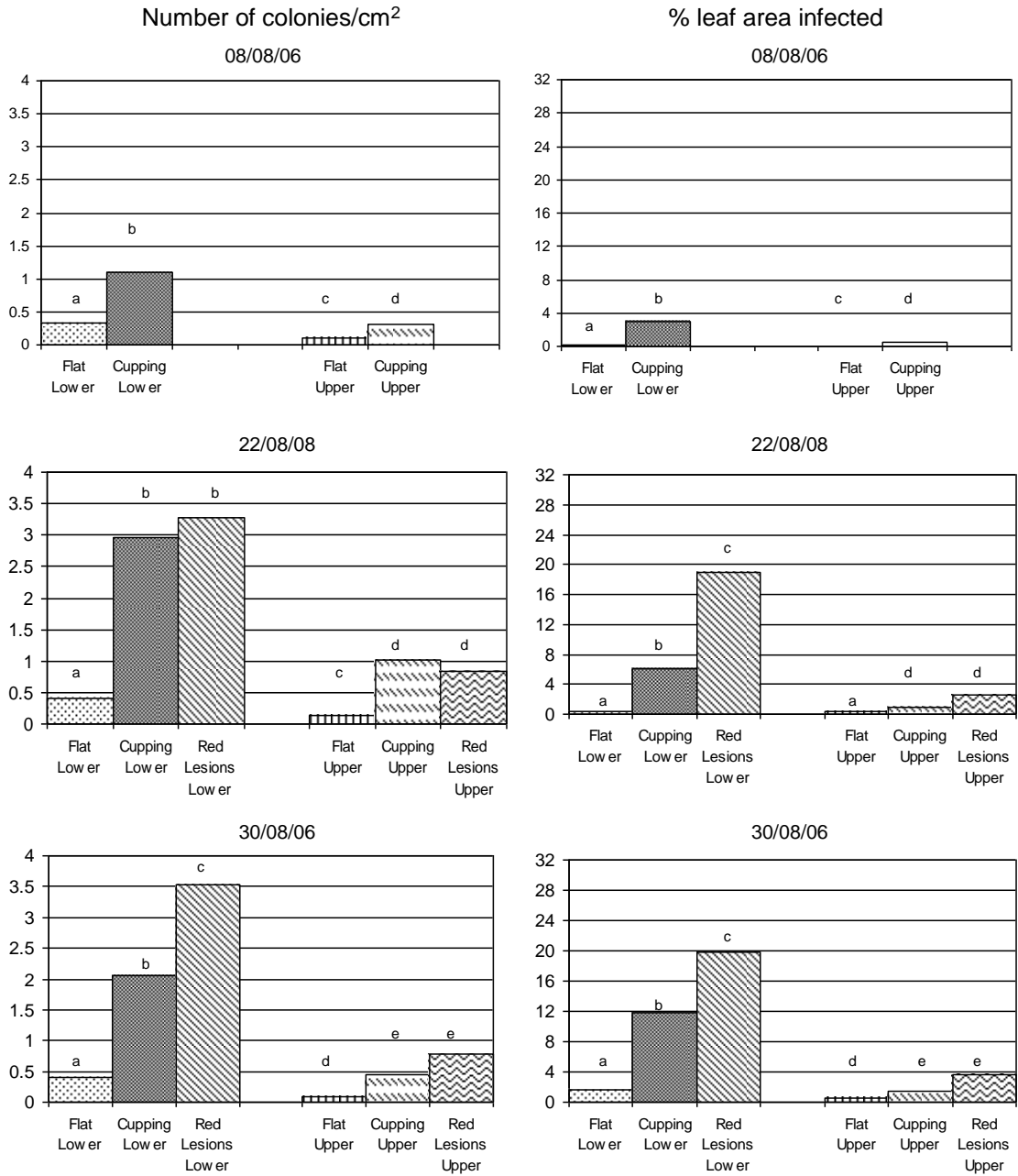
No differences in disease progress were found between the untreated plants, the plants dipped in water or the plants dipped in bicarbonate (Figure 5). However, the onset of symptoms was delayed significantly by dipping the plants in c.p. Systhane (a.i. myclobutanil) The environment for this experiment was especially conducive for the development of powdery mildew infection.



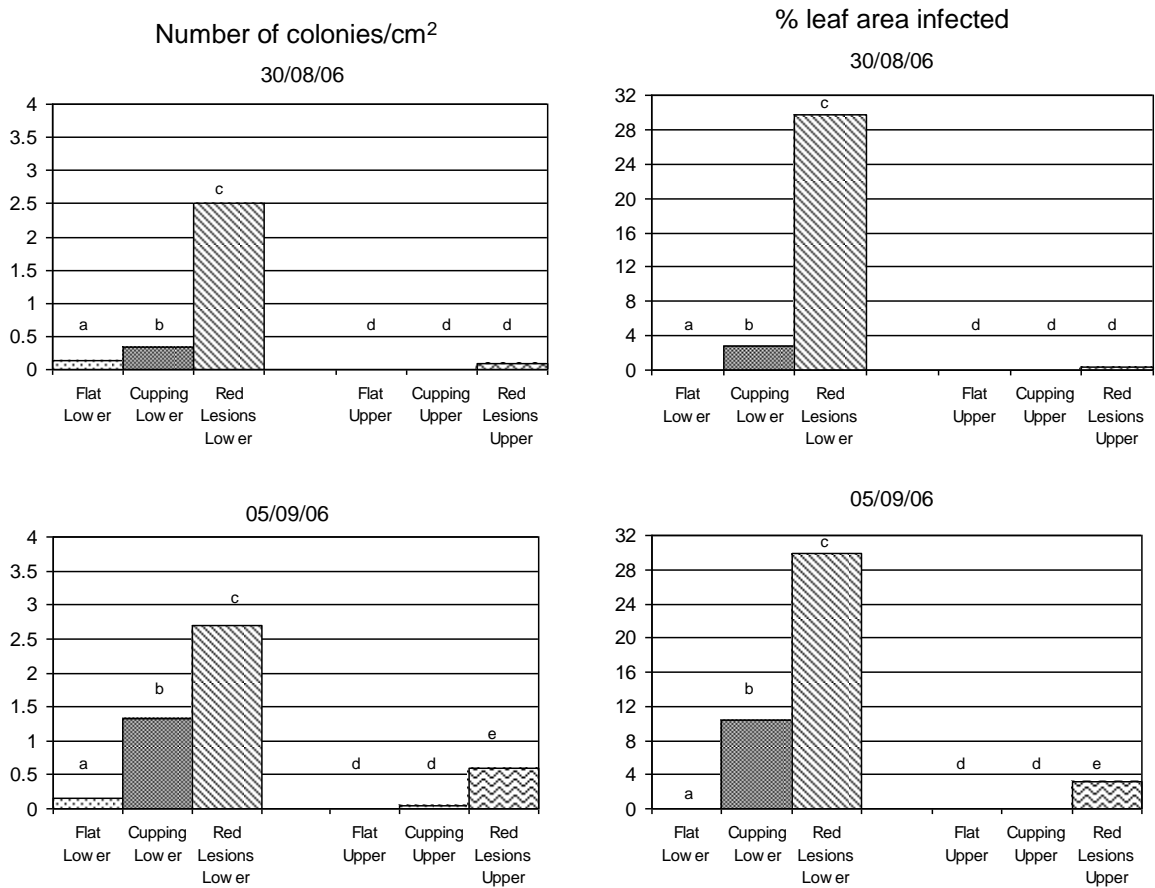
**Figure 5.** Percent of plants with symptoms of powdery mildew after plants were dipped and planted. Lower case letters next to the product name in the key indicate significant differences in the AUDPC at the 5% level.

### ***Inoculum levels linked to cupping and red blotches***

For both fields sampled, more colonies were found on the leaves collected later in the year, and compared with flat leaves, more colonies were found on the leaves that were cupping or red blotched. Figure 6 summarizes the data from field A and Figure 7 summarizes the data from field B, showing the amount of mycelium present for the three symptom types: apparently healthy ('flat'), cupped or red blotched. The data are presented as the number of colonies per cm<sup>2</sup> and as the percentage of the leaf surface covered by mycelium for both the upper and lower leaf surfaces. The Mann-Whitney *U* test was used to detect differences in these measurements at the 5% level (SPSS for windows 11.5.0, SPSS Inc). On all leaves where it was detectable, there was more mycelium on the lower surface than on the upper surface. For leaves collected from Field B, virtually no mycelium was found on the upper leaf surface.



**Figure 6.** Number of colonies per cm<sup>2</sup> and the percentage leaf area (upper and lower surface) covered by mycelium for 3 sampling dates from field A. Lower case letters indicate significant differences at the 5% level as indicated by the Mann-Whitney *U* statistical test.



**Figure 7.** Number of colonies per square centimetre and the percentage leaf area covered by mycelium for 2 sampling dates from field B. Lower case letters indicate significant differences at the 5% level as indicated by the Mann-Whitney *U* statistical test.

### ***Prediction of high risk periods***

A literature search was completed as a preliminary basis for describing the relationship between environmental conditions and development of strawberry powdery mildew. The previous work is summarized in Table 2. The values reported by this table were all obtained under laboratory conditions. Hence, the response to each of the variables is described where other environmental variables are kept constant, and which may not be identical across experiments. These experiments therefore do not describe interactions between the various environmental variables that influence pathogen development and growth. Table 1 details previous laboratory work under optimum conditions for pathogen development showing the time for spore germination. The range of conditions (including the optima) detailed in Tables 1 and 2 were used as the basis for developing a prediction system for strawberry powdery mildew. The time frame for spore development detailed in Table 1 was of particular importance for developing the

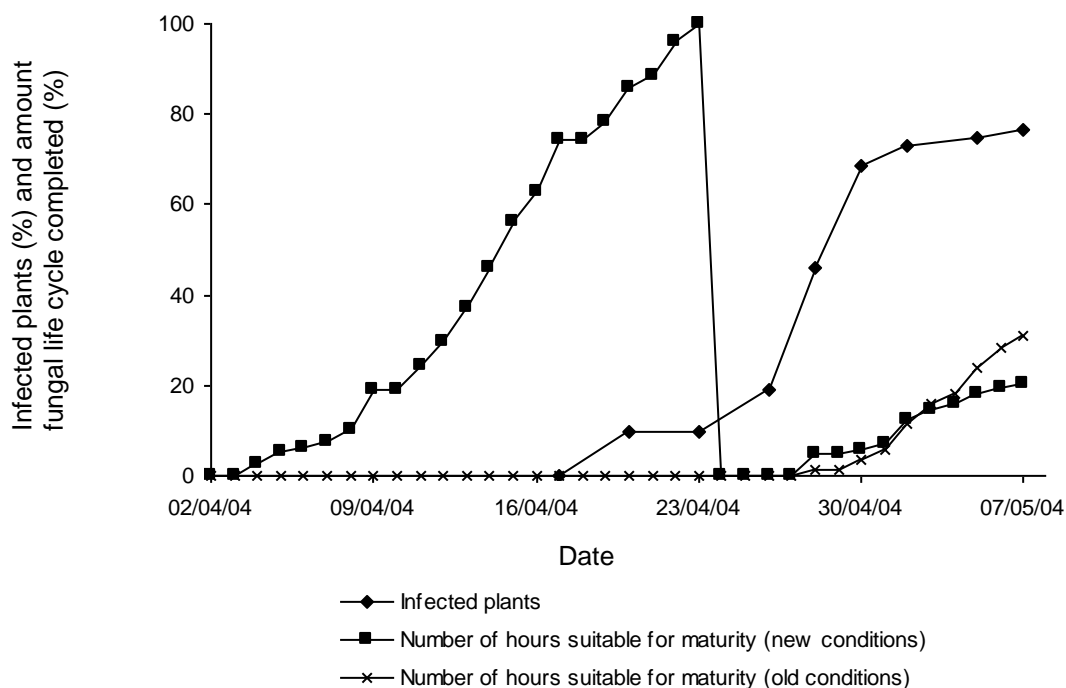


framework of the prediction system. Initial parameter values describing interactions between the environment and pathogen life-cycle were obtained from previous work. These values were modified using observations and grower input obtained in the first 2 years of SF 62 and were further modified on the basis of data collected during the three growing seasons of the project. The parameters developed previously and the new parameters established after work completed this year are presented in Table 4. The (lower) temperature values for growth and germination have been changed slightly between those reported previously (SF 62 Annual Report 2005). The main alteration to the prediction system is to distinguish between plants that have over-wintered in the field and those that were in the cold store. This accounts for the observations in 2004 and 2005, which showed that inoculum over-winters in established fields. Previous work (Smith *et al.*, 1988) has shown that strawberry powdery mildew can over winter as mycelium. As a consequence, initial infections that arise from over-wintered mycelium at the start of a new season could reach maturity and begin producing new inoculum more rapidly than infections arising from conidiospores or cleistothecia. To account for this, the time to reach the first high-risk period in over-wintered crops is reduced. This adjustment assumes that over-wintered infections in established fields are mycelium with developed haustoria and require 84 rather than 144 hours for infection to reach maturity.

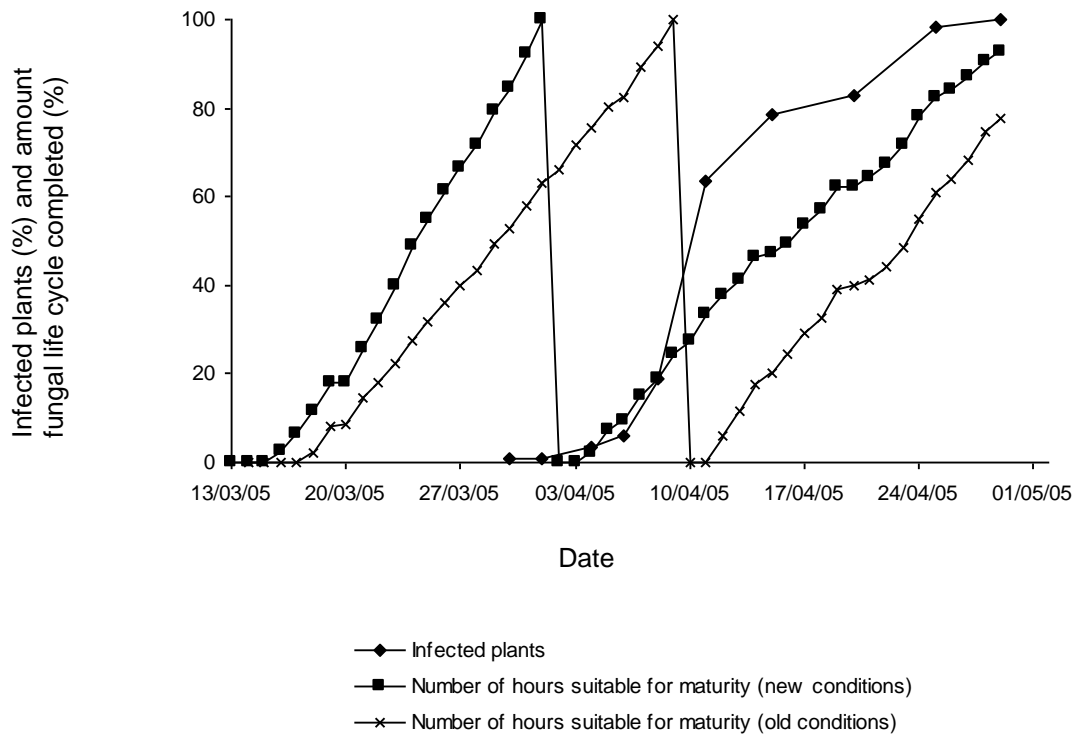
Epidemic development for three established sites (Kent 2004, Wisbech 2005 A and B) and two newly planted sites (Kent 2005 and Wisbech 2006) is shown in Figures 8-12. These figures also show the development of the pathogen [percent of life-cycle complete to spore release] predicted using the original and amended parameters.

**Table 4.** Previously developed parameters for prediction system (old conditions) and parameters developed after analysis of disease development data (new conditions).

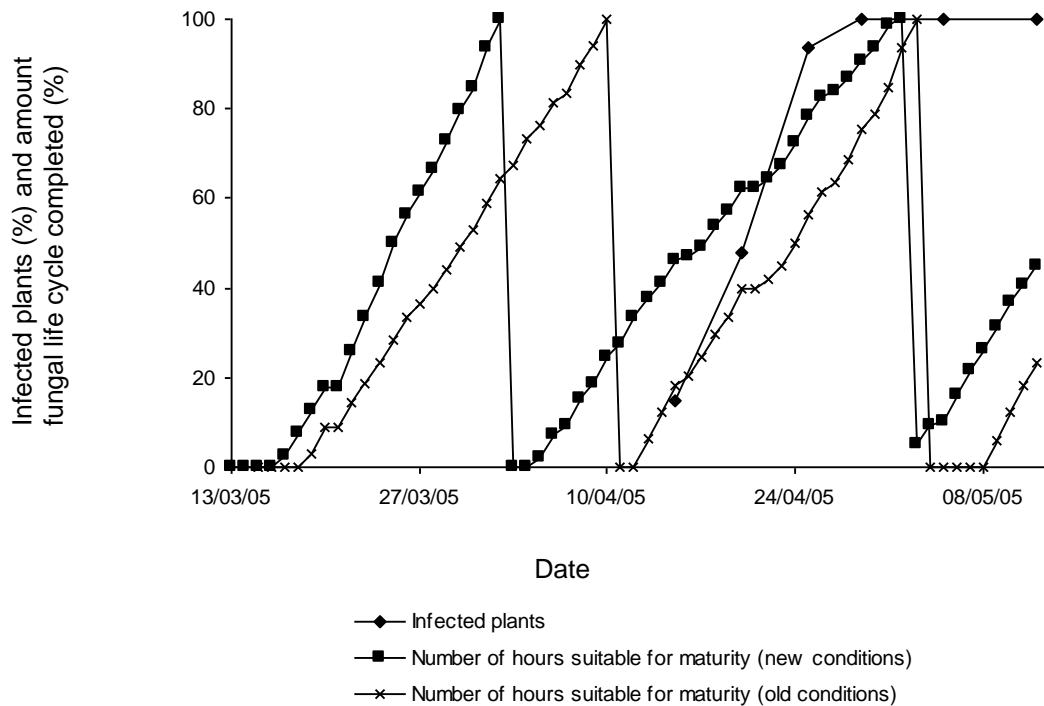
	Old conditions	New conditions
Temperature germination (°C)	17.5	15.5
Temperature growth and spore release (°C)	16	18
Relative humidity (%)	60	60
Leaf Wetness (%)	95	95
Temperature germination upper value (°C)	30	30
Temperature growth and spore release upper value (°C)	30	30
No. of hours to maturity	144	<i>na</i>
No. of hours to maturity <i>established</i> field 1st infection	<i>na</i>	84
No. of hours to maturity <i>established</i> field after 1st infection	<i>na</i>	144
No. of hours to maturity <i>new</i> field all infections	<i>na</i>	144



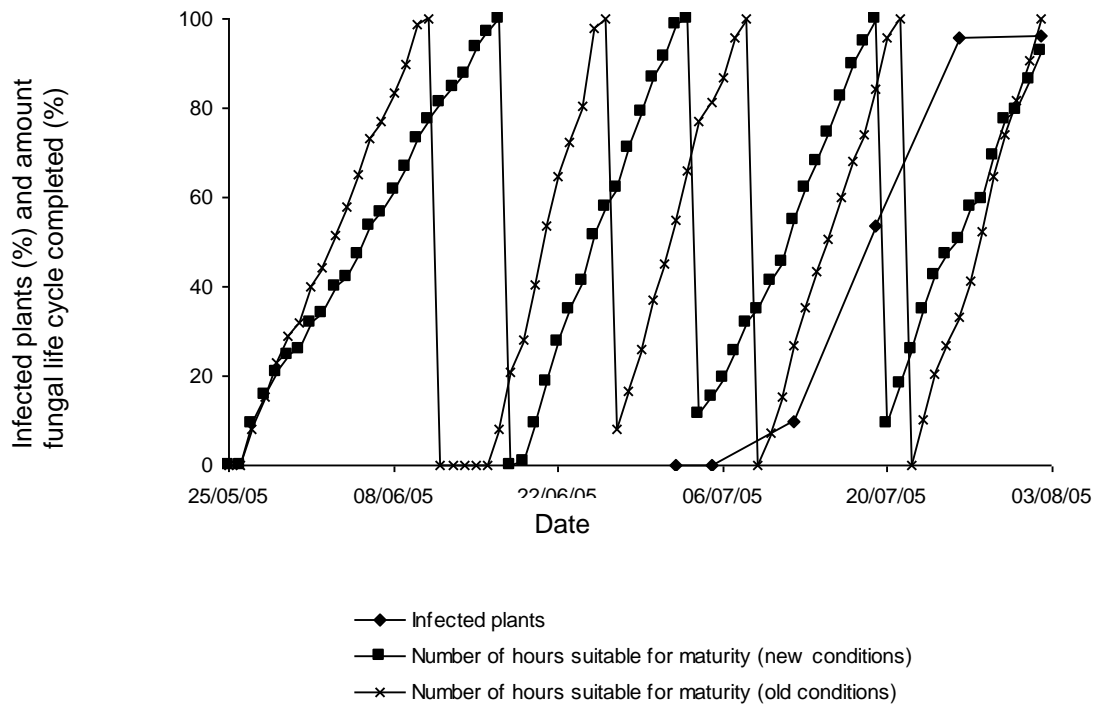
**Figure 8.** Disease development data for Kent 2004 showing plants infected (%) and the amount of the fungal life cycle completed before spore release (%) for the old and new parameters.



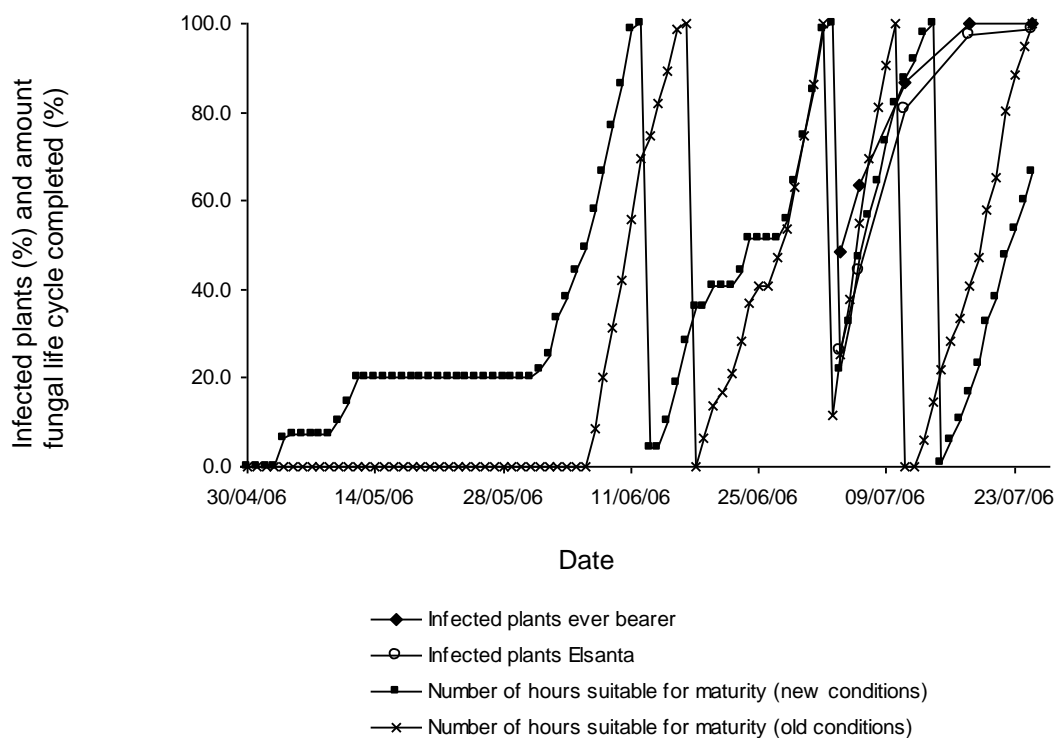
**Figure 9.** Disease development data for Wisbech 2005 A showing plants infected (%) and the amount of the fungal life cycle completed before spore release (%) for the old and new parameters.



**Figure 10.** Disease development data for Wisbech 2005 B showing plants infected (%) and the amount of the fungal life cycle completed before spore release (%) for the old and new parameters.



**Figure 11.** Disease development data for Kent 2005 showing plants infected (%) and the amount of the fungal life cycle completed before spore release (%) for the old and new parameters.



**Figure 12.** Disease development data for Wisbech 2006 showing plants infected (%) and the amount of the fungal life cycle completed before spore release (%) for the old and new parameters.

## Discussion

### *Inoculum and primary disease spread*

Infected plants within newly established crops were distributed throughout the tunnel and were not clustered towards the open ends. This supports the hypothesis, developed in previous reports, that source of primary inoculum in a newly planted fields is from latent infections of the planting material. Hence, symptoms and associated inoculum develop when conditions are suitable for pathogen growth. It is likely that the inoculum on planting stock originates from field infection of during the propagation process, surviving as either conidia or cleistothecia. Either of these survival routes would require time to germinate and infect the host plant, before generating new spores that would posing a threat to uninfected plants. In the experiments reported here, the time between planting and covering the tunnels was sufficient for two

generations of powdery mildew to reach maturity (Figure 12). The first generation would originate from inoculum that over-wintered in the cold store on the planting stock, whereas the second generation develops from the dispersal of inoculum from these primary infections.

Disease is most easily controlled when amount of inoculum present in the crop is small. By implication, more effective control is achieved if treatments are applied before severe disease symptoms are evident. Hence inoculum levels need to be managed throughout the crop cycle and this may entail early treatments early in the season. This is consistent with the adage that prevention is better than cure.

### ***Dipping plants to control initial disease development***

Dipping of plants to control powdery mildew is currently not approved by The Pesticide Safety Directorate, but is approved for some fungicides used specifically to control red core. In this study, an experiment was undertaken to test the hypothesis that dipping of strawberry plants can reduce or remove the inoculum of powdery mildew on planting stocks, so that the build-up of disease will be delayed. The untreated plants developed visible signs of infection between 9 to 14 days after they were planted. In contrast, the plants treated with Systhane (myclobutanil) did not have any visible symptoms until 14 to 21 days after planting. The plants were grown in a high-pressure mildew environment without any other treatments for the control powdery mildew applied. Under commercial conditions, it might therefore be possible to combine the use of dipping, with early season treatments to delay the primary infection of new crops.

### ***Inoculum levels linked to cupping and red blotches***

Strawberry powdery mildew infection progresses through a range of symptoms. Healthy strawberry plants have flat leaves. The leaves begin to cup soon after infection, mycelium may then become visible on the leaves (first on the lower then the upper surfaces) and red blotches begin to form. Finally, if effective treatments are not applied mycelium can form on the fruit. This progression of symptoms is well-established and recognised by crop managers. However, the only symptom that can be linked to strawberry powdery mildew with any certainty is the presence of visible mycelium. Leaf cupping can be caused by heat/water stress, and red blotching is a general stress response of strawberry plants.

The results presented here confirm the veracity of the assumed symptom progression. Cupped and red-blotched leaves both had more mycelium present than the flat leaves. However, very small amounts of mycelium were sometimes present on flat leaves. For each associated symptom, the amount of mycelium measured increased with sample date. The difference in colony size between cupped leaves and to red-blotched leaves was relatively greater than the difference in the number of colonies on cupped compared to red-blotched leaves. This indicates that, over the course of an epidemic the increase in inoculum pressure from individual leaves is primarily due to growth of colonies. Significantly more mycelium was present on the lower than on the upper leaf surfaces, which may have significant implications for crop treatment. Spray deposits to the lower leaf surface are more difficult to achieve than to the upper surface. As a consequence the effective dose may be much smaller than the applied dose at the primary target. Depending on the conditions infection can be very advanced before any visible mycelium is evident on the upper leaf surface. In Field A mycelium was visible on the upper surface of some leaves, but in Field B none was observable with the naked eye. Despite this, leaves from Field B had a larger proportion of their lower surface covered with mycelium than the leaves from Field A.

These results have several implications for disease management. Firstly, crop managers should attempt to target control treatments to control the infection on the lower leaf surface. In addition, programmes to control powdery mildew should be informed by the appearance of leaf cupping and that crops may justify treatment when red-blotches are the only visible symptom.

### ***Prediction of high-risk periods***

The parameters used by the prediction system to identify high-risk periods have been modified from those reported in the previous annual report. The original parameters were defined by reviewing literature. These values were adjusted as a consequence of initial test runs of the prediction system.

Predictions of high-risk using the original (old) parameters were compared to known disease development periods, derived from observations in field experiments. This comparison showed that predicted high-risk periods did not match accurately disease increases observed in the field. Disease in the field increased before the prediction system predicted any high-risk

periods. The parameters were adjusted (new parameter) and the system was tested to ensure that predictions of high-risk were coincident with increases in disease.

The new parameters now predict when the disease will first develop in the season, using measurements of environmental data from the 1 January (of the current growing season) For established crops the first high risk period will develop sooner than in new plantings, because of assumed differences in the sources and possibly levels of primary inoculum. More specifically, to account for the possibility that relatively large amounts of inoculum might survive the winter as mycelium in established crops that over-winters; whereas, in new plantings, primary inoculum is more likely to originate from cleistothecia or conidia, which take longer to each maturity and generate new inoculum.

### ***Integrated control of strawberry powdery mildew***

Results from all three years work have shown that the source of primary inoculum is from within the field rather than from external sources. Inoculum most likely overwinters as mycelium in established fields and could overwinter as either cleistothecia or conidia on plants in cold store. The role of wind-borne inoculum on primary infection is probably negligible. It is therefore unnecessary for Crop Managers to implement tunnel management strategies designed to prevent the immigration of exogenous wind-borne inoculum at the start of the season: primary infections are likely to be present already.

Instead, application of control products is probably justified early in the season, timed to coincide with the covering of tunnels or fleece removal. In these studies Fortress (quinoxifen) was the most effective product tested (SF 62 Annual Report 2004). This is a protectant fungicide with a long effect and long harvest interval (14 days); but has no effect on established infection. It is likely to be especially effective when applied at the start of the season when infection levels are low, under which circumstances it can offer long protection and the relatively long harvest interval is not an issue. In addition, an application of Corbel (fenpropimorph) (outdoor approval only), which acts as a systemic eradicator, will reduce or remove any overwintering inoculum, enabling the application of Fortress to have the greatest effect.

Dipping of planting stock provides a good opportunity to achieve excellent fungicide coverage, certainly greater than provided by foliar sprays. This work has shown the benefit from a dipping



treatment in delaying the initial build-up of disease and hence inoculum pressures. Systhane (myclobutanil), used in the experiment reported here, delayed the onset of disease by over 7 days. Whilst this product is not currently approved for use as a dipping treatment, the observation demonstrates and quantifies the potential benefits from dipping treatment.

Whilst there are clear benefits from managing levels of primary inoculum, the benefits are likely to be a delay rather than a prevention of disease progress. As a consequence, vigilant crop walking is required throughout the season to monitor disease progress. These studies have shown that leaf cupping and red-blotching are associated with the generation of inoculum, and that sprays need to target the lower leaf surface. Given the density of strawberry crop canopies this poses substantial challenges for spray timing and application methodologies.

Strawberry growers have a limited range of active ingredients with which to control powdery mildew. It is crucial that these are within strategies that slow the development of fungicide insensitivity and complete resistance in the target pathogens. Choice of fungicide should include consideration of both its activity (*i.e.*, protectant, curative, anti-sporulant *etc*) and the mode of action. Ideally fungicides with the same modes of action should not be used consecutively or too frequently. Advice on resistance management strategies was provided in SF 62 Annual Report 2004.

This work has shown that bicarbonate (potassium hydrogen carbonate) was as effective as Systhane at controlling established infection. Bicarbonate works by contact, so good leaf coverage from sprays is necessary to achieve the optimum benefit for disease management. Bicarbonate does not have a harvest interval so is an ideal product to use at the time fruit are being picked. However, for crop managers unfamiliar with the treatment it may be prudent to experiment using test plots, because high doses of bicarbonate can cause leaf scorching. Bicarbonate does not provide long lasting protection; it will only kill the mycelium that it contacts. Crop walking therefore remains important following treatment. Sulphur is another product that can be used in an integrated spray program to reduce the pressures on the available modes of action. In addition, some bio-control products might be suitable for use by strawberry growers. They should be used after consulting an independent agronomist.

This work has developed a prototype system for predicting high-risk periods for the development of strawberry powdery mildew. The system is still under development, but when

completed it should provide valuable information to complement observations from crop walking. At the moment it appears that the growers sometimes apply more than the optimal number of treatments necessary to manage powdery mildew. This increases the risk of resistance developing, retailers detecting fungicide residues and is economically wasteful. The prediction system is designed to predict the minimum time necessary between applications of control products.

The benefits to management of strawberry powdery mildew from the use of varieties with quantitative resistance were reported in SF 62 Annual Report 2004. However, retailers have very specific preferences for varieties, limiting the flexibility for crop managers to reduce disease pressures by growing more resistant plants. Some progress towards introducing more resistant stocks might be achieved by education of retailers and consumers about the significant effect crop resistance can have on fungicide dependence.

## Acknowledgements

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