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

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Abstract

Until recently strawberries grown in the United Kingdom were grown in open fields, the plants and fruit were exposed to the British weather. This resulted in a short 6 week harvest period where the fruit was often damaged by rain and infected by *Botrytis cinerea*. Strawberry growers started to use polythene tunnels to extend the cropping season, protect the fruit from rain damage and reduce the incidence of infection by *B. cinerea*. However the conditions produced by the polythene tunnels were ideal for the growth and development of *Podosphaera aphanis* (strawberry powdery mildew). Growers are now under pressure from the retailers to reduce the amount of fungicides that they use to control *P. aphanis*.

The symptoms related to *P. aphanis* infection have been identified (leaf cupping, visible mycelium and red blotches) and a progression has been established. From the symptom progression two new scoring methods for the identification of *P. aphanis* infections were developed which have greater relevance to current cultivation methods than the previous method.

The source of initial inoculum for newly planted and established sites was identified. The inoculum was planted into new sites on the plants coming from the propagators and over wintering on plants within established sites. This was contrary to what the growers believed. They were basing their early season tunnel management on keeping the perceived air borne infection out of their tunnels.

A rule based prediction system has been developed that has the potential to reduce the number of fungicide applications applied by the growers. The prediction system ensures that fungicide applications are not applied too close together. Potassium Bicarbonate has been shown to provide comparable control of *P. aphanis* to that achieved with Systhane (Myclobutanil). Significantly better control of *P. aphanis* was achieved using a new (at the time) product, Fortress (Quinoxyfen). There were significant differences in the resistance to infection by *P. aphanis* displayed by different cultivars of strawberry. Elsanta, the cultivar favoured by the retailers was not one of the most resistant. Control of inoculum already present on plants as they are being planted could be achieved by dipping the plant in Systhane.

Growers are under considerable pressure from the retailers to reduce the amount of fungicides used to control *P. aphanis*. Growers could achieve this by implementing the recommendations made in this report.

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Dodgson J, Hall A and Parker S. 2005. Overwintering *Podosphaera aphanis* as main source of inoculum in a second year Elsanta strawberry crop under Spanish tunnels and relative resistance of seven varieties. The BCPC International Congress - Crop Science and Technology, 2005, 475-478. 31st October - 2nd November 2005, Glasgow, BCPC.

Dodgson J, Hall A and Parker S. 2005. Targeting applications of fungicides effectively to control *Podosphaera aphanis*. Proceedings of BSPP presidential meeting 2005, 28. 19th-21st December 2005, University of Nottingham, UK.

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Dodgson J, Hall A and Parker S. 2007. System to predict high risk periods for *Podosphaera aphanis* infection of strawberries grown in polythene tunnels. Aspects of Applied Biology 83, 59-63.

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Chapter 1 - General Introduction

1.1 Introduction

In the last 18 years the area used for strawberry production has gone down from 5564 hectares in the 1988/89 season to 3782 hectares in the 2005/06 season. In the same time the amount of fruit produced has risen from 42,800 tonnes to 63,900 tonnes and the value of fruit produced has risen from £65 million to over £127 million (Anon., 1999, Anon., 2006d). The improved production per unit area is largely explained by the implementation of polythene tunnels (Anon., 2005b). This increase in production has happened in a sector of the agricultural industry that does not receive government subsidies (Anon., 2005b).

1.2 Strawberry production

Strawberries were traditionally grown in open fields which left them exposed to the vagaries of the British weather. This resulted in a short, 6 week harvest period from late May to early July (Anon., 2005b). Large yield losses were typical, due to infection of the fruit by *Botrytis cinerea* (Maas, 1970, Maas and Smith, 1972) and also fruit was often damaged by heavy rains (Fletcher, 2006). This resulted in a strawberry industry that was reliant on 'good' weather to be able to produce fruit in suitable condition and qualities for the retail market. Today the retail industry demands a constant reliable supply of British fruit

(Anon., 2005b) which the growers could not supply without polythene tunnels (polytunnels).

1.2.1 Polythene tunnel production of strawberries

Growers started to use polythene tunnels around 1994 (Fletcher, 2006). These protect the fruit from rain damage and provide temperatures more conducive to strawberry production than external temperatures, at the start and end of the season. An added benefit is that conditions inside polytunnels are less favourable for dispersal and growth of *B. cinerea*.

The use of polythene tunnels has extended the British strawberry production season to a 5 month harvest period from May to the end of September (Anon., 2005a). This longer cropping season has resulted in a need for different varieties and cultivation methods to produce fruit at different times of the season, because crops will only produce commercially viable fruit for 3 weeks. The strawberry growing season is broken down in to 3 main cropping periods, which are shown schematically in Fig. 1.1. 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. Fields are sometimes covered with horticultural fleece before the tunnel sheets are put on. This is a soft white, non woven, UV stabilised polypropylene material permeable to air and water, which helps protect the plants from late frosts and encourages the plants to produce flowers. The

fleece is removed once flowers have been produced to allow pollination. The main crop is either planted at the start of that season (March to early May) and forced to produce fruit (a 60 day crop) or they are plants that have over wintered either once or twice. The late (ever bearer) crop is produced by special ever bearer varieties, which have been bred to produce fruit over a longer period. Provided they are picked regularly, these produce fruit for up to 10 weeks (personal communication, Harriet Duncalfe, Wisbech). Generally growers have several early, main and ever bearer fields. They are managed so that fruit production by the farm continues throughout the season. When a field is producing fruit it is picked every three or four days.

1.2.2 Polythene tunnel management

Conditions within the tunnels need to be carefully managed, to optimize the fruit production of the plants. The plants need to be irrigated as the tunnels stop rain replenishing soil moisture. Once covered, crops are irrigated at least once each day often more frequently, when conditions are hot. The irrigation system also provides a route to deliver additional nutrients.

Temperatures in polythene tunnels can become too hot for optimal crop production, especially when the sun is shining. To manage the temperature, tunnels can be vented by pushing the polythene sheets up the sides of the tunnels (Fig 1.2 A and B) so the hot air can escape. Whereas when ambient

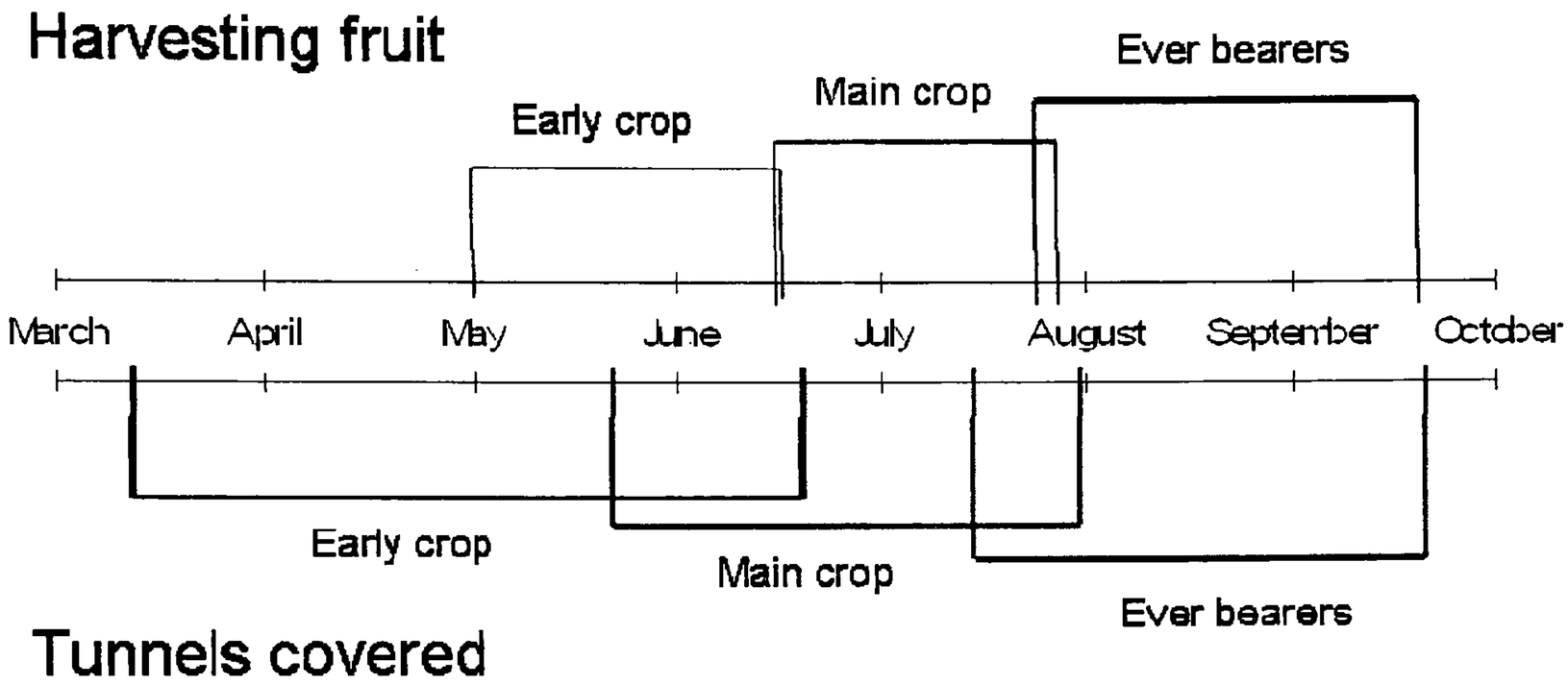

Fig. 1.1 Representation of the strawberry season highlighting when each of the three main crops will be picked and when the corresponding tunnels are covered

temperatures are too cool the sheets can be lowered to keep warm air inside the tunnels. At the start of the season tunnels are only vented when necessary and the sheets are lowered at night. During this period the tunnels are fitted with doors and mypex sheets (woven plastic mulch that is used as a ground cover to suppress weeds) at the edges where the polythene sheets do not reach the ground, so that the tunnel is completely enclosed. These can be removed as the weather gets hotter. As the season progresses in to summer the sheets are fixed in the open position until the conditions cool down again towards the end of the season (personal communication, Harriet Duncalfe, Wisbech).

1.2.3 Strawberry cultivation methods

Commercial strawberries can be grown in beds formed mechanically before the tunnel is erected from the soil present in the field (Fig 1.2 A and B). The bed is covered with a plastic mulch, to prevent weeds growing and help protect the ripe fruit. The irrigation system is placed below the mulch. Alternatively strawberries can be grown in troughs or bags that are filled with peat. This requires more irrigation but reduces the risk of soil borne diseases considerably. The troughs can be either on the ground or placed on raised platforms, to ease picking (Fig. 1.2 C and D). Growers plant new strawberry plants any time from February through to July. When planted in the summer they quite often need to be misted (Fig. 1.2 B) by over head sprinklers, in addition to the irrigation system within the

strawberry bed. Plants are misted little and often so the leaf surface is wet most of the time this is only done for a maximum of two or three weeks after planting.

Strawberries are also produced in glasshouses, these fruit before (April until mid-June) and after (September to December) the plants grown in polytunnels (Anon., 2005a).

1.2.4 Propagation of plants

In addition to producing sexual berries strawberries reproduce asexually by the production of runners. Runners, or stolons, arise from axillary leaf buds on the crown of the plant. The distal end of the runner normally develops into a runner plant. If the runner plant lies on moist soil roots develop quickly (Maas, 1998). Depending on the cultivar the number of runners produced in a season can range from none up to fifty. Where the plant does not produce runners plants can be propagated by the division of the branch crowns bearing roots or by tissue culture, this however is not common (Maas, 1998).

The strawberry plants for commercial production are produced by specialist growers referred to as propagators. 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

Fig. 1.2 A. Tunnel with the polythene sheet pulled down (as indicated by the arrows), the plants are planted into mechanically formed beds. B. Tunnel with the polythene sheet pushed up (as indicated by the arrow), plants grown in mechanically formed beds. C. Plants grown in troughs (on the ground) filled with peat; the drip irrigation system is also visible. D. Plants grown in raised troughs filled with peat

could lead to the transmission of viruses to the daughter plants. There are five levels of approval from Foundation to Super Elite to Elite to A to Approved Health (Anon., 2006f). Usually the propagation of strawberry plants and the production of commercial fruit are carried out by completely different growers in different locations. When harvested, runners are graded for size then stored at 4°C over the winter in a cold store before being planted the following season by strawberry growers.

1.2.5 Supplying the retail industry

The majority of strawberries produced are sold through supermarkets, which are supplied by grower cooperatives or marketing companies. These cooperatives and marketing companies can supply fruit all season; whereas individual growers will have ups and downs in production. The cooperatives and marketing companies are able to smooth out the supply of fruit by sourcing fruit nationally and in some cases internationally.

The dominant position of the UK's supermarkets in the retail sector means that they are in a strong position when dealing with primary producers. As consumers become more aware of the way that food is produced, supermarkets are starting to use pesticide residue data as a marketing tool, rather than a consumer health measure. The supermarkets tell the growers what levels of pesticide residues are acceptable in the fruit they supply. These levels are often well below the legally

permitted maximum residue levels (MRL). Appendix 5 contains the published MRLs permitted in strawberries. Growers are therefore under strong pressure by supermarkets to reduce pesticide use, whilst maintaining fruit of the highest quality.

1.2.6 Pathogens, pests and weeds of strawberry fields

Mass (1998) lists the worldwide infectious diseases of strawberry plants (Table 1.1). Of these, only a few are of significance to UK strawberry growers (Table 1.1) (Anon., 2004a). There are also many arthropod and mollusc pests of strawberries and a large proportion of these can act as vectors for pathogens, in addition to the losses they cause by direct damage to plants (Anon., 2004a, Maas, 1998).

In the UK products for the control of pathogens are licensed for use by the Pesticide Safety Directorate (PSD). Products either have an on label approval, they have approval for use on that crop on the label of the packet as registered by the manufacturer or they can have an off label approval where a Specific Off-Label Approval (SOLA) has been applied for by the grower or the representative organisation, for the product to be used on a crop not included as an on label approval (Anon., 2006b). Products can also be classified as a commodity substance which are chemicals that have a variety of non-pesticidal uses and also have minor uses as pesticides (Anon., 2006c).

Table 1.1 Number of infectious diseases of strawberry plants (worldwide and with just UK distribution) (Anon., 2004a, Maas, 1998)

	Infectious diseases of strawberry plants	
	Worldwide	UK
Bacterial diseases (leaf)	2	1
Fungal diseases (fruit)	17*	2
Fungal diseases (leaf)	20*	5
Fungal diseases (root and crown)	23*	3
Aphid borne viruses	6	1
Nepoviruses	5	1
Other viruses and virus like diseases	5	0
Leafhopper-vectored diseases caused by phytoplasmas	6	1
Bacterium like organisms	2	0

* Some fungal diseases on different parts of the plant are caused by the same causal organism

Table 1.2 Fungal pathogens of strawberry for which there is a control product with either on or off label approval (Anon., 2004a, Anon., 2005d, Anon., 2006e, Maas, 1998, Whitehead, 2006)

On-label approval		Off-label approval	
Common name	Scientific name	Common name	Scientific name
Botrytis	*Botrytis cinerea*	Botrytis	*Botrytis cinerea*
Crown rot	*Phytophthora cactorum*	Crown rot	*Phytophthora cactorum*
Red core	*Phytophthora fragariae*	Red core	*Phytophthora fragariae*
Powdery mildew	*Podosphaera aphanis*	Powdery mildew	*Podosphaera aphanis*
		Blackspot	*Colletotrichum acutatum*
		Leaf spot	*Mycosphaerella fragariae*
		Septoria leaf spot	*Septoria fragariae*
		Alternaria black leaf spot	*Alternaria alternata*

As of the 14/12/06 products were registered with on label uses for 4 fungal pathogens of strawberry and off label uses permitted for 7 fungal pathogens (including the 4 pathogens for which there were on label approvals) (Table 1.2) (Anon., 2005d, Anon., 2006e, Whitehead, 2006).

The Plant Health Propagation Scheme (PHPS) was launched in 1982. Its aim is to provide growers with planting material descended from stock that is proven both in terms of health and vigour (Anon., 2006g) and along with the Nuclear Stock Association provides a scheme that reduces the risk of viruses being passed to growers via plants obtained from colonially propagated stock. The PHPS also issues a list of notifiable diseases of strawberry. Currently 4 diseases are listed: red core disease, strawberry crinkle virus, strawberry mild yellow edge and strawberry blackspot (Anon., 2006g).

In addition to the pathogens and pests of the strawberry plant growers also have to control weeds that grow between and within rows. These are controlled by herbicides (between rows) and hand weeding within the rows. Many of the insect pests of commercial strawberry production are controlled by biological control agents that can be affected by applications of pesticides.

1.2.7 Potential for development of fungicide resistance

Some growers will use as many as 20 fungicide applications for control of just *Podosphaera aphanis* within a season. Generally growers perceive crops to be at

very high risk of infection from *P. aphanis,* so fungicides are applied regularly through out the season and especially when the field is producing fruit, because fruit is picked every three or four days, growers must time these applications very carefully so that the harvest interval expires by the time fruit is next picked. Not all of the products approved for use on strawberries (Table 1.5) have harvest intervals of three days or less. As a consequence products with short harvest intervals tend to be used heavily when the crop is fruiting. Products with longer harvest intervals are used either before or after the field has produced fruit. The author is aware of at least one strawberry grower that has applied 3 successive applications of the same product (personal communication).

The way in which some growers use their fungicides could lead to the development of fungicide resistance in *P. aphanis*. There has been a gradual increase in the occurrence of fungicide resistance since the introduction of systemic fungicides in the early 1970s, and this often occurs with fungicides that have a very specific mode of action (Anon., 2005e). Multiple applications of fungicides with the same mode of action in succession can lead to fungicide resistance developing faster than if products with different modes of action are used (Anon., 2005e). The Fungicide Resistance Action Committee lists different powdery mildews as at a high, medium and low risk of developing fungicide resistance (Anon., 2005f). The powdery mildews that are included in the medium and low risk categories are still regarded as at a high risk of resistance

developing. There is just no evidence of resistance developing or they are of little commercial importance so have not been included in the high risk category.

1.2.8 Current control of *P. aphanis*

As of the 14/12/06 there were 86 products with on label approval for control of fungi on strawberries, 14 products with off label approval and 1 commodity substance (Table 1.3). Of these products 26 on label, 4 off label and the commodity substance were approved for control of *P. aphanis* (Table 1.4). The 31 different products approved for control of *P. aphanis* included only 8 different target sites (Table 1.5) (Anon., 2005d, Anon., 2006e, Whitehead, 2006). Of the 8 products not all will have the same efficacy at controlling *P. aphanis* infections.

1.3 The Horticultural Development Council

The work reported here was funded by the Horticultural Development Council (HDC). The HDC is a statutory body who administer the collection of an 'industry levy' to fund essential near-market research and development for the benefit of UK horticulture. The HDC was established on the 1st July 1986 to meet the needs of near-market research which are decided and funded by the grower. All growers with sales of more than £25,000 a year are required to register with the council. Registered growers are then asked to make an annual return of the

Table 1.3 Control products licensed in the UK for use on strawberries (including on label and off label registrations as well as commodity substances) as of 14/12/06, compiled by the author (Anon., 2005d, Anon., 2006e, Whitehead, 2006)

	Total	Fungicide	Herbicide	Insecticide	Miscellaneous
On label	303	86[a]	112	100[a,b]	20[b]
Off label	35	14	8	11	2
Commodity substance	1	1	0	0	0

[a] four products are classed as fungicidal and insecticidal
[b] eleven products are classed as insecticidal and miscellaneous

Table 1.4 Number of products, active ingredients and modes of action registered for use on strawberries to control *P. aphanis* as of 14/12/06, compiled by the author (Anon., 2005d, Anon., 2006e, Whitehead, 2006)

	No. products	No. of different active ingredients	No. of different modes of action
On label	26	6	4(5)[a]
Off label	4	2	2[b]
Commodity substance	1	1	1

[a] two products have the same mode of action but different target sites
[b] one of these products has the same mode of action but a different target site as an on label product

Table 1.5 Common names of products approved for use on strawberries to control *P. aphanis* including their mode of action and target site as of 14/12/06 (Anon., 2005d, Anon., 2006e, Whitehead, 2006)

Code	Common Name	Mode of Action	Target Site
a2	Bupirimate[a]	Nucleic acids synthesis	Adenosindeaminase
c3	Kresoxim-methyl[a]	Respiration	ComplexIII: cytochrome bc1 (ubiquinol oxidase) at Qo site
c5	Dinocap[a]	Respiration	Uncoupler of oxidative phosphorylation
e1	Quinoxyfen[b]	Signal Transduction	G-proteins in early cell signaling (proposed)
g1	Myclobutanil[a]	Sterol biosynthesis in membranes	C14-demethylase in sterol biosynthesis
g2	Fenpropimorph[b]	Sterol biosynthesis in membranes	Δ^{14}-reductase and $\Delta^{6}\rightarrow\Delta^{7}$-isomerase in sterol biosynthesis
m	Sulphur[a, d]	Multi-site contact activity	Multi-site contact activity
nc	Potassium bicarbonate[c]	Not classified	Unknown

[a] on label products
[b] off label products
[c] commodity substance
[d] also used as an insecticide

value of their sales which if, after specified deductions exceed £50,000, is used to calculate the levy due (Anon., 2006a).

The HDC is divided in to seven sectors that cover over 300 crops. This work was commissioned by the soft fruit sector panel (Anon., 2006a). Therefore the work carried out for this PhD needed to fulfil the requirements of the HDC as well as the requirements of a PhD. The HDC requires that the majority of the results generated by this work should be relevant to the grower and that any recommendations should be practicable for implementation by growers. A draft of the final grower report submitted to the HDC has been included in Appendix 1.

1.4 Literature review

1.4.1 *Podosphaera aphanis*

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, 1961, 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 and 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 powdery mildew on strawberry is *P. aphanis* (Braun, 1982, Braun, 2002). These studies provide further confirmation that the fungi causing strawberry and hop powdery mildew are different. *P. aphanis* (referred to as *S. macularis*) cleistothecia are gregarious or scattered, or caespitose, 60-125µm diameter, dark brown to black, smooth and with numerous hyphal appendages from the lower half and each contains one ascus (Mukerji, 1968). For brevity, when citing previous work the current taxonomic name will be used. The terms 'cleistothecium / cleistothecia' are no longer correct when referring to the fruiting bodies of powdery mildews. The terms 'chasmothecium / chasmothecia' have been suggested as suitable alternatives (Belanger *et al.*, 2002, Kirk *et al.*, 2001). The classification of *P. aphanis* is presented in Fig. 1.3.

1.4.2 Symptoms of infection

Infection by *P. aphanis* causes a progression of symptoms on the leaves and fruit. A healthy strawberry leaf is flat and green (MAFF Strawberry Powdery Mildew Key No. 8.1.1, included in Appendix 4). Infected leaves begin to cup upwards exposing the underside of the leaf. Mycelium first become visible on the abaxial leaf surface and then on the adaxial surface. Red blotches form on

Kingdom	Fungi
Phylum	Ascomycota
Class	Ascomycetes
Order	Erysiphales
Family	Erysiphaceae
Tribe	Cysthotheceae
Sub-tribe	Cystothecinae
Genus	*Podosphaera*
Species	*P. aphanis*

Fig. 1.3 Classification *P. aphanis* within the kingdom Fungi (Belanger *et al.*, 2002, Cook *et al.*, 1997, Kirk *et al.*, 2001)

the leaf (visible on the abaxial and adaxial surfaces) as the amount of visible mycelium reduces (Blanco *et al.*, 2004, Scott *et al.*, 1970, Salmon, 1900). The leaf cupping symptom persists throughout the infection. In the field, visible mycelium is the only symptom that can be identified as *P. aphanis* with any certainty. Leaf cupping is also a symptom of drought stress and there are at least 7 other fungal pathogens that cause red or purple blotches on the leaves of strawberry plants (Scott *et al.*, 1970, Salmon, 1900).

1.4.3 Conditions suitable for growth of *P. aphanis*

The optimum temperature for germination of the conidia is in the range 18°C to 22.5°C with between 85-88% of conidia germinating in this range, when experiments were carried out in the laboratory (Peries, 1962a). Subsequent authors found 20°C to be the optimum temperature for germination of conidia (Jhooty and McKeen, 1965, Miller *et al.*, 2003). Amsalem, *et al.* (2006) suggested that the germination rate was similar over the temperature range 15°C to 25°C. Jhooty and Mckeen (1965) found that the minimum and maximum temperatures for spore germination were 3°C and 38°C respectively. This is supported by Miller *et al* (2003), who found that 8% of spores germinated at 4°C. Spores also germinate, at a greatly reduced frequency, at 36°C. Only 1% germination was observed at 5°C and 35°C (Amsalem *et al.*, 2006). Peries (1962a) found that less than 1% 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 less than

10°C and more than 30°C these temperatures are not conducive for disease development. Between 5°C and 13°C no sporulation was observed after 3 weeks. The amount of infection at 15°C is consistently greater than at 25°C (Jhooty and McKeen, 1965). Radial growth was slower at 15°C than 18°C but colonies reached maturity (production of spores) in the same amount of time (Peries, 1962a) (Table 1.6).

Conidia remain viable even when conditions are not favourable for germination, conidia stored at 0°C in Petri-dish moist chambers for 14 days had a 55% germination rate (Peries, 1962a). Conidia that were on strawberry leaves could retain their viability for appreciable periods when they were stored at 0°C in Petri-dish moist chambers (Table1.6).

Measuring the passage of time related to the temperature has been used extensively in agronomy to predict the lengths of different phases of development of the crop (Bonhomme, 2000) and to a lesser extent to describe epidemiological measurements within plant pathology (Lovell *et al.*, 2004). The unit is often based around the words 'degree' and a measurement of time, e.g. 'degree-day' (Bonhomme, 2000) 'day-degrees' (Norton and Mumford, 1993) or 'degree-hours' (Lovell *et al.*, 2004). Each day (or other unit of time) the physiological time accrued is calculated, the sum of the day-degrees above and below a species-specific threshold are accumulated (Norton and Mumford, 1993). The use of 'day-degrees' can therefore enable a comparison of measurements that have

been taken over a range of sites and years, where as a measurement of just time would mean that the comparisons would not possible (Lovell *et al.*, 2004).

Relative humidity (RH) is also a major influence on the germination and development of *P. aphanis* spores; 100% RH is the most conducive condition for spore germination (Peries, 1962b, Peries, 1961, Jhooty and McKeen, 1965, Jhooty and McKeen, 1964b, Jhooty and McKeen, 1964a, Peries, 1962a). The amount of spores germinating reduces when RH falls below 95%. On detached leaves at 20°C the most germination occurred at 100% RH. The amount of germination reduced until 75% RH, after which the amount of conidia germinating remained constant at 5% (Amsalem *et al.*, 2006). Peries (1962a) found that RH does not affect the development of the fungus after germination. 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 the germination of the majority of conidia (Table 1.6).

Using spore traps, Peries (1962a) found that the majority of conidia are released between 12.00 and 16.00 hours and the least between 20.00 and 08.00 hours. Rain reduces the number of air-borne conidia greatly and 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 from their source and vertically within 3 feet (Peries, 1962a). Relationships between environmental conditions, incidence of powdery mildew in strawberry and concentrations of *P.*

aphanis conidia in the air have been described recently for US conditions (Blanco *et al.*, 2004), they were similar to the results described by (Peries, 1962a). All the conditions affecting the germination and growth of *P. aphanis* are summarised in Table 1.6. Table 1.7 summarises laboratory work by (Peries, 1962b) on development times of *P. aphanis*.

1.4.4 Life cycle of *P. aphanis*

Fungal epidemics fall in to two different types. Either monocyclic, where there is just one generation of the pathogen each season e.g. many soil-borne pathogens, or polycyclic where there are many generations in a season e.g. airborne foliar pathogens, such as rusts, powdery mildews and potato late blight (Lucas, 1998). The faster that a polycyclic pathogen completes each life cycle the faster that disease levels build up. Every species develops at different rates. Therefore the time each cycle would take would depend on the species of pathogen. It is important to understand how long each generation of a polycyclic epidemic would take. This data can be used to model and predict the speed that infection will develop at. The development time for *P. aphanis* was determined from laboratory experiments (Table 1.7). Using the measurements of Peries (1962b), the total development time can be broken down in to the key developmental milestones for the fungus.

Table 1.6. Summary of conditions that effect the life cycle of *P. aphanis* (data obtained from laboratory observations)

		Germination	Infection	Sporulation
Temperature (°C)	Minimum	5[1], 3[4], 2[6]	5[4,5,6]	13[6]
	Optimum	15-25[1,4], 18-25[5] (15*)18-22.5[6]	18-30[6]	20[4]
	Maximum	35[1], 38[4], 30-35[6]	30[4,6]	35[4]
Relative humidity (%)	Minimum	8[2], 12[6]	No effect[5,6]	No effect[5,6]
	Optimum	100[1,3,5], 97[6]	No effect[5,6]	No effect[5,6]
	Maximum	100[1,2,3,6]	No effect[5,6]	No effect[5,6]
Presence of free water (immersion time hours)		Up to 3[6]	No effect[5,6]	No effect[5,6]
Time of day (hours)	Minimum	No effect[6]	No effect[6]	20.00-8.00[6]
	Maximum	No effect[6]	No effect[6]	12.00-16.00[2,6]

[1] (Amsalem *et al.*, 2006), [2] (Blanco *et al.*, 2004), [3] (Jhooty and McKeen, 1964b), [4] (Jhooty and McKeen, 1965), [5] (Miller *et al.*, 2003) and [6] (Peries, 1962a)
* Radial growth is slow at 15°C but maturity is reached in the same time as at 18°C

Table 1.7 Time for development of major stages in fungal infection. Compiled from laboratory work (Peries, 1962b)

Life Cycle Stage	Time since inoculation (hours)	Development time since previous phase
Conidia germinate	4-6	
Appressorium formed	12	6
Host penetration	20	8
Haustoria developed	36	16
Conidiophore start to form	96	60
Conidiophores fully developed	120	24
Lesion visible to naked eye	144	24

1.4.5 Origins and genetics of the cultivated strawberry

The modern strawberry, *Fragaria* x *ananassa* Duchesene, is of recent origin (Maas, 1998). The progenitor of the modern strawberry came into being around 1750 when the North American *F. virginiana* Duchesene and the South American *F. chiloensis* (L.) Duchesene were brought together and hybridized. Following further hybridizations since 1850 the modern *F.* 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 of x = 7 (Sargent *et al.*, 2004). Within the genus there are four groups based on their chromosome numbers; diploid, tetraploid, hexaploid or octoploid (Marta *et al.*, 2004). The cultivated strawberry (*F.* x *ananassa*) is octoploid (2*n*=8*x*=56) (Teruko *et al.*, 2006, Sargent *et al.*, 2004). The majority of genes in modern cultivars came from only 7 nuclear and 10 cytoplasmic sources (Hancock *et al.*, 2002). Wild *Fragaria* species cover a wide geographical range that encompasses a large number of potential biotic and abiotic stresses. This gene pool could act as an extensive reservoir of new flavours, resistance to abiotic stresses and tolerance to diseases and pests (Hancock *et al.*, 2002). The wild *Fragaria* species *F. vesca*, *F. moschata* and *F. viridis* are native species to Britain and Europe (De Rougemont, 1989, Stace, 1991).

In the UK, crop improvement programs have focused on breeding strawberries for the appearance, quality (shelf life) and yield of the fruit produced, these are

the traits that are important to the supermarkets. Breeding for disease resistance is not a high priority of the current breeding programs. Once a variety has been selected the breeder will assess the level of disease resistance in the line, so they can give the growers some information on how much disease control will be needed.

1.4.6 Prediction of disease incidence and disease modelling

Weather-based predictive models can be classed as either mechanistic (based on observed phenomenon, can be very complex) or empirical (derived from data mining) (Pietravalle *et al.*, 2003). Whereas epidemiological models (similar to weather based models as they are based on environmental (weather) data) can be classified in to three types, descriptive (generalized experimental results, do not reveal the underlying mechanisms), predictive (also descriptive but predicts occurrence and severity) and conceptual (representations of underlying biological and ecological processes) (Van Maanen and Xu, 2003). Authors have produced models that aim to predict disease pressures at the end of the season from conditions early in the season for powdery mildew of sugar-beet (Asher and Williams, 1991) and powdery mildew of jujube (Sinha, 2005). For grape powdery mildew detailed models have been produced that model the growth of the plant as well as the fungus (Chellemi and Marois, 1991, Sall, 1980). For other powdery mildews papers have been published that detail work under controlled conditions to produce just one formula that would form part of a larger mechanistic or

conceptual model e.g. effect of temperature on latent period for, rose (Xu, 1999a, Xu, 1999b), apple (Xu, 1996, Xu, 1999c), clematis (Xu and Robinson, 2001) and hawthorn (Xu and Robinson, 2000). A field based model to be used with wheat disease management decision support systems has been developed for 4 foliar diseases including powdery mildew (Audsley *et al.*, 2005). This model was developed from field based observations and only uses inputs that would be available to most growers.

When producing disease models they need to be problem specific, the problem needs to be defined (Van Maanen and Xu, 2003). In addition the source of the inoculum, the latent period, host dynamics, nutrition status and environmental factors need to be considered when modelling fungal infections (Vallavieille-Pope *et al.*, 2000, Van Maanen and Xu, 2003). Polycyclic diseases need to be broken down and modelled as monocyclic diseases where several cycles are joined together to form the model of the polycyclic infection (Vallavieille-Pope *et al.*, 2000).

Unfortunately however complex models are seldom used in disease forecasting. Many of them are probably too complicated for practical use (Vallavieille-Pope *et al.*, 2000). This might suggest that there was a case for a simpler empirical 'model'. To date there appear to be no models or prediction systems for strawberry powdery mildew reported in the literature.

1.5 Aims and objectives

1.5.1 Aims

1. Identification and confirmation of symptom progression linked to *P. aphanis* infections

2. Identify the source of fungal inoculum responsible for initiating primary outbreak of disease and follow the development of the subsequent epidemic

3. Development of rule based prediction system to predict high risk periods for infection by *P. aphanis*

4. Identification of more efficient control methods for *P. aphanis*

1.5.2 Objectives

1st Aim;

1.1. Establish if *P. aphanis* mycelium can be identified on leaves that are cupping or that have red blotches

1.2. Follow the build up and progression of *P. aphanis* symptom(s) on strawberry plants in the field

1.3. Develop a scoring method that incorporates all symptoms of *P. aphanis* infection

2nd Aim;

2.1. Identify the source of primary inoculum in a newly planted field

2.2. Identify the source of inoculum in an over wintered crop

2.3. Establish the rate of disease build up in newly planted and established fields

3rd Aim;

3.1. Identification of the temperature and relative humidity that favours development of *P. aphanis* infection from published literature

3.2. Compare conditions identified in the literature with conditions associated with initiation of disease development in the field

3.3. Development of scheme to predict high risk days for infection by *P. aphanis*

3.4. Compare the high risk periods identified by the prediction system with the dates growers applied control products

4th Aim;

4.1. Quantify the level of disease resistance in strawberry cultivars available to strawberry growers

4.2. Identify new products for the control of *P. aphanis*

4.3. Develop method to reduce initial inoculum in newly planted sites

1.6 Field sites

Data for this project was gathered from four field sites located near Colchester, Kings Lynn, Mereworth and Wisbech (Fig. 1.4). Experimental trials were established on three of the sites (Colchester, Mereworth and Wisbech).

At Mereworth, Kent (Grid reference: TQ 679 536) an experimental field was setup and managed by the grower. This was located several miles away from the other fields of strawberries commercially managed by this grower. The site was used in 2004 and 2005. The environmental conditions were recorded both internally and externally.

The experimental plots on the Colchester, Essex (Grid reference: TM 071 300) and Wisbech, Cambridgeshire (Grid reference: TF 459 037) sites were located within fields that were managed commercially by the growers. The tunnels used for experimental work were located at the end of the site in each occasion and separated by plastic sheets from the commercial parts of the sites. The Colchester site was used for experimental work in 2006. The grower also supplied field management data for the part of the site that was managed commercially. Experimental tunnels were established on the Wisbech site in 2005 and 2006. The grower also supplied field management data from 2004, 2005 and 2006. The internal and external environmental conditions were

recorded in 2006 at the Colchester site and in 2004, 2005 and 2006 in Wisbech site.

The Kings Lynn, Norfolk (Grid reference: TF 724 179) site was a propagation field. The environmental conditions (external only as propagation fields are not covered) were monitored and field management data was obtained from the grower for 2005.

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Fig. 1.4 Locations of field sites from which data was gathered for this project 1) Colchester, 2) Kings Lynn, 3) Mereworth and 4) Wisbech

Chapter 2 - Symptoms and scoring methods

2.1 Introduction

Strawberries infected by *P. aphanis* progress through a range of symptoms. The first symptom visible in the field is leaf cupping, followed by mycelium on the abaxial surfaces, development of red blotches and finally leaf senescence (Blanco *et al.*, 2004, Scott *et al.*, 1970, Salmon, 1900). In the literature mycelial growth and red blotches have only been reported as present on the abaxial leaf surface. However, strawberry growers have witnessed mycelium and red blotches on the adaxial surfaces. Mycelium is the only definite symptom measurable in the field. Leaf cupping is also a symptom of drought stress and there are other fungal pathogens that cause red or purple blotches on the leaves of strawberry plants (Anon., 2004a, Maas, 1998, Salmon, 1900). Mycelium can develop on the fruit if the infection if not treated.

Growers need to be able to identify infection by *P. aphanis* as soon as possible if they are to control the infection in the most efficient and cost effective way. They often identify that their crop has an infection when they see mycelium. They often see that there is leaf cupping in their fields but some times misdiagnose this as drought stress. The early stages of an epidemic, are where there is the greatest multiplication of the pathogen (Zadocks and Schein, 1979). The initial lag phase of an epidemic is where the inoculum spreads throughout the field. When the

grower sees that a few plants have mycelium they start to treat the infection believing they are applying products when there is only minimal inoculum, but the inoculum is much more widely spread throughout the tunnel, so the grower is much less likely to be able to control it. The initial lag phase is the stage where fungicidal treatments would be most effective. If growers are not starting to treat infection until they can see visible mycelium they are losing all the time from when the leaf cupping first develops, in which they could be treating their crop.

2.1.1 MAFF Strawberry Powdery Mildew Key 8.1.1

The MAFF Strawberry Powdery Mildew Key 8.1.1 (Appendix 4) was produced in 1976 based on observations from cultivar Royal Sovereign. The key is designed to measure disease severity on the field scale using the red blotch symptom which the key states, is visible at harvest. As a consequence the key is most usefully deployed for measuring the impacts on yield. Its value for detailed epidemiological studies or for monitoring disease for the purposes of crop management is less certain. Red blotching is associated with well established *P. aphanis* infection. The key identifies leaf cupping as first symptom of infection, but does not use mycelium on the basis it is very difficult to see. No matter how much leaf cupping there was present, use of the key would only result in the infection level being classified as 5% and the infection level would have been classified as a lot lower if only a proportion of the leaves sampled were cupping,

for infection to be classified as greater than 5% red blotches needed to be present.

2.2 Aim + objectives

2.2.1 Aim

Identification and confirmation of symptom progression linked to *P. aphanis* infections (1st aim, page 27)

2.2.2 Objectives

1. Establish if *P. aphanis* mycelium can be identified on leaves that are cupping or that have red blotches

2. Follow the build up and progression of *P. aphanis* symptom(s) on strawberry plants in the field

3. Develop a scoring method that incorporates all symptoms of *P. aphanis* infection

2.3 Methods

2.3.1 Identification of *P. aphanis* symptoms in the field

Strawberry leaves were collected from cultivar Elsanta plants from three fields at the Wisbech site in 2006. Field A had extensive visible mycelium when the leaf samples were taken, field B had very little mycelium visible and field C had no visible mycelium or symptoms. From each field, eight leaves were sampled for each of the three following conditions: flat (asymptomatic), cupping and red blotches. Samples were taken from field A on the 08/08/06, 22/08/06 and 30/08/06. On the first sample date only flat and cupping leaves were taken (no red blotches present). Samples were taken from field B on the 30/08/06 and 05/09/06. Samples were frozen in liquid nitrogen then stored in a freezer at -70°C.

Leaves were removed from the -70°C freezer as they were scored. Each leaf was separated in to leaflets (3 per leaf) which were 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 in to 4 longitudinal strips. Of the 4 strips 2 were placed on microscope slides adaxial surface up and 2 were placed abaxial surface up. The length of the centre of each strip was measured. 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 distinct *P. aphanis* colonies and area of the leaf surface covered by *P. aphanis* mycelium was recorded. This was repeated for the remaining 3 strips of leaf material. The process was repeated with the next 2 leaflets.

The data were analyzed for statistical differences between the amount of symptom on the adaxial and abaxial leaf surfaces and for differences between the different symptom types collected at each sampling date at the 5% level using the Mann-Whitney *U* Test in SPSS for windows 11.5.0, SPSS Inc.

2.3.2 Development of alternative scoring method

One healthy (symptomless) newly developed leaf was tagged on 28/07/04 using small plastic cable ties on each of 80 strawberry plants, cultivar Elsanta, grown in a polythene tunnel on the Mereworth site 2004. The tunnel was managed commercially except that there were no applications of fungicides. Disease symptoms were scored on the leaves for the first time on the 11/08/04 and for the last time on the 01/09/04. The leaves were scored on a total of 4 occasions, one week apart.

The leaves were scored for the presence or absence of leaf cupping, the percentage of the adaxial and abaxial leaf surfaces covered with mycelium and

the percentage of the adaxial and abaxial leaf surfaces covered with red blotches.

Area under the disease progress curve (AUDPC) was used to identify statistical differences at the 5% level between the disease progress curves when infection development was measured by just red blotching or red blotching and mycelium in Genstat, 8th Edition, VSN International Ltd. Differences between the percentage of the adaxial and abaxial leaf surfaces covered with either red blotches or mycelium were tested using the Mann-Whitney *U* Test in SPSS for windows 11.5.0, SPSS Inc. The data were also analyzed to identify if the amount of red blotching or mycelium on the adaxial surface was related to the amount on the abaxial surface using a Spearman's rank-order correlation coefficient in SPSS for windows 11.5.0, SPSS Inc.

2.3.3 Progression of *P. aphanis* symptoms

The progression of symptoms from mycelium to red blotches on leaves was analyzed. Using the data collected from the 80 tagged leaves described above (section 2.3.2). Spearman's rank-order correlation coefficient (SPSS for windows 11.5.0, SPSS Inc) was used to test whether any relationship existed between the presence of mycelium on the 11/08/04 (1st date scored) and red blotches present on the 18/08/04, 25/08/04 and 01/09/04.

The location of plants with symptoms were recorded in a newly planted tunnel on the Mereworth site 2005 (see section 3.3.2.3 including Fig 3.7). At each sample date each plant was scored as either healthy (no symptoms), cupped leaves, mycelium present or red blotches. The symptoms were assumed to be progressive, so only the most advanced was scored per plant per sample date, e.g. if a plant had cupped leaves and mycelium present, only the later was recorded. Disease patterns were mapped using ArcGis (ESRI Corporation, Redland California, USA), which is a geostatistical software system. A map for each symptom was produced for each sample date. The spatial patterns of each symptom were analysed using SADIE (spatial analysis by distance indices) developed and supplied by J. N. Perry (Rothamsted Experimental Station). The suite of SADIE programs was developed to analyse the distribution of count based ecology data but has found a use in plant pathology (Winder *et al.*, 2001, Xu and Madden, 2003). SADIE analyzes the degree of clustering in the data, in the form of patches and gaps. The software randomly produces disease maps and compares them with the observed data. From this the program calculates the likelihood that the spatial pattern arose by chance (Perry *et al.*, 1996). The SADIE outputs were analysed for associations between different dates and/or symptoms using the association function of the SADIE program (Perry and Dixon, 2002, Winder *et al.*, 2001).

Within the tunnels each bed was divided into $1m^2$ quadrats, so that each quadrat contained 7 plants. This transformed the data so the data was in a form suitable

for analysis by the SADIE program by reducing the total number of data points (there would have been too many data points if each plant had been an individual data point). The maps of the symptoms are representations of individual plants rather than the quadrat (Fig 2.9).

2.4 Results

2.4.1 Identification of *P. aphanis* symptoms in the field

The data from the leaves that were collected and stained is presented in Fig 2.1 for field A and Fig. 2.2 for field B. Each figure shows the amount of mycelium present on the adaxial and abaxial leaf surfaces that were flat, cupped or had red blotches. For both the adaxial and abaxial leaf surfaces, data are presented as the number of colonies per square centimetre, and the percentage of the leaf surface covered by mycelium. The number of colonies per square centimetre was calculated from the total number of colonies observed divided by the total leaf area scored.

At both sites there were more colonies on the leaves the later in the year the sample was collected (Fig 2.1 and 2.2). At both sites and on all sample dates there were more colonies on the leaves that were cupping or had red blotches than there were on the flat leaves (Fig 2.1 and 2.2). There was more infection on the abaxial surface of the leaves than the adaxial surface (Fig 2.1 and 2.2). In

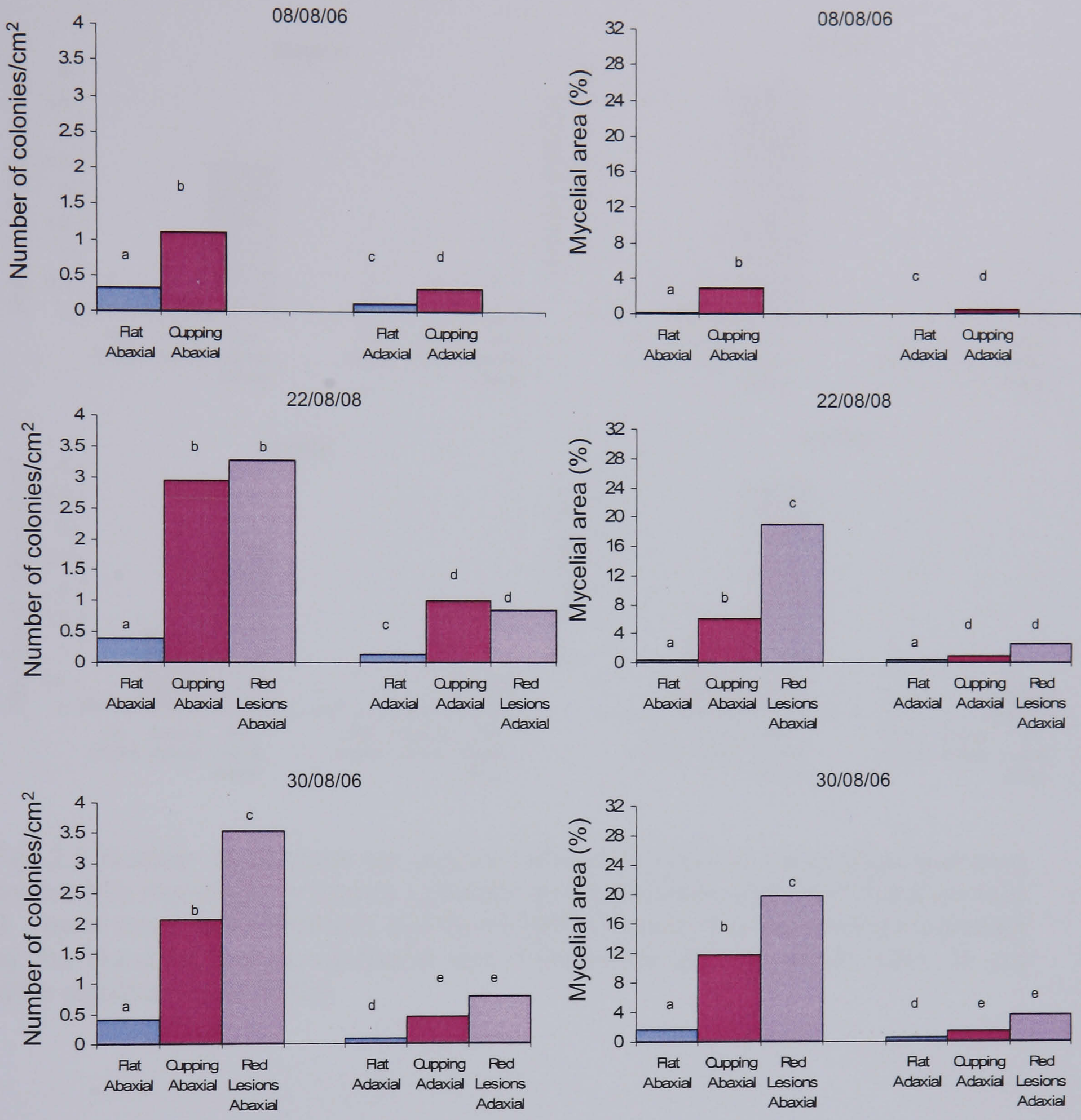


Fig. 2.1 Number of colonies per square centimetre and the percentage leaf area covered by mycelium for leaves collected on the 08/08/06, 22/08/06 and 30/08/06 from field A. Lower case letters indicate significant differences at the 5% level as indicated by the Mann-Whitney *U* statistical test. Treatments with the same letter do not differ significantly (P=0.05)

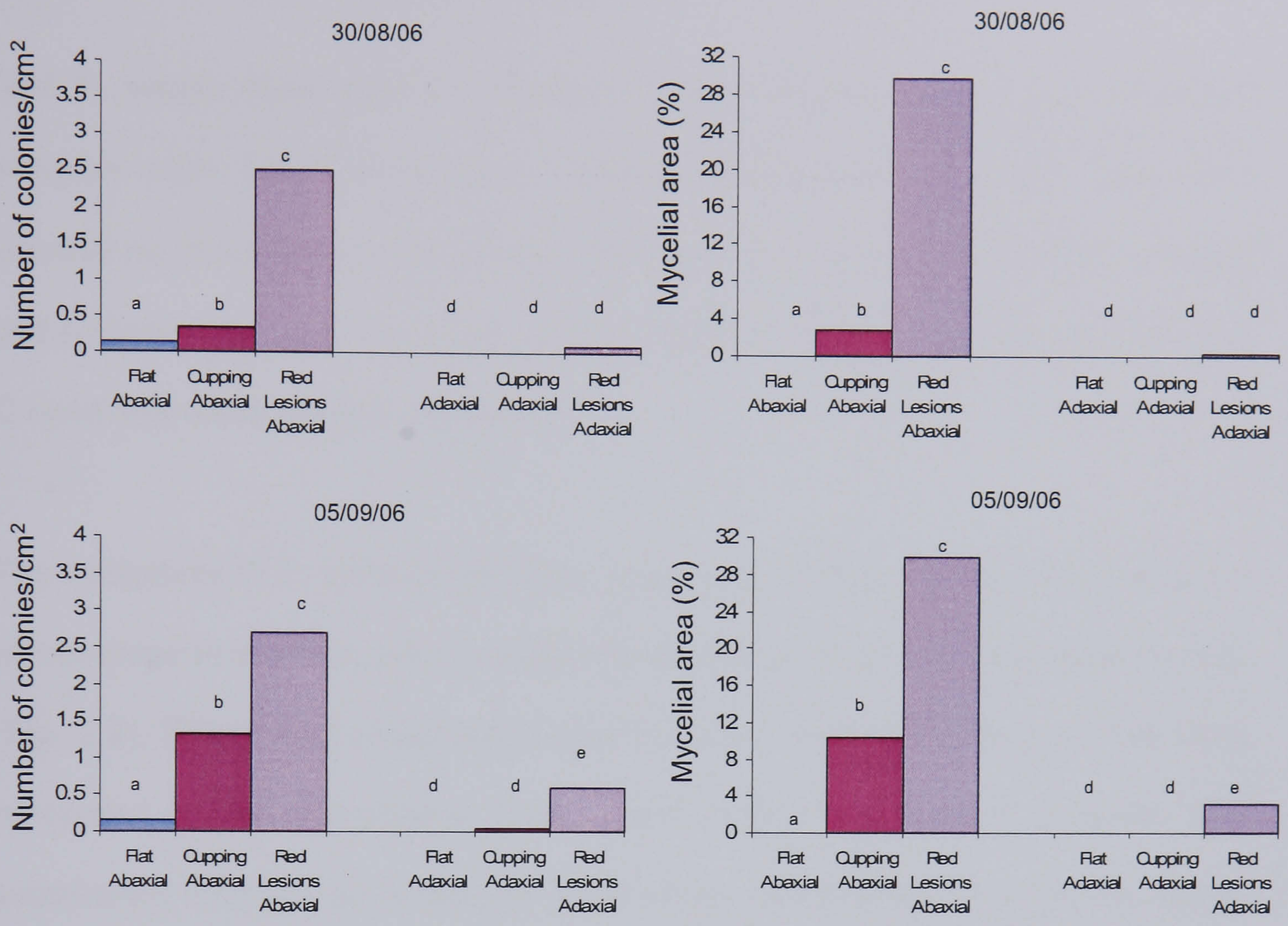


Fig. 2.2 Number of colonies per square centimetre and the percentage leaf area covered by mycelium for leaves collected on the 30/08/06 and 05/09/06 from field B. Lower case letters indicate significant differences at the 5% level as indicated by the Mann-Whitney *U* statistical test. Treatments with the same letter do not differ significantly (P=0.05)

field B, where there was no mycelium visible with the naked eye when the samples were taken microscopic examination showed that whilst there was virtually no mycelium on the adaxial leaf surface, it was present on the abaxial leaf surfaces (Fig 2.2). No mycelium was visible on the leaves collected from field C once they were stained and scored.

The symptoms of *P. aphanis* infection, cupping, mycelium and red blotches were all observed in the field, both in experimental and commercially managed tunnels (Fig. 2.3). These field observations are consistent with the symptoms that were measured in the microscope study. Once a field was heavily infected with established infection all symptoms were easily visible within the field on plants with differing levels of infection. Mycelium was visible even if it was on the lower leaf surface as the leaf had cupped up by that point. Mycelium was very rarely seen on fruit as the growers controlled infection in the commercial tunnels before the infection progressed to the fruit and the fruit was removed from the experimental tunnels to stop it from rotting on the plant.

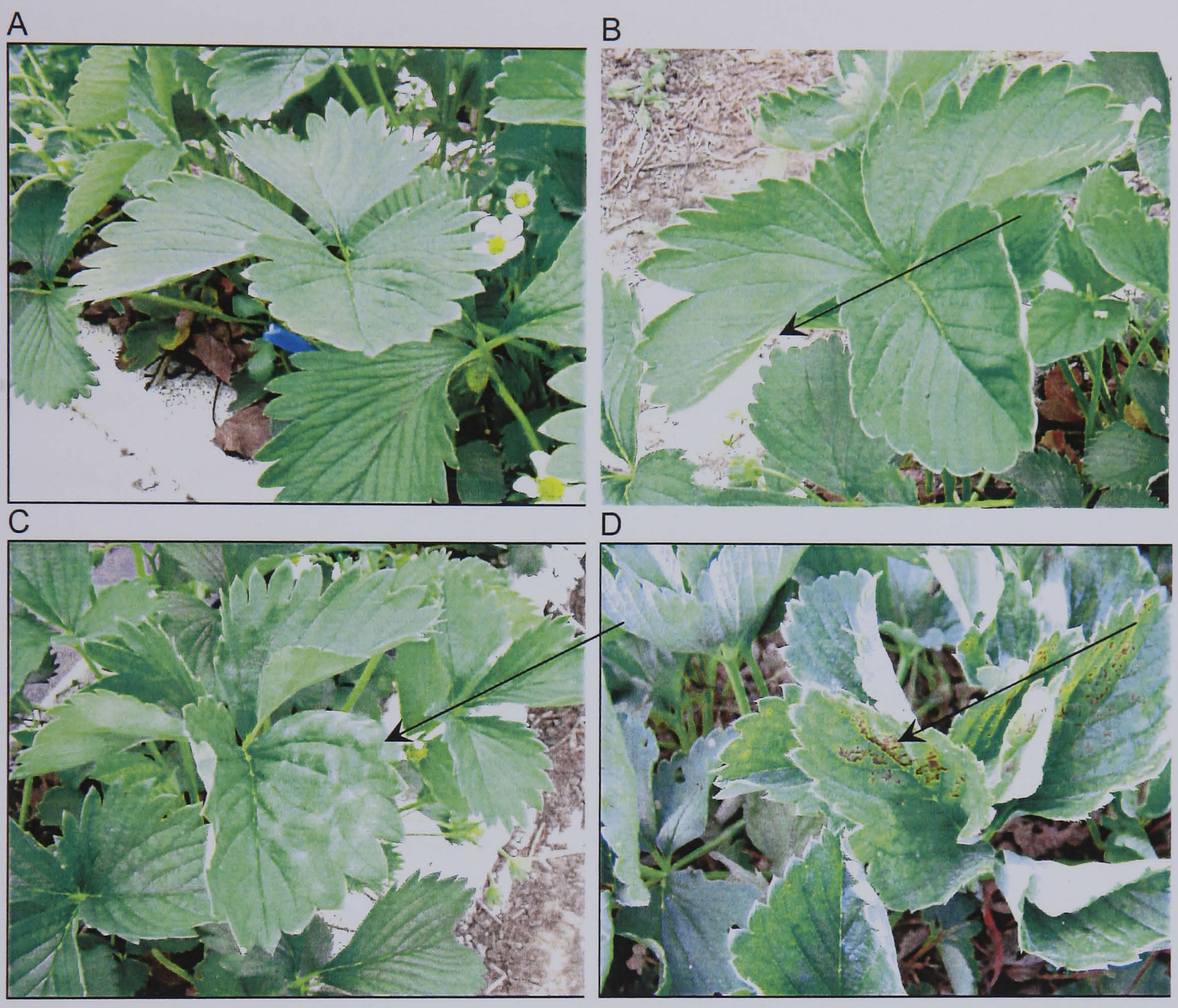


Fig. 2.3 A. Healthy leaf on cultivar Elsanta B. Cultivar Elsanta showing cupping C. Cultivar Elsanta showing mycelium D. Cultivar Elsanta showing red blotches. (Arrows identify the symptom)

2.4.2 Development of alternative scoring method

92.5% of the tagged leaves were cupping at the first sample date. The last time the leaves were scored 96.25% of the leaves were cupping. Fig 2.4 shows the development of infection when scored by either red blotches or red blotches and mycelium. When scored by just red blotches the infection developed at a significantly slower rate (AUDPC) than when scored by both red blotches and mycelium (Fig 2.4). When infection was measured by red blotches and mycelium the detectable onset of the epidemic occurred 16 days sooner than if the development of infection was measured by just red blotches (Fig 2.4).

Mycelium was observed before the development of red blotches. Mycelium was present on both the adaxial and abaxial leaf surfaces on the 11/08/04 (Fig 2.5) but no red blotches were present (Fig 2.6). More mycelium was present on the 18/08/04 than on the 11/08/04. The level of mycelial infection decreased by the 25/08/04 to an amount similar to that recorded on the 11/08/04. By the 01/09/04 the amount of mycelium was much smaller than that present on the other sample dates (Fig 2.5). There was significantly more mycelium on the abaxial leaf surface than the adaxial leaf surface on the first 3 sample dates (11/08/04, 18/08/04 and 25/08/04), but there was no difference in the amounts on these surfaces by the 01/09/04.

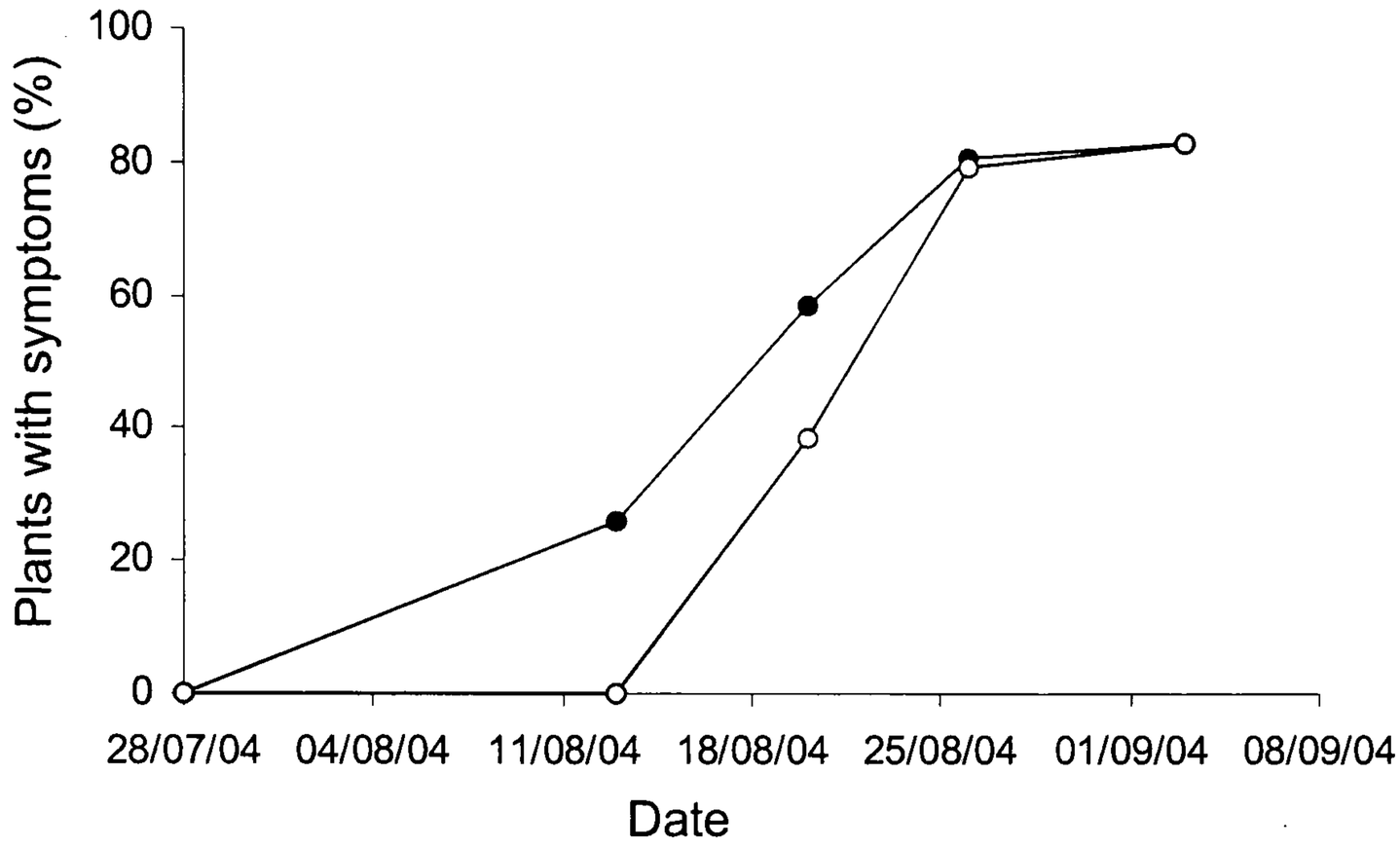


Fig. 2.4 Percentage of plants with red blotches (○) and percentage of plants with mycelium and red blotches (●) from the Mereworth site 2004. Infection development was significantly slower when measured by just red blotches using AUDPC

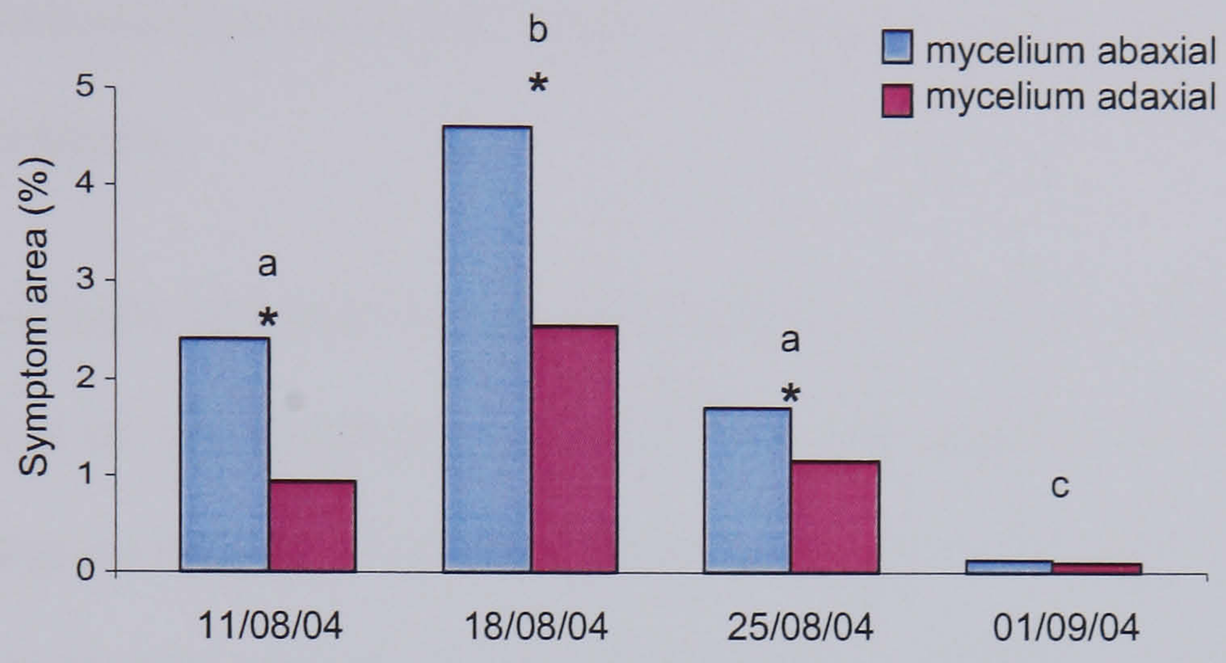


Fig. 2.5 Percentage of abaxial and adaxial leaf surfaces covered by mycelium from Mereworth site 2004. * indicates significant differences between the amount of mycelium on the abaxial and adaxial surfaces at the 5% level as indicated by the Mann-Whitney *U* test. Lower case letters indicate significant differences between the total amounts of mycelium on each sample date at the 5% level as indicated by the Mann-Whitney *U* test

Fig. 2.6 Percentage of abaxial and adaxial leaf surfaces covered by red blotches from Mereworth site 2004. There were no significant differences between the amount of red blotches on the abaxial and adaxial surfaces. Lower case letters indicate significant differences between the total amounts of red blotches on each sample date at the 5% level as indicated by the Mann-Whitney *U* test

Red blotches were first observed on the 18/08/04 (Fig 2.6). There were no significant differences between the amount of infection visible on the abaxial and adaxial leaf surfaces.

For both the amount of mycelium on the abaxial and adaxial surfaces (Fig. 2.7) and the amount of red blotches on the adaxial and abaxial surfaces (Fig. 2.8) there is a strong correlation. The amount of mycelium on the abaxial and adaxial leaf surfaces is strongly correlated (positively) ($P<0.01$) for the samples taken on the 11/08/04 ($r=0.624$), 18/08/04 ($r=0.760$) and 25/08/04 ($r=0.488$). The amount of red blotches present on the adaxial and abaxial leaf surfaces are also very strongly correlated (positively) ($P<0.01$) for the samples taken on the 18/08/04 ($r=0.899$), 25/08/04 ($r=0.974$) and 01/09/04 ($r=0.992$). The statistic given by Spearman's rank-order correlation is called r. The r value ranges from -1 (perfect negative correlation) to 0 (no correlation) to 1 (perfect positive correlation) so the r values can also be used to refine the level of correlation. The correlation is weaker the closer the r value is to 0.

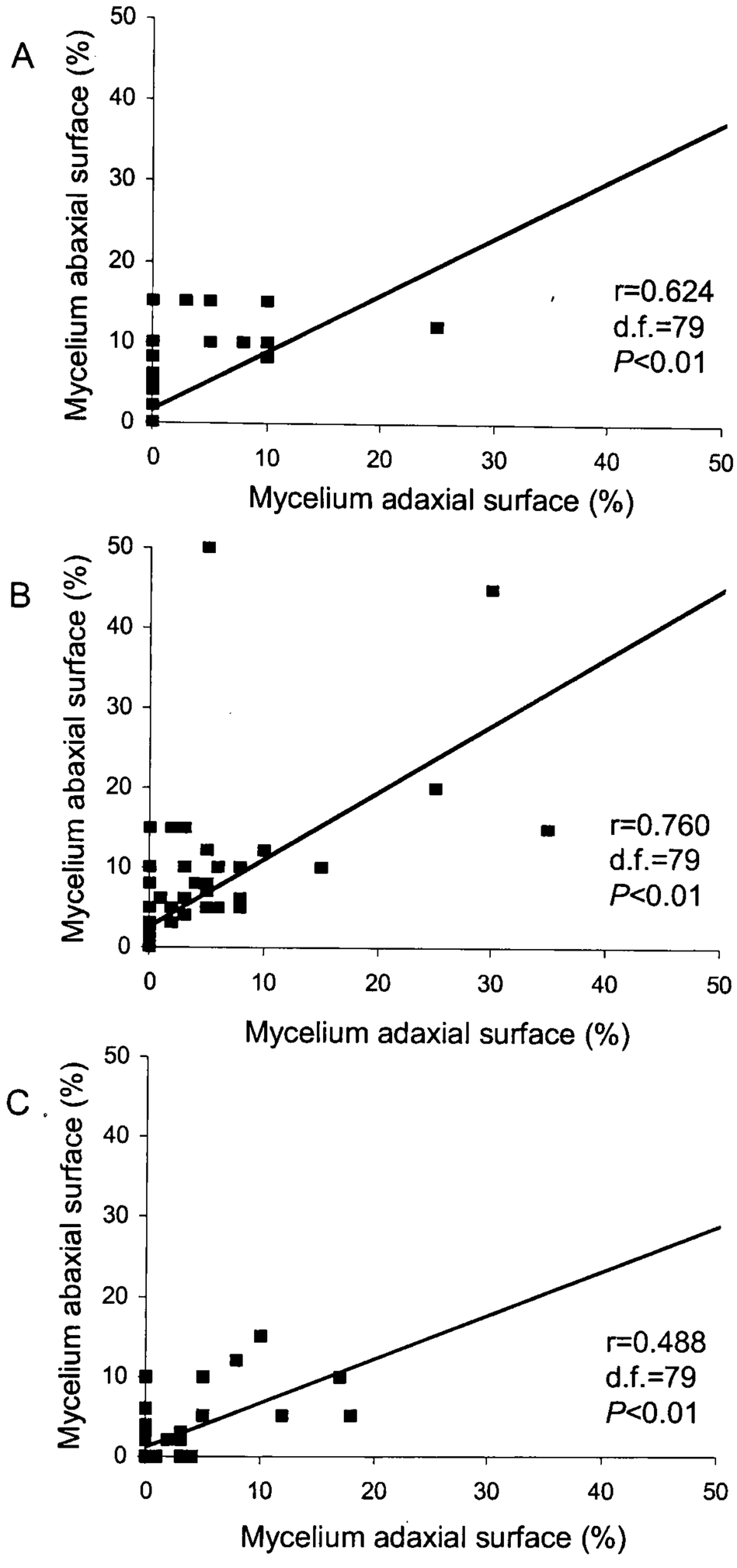


Fig. 2.7 Relationship between percentage of abaxial leaf surface covered with mycelium and the percentage of the adaxial leaf surface covered with mycelium for A) 11/08/04, B) 18/08/04 and C) 25/08/04 quantified using a Spearman's rank-order correlation coefficient

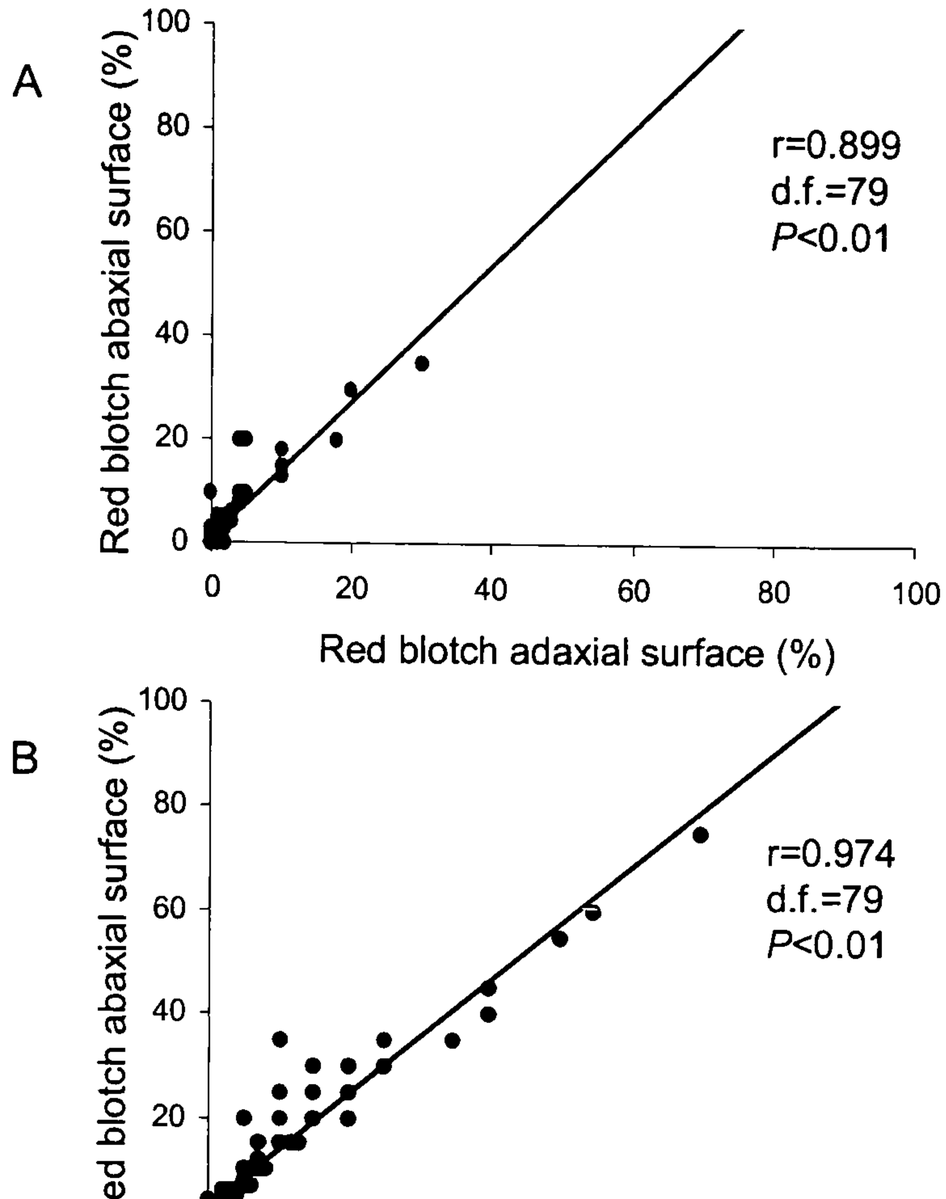


Fig. 2.8 Relationship between percentage of abaxial leaf surface covered with red blotches and the percentage of the adaxial leaf surface covered with red blotches for A) 18/08/04, B) 25/08/04 and C) 01/09/04 quantified using a Spearman's rank-order correlation coefficient

2.4.3 Progression of *P. aphanis* symptoms

The amount of red blotches present on the abaxial and adaxial surfaces of the tagged leaves on the 18/08/04, 25/08/04 and 01/09/04 were correlated with the amount of mycelium present on both the adaxial and abaxial surface on the 11/08/04 (Table 2.1). All possible combinations were significantly correlated when considering the *P* value ($P<0.01$). The 'r' values show that the amount of red blotches on the 18/08/04 were, correlated more strongly with the amount of mycelium on the 11/08/04 than the red blotches were on the 25/08/04 and 01/08/04. Also the amount of mycelium on the abaxial leaf surface was more strongly correlated with the amount of red blotches than the amount of mycelium on the adaxial leaf surface was. This shows that the leaves on which red blotches develop on are associated with the leaves where mycelium was present.

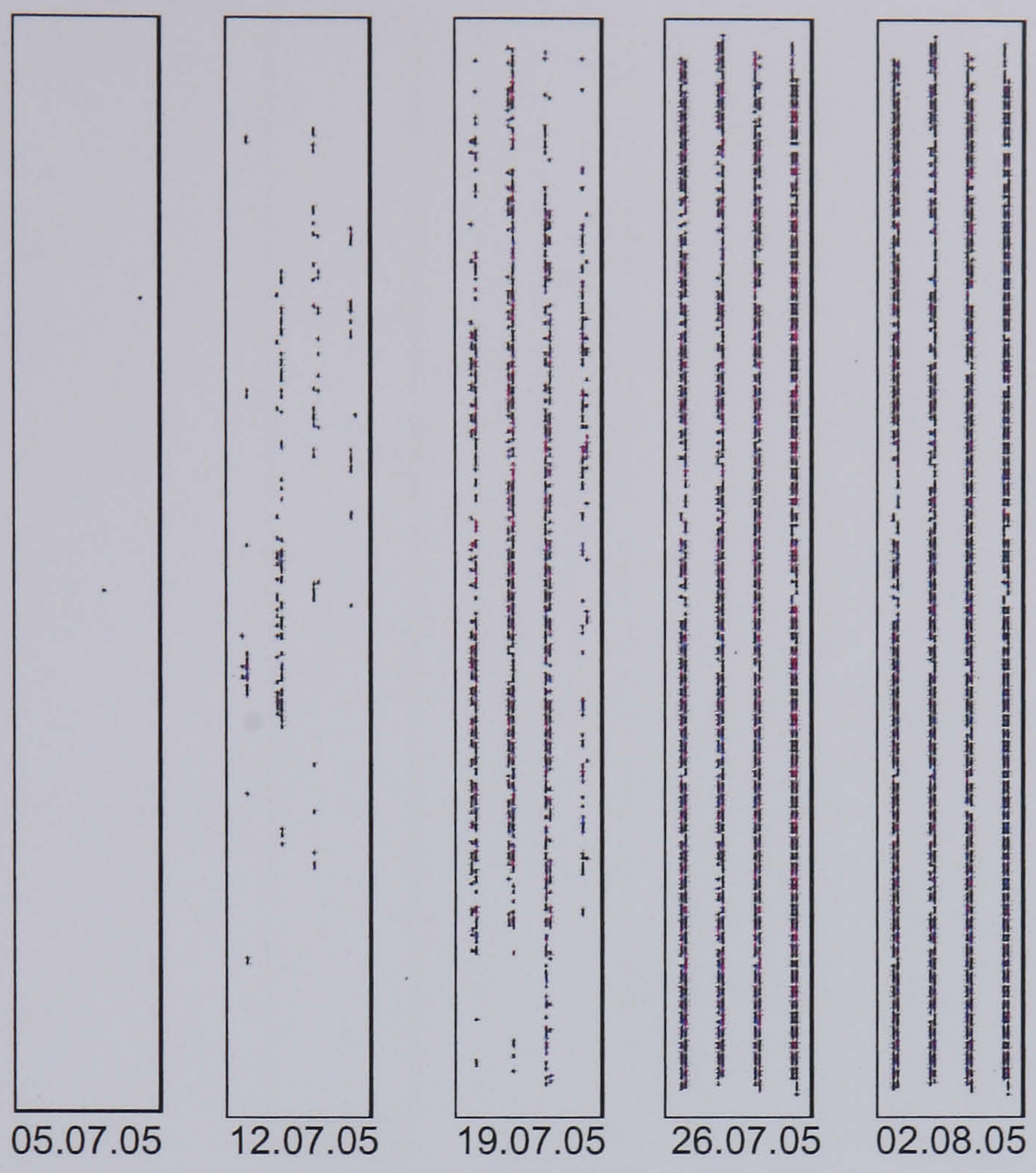
The location of plants with symptoms of *P. aphanis* were mapped for a tunnel of strawberry plants (Mereworth site 2005), on five dates and different symptoms, cupping, visible mycelium and red blotches. The three symptoms scored were assumed to be progressive, so once mycelium was visible on a cupping leaf it was only scored for mycelium. A representation of the tunnel has only been drawn for each symptom when there were plants with that symptom present. Table 2.2 details the number and percentage of plants with each symptom at

Table 2.1 Relationship between the leaves with mycelium present on the 11/08/04 and the leaves that had red blotches present on the 18/08/04, 25/08/04 and 01/09/04 using a Spearman's rank-order correlation coefficient

	Mycelium adaxial leaf surface 11/08/04		Mycelium abaxial leaf surface 11/08/04	
	r	*P*	r	*P*
Red blotches adaxial leaf surface				
18/08/04	0.443	0.000	0.752	0.000
25/08/04	0.378	0.001	0.547	0.000
01/09/04	0.360	0.001	0.445	0.000
Red blotches abaxial leaf surface				
18/08/04	0.362	0.000	0.738	0.000
25/08/04	0.331	0.003	0.496	0.000
01/09/04	0.348	0.002	0.437	0.000

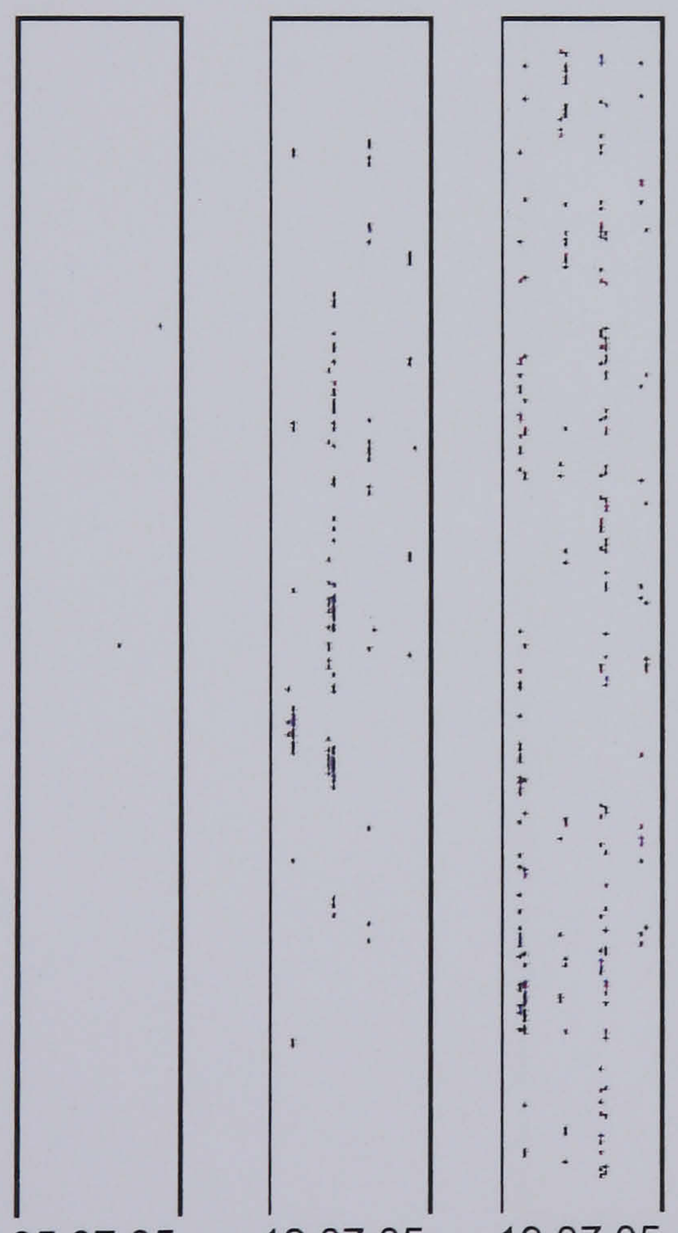
each sample date. Associations between the locations of plants with symptoms and between each sample date are presented in Table 2.3. The locations of the first plants with the cupping symptom (05.07.05 and 12.07.05) were associated with the location of the first mycelium on the 12.07.05 and 19.07.05 as well as the location of the first red blotches 19.07.05 and 26.07.05. The development of the red blotches on the 19.07.05, 26.07.05 and 02.08.05 were associated with the locations of the mycelium symptoms on the 12.07.05.

A - all symptoms (cupping, mycelium and red blotches)



05.07.05 12.07.05 19.07.05 26.07.05 02.08.05

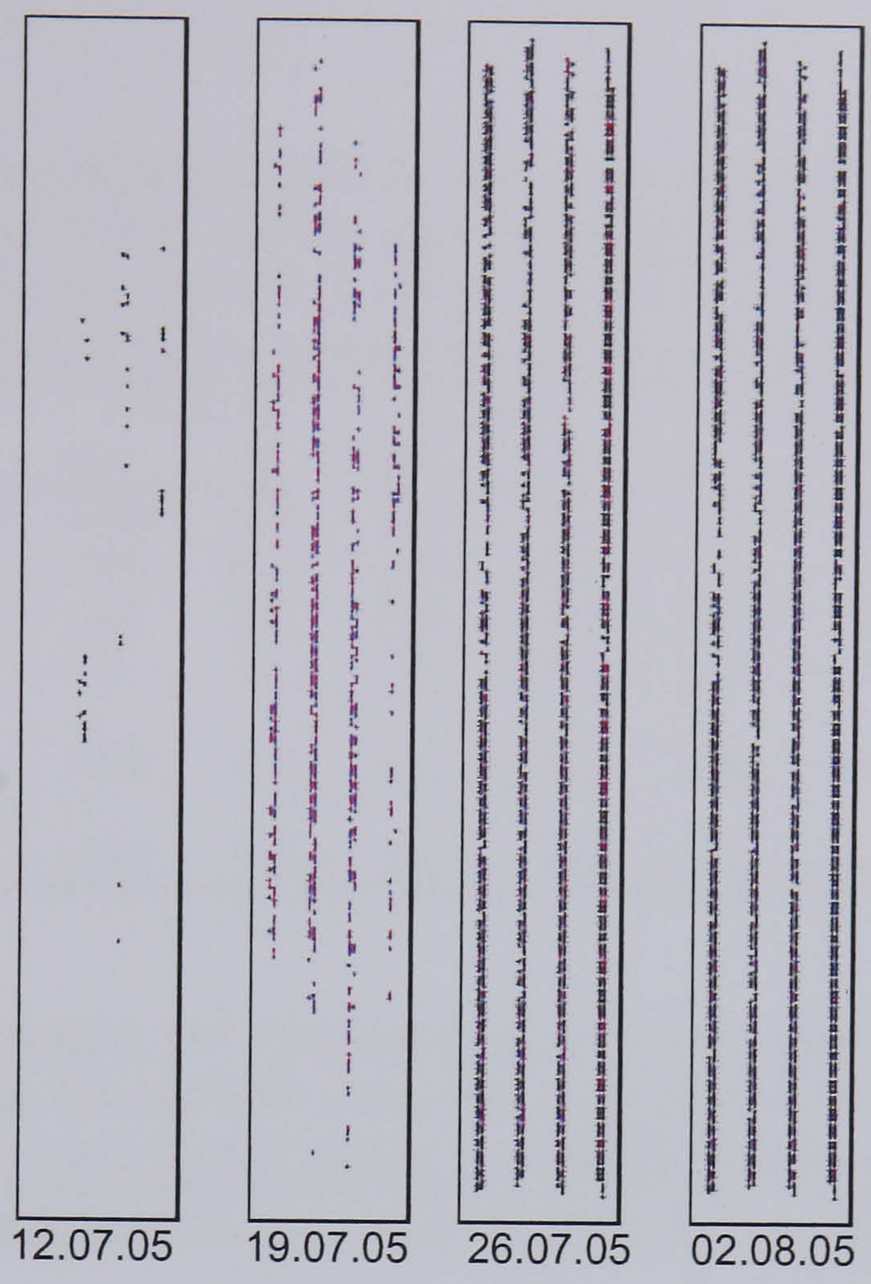
B - Leaf cupping



05.07.05 12.07.05 19.07.05

Fig. 2.9 continued on next page

C - Mycelium



D - Red blotches

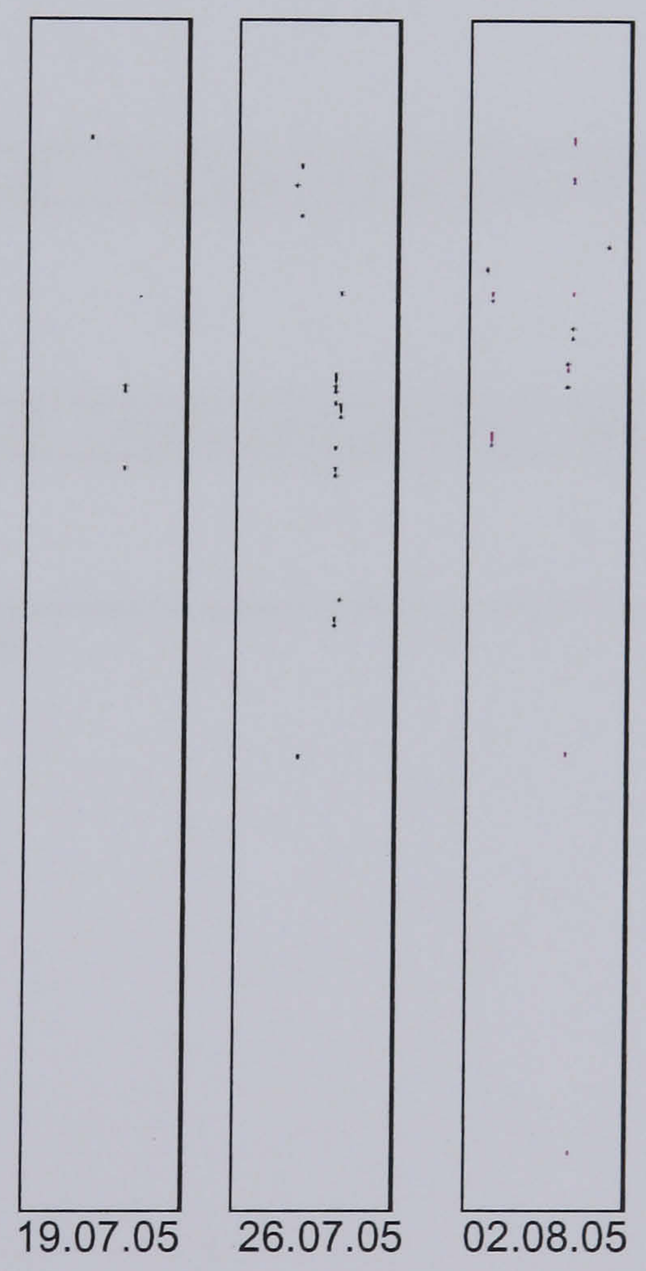


Fig. 2.9 (continued) Representation of a tunnel (Mereworth 2005) showing the location of plants with symptoms of strawberry powdery mildew. A) all symptoms combined, B) leaf cupping, C) mycelium and D) red blotches

Table 2.2 Number and percentage of plants at each sample date from Mereworth 2005 site with strawberry powdery mildew symptoms including plants that died (not necessarily due to strawberry powdery mildew). The information contained in this table is a numerical representation of the data presented in Fig. 2.9

	05.07.05		12.07.05		19.07.05		26.07.05		02.08.05	
	No.	%	No.	%	No.	%	No.	%	No.	%
No symptoms	1555	91.9	1404	83.0	723	42.5	72	3.8	64	2.5
Cupping only	2	0.1	108	6.42	224	13.32	1	0.1	0	0
Mycelium only	0	0	44	2.6	604	35.9	1463	87.0	1472	87.5
Red blotches only	0	0	0	0	4	0.24	19	1.13	19	1.13
Combined (all symptoms)	2	0.1	152	9.0	832	49.5	1483	88.2	1491	88.6
Dead	133	8.0	134	8.0	135	8.0	135	8.0	135	8.9

Table 2.3 Associations between the locations of different strawberry powdery mildew symptoms within the Mereworth site 2005 (as presented in Fig. 2.9), at the 5% level using the quick association tool in the SADIE software package

	Cupping 05.07.05	Cupping 12.07.05	Cupping19.07.05
Mycelium 12.07.05	*	*	
Mycelium 19.07.05	*	*	
Mycelium 26.07.05			
Mycelium 02.08.05			
	Red blotches 19.07.05	Red blotches 26.07.05	Red blotches 02.08.05
Mycelium 12.07.05	*	*	*
Mycelium 19.07.05		*	
Mycelium 26.07.05			
Mycelium 02.08.05			
	Red blotches 19.07.05	Red blotches 26.07.05	Red blotches 02.08.05
Cupping 05.07.05	*	*	*
Cupping 12.07.05	*	*	*
Cupping 19.06.05			

* indicates association at the 5% level

2.5 Discussion

2.5.1 Identification of *P. aphanis* symptoms in the field

Strawberry powdery mildew can express a range of symptoms, which appear as a progressive sequence (Blanco *et al.*, 2004, Salmon, 1900, Scott *et al.*, 1970). Healthy strawberry plants have flat leaves. The leaves start to cup as infection first establishes, sometime later visible mycelium appears on the leaves and finally red blotches form on the leaves (Fig. 2.3). If the infection is not treated the leaf may senesce prematurely. Of these symptoms the only one that can be linked to *P. aphanis* with certainty is visible mycelium, as cupping and red blotches could have other causes (Anon., 2004a, Maas, 1998, Salmon, 1900). Leaf cupping or leaf curl is also associated with infections of other hosts by powdery mildews. Leaves of apricots fold longitudinally when infected by powdery mildew and leaves of vine and peach curl up when infected (Spencer, 1978) as do the leaves of rose (Xu, 1999b). Red or rust coloured areas on the leaves are also associated with infection by powdery mildew on blueberry, mango and cherry (Spencer, 1978), soybean (Spencer, 1978, Yorinori *et al.*, 2004) and peach (Kable *et al.*, 1980).

Leaves with cupping and with red blotches were both found to have mycelium present (Figs. 2.1 and 2.2). More mycelium was present on cupping leaves than the flat leaves. When the amount of mycelium on the leaves with red blotches

was measured the number of colonies present was the same as on the cupping leaves but the amount of the leaf surface covered with mycelium was greater due to each being colony larger (Figs. 2.1 and 2.2). This is very important for the growers as it shows that control measures need to be taken to control the initial inoculum at the leaf cupping stage. The increase in colony size from cupping leaves to leaves with red blotches is comparably greater then the increase in the number of colonies from cupping leaves to leaves with red blotches (Figs. 2.1 and 2.2). Showing that while there is new infection of cupping leaves the increase in infection is mainly due to colony growth.

There was more infection on the abaxial side of the leaves than the adaxial side, $P>0.05$ (Figs. 2.1 and 2.2). Depending on the conditions, infection can be in an advanced stage before there are any visible signs of mycelium on the adaxial leaf surface. In field A mycelium was visible on the adaxial leaf surface (of some leaves) but in field B mycelium was not visible with the naked eye. The leaves from field B had a larger percentage of their abaxial surface covered with mycelium than the leaves from field A. Both fields were planted with cultivar Elsanta. Field B was a 3rd year crop that had been picked where as field A was a 1st year crop which was still being picked.

Leaf cupping is linked with *P. aphanis* infection. This should be the first symptom that growers and agronomists look for when assessing if a field of strawberries is infected by *P. aphanis*. The MAFF Strawberry Powdery Mildew Key 8.1.1 gave

leaf cupping as the first symptom of *P. aphanis* infection but no matter how much cupping there was it would have only resulted in a 5% infection level. To classify the presence of one symptom as indicative of a specific level of infection is not helpful when the symptoms form in a progression. This resulted in not enough significance being placed on leaf cupping. Leaf cupping is an indicator of the early stages of the epidemic, where there is the greatest multiplication of disease (Zadocks and Schein, 1979) and so a very important indicator of epidemic initiation.

The majority of infection is linked to the abaxial leaf surface (Figs. 2.1 and 2.2). When the leaf is cupping good spray coverage of the abaxial leaf surface would be required to effectively combat an infection at this stage. Even when the visible mycelium has disappeared and only red blotches are left there is still mycelium on the leaf surface. Once a grower has applied control products and the infection appears to be reducing, because only red blotches are left, they still need to apply control products as there is mycelium still on the leaves. *P. aphanis* infection is linked to leaves that are cupping and leaves that have red blotches.

2.5.2 Development of an alternative scoring method

The MAFF Strawberry Powdery Mildew Key 8.1.1 considered only cupping and red blotches as key factors in quantifying a *P. aphanis* infection. A grower needs to identify an infection as soon as possible so that they can start to apply suitable

control products and so slow the overall rate at which the epidemic progresses. If the presence of mycelium is used as the first indicator of an infection developing, the start of the epidemic could be identified two weeks sooner than if just red blotches were used (Fig. 2.4). There might just be a small amount of mycelium (Fig. 2.5) present at the start of the epidemic so the strawberry field would have to be monitored closely. If a grower could apply a control product when there was mycelium present rather than when there was red blotching (as recommended by the MAFF Strawberry Powdery Mildew Key 8.1.1) they might be able to slow the epidemic development and so extend the lag phase (Lucas, 1998), therefore reducing crop losses at harvest.

Mycelium developed before the red blotches (Figs. 2.5 and 2.6). There was significantly more mycelium on the abaxial leaf surface than the adaxial leaf surface but there were no significant differences between the amount of red blotches on the abaxial leaf surface and the adaxial leaf surface. For both mycelium and red blotches the amount of symptoms on the abaxial surface were correlated to the amount of symptoms on the adaxial surface (Figs. 2.7 and 2.8). While both were correlated the amount of red blotches, on the adaxial and abaxial surfaces were much more strongly correlated when considering the r statistic from the Spearman's rank-order correlation.

Initially the first symptom was mycelium which increased before reducing (Fig. 2.5) as the amount of red blotches increased (Fig 2.6). Therefore mycelium is an

important indicator of infection that can be used to identify when control is required before red blotching develops. Therefore it is possible to develop a scoring method that incorporates all symptoms of *P. aphanis* infection (see section 2.6).

2.5.3 Progression of *P. aphanis* symptoms

The amount of mycelium on the adaxial leaf surfaces on the 11.08.04 (1st sample date) was correlated ($P<0.01$) with the amount of red blotches on the adaxial and abaxial surfaces (Table 2.1) on the 3 later sample dates (18.08.04, 25.08.04 and 01.09.04). The mycelium on the adaxial leaf surface on the 1st sample date was also correlated ($P<0.01$) with the amount of red blotches on the adaxial and abaxial surfaces on the 3 later sample dates. This however was a much stronger correlation (r value). The red blotching that developed later in the infection was correlated to the amount mycelium present at the start of the epidemic.

When scoring the whole plant for symptoms of *P. aphanis* the 3 symptoms develop in a progression throughout the whole tunnel. The first symptom to develop is cupping then mycelium followed by red blotches (Fig. 2.9 and Table 2.2). The locations that the symptoms developed in were associated (Table 2.3). The locations of the first plants with mycelium and plants with red blotches were associated with the locations that the first cupping developed. The locations of the plants that first developed the red blotching symptom were associated with

the location of the first plants to develop mycelium. The 3 symptoms developed in a progression from cupping to mycelium to red blotches. The first plants with each symptom were located in the same locations, within the tunnel that the previous symptom developed in.

2.6 New *P. aphanis* scoring methods

2.6.1 Method for growers to use

When a grower is trying to identify an infection by *P. aphanis* they should be looking for leaf cupping as the first symptom of infection. This is the stage where the grower has the first and best chance of slowing the epidemic. Then growers should look for mycelium on the abaxial or adaxial leaf surface (more often found on younger leaves) as the second symptom of *P. aphanis* infection and red blotches should be used as the third symptom of *P. aphanis* infection. If the conditions are conducive for infection progression from cupping can occur within a few days. The progression from mycelium to red blotches generally takes a longer time (about 14 days). The grower can use control products to control the infection at any point but the sooner the grower can control the infection the slower the build up of inoculum will be (Lucas, 1998).

2.6.2 Method for use in fungicide trials

When carrying out experiments to test the effects of fungicides and other products that might be able to control a *P. aphanis* infection individual leaves should be tagged so that the effects of the fungicidal control products can be recorded more specifically. The tagged leaves should be scored for;

- presence or absence of cupping
- percentage of abaxial leaf surface covered with mycelium*
- percentage of adaxial leaf surface covered with mycelium*
- percentage of adaxial leaf surface covered with red blotches

* While the amount of mycelium present on the abaxial and adaxial leaf surfaces is correlated (Fig. 2.7) there are significant differences between the levels of infection found on the abaxial and adaxial leaf surfaces (Fig. 2.5). It is recommended that the amount of mycelium on both leaf surfaces is recorded.

The leaves should be tagged and scored prior to application of the product, so that the product could be tested for, either its ability to combat an established infection (a tagged leaf with visible symptoms) or its ability to slow the development of new infections (a tagged leaf with no visible symptoms). Then the leaves can be scored at regular intervals after the application of the product to measure the amount of symptom development and therefore the amount of

inoculum present, and then compared to an untreated control to establish whether the product has any control capabilities against *P. aphanis*.

2.7 Conclusion

There are 3 symptoms that can be linked to a *P. aphanis* infection. These are leaf cupping, mycelium present on either leaf surface, and red blotches present on either leaf surface, which could lead to leaf death. The 3 symptoms form a progression, cupping first (this persists through out the infection), then mycelium appears and finally red blotches appear as the amount of mycelium starts to decrease.

When scoring a whole tunnel or even a whole field for the development of a *P. aphanis* epidemic leaf cupping should be regarded as the first symptom as stated in the MAFF Strawberry Powdery Mildew Key 8.1.1. However the presence or absence of cupping should be scored, rather than it contributing to an overall percentage (level) of infection as it does in the MAFF Strawberry Powdery Mildew Key 8.1.1. Cupping should be identified as the first symptom, which should act as a trigger for the grower to start to apply fungicides when the infection is in it's early stages so that the lag phase can be extended (Lucas, 1998, Zadocks and Schein, 1979). In this study it has been found that the second symptom used to identify a *P. aphanis* infection should be the presence of mycelium as this is correlated with the amount of red blotching that is likely to

form. This is contrary to the information in the MAFF Strawberry Powdery Key 8.1.1. It however has to be remembered that visible mycelium is a passing symptom and it will reduce as the amount of red blotching increases. This does not mean the epidemic is ending; the infection has just progressed on to the next symptom. The final symptom that is used to identify a *P. aphanis* infection is red blotching. When quantifying the level of infection, either the percentage of leaf surface area covered with symptoms (mycelium and or red blotches) or the total number of plants with symptoms can be used. Growers could well be more interested in the number of plants with symptoms and their location within the tunnel. The more infected plants there are the more sources of infection there will be. If these plants were spread throughout the tunnel there would be the biggest chance of the infection spreading to the neighbouring plants.

The 3 symptoms associated with *P. aphanis* infection have been identified. These have been used to develop two new scoring methods that are specific to the situation that they will be used in and so offer benefits over the MAFF Strawberry Powdery Key 8.1.1. One scoring method is designed for use by the grower to help them identify infections of *P. aphanis* and so help them apply fungicides at the right time. The other is designed for use in fungicide trials. It generates much more information on the development and progression of the symptoms so the effects of the fungicides can be quantified in relation to each of the symptoms that the grower might see in the field.

Chapter 3 - Epidemiology

3.1 Introduction

Epidemiology is the 'study of factors affecting outbreaks of disease and spread of infectious diseases' (Waller *et al.*, 2002) or more broadly, 'the study of disease incidence, distribution and control' (Kirk *et al.*, 2001). Development of more effective and reliable disease management programmes depends upon understanding the epidemiology of *P. aphanis*. In particular the source of the initial inoculum needs to be identified for both newly planted fields and established fields so that the control strategies employed by the growers match the situation in the field. The patterns formed by the infected plants within the field can be used to identify the source of the initial inoculum, either air borne or established in the field. Growers believed that the initial inoculum was wind borne (personal communication, Harriet Duncalfe, Wisbech). Once these factors have been identified they can be used to develop an integrated control program. Identification of the initial source of inoculum will enable the grower to tailor their tunnel management and fungicide applications so that they are applied when they would result in the maximum benefit.

3.1.1 Over wintering infection

An obligate biotroph *P. aphanis* (Green *et al.*, 2002) requires living plant material for growth. Hence when conditions are unsuitable for survival of the host, the pathogen needs a strategy to survive. This could be either as sexual overwintering bodies (chasmothecia), dormant mycelium for example within leaf buds or some pathogens over winter as an infection on an alternative host.

Chasmothecia can provide a route for inoculum to survive across strawberry production seasons (Gourley, 1979). Strawberry powdery mildew chasmothecia have been observed on strawberry plants in UK fields (Farooq *et al.*, 2007, Rashid Khan, 1960, Salmon, 1900). Chasmothecia have been reported in a field in Florida (Howard and Albregts, 1982). Peries (1961) witnessed chasmothecia (referred to as perithecia) under one set of conditions, in a glasshouse, in specially built chambers covered with muslin, which provided a 75-90% reduction in light intensity. Natural dehiscence of the chasmothecia was not observed by Peries (1961). Currently growers and strawberry agronomists do not believe that chasmothecia play an important role in the over wintering of *P. aphanis* infection in the UK crop.

There is also evidence that *P. aphanis* can survive as mycelium on over wintering strawberry leaves and that this produced conidia in the spring, which infected the young leaves (Peries, 1961). Grape powdery mildew (*Uncinula*

necator) overwinters as mycelium within buds on the grapevine (Pearson and Gartel, 1985, Sall and Wrysinski, 1982, Van der Spuy and Matthee, 1977). As the conditions become suitable the infected buds form flag shoots which act as foci from which subsequent disease originates. When strawberries over winter there is usually green leaf material on the plants until the start of the next season, on which it could be possible for the mycelium to over winter (Smith *et al.*, 1988). *P. leucotricha* over winters in apple buds that were formed the previous season (Xu, 1996, Xu, 1999c), *S. pannosa* perennates as mycelium in rose buds (Price, 1970) as does *P. clandestina* on hawthorn (Khairi and Preece, 1978).

3.1.2 Disease progression in relation to source of inoculum

'The natural world is a patchy place' (Dale, 1999), with patches forming on many different scales. The agricultural landscape is made up of patches; cropped fields contain a single species where other species are excluded. As far as pathogens are concerned this results in areas that are susceptible to infection, surrounded by comparatively large areas that are not susceptible. One of the first questions that needs answering when considering an infection is 'where did the primary inoculum come from'? A strawberry plant will usually be cropped 3 times within 3 years. Therefore a plant will overwinter in the field twice. This production method means that there are two different situations to consider when identifying the source of initial inoculum. Firstly where does the primary inoculum come from

when a field is first planted? Secondly what is the source of inoculum in an established over wintered crop?

Spatial patterns (points of infection) can be described in 3 ways. Random patterns, the location of one infected plant does not have an effect on the location of another infected plant. Clumped patterns, the presence of an infected plant increases the probability of finding another. Over dispersed patterns, the presence of one infected plant decreases the probability of finding another (Dale, 1999). The larger the area of susceptible plants the easier the analysis of the patterns will be (Fletcher, 1984). Occurrence of infected plants in greenhouses (and also polythene tunnels, in this instance) can often be linked to their location within the greenhouse (location near a tap, a telephone or on a larger infection scale the vents and or topography of the greenhouse) (Fletcher, 1984).

The patterns of plants showing first symptoms can be analysed for information on the source of infection. Plants showing the first symptoms can act as foci for further infection, especially when the disease is a polycyclic fungal pathogen (Zadocks and Van der Bosch, 1994) such as powdery mildews (Lucas, 1998). The development of grape powdery mildew has been shown to cluster around initially infected plants which acted as foci (Cortesi *et al.*, 2004). Hop powdery mildew (*S. humuli*) infections show 'nearly random distribution' suggesting that epidemics are initiated from well distributed or readily dispersible overwintering populations (Turechek and Mahaffee, 2004). It is possible an infection by wheat

powdery mildew developed from a few successful infections which acted as foci for disease development (Parker *et al.*, 1997).

Disease maps of the location of infected cucumber plants in a glasshouse have been produced (Ruiz *et al.*, 2006). Two virus, *Cucurbit yellow stunting disorder virus* (CYSDV) and *Cucumber vein yellowing virus* (CVYV) were studied; they were both transmitted by the same vector, sweet potato whitefly *Bemisia tabaci*. The authors demonstrated that the disease distribution was non-random and the limiting factors were the number of whiteflies and temperature (by affecting the vector dynamics). Spatial pattern maps of *Phytophthora* epidemic development in commercial bell pepper fields have been produced over several years (Larkin *et al.*, 1995). The disease severity developed over the course of the experiment on all 3 fields that were sampled. In the majority of the fields the distribution of the natural inoculum was not random but distinctly aggregated. The degree of aggregation increased over time as the clusters of diseased plants expanded. Disease spread was greater 'down' rows than across rows suggesting that inoculum could have been carried in surface water.

Disease maps for potato blight (*Phytophthora infestans*) which illustrated the development of infections have also been produced (Cragg, 1971). Rows of infected plants were planted between rows of clean (uninfected) plants. Infection developed first on the infected plants, then on the plants closest to the infected plants and from there the disease spread to the rest of the field (Fig. 3.1). The

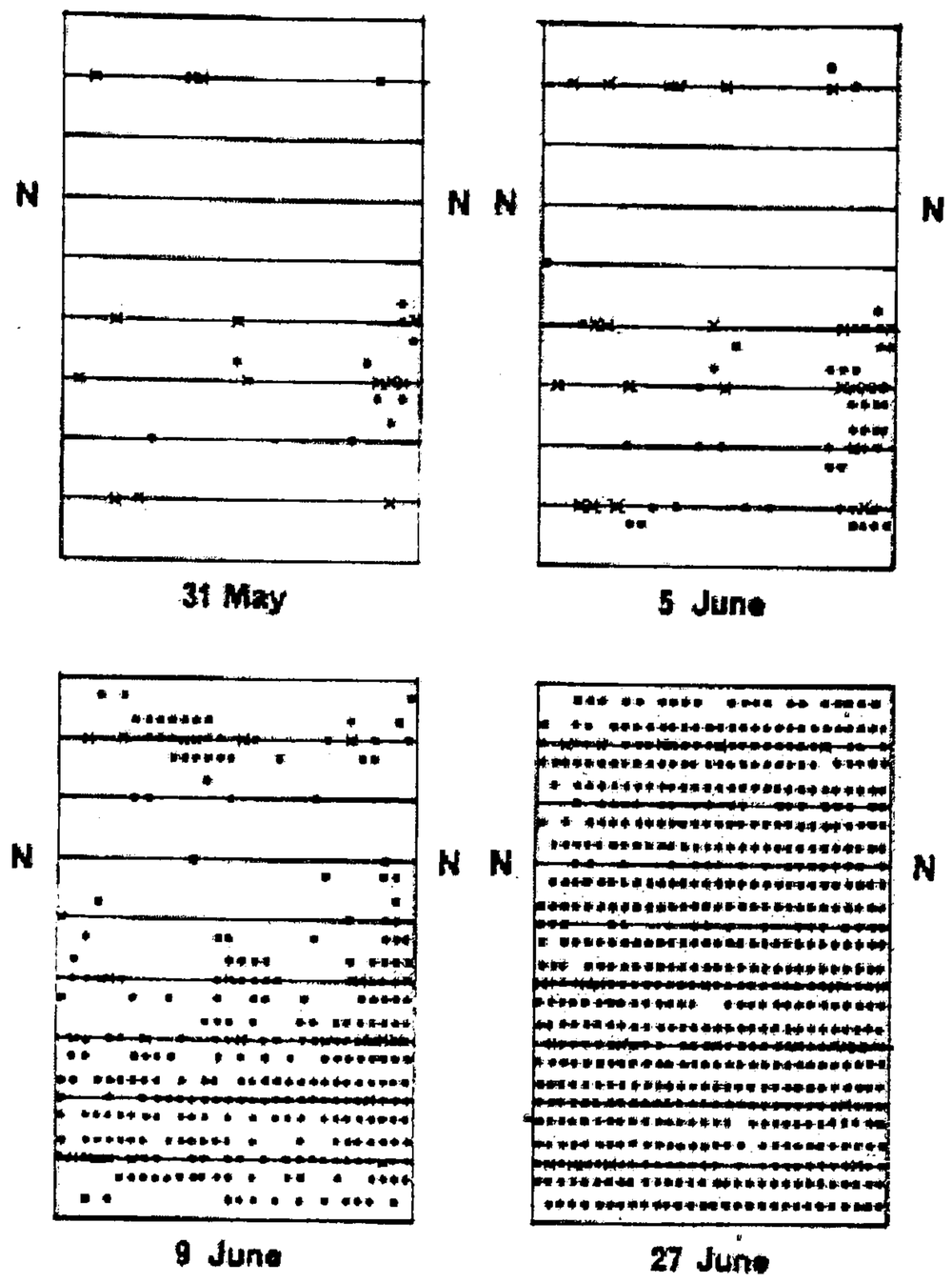


Fig. 3.1 Occurrence of primary infectors (infected with potato blight, *Phytophthora infestans*) and the subsequent spread to healthy plants.

N-N=row of naturally infected tubers

-=rows of artificially infected tubers

x=Primary infector

o=Secondary infections

Reproduced from (Cragg, 1971)

disease spread from neighbouring plant to neighbouring plant until the majority of the plants were infected. The infection on the first plants to show symptoms did not directly spread to infect all the plants in the field.

Generally when the first symptoms of disease develop in a field there will only be a few infected plants. These infected plants act as the source of inoculum for the closest neighbouring plants to them, which will then act as the source of inoculum for their neighbours. The disease spreads over all the plants in waves. Not all the plants will show symptoms as soon as the conditions are suitable for development of the pathogen. The pathogen needs time for secondary inoculum to develop from the plant infected with the primary inoculum. The secondary inoculum will start to spread once the latent period has passed.

Aerial photographs showing disease development have been published (Brenchley, 1968), two of these photographs have been reproduced as Figs. 3.2 and 3.3. Fig. 3.2 shows a potato field infected with late blight (an air-borne disease). The dark patches represent infection. The majority of infection was located towards the north of the field. The original author identified a cull pile located towards the north of the area covered by the photograph. The infection there was much further advanced so the author concluded that it acted as a source of air-borne inoculum. There are more patches of infection toward the north of the field (closer to proposed source of inoculum) than towards the south. This shows that the concentration of inoculum in the air gets less the further

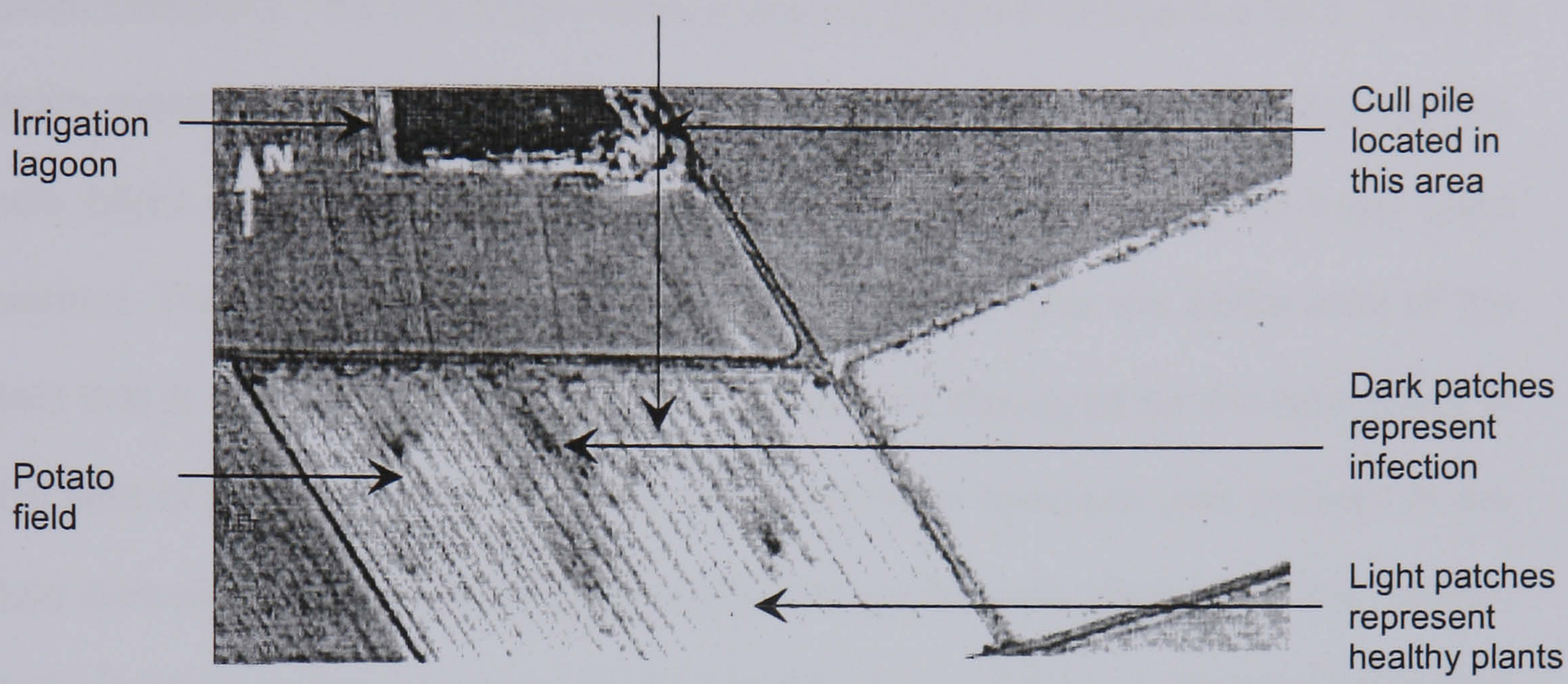


Fig. 3.2 Spread of potato late blight (infra-red photograph). Reproduced from (Brenchley, 1968)

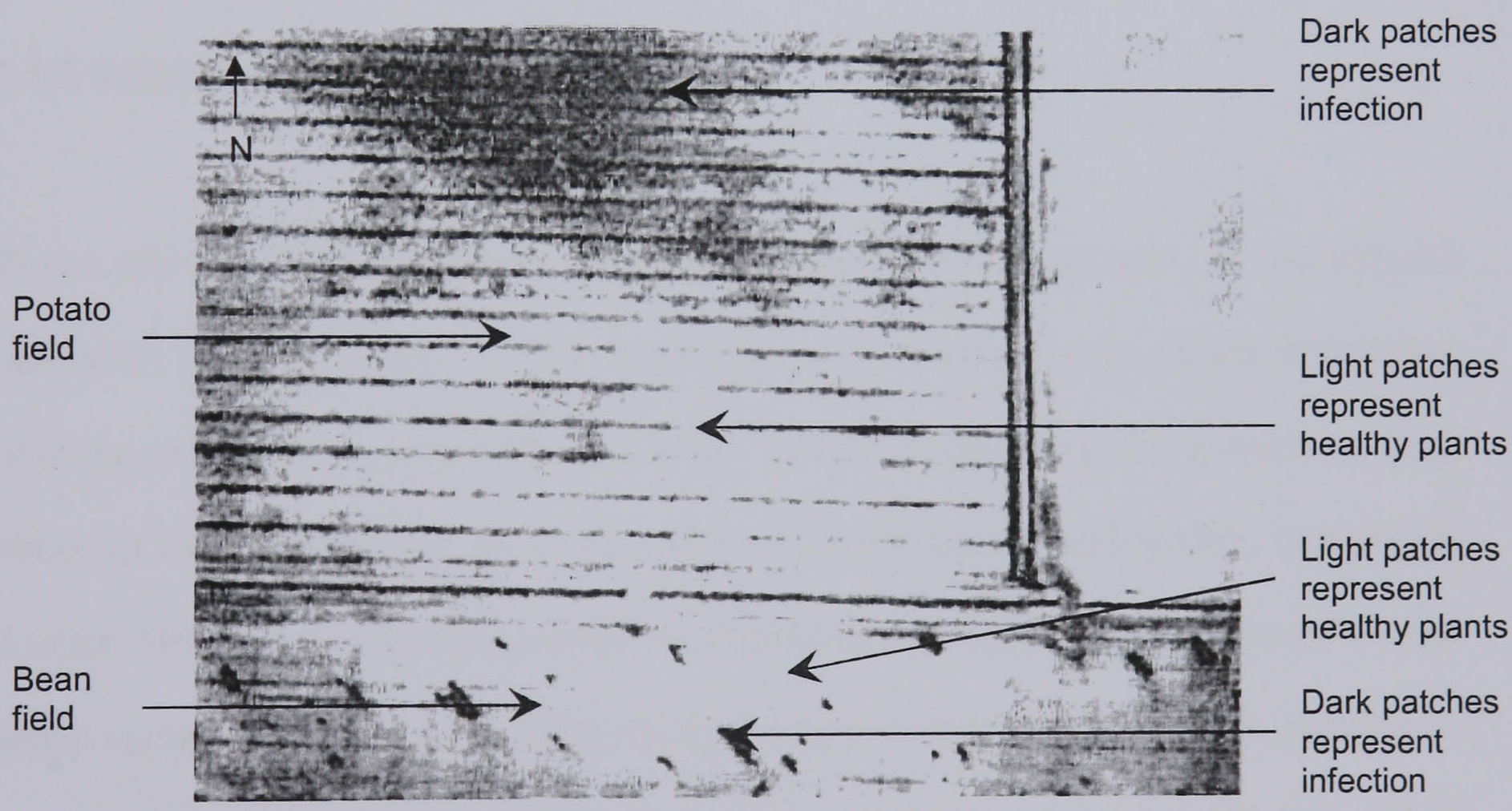


Fig. 3.3 Halo blight of beans (below) and potato late blight (infra-red photograph). Reproduced from (Brenchley, 1968)

away it is from the proposed source of inoculum. Fig. 3.3 shows another field of potatoes infected with late blight and a field of beans infected with halo blight (seed infection). The late bight shows a distinct gradient across the field. There is much more infection towards the north of the field than the south, whereas the halo blight occurs in distinct (dark) patches, where infected seeds have been planted. The patches appear to be distributed evenly over the entire area of the field that is visible in this photograph. The pattern displayed by the halo blight in the field of beans is what would be expected if the inoculum was present in the field (infection distributed through out) where as the late bight show the pattern that would be expected in an infection distributed in the wind (gradient from high to low).

3.1.3 Infection rate

There are two distinguishing types of epidemiological growth. A monocyclic epidemic, one generation of the pathogen each season (e.g. many soil-borne pathogens) and a polycyclic epidemic, many generations in a season (e.g. airborne foliar pathogens, such as rusts, powdery mildews and potato late blight) (Lucas, 1998). A polycyclic infection is composed of many cycles after each other (each cycle is similar to a monocyclic epidemic). In each cycle spores germinate, grow, reach maturity and produce more spores to start the next cycle. With each cycle the amount of infection increases (Zadocks and Schein, 1979).

The rate at which the population of a pathogen increases is known as the apparent infection rate *r* (Van der Plank, 1963). The formula for infection rate is similar to that used for compound interest of money with a correction factor added to take into account that the disease level can reach a maximum (all plants infected) whereas monetary interest would keep on increasing (Tainter and Baker, 1996, Van der Plank, 1963).

Van der Plank (1963) describes how to construct the formula for calculating the apparent infection rate. When one hundred percent infection has been reached x=1. At low levels of disease the pathogen can spread almost unhindered and the equation to describe this can be written as,

$$\frac{dx}{dt} = rx \qquad (1)$$

In equation 1 the rate of increase of disease dx/dt is proportional to x, the increase is logarithmic. As x increases the proportion of susceptible tissue decreases (1-x). Equation 1 can be rewritten to incorporate this,

$$\frac{dx}{dt} = rx(1-x) \qquad (2)$$

The increase of disease will no longer be logarithmic. Equation 2 defines r. To be able to estimate r the disease levels need to be known for two dates. From this r can be estimated. So that equation 2 can be written as,

$$r = \frac{2.3}{t_2 - t_1} \log_{10} \frac{x_2(1-x_1)}{x_1(1-x_2)} \quad (3)$$

r = apparent infection rate
t_1 = date 1
t_2 = date 2
x_1 = disease level at t_1
x_2 = disease level at t_2

An excellent summary of how to calculate 'r' is provided by Tainter and Baker (1996). The apparent infection rate, as a single figure tells much about an epidemic and can be extremely useful for rapid comparison of several epidemics by the same pathogen (Zadocks and Schein, 1979). An apparent infection rate of r=0.5 per unit per day is a fast rate (Van der Plank, 1963). Infection rates are important for modern plant pathology, but must be used with caution and common sense (Van der Plank, 1982).

A polycyclic epidemic develops in three phases. The first is the lag phase. In this phase the greatest multiplication of pathogen numbers occurs, most often this occurs below the visibility threshold. The second is the logistic phase. This goes from the end of the lag phase until the mid point (time = 0.5). The final phase is the terminal phase. This lasts from the end of the logistic phase until the end of the epidemic (Zadocks and Schein, 1979). These 3 phases join together to form a S-shaped (sigmoid) growth curve (Fig. 3.4) (Lucas, 1998, Zadocks and Schein, 1979).

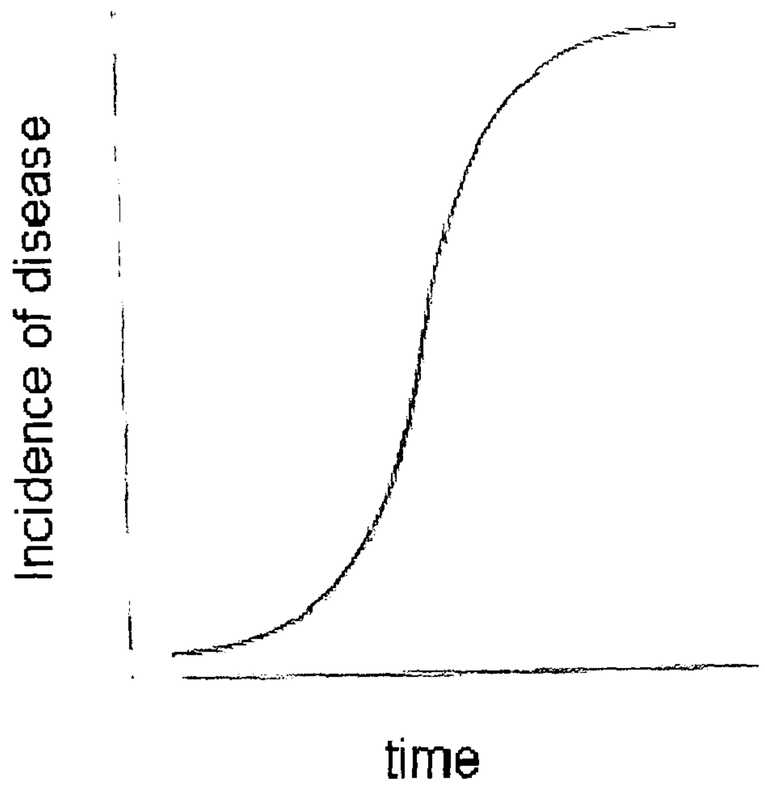


Fig. 3.4 Idealized polycyclic growth curve reproduced from (Lucas, 1998)

An S-shaped growth curve can be transformed. The logit of *x* is linear with time. An S-shaped disease progress curve becomes a straight line when plotted on double linear graphics (Zadocks and Schein, 1979). At low values of *x* the correction factor approaches 1, so the values of log 10 and logit are practically equal and are interchangeable at values of *x* <0.05. When plotted on semilogarithmic scales the resulting line is straight up to *x* = 0.05. At higher values of *x* the line asymptotically approaches a horizontal line (Zadocks and Schein, 1979).

Polycyclic disease progress curves have been produced for foliar anthracnose of yams where the different rates of disease development resulted from differences in climatic factors. Early and careful applications of fungicides delayed the pathogen so that new leaf material was not overtaken by infection (Sweetmore *et al.*, 1994). Disease severity against time has been plotted for pea powdery mildew. On the lower nodes disease progress followed a sigmoid pattern and could be used in the early part of the experiment to differentiate cultivars with varying susceptibility (Viljanen-Rollinson *et al.*, 1998). The development of the logarithmic phase of disease progress was delayed when early blight free (*Apium graveolens* L. var. *dulce* DC.) celery was planted compared with severely infected transplants (Berger, 1973).

3.2 Aim and objectives

3.2.1 Aim

Identify the source of fungal inoculum responsible for initiating primary outbreak of disease and follow the development of the subsequent epidemic (2nd aim page 27)

3.2.2 Objectives

1. Identify the source of primary inoculum in a newly planted field

2. Identify the source of inoculum in an over wintered crop

3. Establish the rate of disease build up in newly planted and established fields

3.3 Methods

3.3.1 Detection of *P. aphanis* symptoms

Plants were scored visually for the presence or absence of *P. aphanis* symptoms. The symptoms that were scored were leaf cupping, presence of

mycelium and or presence of red blotches (Chapter 2.6.1). If any of these symptoms were visible without the aid of magnification the plant was classed as infected. Strawberry plants are grown in a regular pattern within tunnels, so it was possible to score every plant multiple times. As plants were scored more than once it was possible to record the progression of disease foci.

3.3.2 Field sites

3.3.2.1 Established site Mereworth 2004

Experimental tunnel D was used for this work (Fig. 3.5). The tunnel was planted with 2248 strawberry cultivar Elsanta plants supplied by Hugh Lowe Farms. The plants were lifted the previous winter, in the UK, and kept as bare root plants in a cold store until they were planted in early August 2003, the site was not fleeced. The plants were scored for the first time on the 17/04/04 (the day after the tunnels were covered) and scored for the last time on the 11/05/04. In total they were scored 10 times. All the plants in the tunnel were scored as described previously.

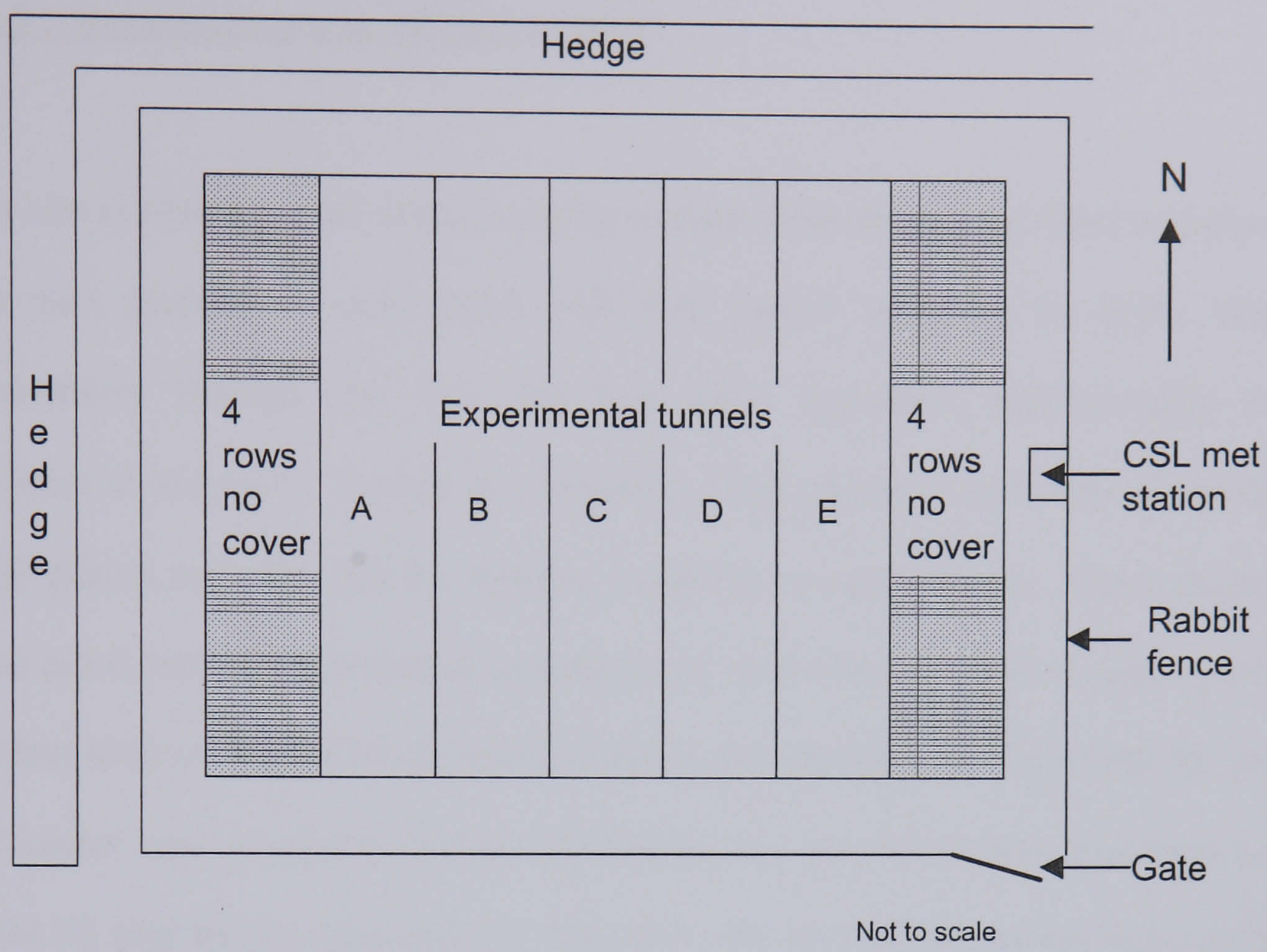


Fig. 3.5 Plan of the Mereworth field site 2004. The site consisted of 5 tunnels of 4 beds each with 4 further beds at each side that were not covered by polythene sheets. There were established hedgerows on the north and west sides of the site. There was a hop garden to the south and a wheat field to the east. The site was surrounded by a rabbit proof fence

3.3.2.2 Established site Wisbech 2005

The site contained third season plants cultivar Elsanta in peat filled troughs. The site was planted in July 2003 with tray plants supplied by R.W. Walpole (Strawberry Plants) Ltd. The site had been managed commercially in the previous 2 seasons. Tunnel A contained 1464 plants and tunnel B contained 1458 plants see Fig. 3.6 for relative locations of each tunnel. The symptom(s) each plant had were recorded as previously described. Tunnel A was scored for the first time on the 30.03.05 and scored for the last time on the 05.05.05. In total the tunnel was scored 11 times. Tunnel B was scored for the first time on the 15.04.05 and for the last time on the 12.05.05. In total the tunnel was scored 6 times. Both tunnels were fleeced in mid February 2005 and the tunnels were covered on the 12.03.05. The fleece was removed from tunnel A on the 30.03.05 and the fleece was removed from tunnel B on the 15.04.05.

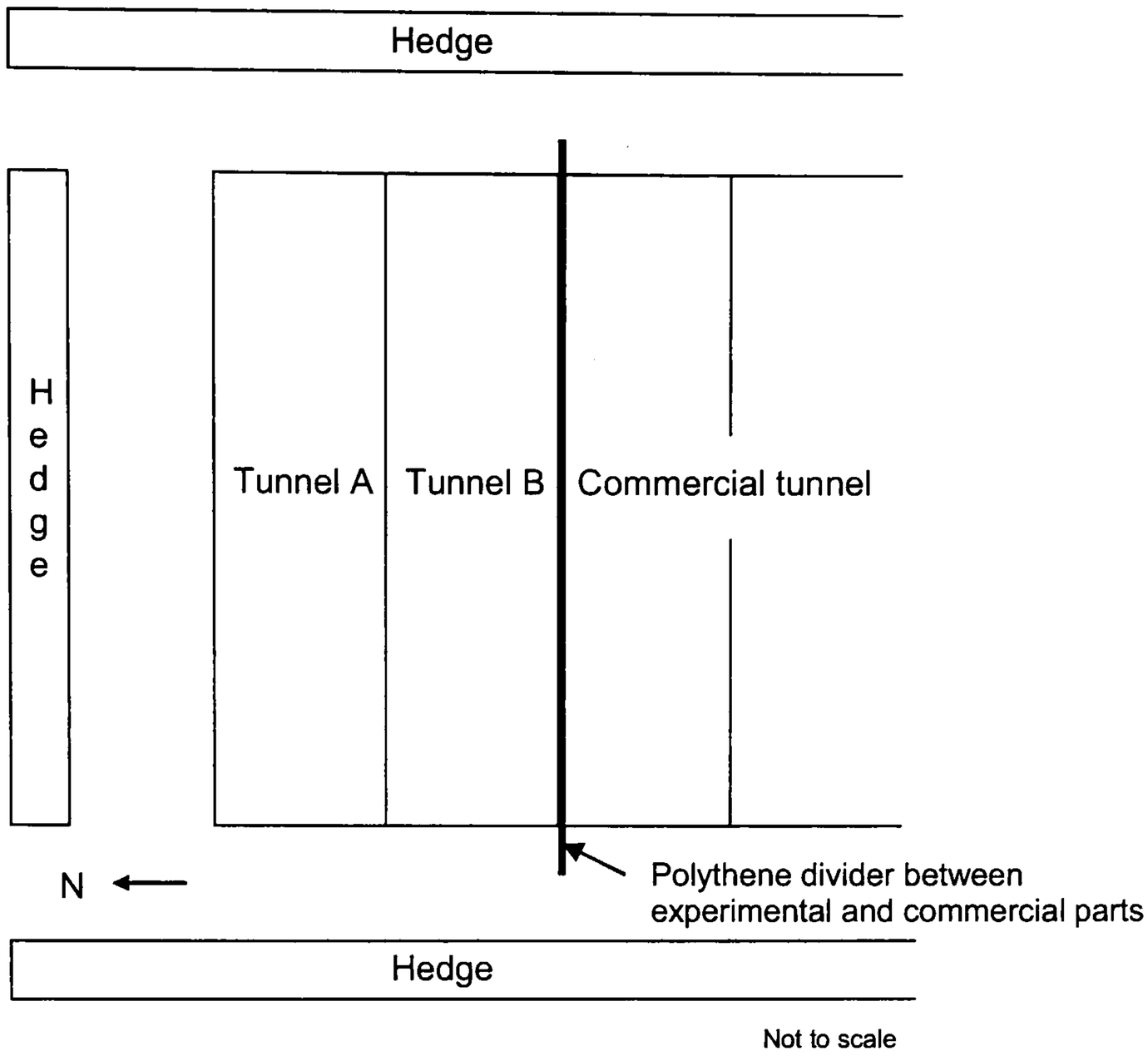


Fig. 3.6 Plan of the Wisbech field site 2005. Location of the two experimental tunnels in the Wisbech site 2005. Each tunnel contained 3 rows of plants in peat filled troughs. The site was surrounded by large established hedges on three sides and the commercially managed tunnels were on the other side. A plastic sheet was installed between the experimental tunnels and the rest of the site to reduce the amount of inoculum spreading to the commercial part and to reduce any drift of fungicides on to the experimental part

3.3.2.3 Newly planted site Mereworth 2005

Experimental tunnel B was used for this work (Fig. 3.7). The tunnel was planted with cultivar Flamenco supplied by R.W. Walpole (Strawberry Plants) Ltd. The plants were lifted the previous winter, in France, and kept as bare root plants in a cold store until they were planted on the 24th May 2005. The symptom(s) each plant had were recorded as previously described. The tunnel contained 1682 plants. The tunnel was scored for the first time on the 14.06.05 and scored for the last time on the 02.08.06. In total the tunnel was scored 8 times. The tunnel was already covered when the plants were planted. The site was not covered with fleece.

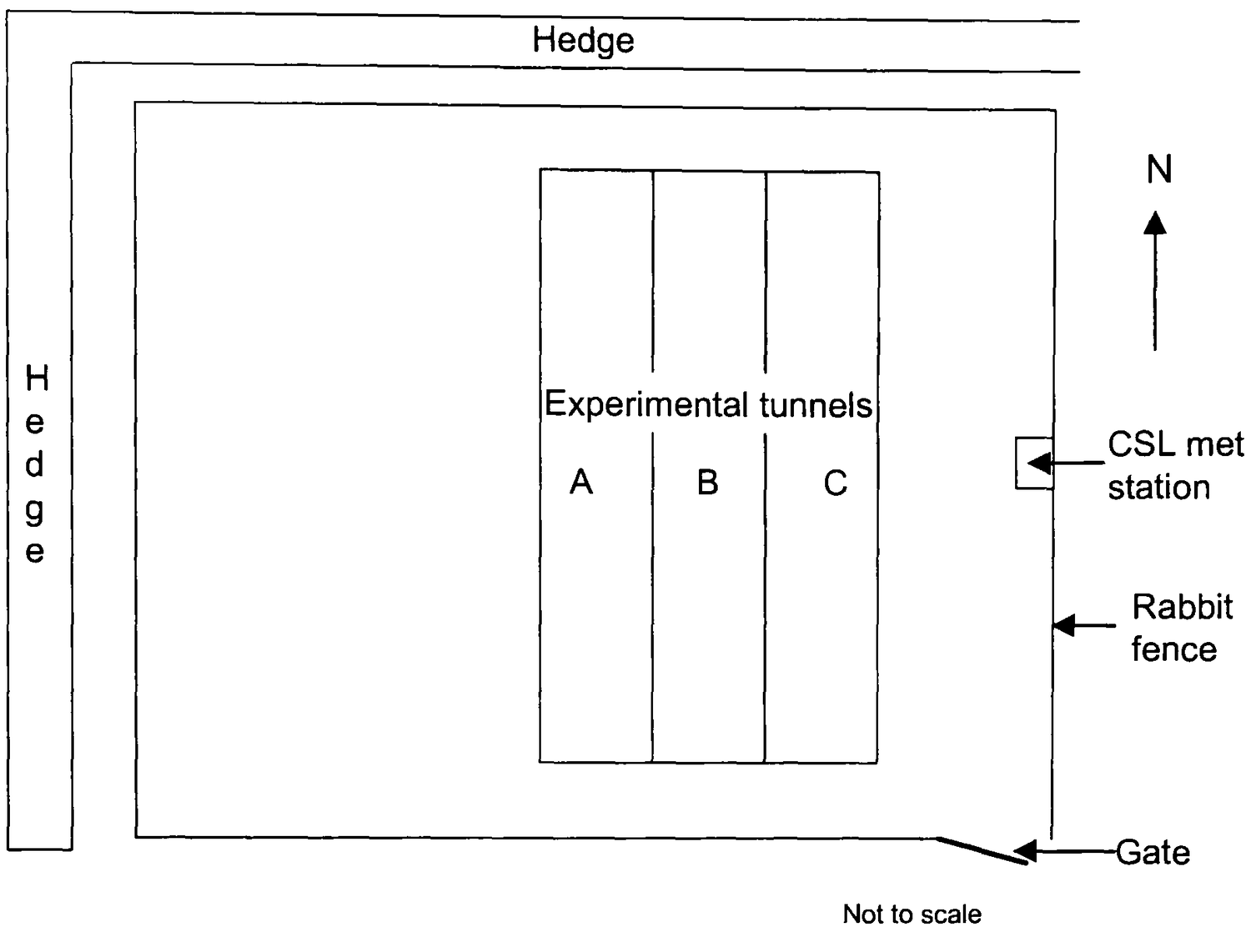


Fig. 3.7 Plan of the Mereworth field site 2005. The site consisted of 3 tunnels of 4 beds each. The parts of the site that were not used for the tunnels were unmanaged. There were established hedgerows on the north and west sides of the site. There was a hop garden to the south and a field of oilseed rape to the east. The site was surrounded by a rabbit proof fence

3.3.2.4 Newly planted site Wisbech 2006

Experimental tunnels A and B were used for this work (Fig 3.8). Tunnel A was planted with first season cultivar Elsanta and tunnel B was planted with a first season ever bearer supplied by Stefan Kraege. The Elsanta plants were planted in May 2006 and the ever bearers were planted in the 1st week of March 2006. The symptom(s) each plant had were recorded as previously described. The tunnels were scored for the first time on the 04.07.06 and scored for the last time on the 25.07.06. In total the tunnels were scored 5 times. Both tunnels were covered on the 3rd July 2006. The ever bearer tunnel was fleeced the 1st week of March 2006 and the fleece was removed the 3rd week of April 2006. The tunnels were located in a commercial field. Only a small part of each tunnel was scored. This was located to the north of the each tunnel 5m from the end of the tunnels.

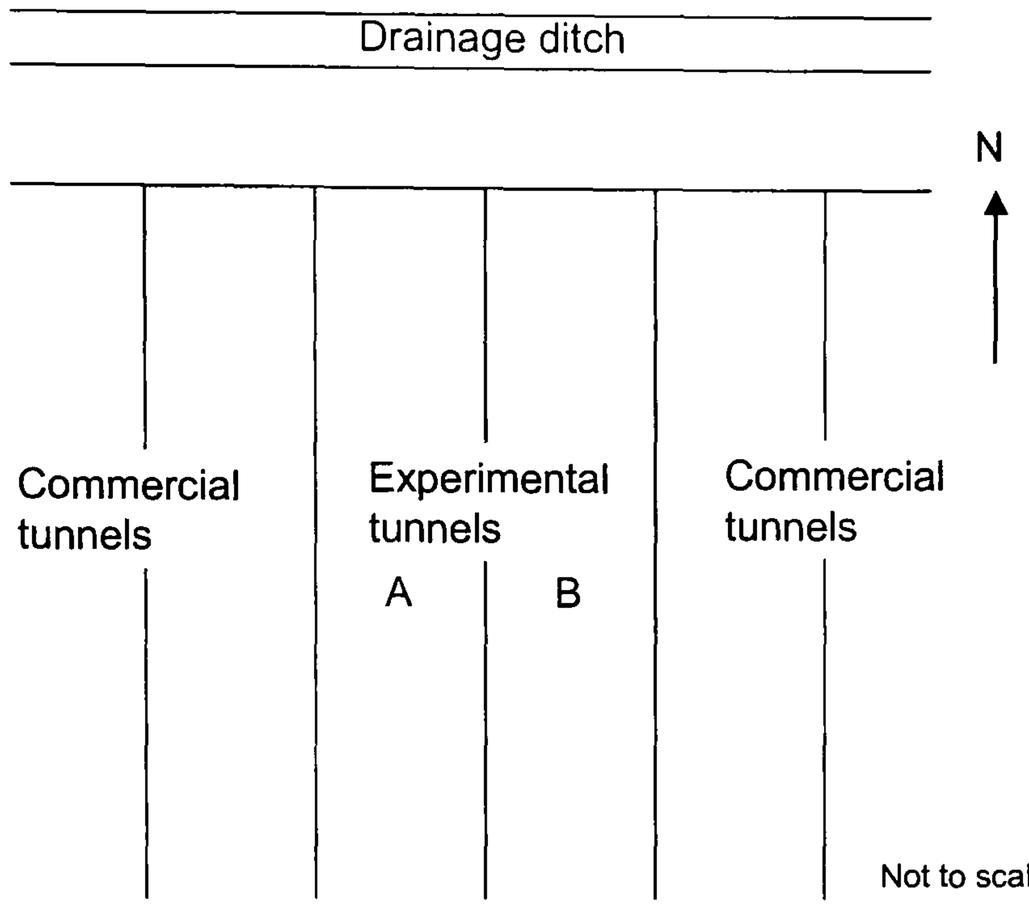


Fig. 3.8 Plan of the Wisbech field site 2006. The site consisted of tunnels with 5 beds each; parts of two tunnels were used for this work. The commercial tunnels to the west of experimental tunnel A were planted with cv. Elsanta and the commercial tunnels to the east of experimental tunnel B were planted with an ever bearer. There was a drainage ditch to the north of the site with an access track between the ditch and the tunnels

3.3.3 Analysis of disease patterns

The distribution of infected plants and then the subsequent build up of disease can reveal a lot about the source of an inoculum. Strawberry growers believed that *P. aphanis* inoculum was air-borne, and believed that at the start of each season their fields were not infected with *P. aphanis*. They directed all their efforts to trying to keep the pathogen out of their tunnels (personal communication, Harriet Duncalfe, Wisbech). If this was the case the first plants to shows symptoms most likely would be at the ends of the polythene tunnels. The spores could have been deposited on to the first plants in the tunnel as the wind velocity dropped and could no longer hold the spores in the air. There would then have been a lag phase as these spores germinated and reached maturity before producing more spores that would then infect the next plants into the tunnel. This would then repeat as the infection progressed up the tunnel in waves of infection. If the inoculum was already present in the field when the tunnels were covered the first plants showing symptoms would be distributed through out the tunnel. The infected plants would then act as foci for disease development, as shown in Fig. 3.3. The potato blight is air-borne so shows a concentration gradient away from the source of inoculum where as the halo blight of beans is in distinct patches as the inoculum was planted into the field, so developed within the site (Brenchley, 1968) as might an established infection that had over wintered within a site.

Maps of the tunnels were produced at each sample date with the location of each plant that had symptoms of powdery mildew infection marked. The spread of disease could be followed by comparing the location of plants with symptoms at each subsequent sample date.

3.3.4 Collection of internal and external environmental conditions

The environmental conditions within the tunnels were monitored. Tinytag data loggers, supplied by Gemini Data Loggers (UK) Ltd, Chichester were used to monitor relative humidity, temperature and leaf wetness. The Tinytag Plus TGP-0903 was used to monitor leaf wetness and the Tinytag Plus TGP-1500 was used to monitor temperature and relative humidity. All three parameters were recorded every 60 minutes. The external environmental conditions were recorded using a DL2e meteorological station supplied by Delta-T-Devices Ltd, Cambridge. The meteorological stations recorded a range of variables including temperature, relative humidity and rainfall; variables were recorded every 10 minutes.

3.3.5 Comparing results from different sites and years

When results are collected from different sites and years a common unit of 'time' needs to be used. A unit of physical time (e.g. hours or days) is not ideal as pathogens and hosts often respond strongly to the temperature of their

environment and recording time in days or hours does not take the temperature in to account; so a unit of thermal time (e.g. degree-days or degree-hours) is better suited for most pathogens (Lovell *et al.*, 2004).

A *P. aphanis* conidiospore takes 120 hours (under ideal conditions in the laboratory) to germinate and form a mature colony; it takes 144 hours (under ideal conditions in the laboratory) for a germinating conidiospore to form a colony that is visible to the naked eye (Peries, 1962b). The lowest temperature at which a *P. aphanis* conidiospore could form a small but mature colony in 120 hours is 15°C (Table 1.3). There were no observable differences in the growth and development of colonies between 18°C and 30°C. Radial growth was slower at 15°C than 18°C but maturity was reached in the same amount of time (Peries, 1962a). Development of *P. aphanis* slows once the temperature reaches 30°C (Peries, 1962a). Therefore 15°C was taken as the temperature under which no growth would occur and over that temperature development would take place. Once the temperature had reached 30°C growth of the fungus would stop. Therefore to be able to compare the development of *P. aphanis* between sites and years 15°C was used as the lower trigger for when development would take place and 30°C was used as the upper level. The fungus would develop to maturity in 120 hours whether those 120 hours were spread over 1 week or one month as long as the temperature was between 15°C and 30°C.

Thermal time as described by Lovell (2004) uses the accumulation of the temperature per unit as the measure time e.g. the temperature recorded in hour one is added to the temperature in hour two, the total of which is added to the temperature in hour three and so on. The affect of temperature on the development of the pathogen is considered to be linear. The greater the temperature the faster the pathogen will develop. As *P. aphanis* develops at the same rate as long as the temperature is >15°C but <30°C thermal time would not be a suitable measure of time for this pathogen.

Hours >15°C but <30°C has been used as the unit of time when comparing results from different sites and years. If the temperature in the polythene tunnel was equal to or over 15°C and under 30°C the hour was scored as 1. If the temperature was under 15°C or equal to or over 30°C the hour was scored as 0. The sum of all the 1's and 0's was used to quantify the time. This will be referred to as hours of suitable conditions (hours s.c.).

3.4 Results

3.4.1 Established site Mereworth 2004

Fig. 3.9 shows the development of infection in tunnel D from the Mereworth site 2004, over a period of 11 days (83 hours suitable conditions (s.c.)). At the first assessment only 4 plants had visible powdery mildew symptoms, these were distributed throughout the tunnel. Within 15 hours s.c. infected plants were distributed throughout the tunnel. Infection continued to develop throughout the tunnel. By the 5th assessment (83 hours s.c.) the majority of the plants had symptoms of *P. aphanis*.

When the tunnel was first covered 4 plants (<1%) had symptoms of powdery mildew infection (Fig. 3.10). After 15 hours s.c. 10% of the plants had symptoms. The number of plants with symptoms remained stationary until 39 hours s.c. after the tunnel was covered. The number of plants that had symptoms increased until 105 hours s.c. after the tunnels had been covered, when a total of 75% had symptoms. The apparent rate of increase from the time the tunnel was covered until the plateaux was reached was r=0.06 per hour s.c. (Fig. 3.10).

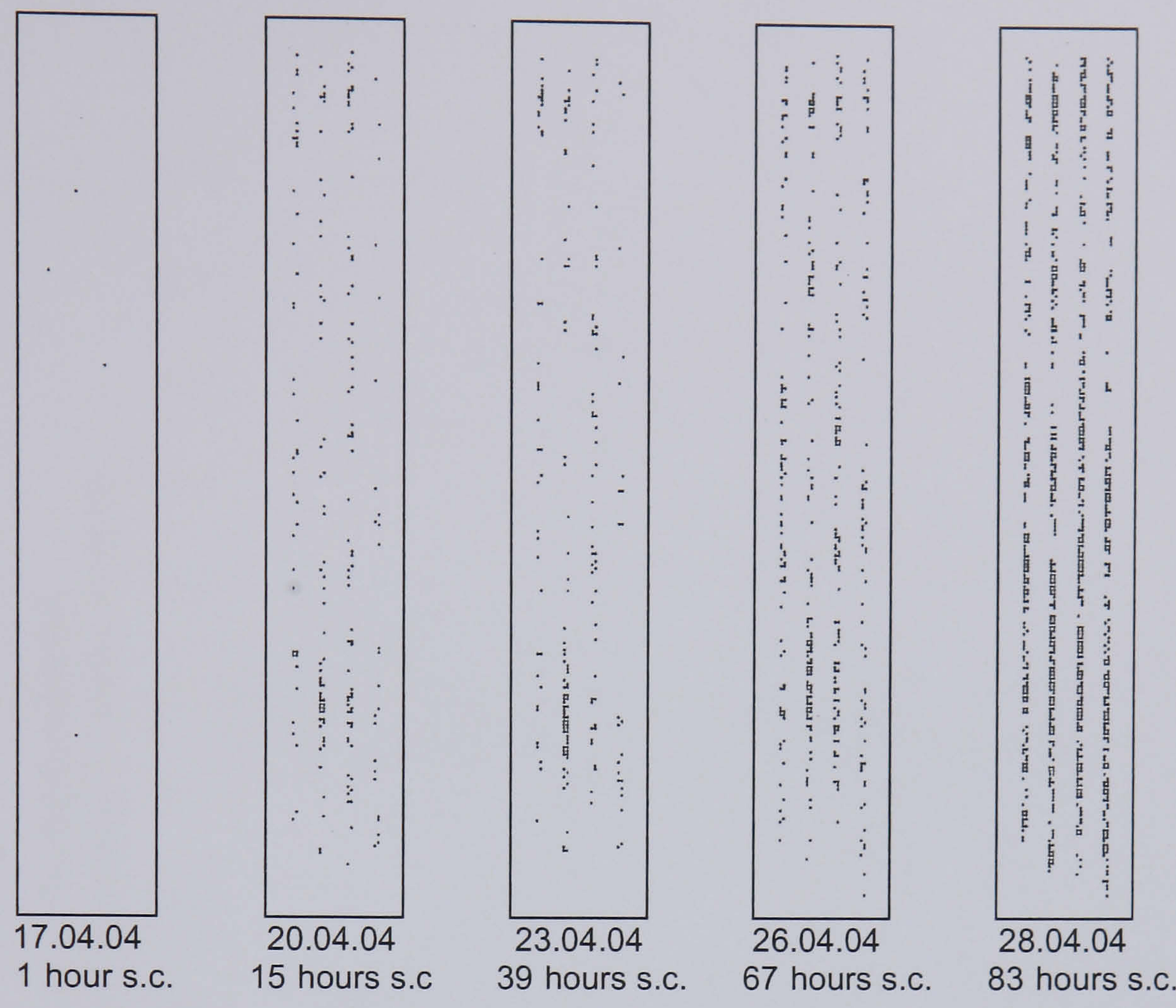


Fig 3.9 Distribution of plants with *P. aphanis* symptoms in the Mereworth tunnel D 2004, from 5 sample dates. The dots within each tunnel plan represent the location of infected plants. Infection developed throughout the tunnel in less than 83 hours of suitable conditions

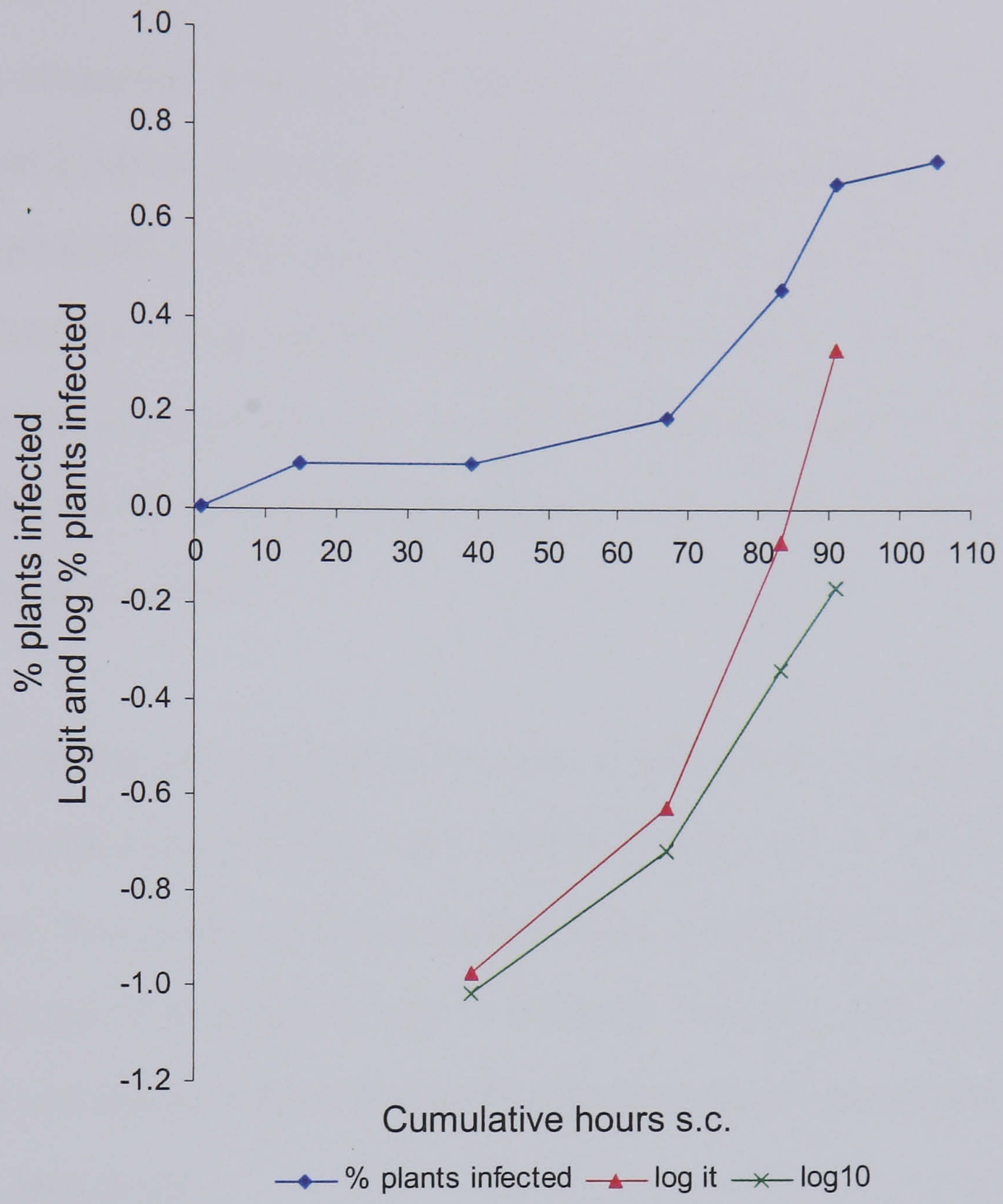


Fig. 3.10 Disease progress curve for percentage plants with symptoms, logit and log 10 of, percentage of plants with symptoms Mereworth site 2004 (tunnel D), r=0.06 per hour s.c.

3.4.2 Established site Wisbech 2005

Fig. 3.11 shows the development of infection in tunnel A from the Wisbech site 2005, over a period of 13 days (83 hours s.c.) after the fleece was removed. At the first assessment only 8 plants had visible powdery mildew symptoms, these were distributed throughout the tunnel. Within 33 hours s.c. infected plants were distributed throughout the tunnel. Infection continued to develop throughout the tunnel. By the 6th assessment (83 hours s.c.) the majority of the plants had symptoms of *P. aphanis*.

When the fleece was removed from tunnel A, 8 plants (<1%) had symptoms of powdery mildew infection (Fig. 3.12). Within 46 hours s.c. 6% of the plants had symptoms. The number of infected plants grew steadily until 79% of the plants were infected. The apparent rate of increase from the time the fleece was removed until the majority of the plants were showing symptoms was r=0.06 per hour s.c. (Fig. 3.12).

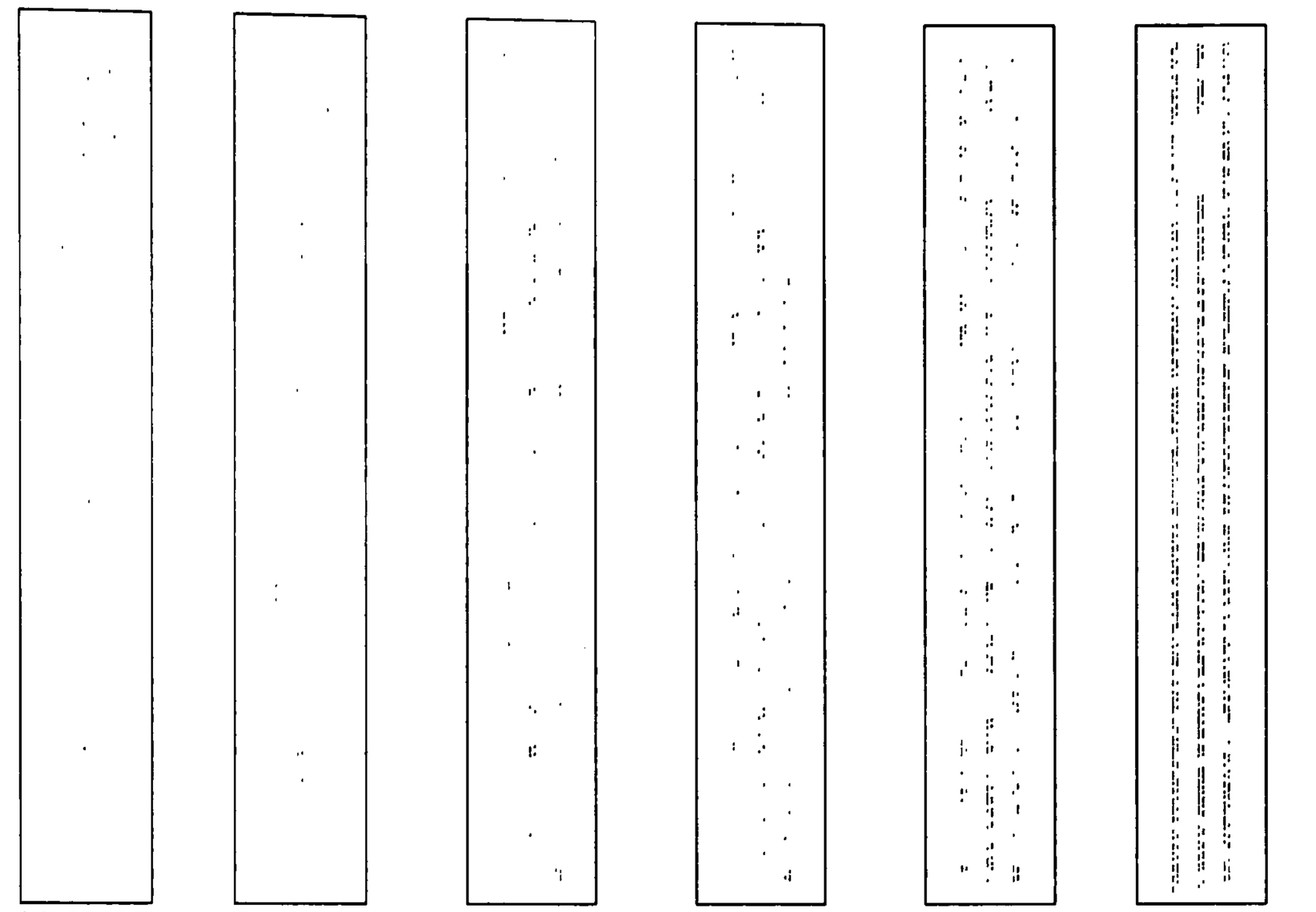


Fig 3.11 Distribution of plants with *P. aphanis* symptoms in the Wisbech tunnel A 2005, from 6 sample dates. The dots within each tunnel plan represent the location of infected plants. Infection developed throughout the tunnel in less than 83 hours of suitable conditions

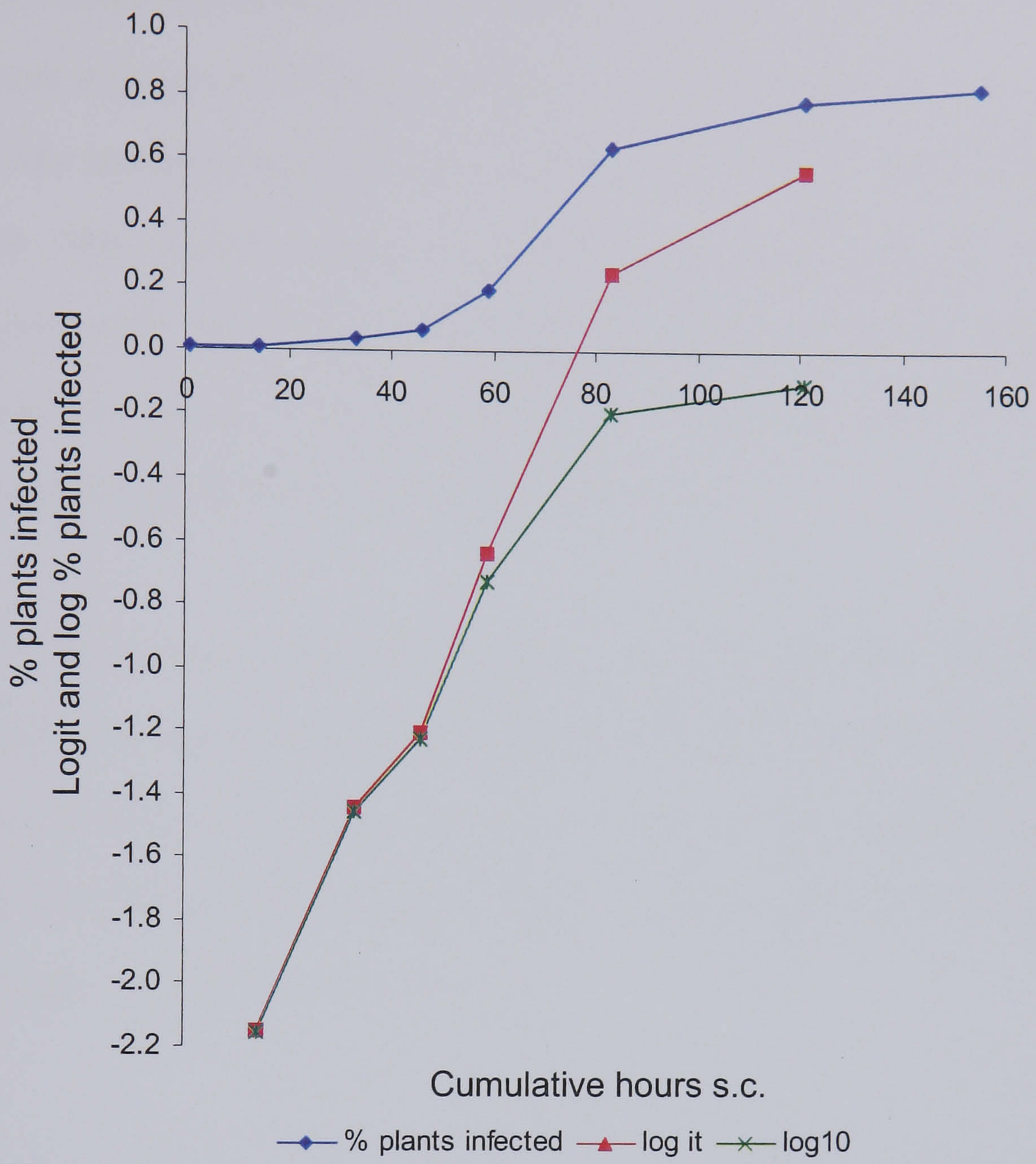


Fig. 3.12 Disease progress curve for percentage plants with symptoms, logit and log 10 of, percentage of plants with symptoms from tunnel A Wisbech 2005, after the fleece had been removed from the tunnel, r=0.06 per hour s.c.

Fig. 3.13 shows the development of infection in tunnel B from the Wisbech site 2005, over a period of 10 days (64 hours s.c.) after the fleece was removed. The fleece was removed from tunnel B 17 days after the fleece was removed from Wisbech 2005 tunnel A, which was adjacent to tunnel B (Fig 3.6). At the first assessment 217 plants had visible powdery mildew symptoms these were distributed throughout the tunnel. By the 2nd assessment (34 hours s.c.) the majority of the plants had symptoms of *P. aphanis*.

When the fleece was removed from tunnel B Wisbech site 2005 (121 hours s.c. after the fleece was removed from tunnel A), 217 plants (15%) had symptoms of powdery mildew infection. The number of plants showing symptoms increased steadily until 93% of the plants were infected. The apparent rate of increase grew at a rate of r= 0.11 per hour s.c. (Fig. 3.14).

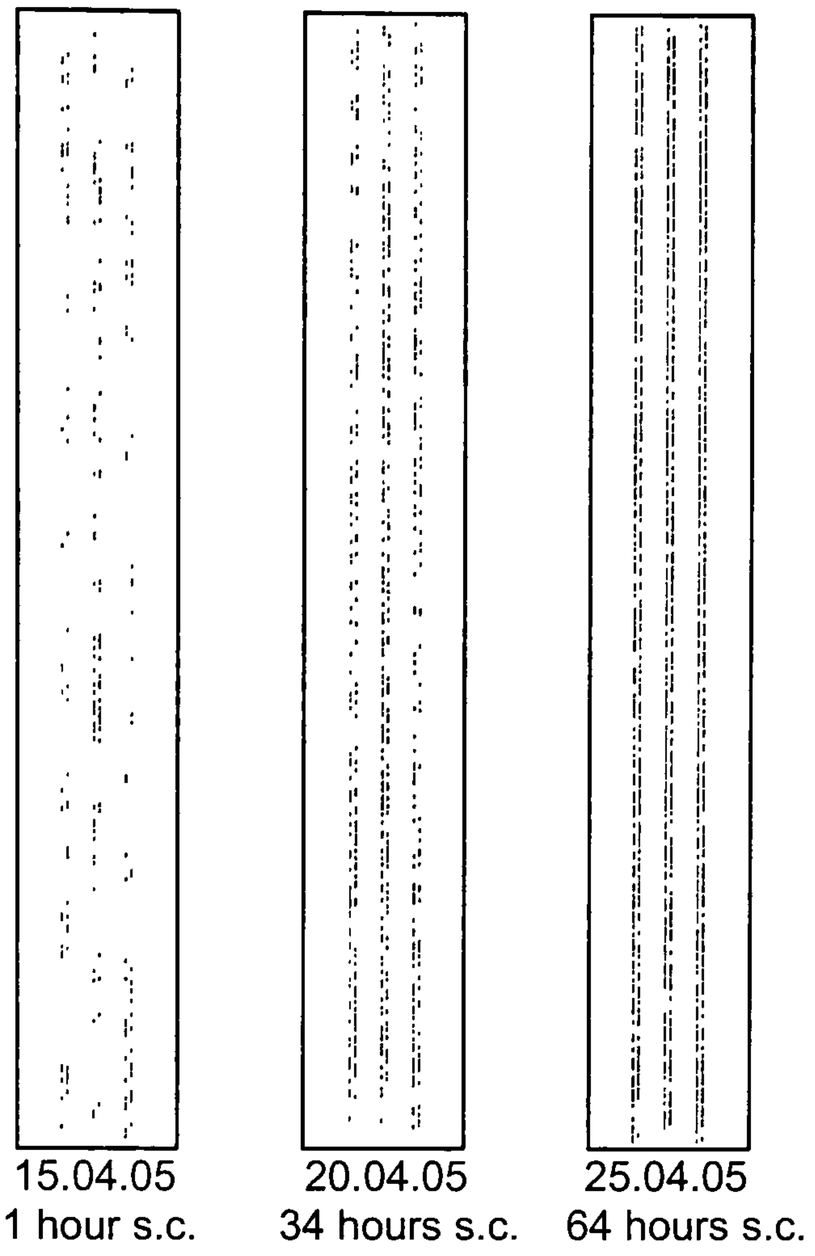


Fig 3.13 Distribution of plants with *P. aphanis* symptoms in the Wisbech tunnel B 2005, from 3 sample dates. The dots within each tunnel plan represent the location of infected plants. Infection developed throughout the tunnel in less than 34 hours of suitable conditions

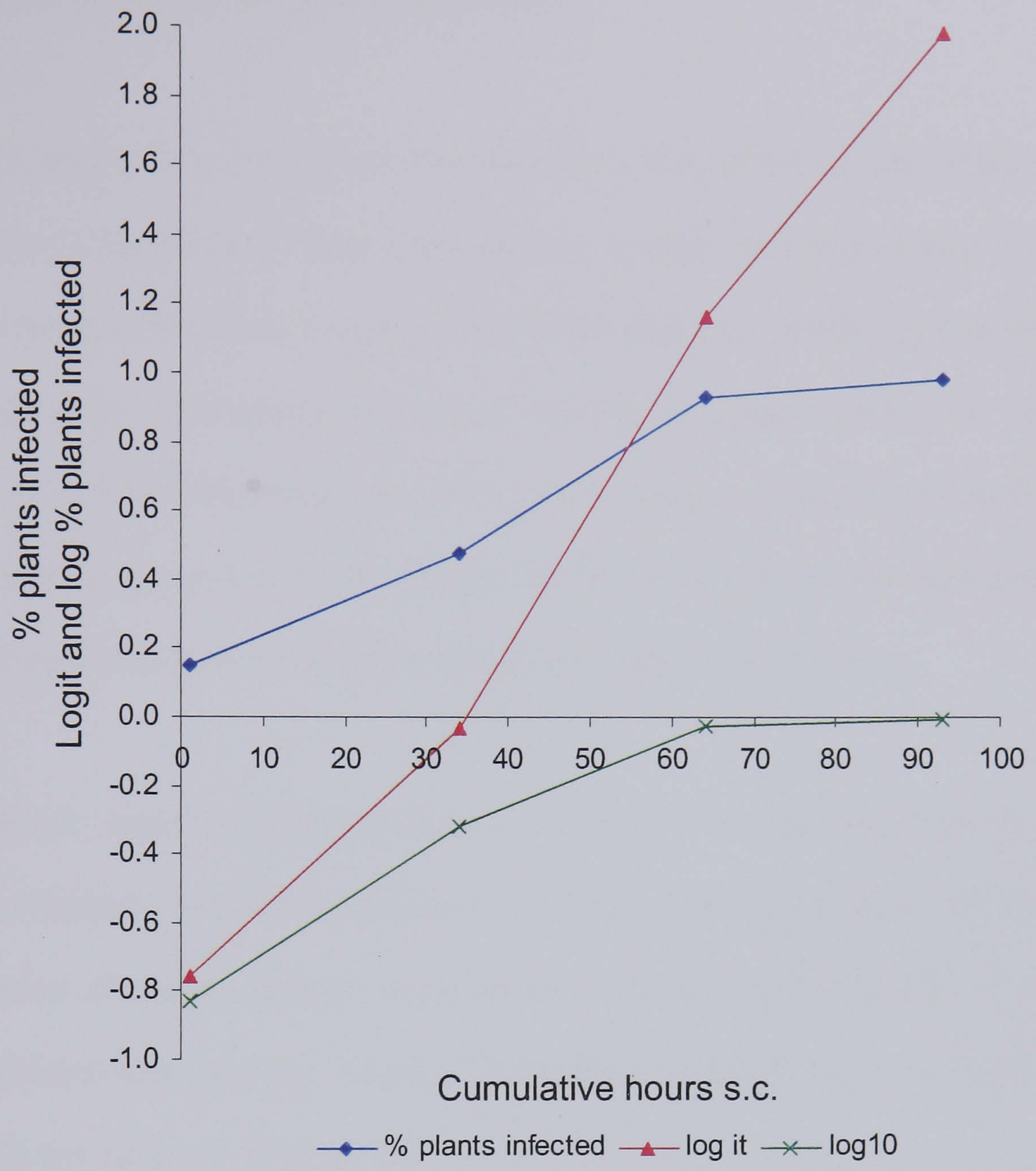


Fig. 3.14 Disease progress curve for percentage plants with symptoms, logit and log 10 of, percentage of plants with symptoms from tunnel B Wisbech 2005, after the fleece had been removed from the tunnel, r= 0.11 per hour s.c.

3.4.3 Newly planted site Mereworth 2005

Fig. 3.15 shows the development of infection in tunnel B from the Mereworth site 2005, over a period of 21 days (340 hours s.c.) after the misting was stopped. At the first assessment only 2 plants had visible powdery mildew symptoms, these were distributed throughout the tunnel. By the 3rd assessment (234 hours s.c.) the majority of the plants had symptoms of *P. aphanis*. The symptoms developed much more slowly in this newly planted site than they did in the established sites. The first patches of infected plants developed after 135 hours s.c.

36 hours s.c. after the misting ended, 2 plants (0.1%) had symptoms of powdery mildew infection. Within 135 hours s.c. 10% of the plants had symptoms. Then the number of plants showing symptoms increased until over 95% of the plants were infected with powdery mildew. The apparent rate of increase grew at a rate of r=0.03 per hour s.c. (Fig. 3.16).

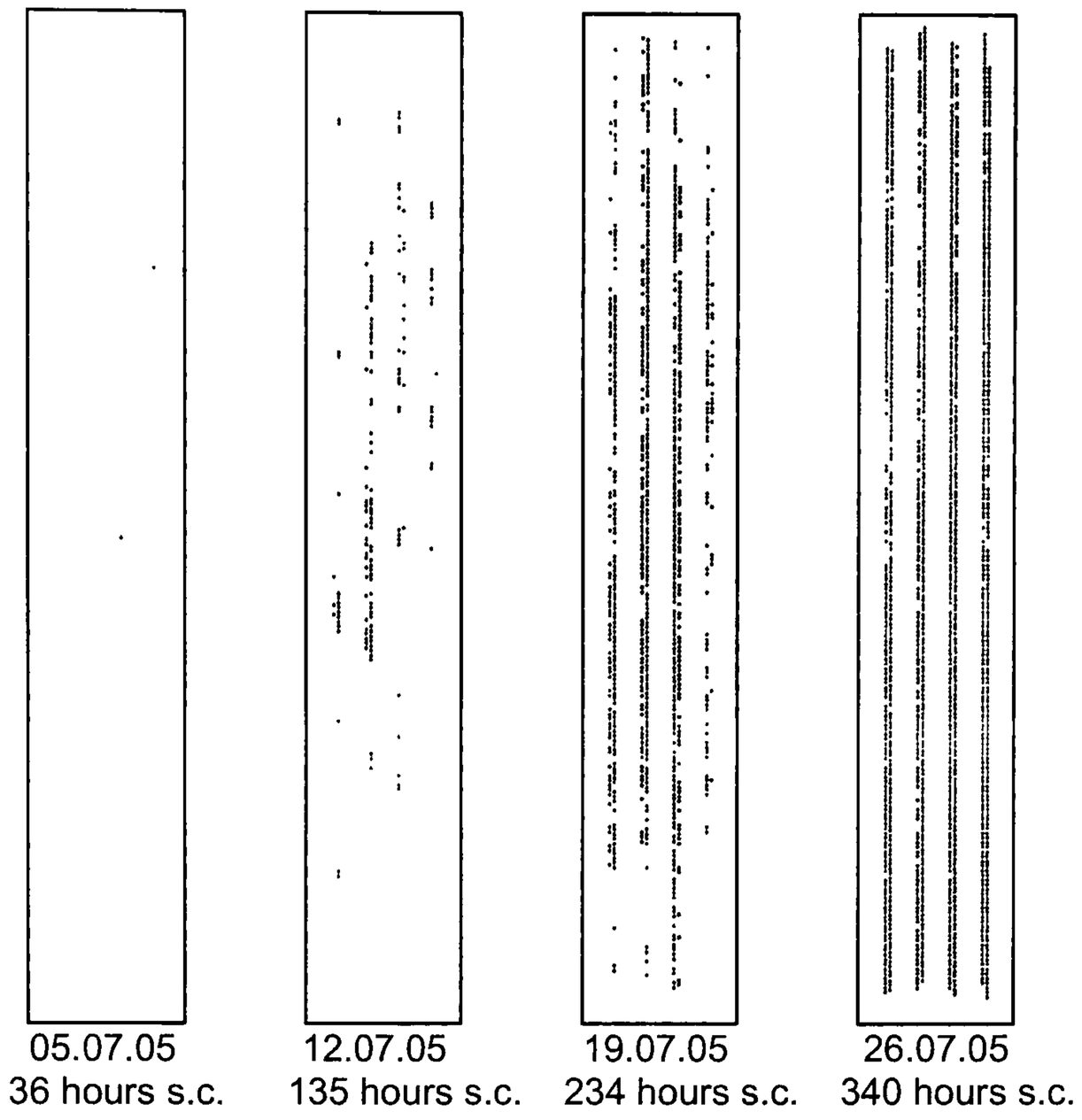


Fig 3.15 Distribution of plants with *P. aphanis* symptoms in the Mereworth tunnel B 2005, from 4 sample dates. The dots within each tunnel plan represent the location of infected plants. Infection developed throughout the tunnel in 234 hours of suitable conditions

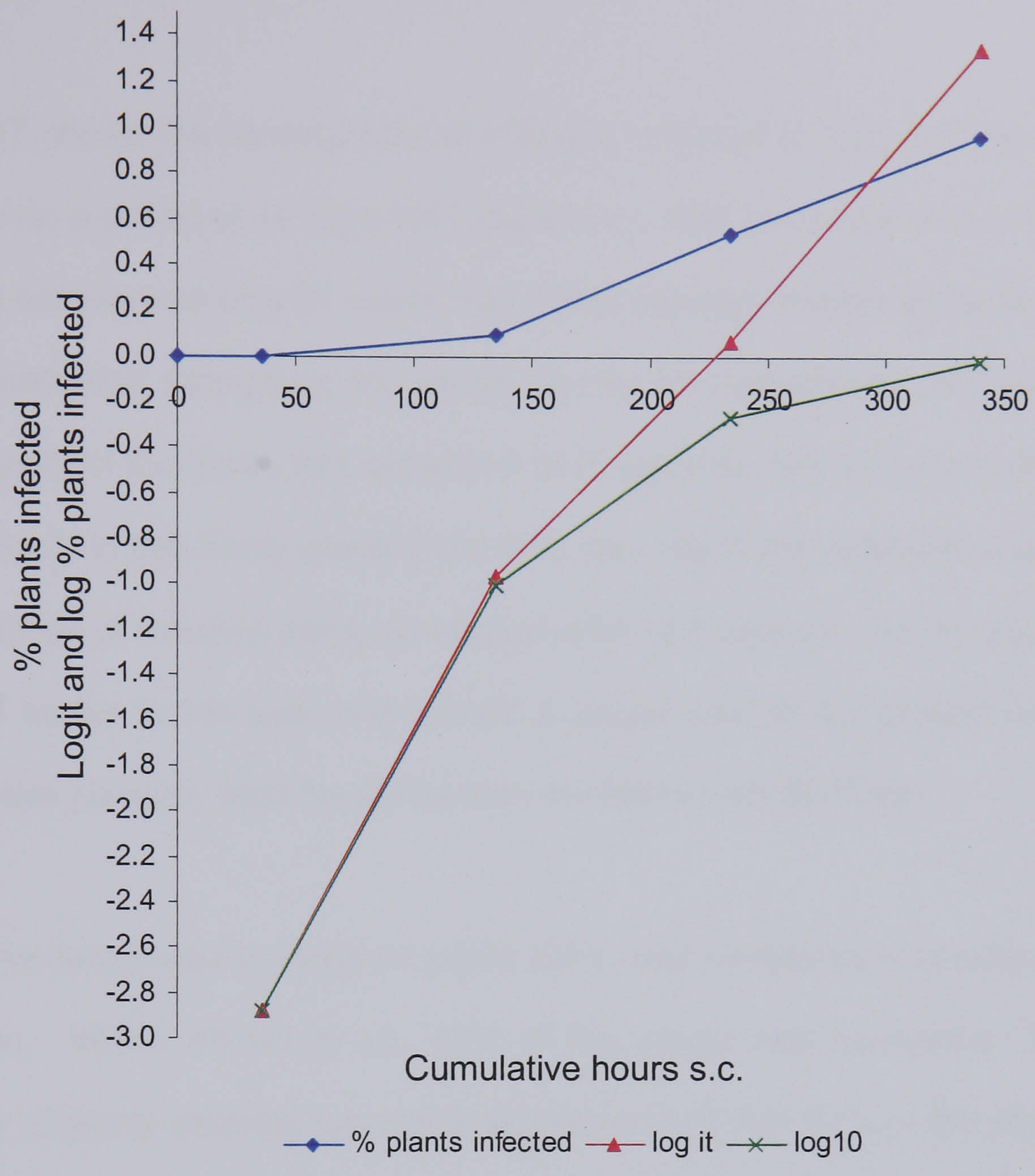


Fig. 3.16 Disease progress curve for percentage plants with symptoms, logit and log 10 of, percentage of plants with symptoms Mereworth 2005 tunnel B, after the misting had ended, r=0.03 per hour s.c.

3.4.5 Newly planted site Wisbech 2006

Fig. 3.17 shows the development of infection in tunnel A from the Wisbech site 2006, over a period of 14 days (331 hours s.c.), after the tunnel was covered. At the first assessment only 91 plants had visible powdery mildew symptoms, these were distributed throughout the tunnel. By the 3rd assessment (165 hours s.c.) the majority of the plants had symptoms of *P. aphanis*. The symptoms developed more slowly in this newly planted site than they did in the established sites. The first patches of infected plants developed after 45 hours s.c. but the plants were planted earlier in the year and so had a longer time in the ground before the tunnel was covered, than the plants from the Mereworth 2005 site.

When the tunnel was covered 91 plants (26%) had symptoms of powdery mildew infection. Within 45 hours s.c. 44% of the plants had symptoms. Then the number of plants showing symptoms increased until over 97% of the plants were infected with *P. aphanis*. The apparent rate of increase grew at a rate of r=0.02 per hour s.c. (Fig. 3.18).

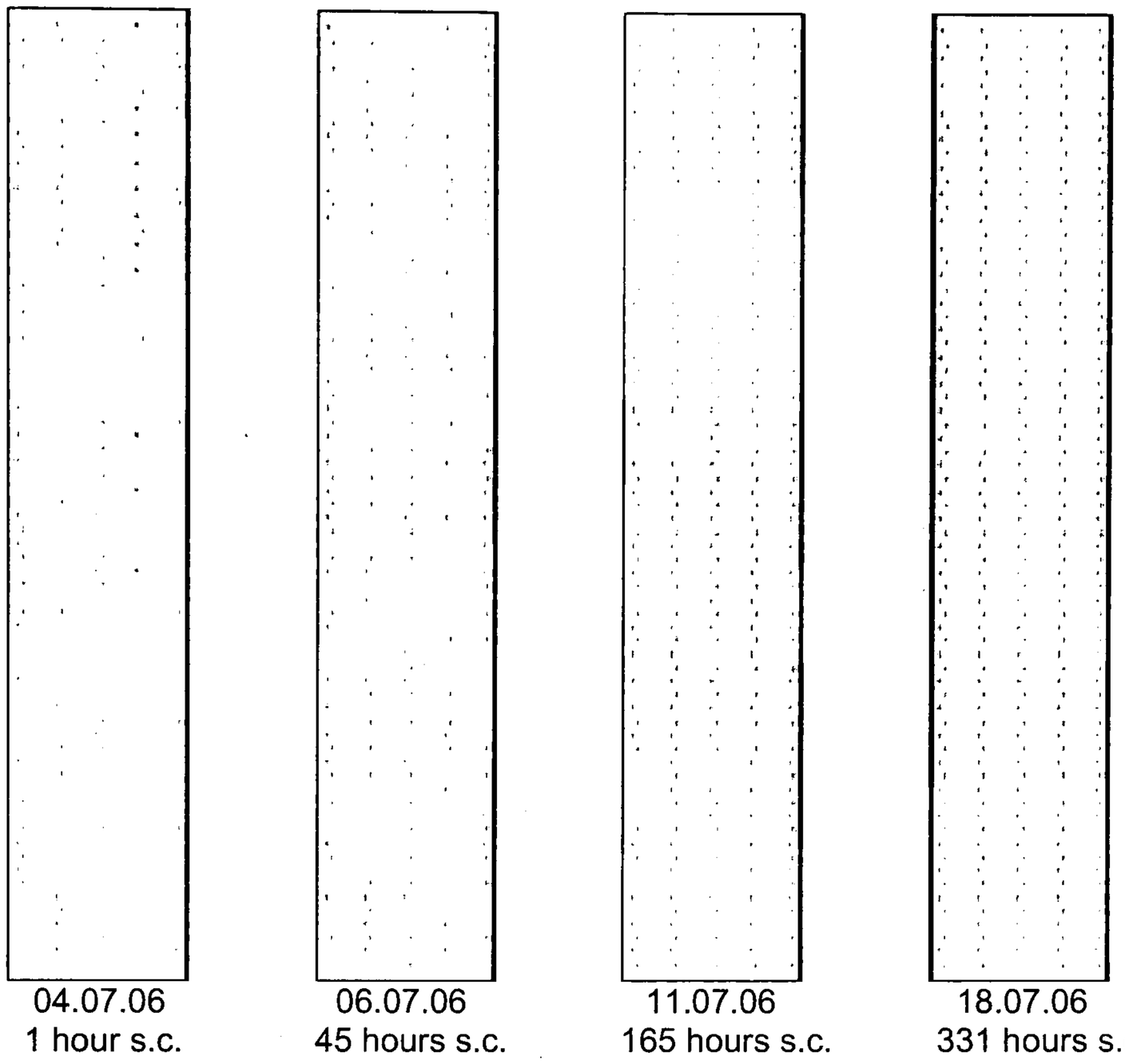


Fig 3.17 Distribution of plants with *P. aphanis* symptoms in the Wisbech tunnel A 2006, from 4 sample dates. The dots within each tunnel plan represent the location of infected plants. Infection developed throughout the tunnel in less than 45 hours of suitable conditions

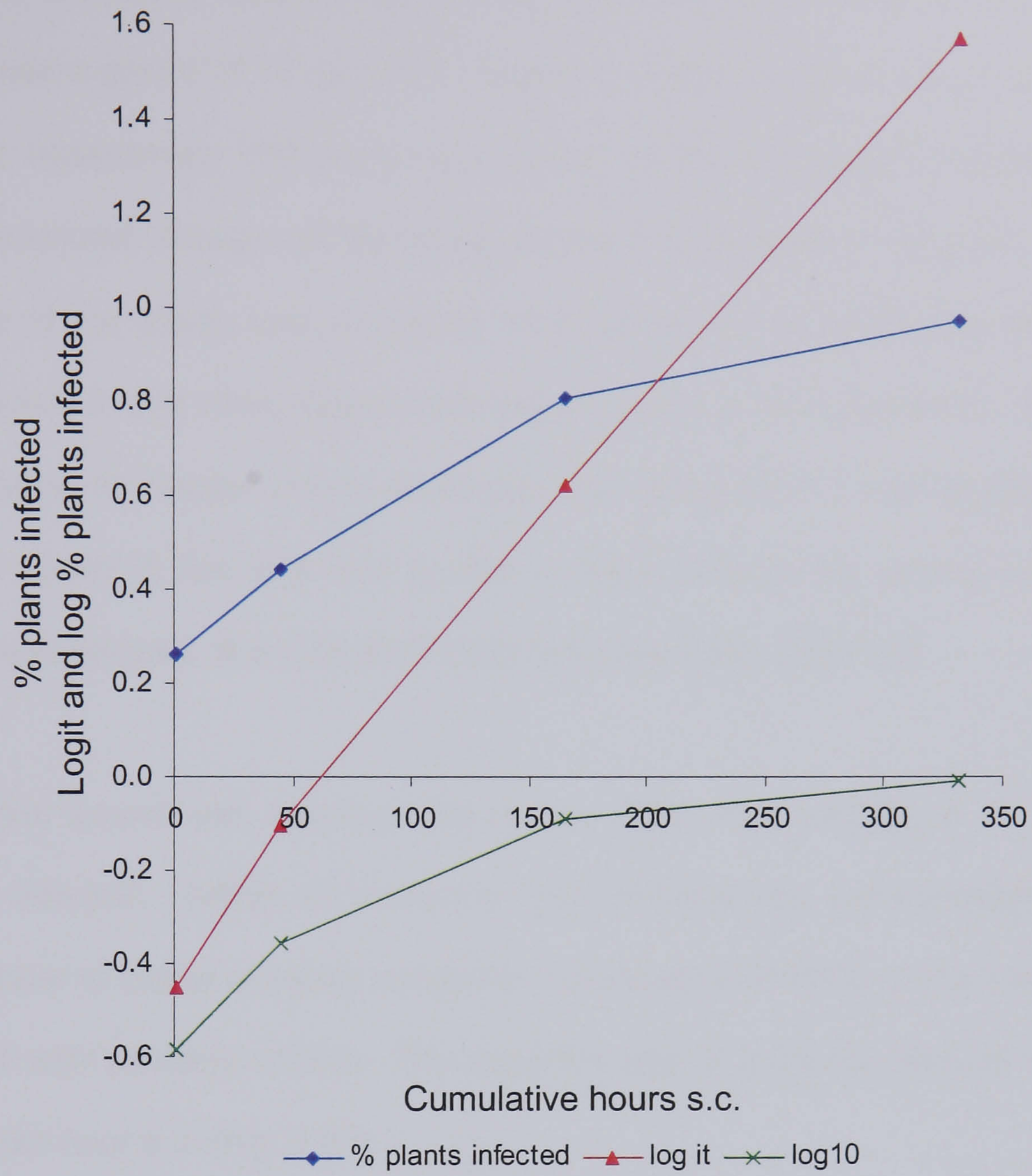


Fig. 3.18 Disease progress curve for percentage plants with symptoms, logit and log 10 of, percentage of plants with symptoms tunnel A Wisbech 2006, after the tunnel was covered, r=0.02 per hour s.c.

Fig. 3.19 shows the development of infection in tunnel B from the Wisbech site 2006, over a period of 14 days (331 hours s.c.) after the tunnel was covered. At the first assessment 163 plants had visible powdery mildew symptoms these were distributed through out the tunnel. By the 2nd assessment (45 hours s.c.) the majority of the plants had symptoms of *P. aphanis*. The symptoms developed more slowly in this newly planted site than they did in the established sites. The first patches of infected plants developed after 45 hours s.c. but the plants were planted earlier in the year and so had a longer time in the ground before the tunnel was covered, than the plants from the Mereworth 2005 site.

When the tunnel was covered 163 plants (48%) had symptoms of powdery mildew infection. Within 45 hours s.c. 64% of the plants had symptoms. Then the number of plants showing symptoms increased until 100% of the plants were infected with powdery mildew. The apparent rate of increase grew at a rate of r=0.01 per hour s.c. (Fig. 3.20).

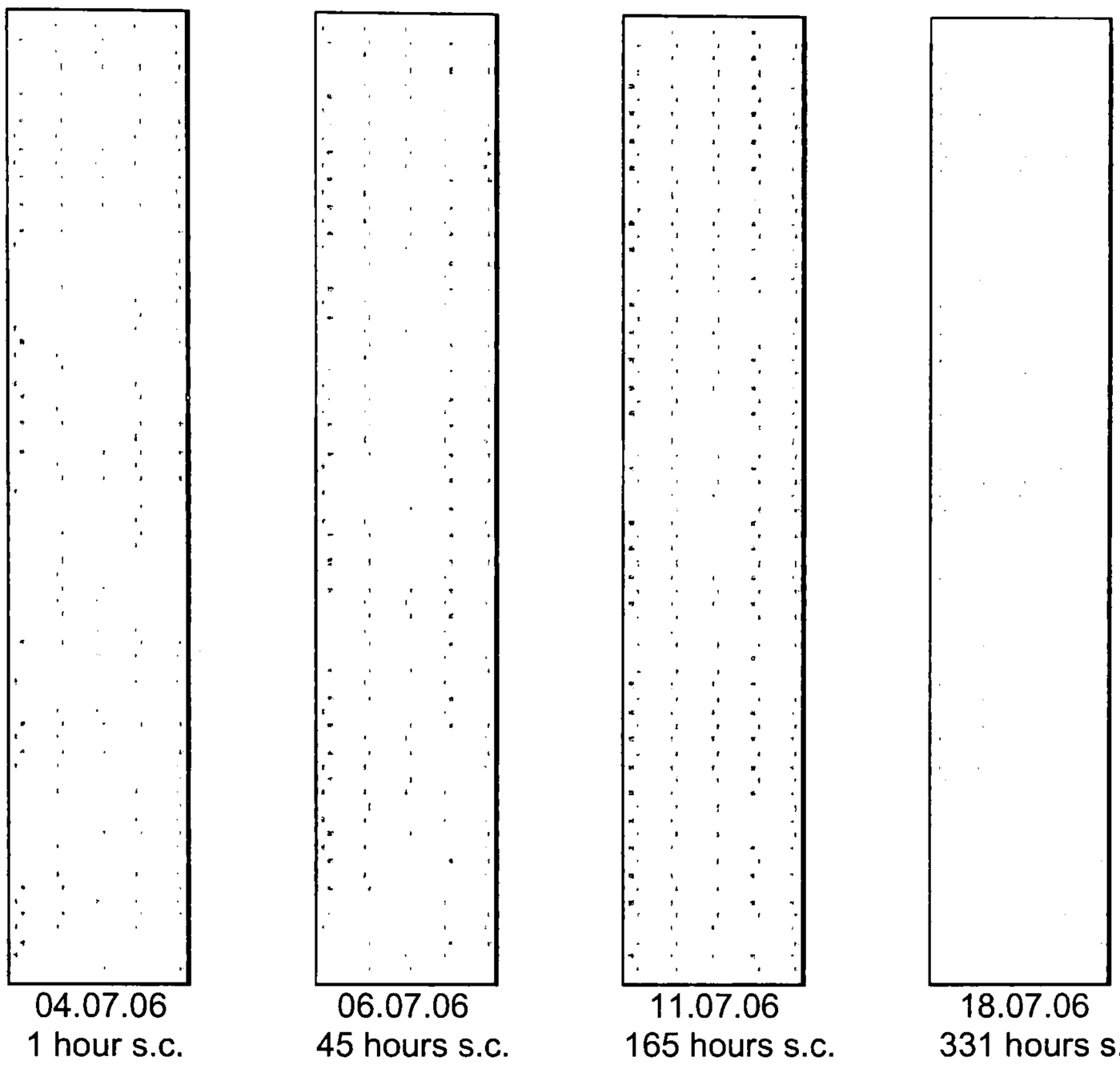


Fig 3.19 Distribution of plants with *P. aphanis* symptoms in the Wisbech tunnel B 2006, from 4 sample dates. The dots within each tunnel plan represent the location of infected plants. Infection developed throughout the tunnel in less than 45 hours of suitable conditions

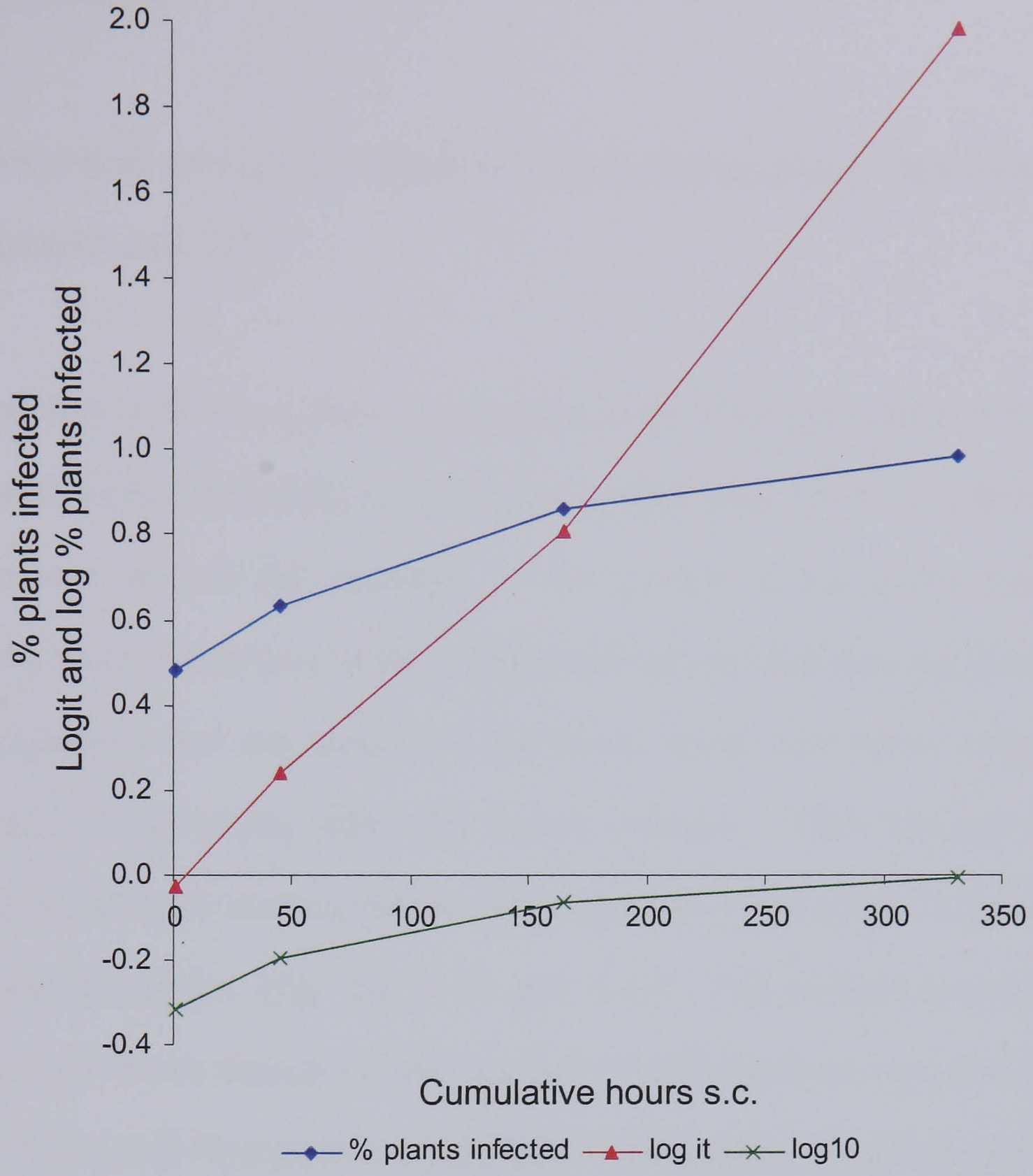


Fig. 3.20 Disease progress curve for percentage plants with symptoms, logit and log 10 of, percentage of plants with symptoms tunnel B Wisbech 2006, after the tunnel was covered, r=0.01 per hour s.c.

3.5 Discussion

3.5.1 Source of primary inoculum in overwintering crops - Mereworth 2004 and Wisbech A+B 2005

Within all 3 over wintered crops, the first plants on which symptoms of *P. aphanis* developed, were distributed throughout the tunnels (Fig. 3.9, 3.11 and 3.13), and not clustered around the openings in the tunnels. If the plants had shown clustering around a feature of the tunnel, such as the entrance and exit it would have suggested that the feature of the tunnel could have been linked to the distribution of the plants within the tunnel (Fletcher, 1984). As the epidemic progressed, disease clusters formed as neighbours of the initially infected plants developed symptoms (Fig. 3.9, 3.11 and 3.13). This pattern developed in a similar way to those shown for potato blight (*P. infestans*) on potatoes in Fig 3.1 (Cragg, 1971) and *Phytophthora* epidemics in bell peppers (Larkin *et al.*, 1995).

The results support that *P. aphanis* inoculum is present in the tunnels at the start of the season. As the vast majority of *P. aphanis* spores travel less than 10 feet (about 3 metres) (Peries, 1962a) it is unlikely that the inoculum was deposited on to the site before the tunnels were erected but not impossible as it is possible for a small number of spores to travel significantly longer distances than the majority of spores (Shaw, 1994). Also there would probably not have been any sources of *P. aphanis* inoculum early in the season from which the air borne spores could

have come. Even if a small amount of inoculum was deposited on the site before the tunnels were covered the infection initiated by it would behave in a similar manor to over wintering infection. The most likely source of over wintering inoculum is either *P. aphanis* mycelium on the plant (Peries, 1961) or chasmothecia (Farooq *et al.*, 2007, Rashid Khan, 1960, Salmon, 1900). This inoculum then develops as the tunnels are covered at the start of the season once the conditions become more conducive for growth after the winter.

3.5.2 Source of inoculum in newly planted sites - Mereworth 2005 and Wisbech 2006 A+B

The first plants to develop symptoms were located in the centre of the Mereworth tunnel 2005 (Fig. 3.15). At this site the infection, especially after 135 hours s.c. appeared to be located as patches within rows. The plants came from the propagator in boxes. Due to the harvesting and grading process all the plants in one box were probably from the same part of the propagation field. Hence, if there was a patch of infection in the propagators field all the plants from that patch could be graded and sorted together within a small number of boxes. So it would be possible for the grower to plant a box containing a majority of healthy plants followed by a box containing a majority of infected plants. The pattern of infected plants (Fig 3.15) closely follows the distribution of infected plants within a glasshouse where the infection was being spread by workers (Fletcher, 1984). Only in this case the infection was already present on the plants within a box, so

that a worker would start to plant from a new box of plants that had infection present, so forming what would appear to be the initial point of infection from which a 'tail' of infected plants would form. The level of infection on planting stock is known to influence the levels of disease development. For example the planting of *A. graveolens* L. var. *dulce* DC. free celery transplants resulted in less infection developing than when compared to transplants that had 5% and 40% blighted foliage (Berger, 1973). The tunnel was already covered when the plants were planted so it is unlikely that any air-borne inoculum would be deposited into the tunnel. The Mereworth experimental site was not located close to other fields with strawberry plants in them (about 2 miles from the rest of the farm). This shows that the inoculum must have been present on the plants when they were planted.

Plants with symptoms were distributed throughout the parts of the two tunnels scored at the Wisbech site 2006 (Figs 3.17 and 3.19). These tunnels were covered much later in the season. At the time of covering the percentage of plants showing symptoms in these tunnels was greater than in the other experiments. This indicates that inoculum was building up at the site before the tunnel was covered.

3.5.3 Rate of disease development

P. aphanis symptoms developed quickly and the curve followed the S-shape

pattern in both the Mereworth tunnel 2004 and Wisbech tunnel A 2005 (Figs. 3.10 and 3.12) (Lucas, 1998, Zadocks and Schein, 1979). In both tunnels there was a lag phase followed by rapid disease build up which slowed as the number of diseased plants reached a plateau. In the Mereworth tunnel 2004 the maximum infection was reached after 110 hours s.c. and in the Wisbech tunnel A 2005 maximum infection was reached after 155 hours s.c. The apparent rate of infection for both tunnels was r=0.06.

When the fleece was removed from Wisbech tunnel B 2005 (Fig 3.14) a larger percentage of the plants had symptoms, than in either Wisbech tunnel A 2005 (Fig. 3.10) or Mereworth 2004 (Fig. 3.12) at the start of the season. However the amount of inoculum had built up in the time between the fleece being removed from Wisbech tunnel A 2005 and the fleece being removed from Wisbech tunnel B 2005, so that there was a higher percentage of plants with symptoms of *P. aphanis* in Wisbech tunnel A 2005 when the fleece was removed from Wisbech tunnel B 2005. This suggests that the inoculum present in tunnel B had matured in the time between the fleece being removed from Wisbech tunnel A 2005 and the fleece being removed from Wisbech tunnel B 2005. The amount of infection in Wisbech tunnel B 2005 then increased rapidly until the majority of the plants in the tunnel had symptoms after 93 hours s.c. (r=0.11). The inoculum that was present in the tunnel before the fleece was put on was able to develop slowly under the fleece but not spread extensively, possibly due to reduced air flow.

Infection developed more slowly in the Mereworth 2005 tunnel but it still followed the classic epidemiological development pattern, with a lag phase followed by disease build up which resulted in a majority of plants with symptoms (Fig. 3.16). Maximum infection was reached after 350 hours s.c. (r=0.03). This was a slower disease build up than in the sites where the plants over wintered in the ground (Mereworth 2004 and Wisbech A+B 2005). The plants in Wisbech tunnels A and B 2006 were planted early in the season but the tunnels were not covered until the middle of the season (July). There had been time for the initial inoculum to develop so that when the tunnels were covered infection was already established (Figs. 3.18 and 3.20). In both tunnels infection developed until a majority of the plants were infected, 330 hours s.c. after the tunnels was covered. Wisbech tunnel A 2006 had an apparent rate of infection of r=0.02 and Wisbech tunnel B 2006 was r=0.01 over the timescale that the tunnels were scored for *P. aphanis* symptoms.

Disease development was much slower in the three newly planted sites (Mereworth 2005 and Wisbech A+B 2006) compared to the three established sites (Mereworth 2004 and Wisbech A+B 2005). It took between two and a half and three times as many hours s.c. for maximum infection levels to be reached in the newly planted sites, which meant the apparent rates of infection was less. It is possible that the inoculum over wintering on the plants in the field was already starting to develop before the tunnels were covered at the start of the season, so when the tunnels were covered the inoculum was ready to develop quickly.

Whereas the newly planted plants had been in cold store so there would have been no development of the inoculum until the plants were removed from the cold store and planted. So the development of the epidemic on newly planted plants would be much slower than the epidemic that would form on plants that had over wintered in the field.

3.5.4 Implications for tunnel management

The growers managed their tunnels (newly planted and established) believing that initial inoculum of *P. aphanis* is entirely wind borne from distant sources. Therefore they kept the tunnels as enclosed as possible for as long as possible, often resulting in a detrimental effect on fruit production, and only started to control *P. aphanis* once they saw the first signs of infection. Where as growers should open the tunnels up as soon as the conditions become sub optimal for fruit production and apply early applications of fungicides to eradicate as much inoculum as possible, to extend the lag phase of the epidemic as much as possible (Lucas, 1998). By reducing the initial inoculum, as or even before first symptoms are shown the rate of disease increase will be slowed as there would be less infection form which inoculum could be produced. Even when tunnels have not been covered, due to cropping period of the field, the growers should also monitor the field for signs of infection and apply an early fungicide application to slow disease build up and reduce inoculum levels.

3.6 Conclusions

Plants supplied from the propagators are the main source of initial *P. aphanis* inoculum in newly planted sites and the plants over wintering in the field are the source for established sites. The distribution of the infected plants within the tunnels, show that the inoculum was established, throughout the tunnels at the start of the season. The source of the inoculum was not from air borne spores once the season had started as the growers believed. If it had been air borne once the tunnels were covered the first plants to show symptoms would have been clustered around the ends of the tunnels where the inoculum would have been deposited. Especially if there had been a mild end to the winter and start of spring the inoculum present on the plants that over wintered in the field could develop much faster than the inoculum that would be present on plants that had been in the cold store all the winter.

This information could be integrated into the growers control programs. They need to manage their tunnels for optimum fruit production conditions at the start of the season rather than trying to use the tunnels as an enclosure which can keep the inoculum from infecting the tunnel. The tunnel is already infected with inoculum. The plants need to be treated early in the season so that the inoculum present is reduced as much as possible therefore extending the initial lag phase of the epidemic as long as possible.

Chapter 4 - Rule based prediction system

4.1 Introduction

Writing in 1990 Hau stated that during the previous 20 years several epidemiological models had been developed that described the development of plant diseases in time and/or space (Hau, 1990). A model for the epidemiology of grape powdery mildew based on Vanderplank's compound interest equation (Van der Plank, 1963) was published in 1980 (Sall, 1980) and a model for the demographic growth of *U. necator* was developed using spreadsheet software by Chellemi and Marios (1991) who stated that 'with the advent of microcomputers and their associated software, it is now possible to develop even complex models without a significant investment of time to learn a programming language'. There are now many dynamic models that, intuitively, might be expected to be useful for disease forecasting. But they are seldom used for this (Parker, 2001); possibly because they are too complex for practical use (Vallavieille-Pope *et al.*, 2000).

Models are representations of systems. They attempt to mimic the essential features of a particular system where that system is taken as a limited part of reality (Dent, 1995). Models strive to represent the growth and development of the pathogen and host as a series of ever more complex formulae, where as a rule based prediction system uses subjective knowledge about a system to

identify when a plant will be at highest risk of attack from a pathogen (Norton and Mumford, 1993). When the point of highest risk has been identified the grower can be alerted to the possible need for an application of a fungicide. The relationships between components of a system are represented with rules rather than formulae.

4.1.1 Previously developed modelling systems

Models have been developed for various different powdery mildews. Models for sugar beet (Asher and Williams, 1991) and jujube (Sinha, 2005) exist to predict end of season disease pressures. Both of these models result in an output that can be used to advise growers about the amount and timing of fungicide applications that will be needed. There have been models developed that describe both the growth of the plant and of the pathogen for grape powdery mildew (Chellemi and Marois, 1991, Sall, 1980). For other powdery mildews extensive work has been done in the laboratory under controlled conditions trying to develop just one formula that will form part of a larger complex model for the effect of temperature on latent period for, rose (Xu, 1999a, Xu, 1999b), apple (Xu, 1996, Xu, 1999c), clematis (Xu and Robinson, 2001) and hawthorn (Xu and Robinson, 2000). A field based model for 4 foliar diseases of wheat (including powdery mildew) has been developed to be used with a management decision support system (Audsley *et al.*, 2005). However despite substantial investment from funders, this is not used in commercial crop production.

4.1.2 Problems with modelling

Many apparently good models seldom get used practically in the field by the grower (Van Maanen and Xu, 2003) as in practice they are often too complicated. This belief is reiterated by some strawberry growers, who are concerned that models for *P. aphanis* which they have seen the initial development of, have been too complex (personal communication, Harriet Duncalfe, Wisbech). This resulted in the growers having insufficient confidence in the model to participate in its development.

A model's predictions are only as good as the weakest part of data that the model is run with. The weather data that is used in the model must be representative of the site for which the model is producing a disease warning. If the data is not representative of the site the warning will not reflect the characteristics of the site. Only very recently have farmers and growers started to invest in their own weather stations (personal communication, Simon Turner, Agri-Tech). In the past models had to use data sourced from the closest commercial weather station which could have been many miles away. This could have resulted in discrepancies between the predicted and observed disease levels for crops grown externally, but for crops grown in enclosed structures such as polythene tunnels the predicted and observed disease levels could bear no resemblance at all to each other. This acted as a further reason why strawberry

growers lacked confidence in previous models and prediction systems as these have often used external weather conditions to predict the internal conditions. The weather stations available now are sophisticated enough to have sensors in several tunnels at the same time.

4.1.3 Rule based prediction systems

Many different types of model have been developed for the study of plant pathogens. There are analytical models where explicit formulae are derived for predicted values or distributions (written as algebraic expressions). Simulation models, these simulate the different population dynamics of the pathogen and host (less mathematical sophistication is required). Also expert systems which mimic the processes employed by a human expert and finally rule based models (Dent, 1995, Norton and Mumford, 1993). Rule based systems and expert systems are very similar in design. Expert systems are generally designed to supplant some aspects of an experts role while rule based systems support decision makers (Parker and Sinclair, 2001). Many plant disease prediction systems utilize a rule based approach, in their simplest form to predict the occurrence of the pathogen (Yuen and Hughes, 2002). Rule based systems use 'IF-THEN' rules to progress through a number of discrete states to describe disease development (Dent, 1995, Norton and Mumford, 1993). As with other types of modelling systems, rule based prediction systems need to be problem specific (Travis and Latin, 1991, Van Maanen and Xu, 2003). The rules that will

govern the system need to be developed by an expert before the system is evaluated and then possibly adopted by the growers (Travis and Latin, 1991). The more complex the systems (analytical) are, the more precisely the parameters need to be developed. When many precise parameters are used to develop a system they all need to integrate or the system will fall down. Where as the less complex systems (rule based) are more robust but may not represent the problem as completely as a more detailed system.

More predictive systems have been developed for late potato blight than any other pathogen (Krause and Massie, 1975). Rule based prediction/forecasting system have been used by potato growers to predict the onset of conditions suitable for growth and development of late potato blight since 1920's (Taylor, 2000). The Blitecast system developed in 1975 went a stage further and as well as predicting the first application of a fungicide also predicted the interval between subsequent applications (Krause *et al.*, 1975, Taylor, 2000).

A rule based prediction system will be developed for *P. aphanis*. The system will use the temperature and relative humidity measured from within the polythene tunnels, so the output from the system will be specific to the site and field from which the measurements were taken. A set of rules will be developed that will enable the prediction of the periods when a strawberry field is at highest risk of infection by *P. aphanis* and so the optimal time for a grower to apply a fungicidal control product for the control of *P. aphanis*. A rule based prediction system was

chosen so that the system would be robust enough to cope with changes in the cultivar of strawberry plants that are grown. Also so that when the grower fails to apply a fungicide when prompted to by the system it is easy to reset so the rules restart when the grower manages to apply the fungicide treatment.

4.1.4 Sensitivity analysis

Sensitivity analysis was carried out on the parameters of the prediction system to determine which of the parameters has the greatest effect on the output (Norton and Mumford, 1993, Sgrillo *et al.*, 2005). The parameters of the model or prediction system can be screened to determine their influence on the model (Gilligan *et al.*, 1994). Some of the prediction system parameters are kept constant while the other parameters are altered. The prediction systems outputs are then analysed for differences in the number of predictions, for whatever the prediction system is predicting (Andrade-Piedra *et al.*, 2005, Berger *et al.*, 1995, Willocquet and Savary, 2004). The parameters can then be ranked for importance. The parameters that cause a large change in the output are the most sensitive while those that cause the smallest change are the least sensitive parameters.

4.2 Aim + objectives

4.2.1 Aim

Development of rule based prediction system to predict high risk periods for infection by *P. aphanis* (3rd aim page 27)

4.2.2 Objectives

1. Identification of the temperature and relative humidity that favours development of *P. aphanis* infection from published literature.

2. Compare conditions identified in the literature with conditions associated with initiation of disease development in the field.

3. Development of scheme to predict high risk days for infection by *P. aphanis*.

4. Compare the high risk days identified by the prediction system with the dates growers applied control products.

4.3 Methods

4.3.1 Development of rule based prediction system

The literature was reviewed to examine the range of conditions known to effect the development of *P. aphanis* infections. This information was used to estimate the duration of a complete disease cycle. The conditions identified from the literature were compared to those collected from within polythene tunnels as part of this project. From this comparison the initial parameters for use in the prediction system were developed. The prediction system calculations were run in an Excel spreadsheet (2003), Microsoft Corporation.

4.3.2 Comparison of predicted high risk periods with first symptoms

The conditions (temperature, relative humidity and leaf wetness) within commercially managed tunnels along with the dates that first symptoms of *P. aphanis* developed had been collected as part of the work presented in Chapter 3. The method and details of the conditions which were collected can be found in the methods section of Chapter 3. The conditions from within the commercially managed tunnel were input into the prototype prediction system, which used the initial, parameter estimates (Table 4.3). From this, the first high risk period predicted by the prediction system was compared to the dates that the initial symptoms of *P. aphanis* infection developed.

The predicted high risk periods were close to the actual dates that symptoms developed on but did not fully correspond with them. In light of this the initial parameters were then revised so that the first predicted high risk period matched with the observed development of symptoms of *P. aphanis* in the field. This revised version of the prediction was used in subsequent evaluations.

4.3.3 Sensitivity analysis of prediction system parameters

Sensitivity analysis was carried out on the prediction system new parameters to determine which of the parameters has the greatest effect on the output (the number of predicted high risk periods). Environmental data collected from the field was input into the prediction system (new parameters). All parameters but the one being tested were kept constant while the parameter under analysis was altered (Berger *et al.*, 1995, Willocquet and Savary, 2004). The parameters and range of values over which the sensitivity analysis was tested are presented in Table 4.1. All the parameters were tested through the prediction with and with out leaf wetness data present as this is the measurement that growers are most likely to be missing.

Sensitivity analysis was also carried out with selected pairs of parameters (Table 4.2). The same values were used as when a single parameter was being tested

Table 4.1 Prediction system parameters, range of values and increments of increase that were used for sensitivity analysis of the prediction system

	Range of values	Increment of increase
Germination temperature (°C)	13-25	0.5
Growth temperature (°C)	13-25	0.5
Relative humidity (%)	10-100	5
Leaf wetness (%)	60-100	5
Maximum germination temperature (°C)	25-35	0.5
Maximum growth temperature (°C)	25-35	0.5

Table 4.2 Selected pairs of prediction system parameters that were used for the second sensitivity analysis

Germination temperature (°C)	and	Relative humidity (%)
Germination temperature (°C)	and	Leaf wetness (%)
Relative humidity (%)	and	Leaf wetness (%)
Relative humidity (%)	and	Growth temperature (°C)
Relative humidity (%)	and	Maximum growth temperature (°C)
Relative humidity (%)	and	Maximum germination temperature (°C)
Germination temperature (°C)	and	Growth temperature (°C)
Germination temperature (°C)	and	Maximum growth temperature (°C)
Germination temperature (°C)	and	Maximum germination temperature (°C)
Growth temperature (°C)	and	Maximum growth temperature (°C)
Growth temperature (°C)	and	Maximum germination temperature (°C)
Leaf wetness (%)	and	Maximum growth temperature (°C)
Leaf wetness (%)	and	Growth temperature (°C)
Leaf wetness (%)	and	Maximum germination temperature (°C)

(Table 4.1). At each increment of the first parameter out of the pair the second parameter was altered so that the number of predicted high risk periods, were obtained for each increment of the second parameter. Again each pair of parameters was tested with and with out leaf wetness data, where one of the parameters in the pair was leaf wetness this pair was not retested.

4.3.4 Comparison of predicted high risk periods with grower applications

The high risk days predicted by the prediction system would be when the grower would achieve maximum benefit from applying a fungicide application. The high risk day predicted by the prediction system corresponds to the onset of the perception threshold of the epidemic often at the start of the exponential phase (Lucas, 1998, Zadocks and Schein, 1979). The infection present in the site would have reached maturity and so would therefore be about to produce more inoculum so the grower needs to control it at this point, when the prediction system predicts a high risk day that should act as a trigger for the grower to apply a fungicide.

Spray schedules were obtained for commercially managed fields along with the corresponding measurements from on farm weather stations. The data from the weather stations (starting from 1st January) was input into the prediction system (new parameters, Table 4.4), the number and dates of the predicted high risk days (predicted fungicide applications) were compared with the number and

dates of the treatments applied for control of *P. aphanis*, by the growers, so that any reductions in fungicide use could be identified if the grower had applied products when prompted by the prediction system.

4.4 Results

4.4.1 Prediction system parameters

The literature review provided a large amount of information about the range of conditions affecting the infection by and growth of *P. aphanis*. Germination of conidia is limited by temperature, relative humidity and leaf wetness, whereas the rate of mycelial growth and sporulation is only limited by the temperature (Table 1.3) (Amsalem *et al.*, 2006, Blanco *et al.*, 2004, Jhooty and McKeen, 1964b, Jhooty and McKeen, 1965, Miller *et al.*, 2003, Peries, 1962a). The development time for a fungal infection by *P. aphanis* is 144 hours of suitable conditions from conidial germination to visible symptoms. Infections established as viable mycelium can generate further inoculum after 84 hours of suitable conditions (Table 1.4) (Peries, 1962*b*). The values identified as the minimum, maximum and optimums for the germination and growth of *P. aphanis* were obtained from laboratory experiments. They therefore needed to be transferred to the field with care as the conditions within the field would not always be ideal for the germination or growth of *P. aphanis*.

From the literature review, information regarding the development time of *P. aphanis* and field observations, it was possible to develop an initial set of conditions (Table 4.3), the parameters for a rule-based prediction system, to identify when strawberry plants would be at greatest risk of infection by *P. aphanis*. The life cycle of *P. aphanis* is divided into two parts, germination of the conidia and then growth of the fungus including sporulation. Germination of a spore requires a total of 6 hours (Table 1.4) where the temperature is greater than 17.5°C and less than 30°C, the relative humidity is greater than 60% and the leaf wetness is less than 95% (Table 4.3). Further growth of the spore (up to and including spore release), that has germinated requires a further 138 hours (Table 1.4) where the temperature is greater than 16°C and less than 30°C (Table 4.3). The prediction system calculates the amount of time elapsed when conditions are suitable for a conidium to germinate, reach maturity and generate new inoculum. If conditions are not suitable for germination or growth of *P. aphanis* this does not reduce the level of development the fungus has achieved. Fungal growth starts again from the point it had reached previously when conditions are suitable. The output of the prediction system is presented as percent completion of the total hours needed for a conidium to reach maturity.

Table 4.3 Parameters from literature and initial field observations (initial parameters) for the prediction system

	Initial parameters[1]
Germination temperature (minimum) (°C)	17.5
Growth (and spore release) temperature (minimum) (°C)	16
Relative humidity (minimum) (%)	60
Leaf Wetness (maximum) (%)	95
Maximum germination temperature (°C)	30
Maximum growth (and spore release) temperature (°C)	30
No. of hours[2] to maturity germination and growth (hours)	6 + 138
No. of hours[2] to maturity germination and growth *established* field 1st infection	*Na*
No. of hours[2] to maturity germination and growth *established* field after 1st infection	*Na*
No. of hours[2] to maturity germination and growth *new* field all infections	*Na*

[1]Amsalem, *et al*., 2006, Blanco, *et al*., 2004, Jhooty and McKeen, 1964, 1965, Miller, *et a*l., 2003, Peries, 1962a

[2]Number of hours of suitable conditions (temperature, relative humidity and leaf wetness)

4.4.2 Comparison of predicted high risk periods with first symptoms

Environmental conditions within 4 commercially managed tunnels were recorded (data and collection methods are detailed in chapter 3). Two tunnels had established crops (Mereworth 04 and Wisbech A 05) and two had newly planted crops (Mereworth 05 and Wisbech 06). This data was input into the prediction system and the first predicted high risk days were compared with the actual development of first symptoms within each tunnel (Figs. 4.1, 4.2, 4.3 and 4.4).

In both of the established fields Mereworth 04 (Fig. 4.1) and Wisbech A 05 (Fig 4.2) the predicted high risk days were later than the actual development of first symptoms. In Mereworth 04 (Fig. 4.1) the predicted high risk day had not developed by the time that the majority of the plants had symptoms. A predicted high risk day is indicated by when the line representing number of hours suitable for maturity reaches 100% within each figure. The predicted high risk day for Wisbech A 05 (Fig. 4.2) developed when there was about 40 % infection within the site. In both cases the predicted high risk day is not close enough to the actual date when symptoms developed for the prediction to be useful to the grower.

In both the newly planted fields Mereworth 05 (Fig. 4.3) and Wisbech 06 (Fig. 4.4) the predicted high risk days occurred before the first symptoms were visible in the tunnels. There were two predicted high risk days for Mereworth 05 (Fig.

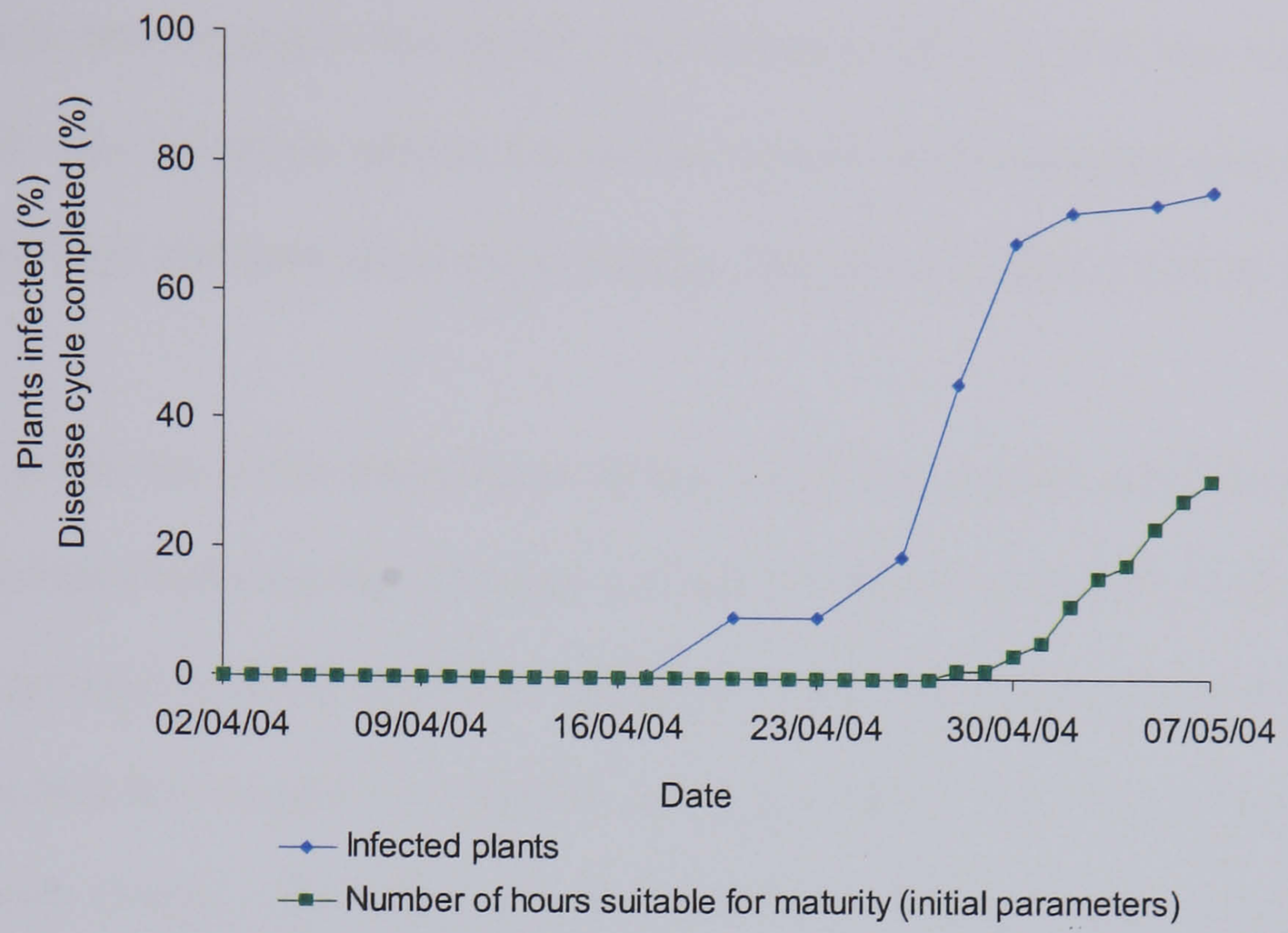


Fig. 4.1 Disease development data for Mereworth 04 (established field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed

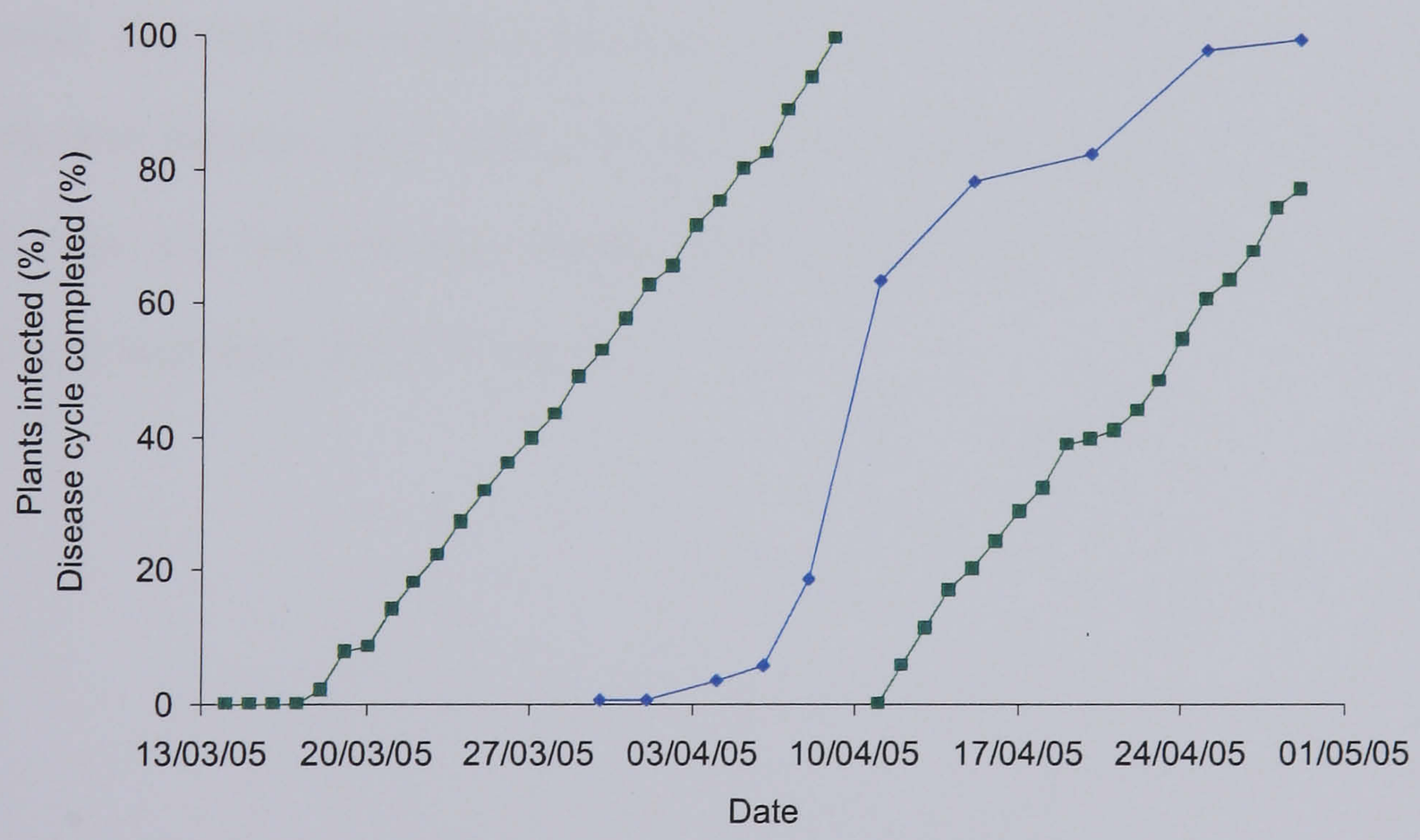


Fig. 4.2 Disease development data for Wisbech A 05 (established field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed

4.3) before the third predicted high risk day coincided with the first actual symptoms developing in the tunnel. For Wisbech 06 (Fig. 4.4) the first predicted high risk day occurred before the actual symptoms developed and the second predicted high risk period occurred slightly after the infection became visible.

In light of this the initial parameters of the prediction system were revised slightly until the first predicted high risk day corresponded to the development of the first visible symptoms of *P. aphanis* infection in the field (new parameters) (Table 4.4). So that the conditions required are; germination of a spore requires a total of 6 hours (Table 1.4) where the temperature is greater than 15.5°C and less than 30°C, the relative humidity is greater than 60% and the leaf wetness is less than 95% (Table 4.4). Further growth of the spore (up to and including spore release), that has germinated requires a further 78 hours in an established field for the first infection and 138 hours for newly established fields and established fields after the first infection (Table 1.4) where the temperature is greater than 18°C and less than 30°C (Table 4.4).

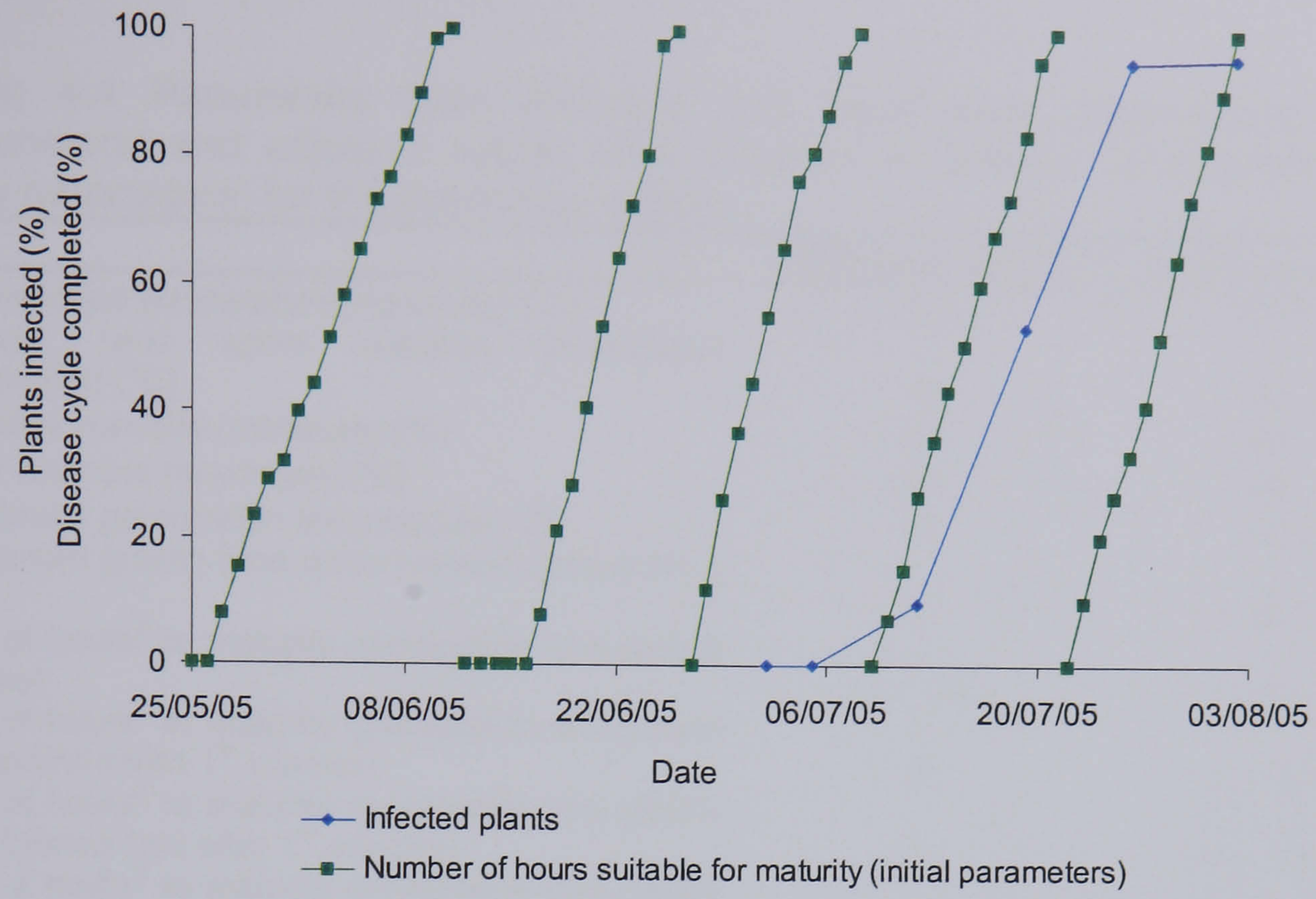


Fig. 4.3 Disease development data for Mereworth 05 (newly planted field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed

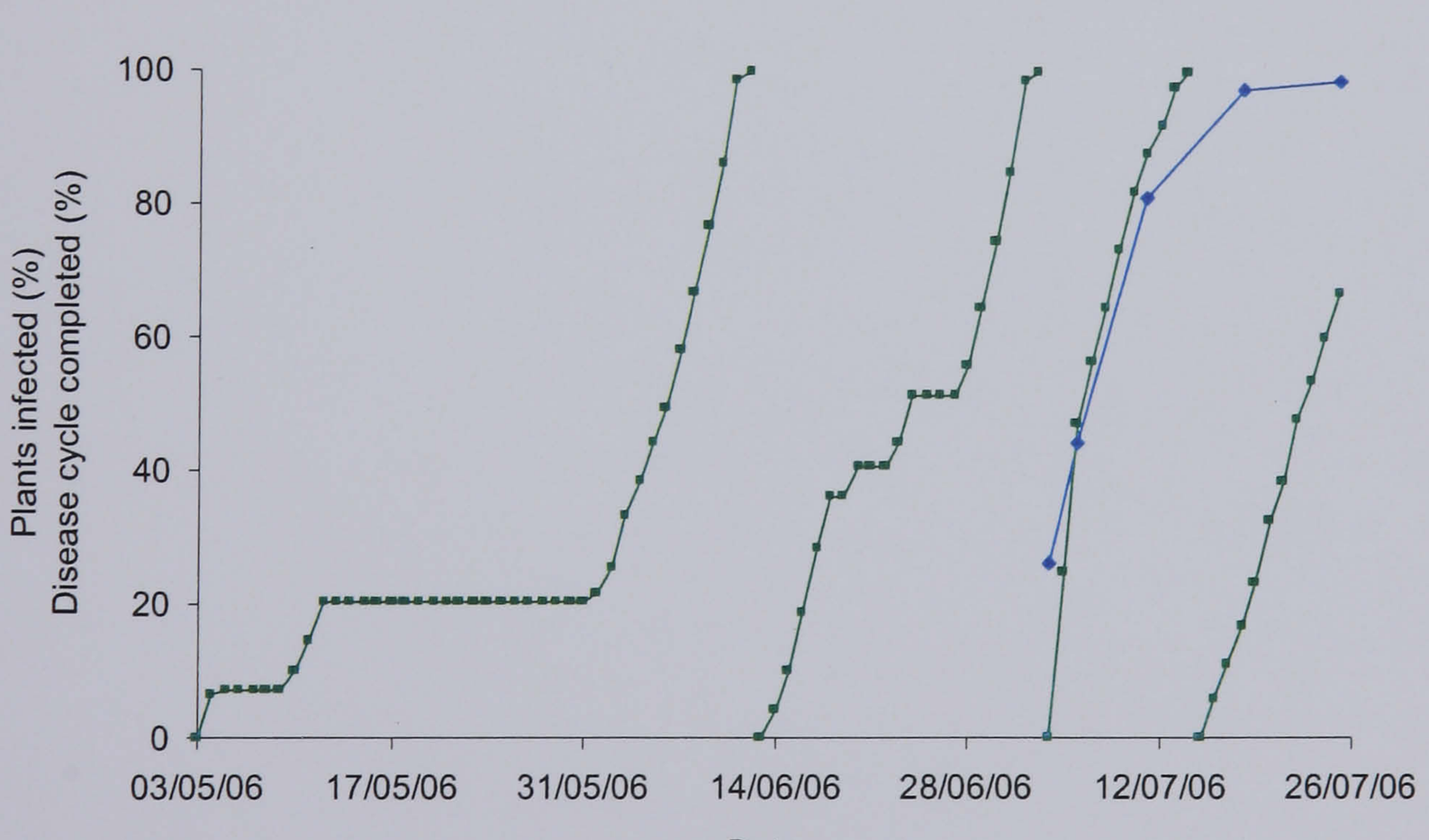


Fig. 4.4 Disease development data for Wisbech 06 (newly planted field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed

Table 4.4 Parameters from literature and initial field observations (initial parameters) and adjusted values after analysis of disease development data (new parameters) for the prediction system

	Initial parameters[1]	New parameters
Germination temperature (minimum) (°C)	17.5	15.5
Growth (and spore release) temperature (minimum) (°C)	16	18
Relative humidity (minimum) (%)	60	60
Leaf Wetness (maximum) (%)	95	95
Maximum germination temperature (°C)	30	30
Maximum growth (and spore release) temperature (°C)	30	30
No. of hours[2] to maturity germination and growth (hours)	6 + 138	*na*
No. of hours[2] to maturity germination and growth *established* field 1st infection	*na*	6 + 78
No. of hours[2] to maturity germination and growth *established* field after 1st infection	*na*	6 + 138
No. of hours[2] to maturity germination and growth *new* field all infections	*na*	6 + 138

[1]Amsalem, *et al.*, 2006, Blanco, *et al.*, 2004, Jhooty and McKeen, 1964, 1965, Miller, *et a*l., 2003, Peries, 1962a

[2]Number of hours of suitable conditions (temperature, relative humidity and leaf wetness)

The weather data was input into the prediction system (using the new parameters) again. This resulted in the predicted high-risk days and the dates when disease actually developed being closer than when the initial parameters were used (Figs. 4.5, 4.6, 4.7 and 4.8).

The first predicted high risk days for the established sites Mereworth 04 (Fig. 4.5) and Wisbech A 05 (Fig. 4.6) were within a few days of the first observed disease symptoms. In both cases the new parameters provide a good prediction of the first high risk day.

For the newly planted sites Mereworth 05 (Fig. 4.7) and Wisbech 06 (Fig. 4.8) the observed symptoms appeared in the field as the second predicted high risk day developed

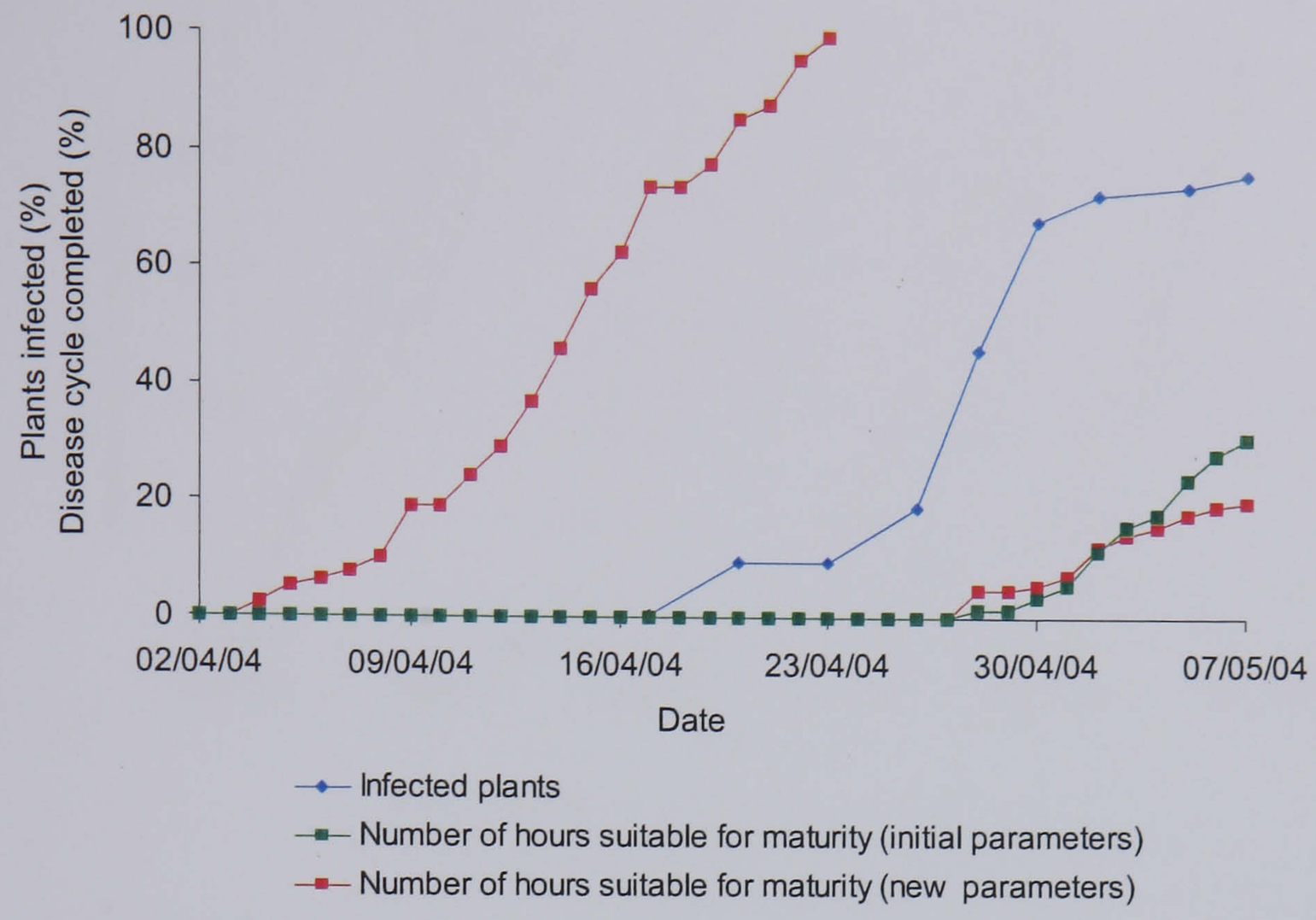


Fig. 4.5 Disease development data for Mereworth 04 (established field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed

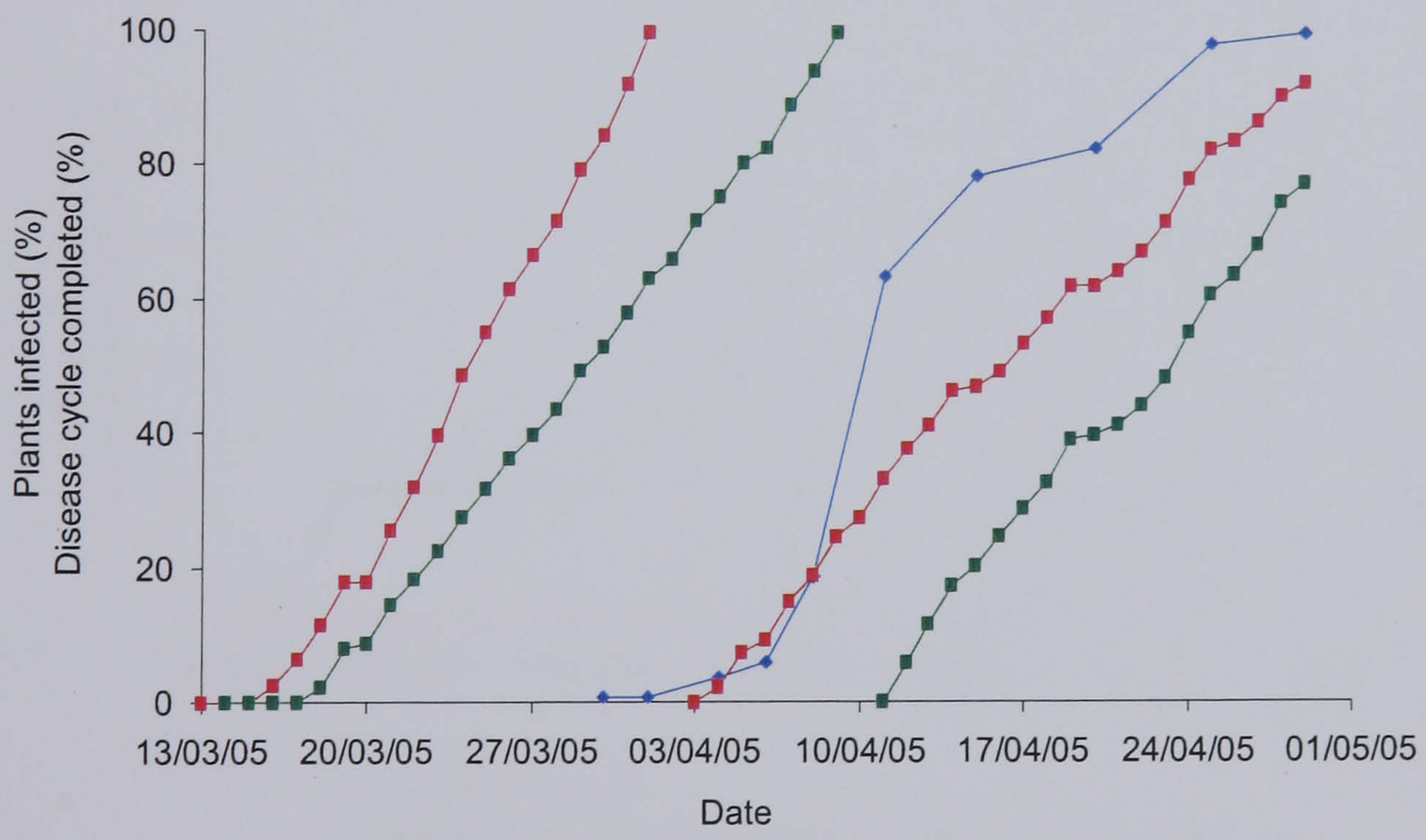


Fig. 4.6 Disease development data for Wisbech A 05 (established field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed

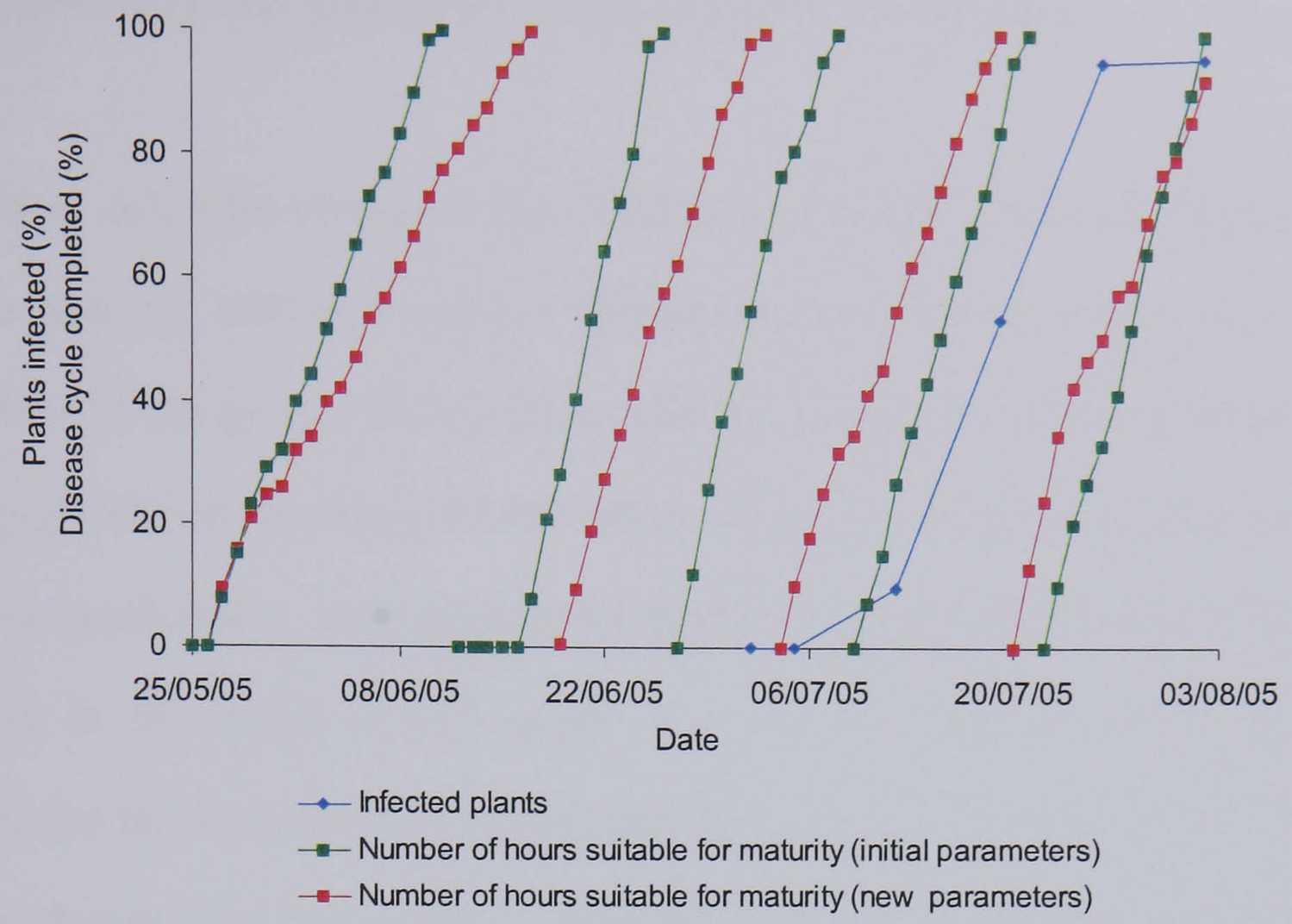


Fig. 4.7 Disease development data for Mereworth 05 (newly planted field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed

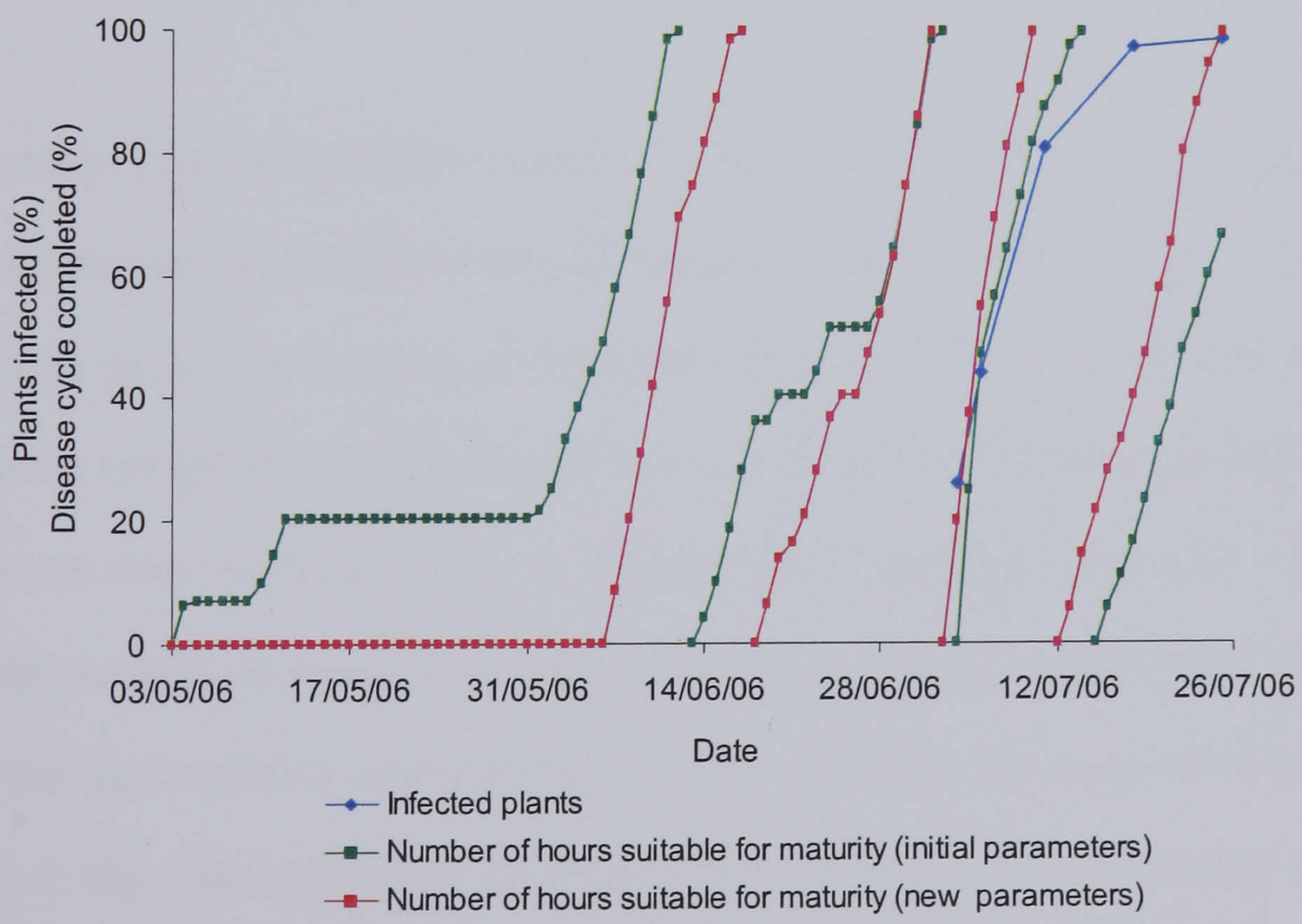


Fig. 4.8 Disease development data for Wisbech 06 (newly planted field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed

4.4.3 Sensitivity analysis of prediction system parameters

There was very little change in the number of predicted high risk days when the system was run with and without leaf wetness data (Figs 4.9, 4.10 and 4.11). Alterations in the growth temperature parameter were the only ones that resulted in a curved line on the figure for the whole range of values tested (Fig 4.9). When the other parameters were altered the lines produced were mainly flat with small changes in the slope at the upper end of the range of values tested, for germination temperature and relative humidity (Figs 4.9 and 4.10). For maximum growth temperature there was a slight slope of the line at the lower end of he range of values tested and no change at all in the slope of the line for maximum germination temperature (Fig 4.11).

The number of predicted high risk days went from 11 to 5 when the germination temperature was varied (Fig 4.9) and without leaf wetness data it went from 11 to 7 high risk days. The number of predicted high risk days went from 22 to 4 when the growth temperature was varied (Fig 4.9) and without leaf wetness the number of high risk days went from 23 to 4. The number of high risk days went from 11 to 4 (5 with out leaf wetness data) when the relative humidity was altered (Fig 4.10). When the leaf wetness was altered the number of high risk days went form 10 to 11 (Fig 4.10). The number of predicted high risk days did not change when the maximum germination temperature (with or with out leaf wetness data) was altered it stayed at 11 (Fig 4.11). The number of predicted high risk days

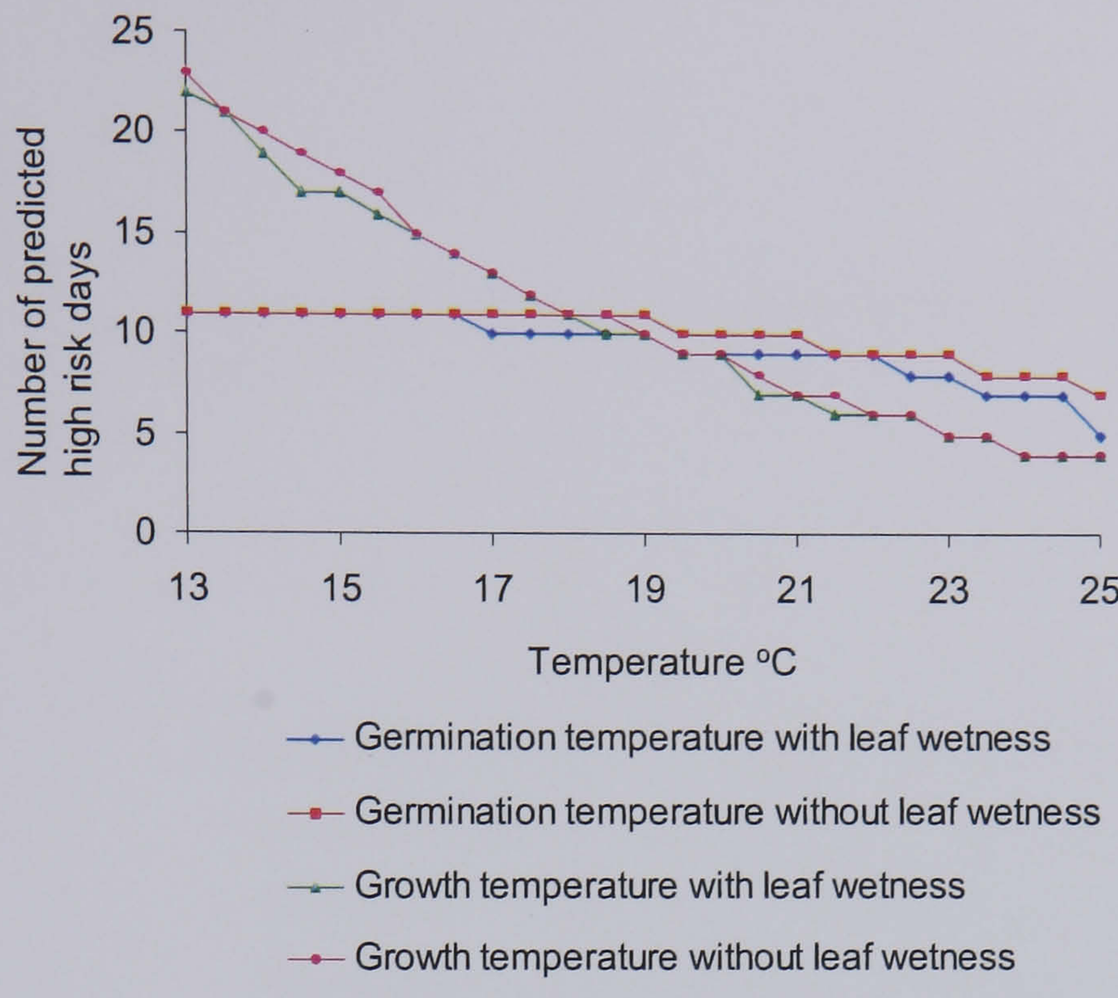


Fig. 4.9 Number of high risk days predicted by the prediction system when germination and growth temperatures were altered with and with out leaf wetness data

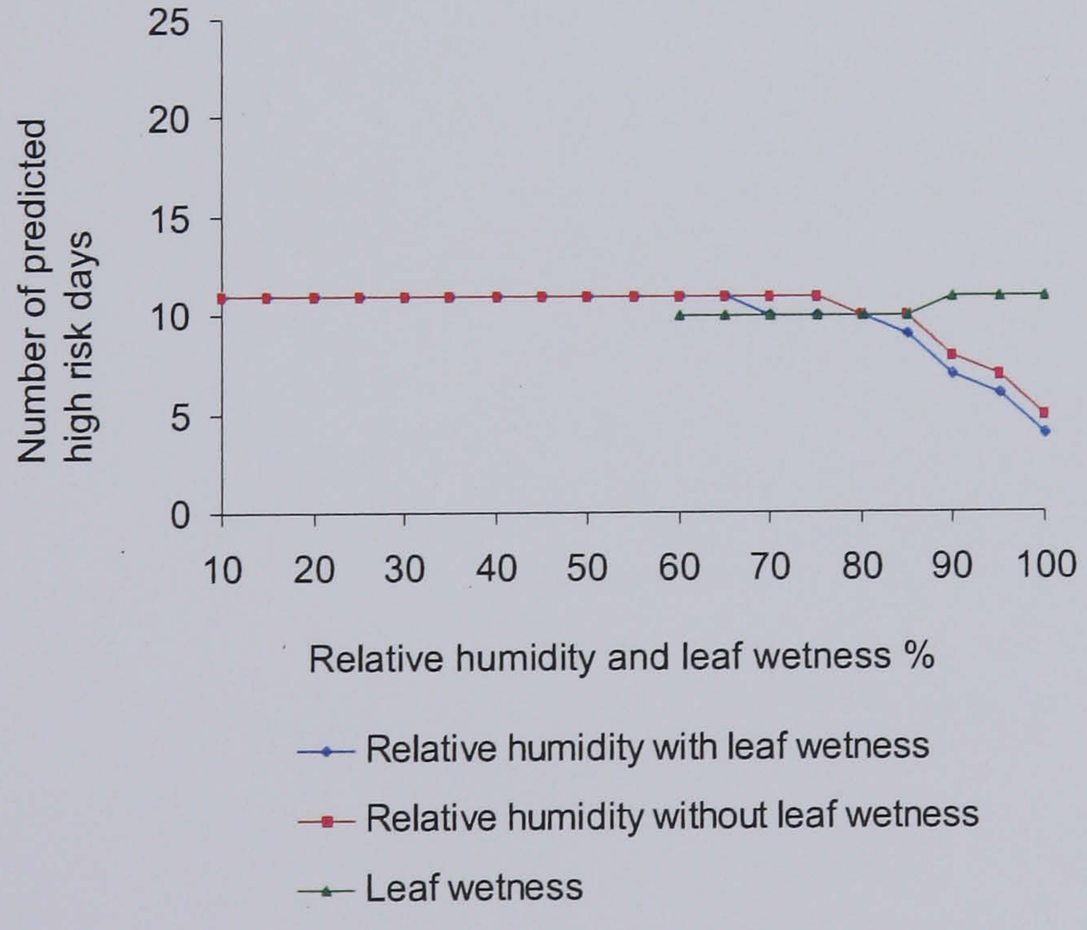


Fig. 4.10 Number of high risk days predicted by prediction system when the relative humidity was altered with and with out leaf wetness data and for when leaf wetness was altered

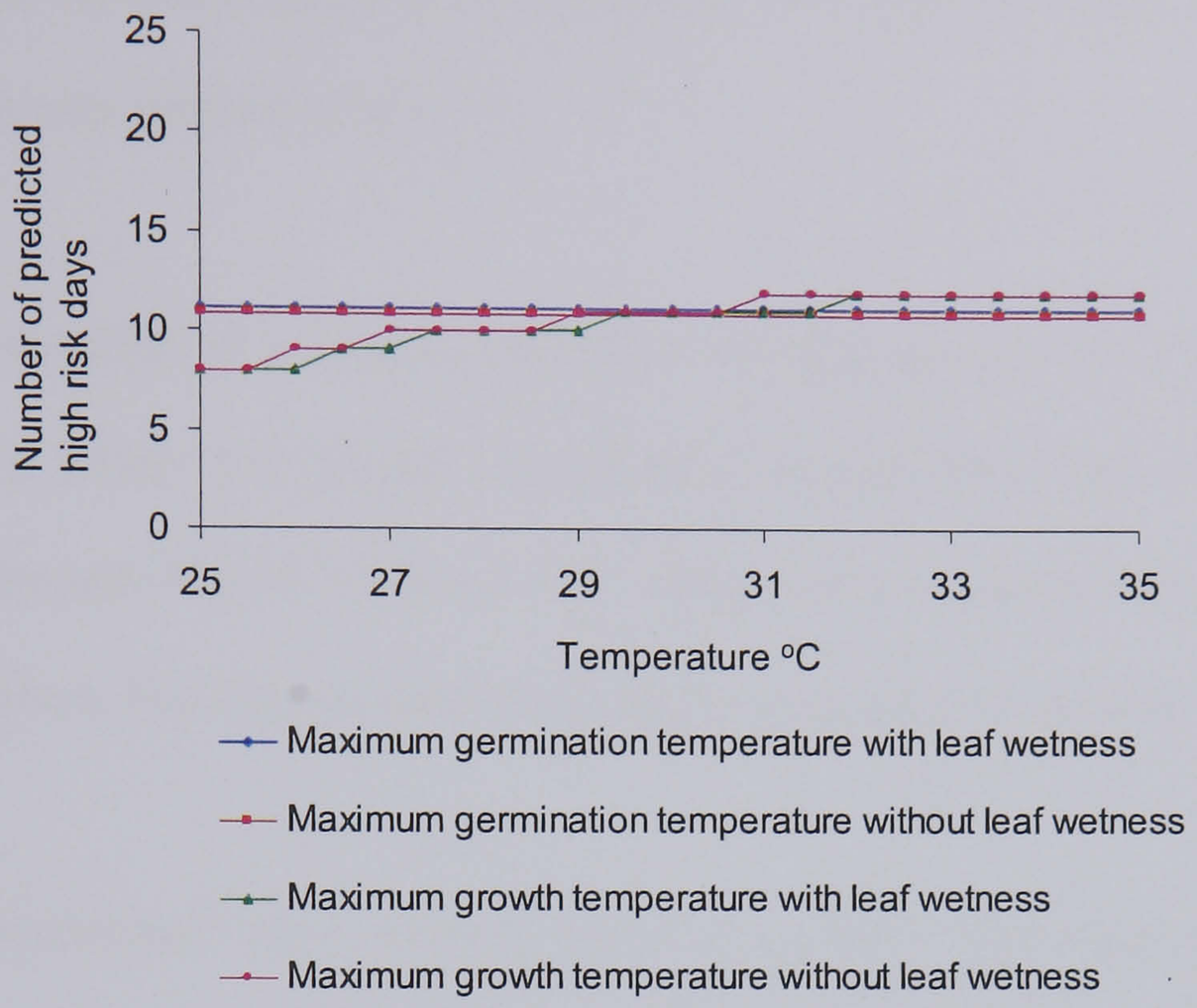
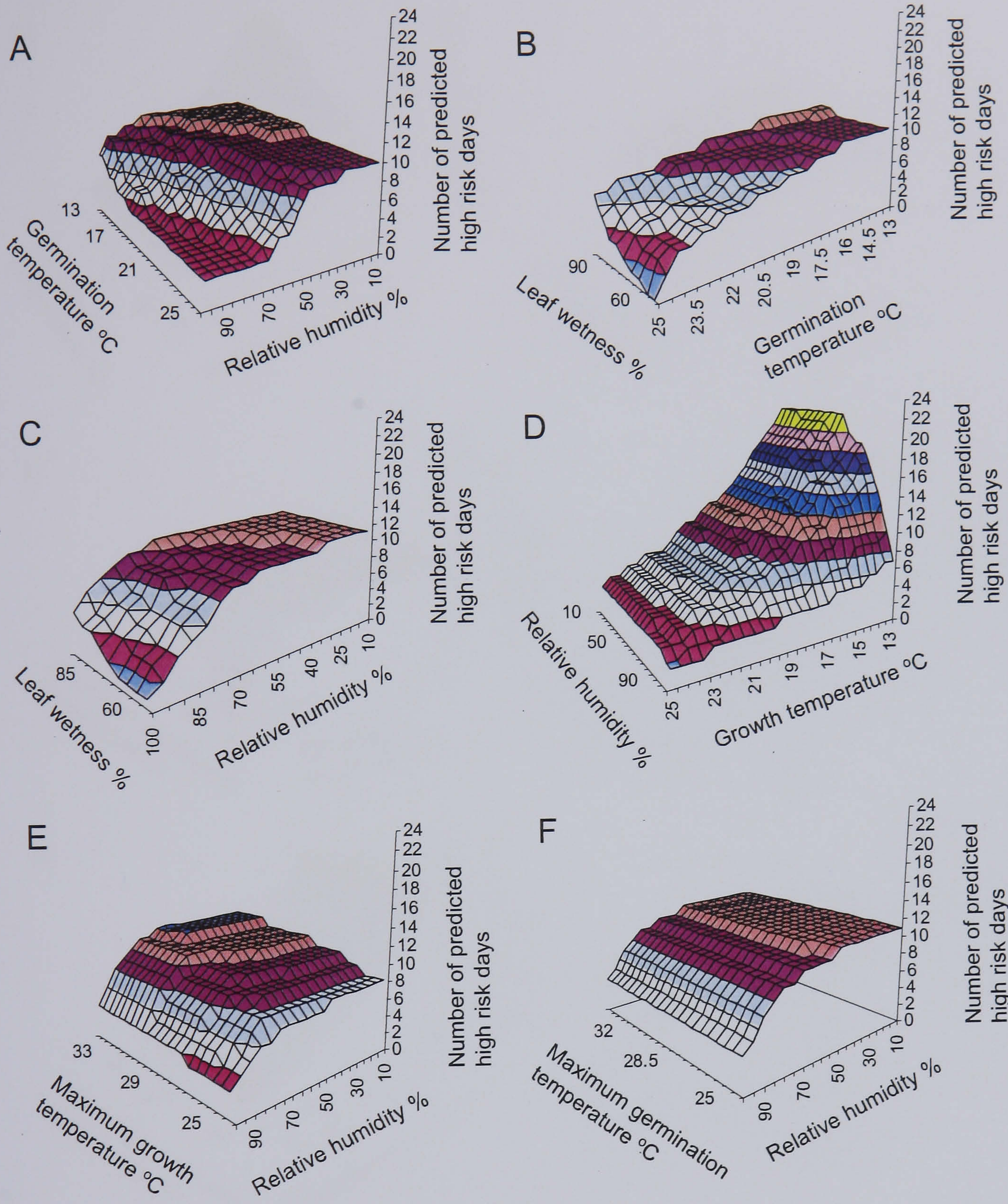


Fig. 4.11 Number of high risk days predicted by prediction system when maximum germination and growth temperatures were altered with and with out leaf wetness data

went from 8 to 12 when the maximum growth temperature (with or with out leaf wetness data) was altered (Fig 4.11).

As with the alteration of a single parameter the greatest constant shape change was produced when the growth temperature parameter was one of the two parameters altered (Figs 4.12 and 4.13). When growth temperature was not one of the parameters, the figures often formed plateau (Figs 4.12 and 4.13).

When two parameters were altered at the same time the maximum number of predicted high risk days remained in the same ranges (11 to 12 high risk days when a germination parameter was altered and 22 to 23 high risk days when a growth parameter was altered) as when one parameter was altered (Figs. 4.12 and 4.13). There was greater variability in the minimum number of high risk days predicted, with several combinations resulting in no predicted high risk days (Figs. 4.12 and 4.13). The majority of the other combinations resulted in between 1 and 5 high risk days with one parameter pair resulting in 10 high risk days and another resulting in 12 predicted high risk days. Alteration of the leaf wetness parameter resulted in the smallest variation in the number of predicted high risk days while alteration of the growth temperature resulted in the greatest variation in the number of predicted high risk days.



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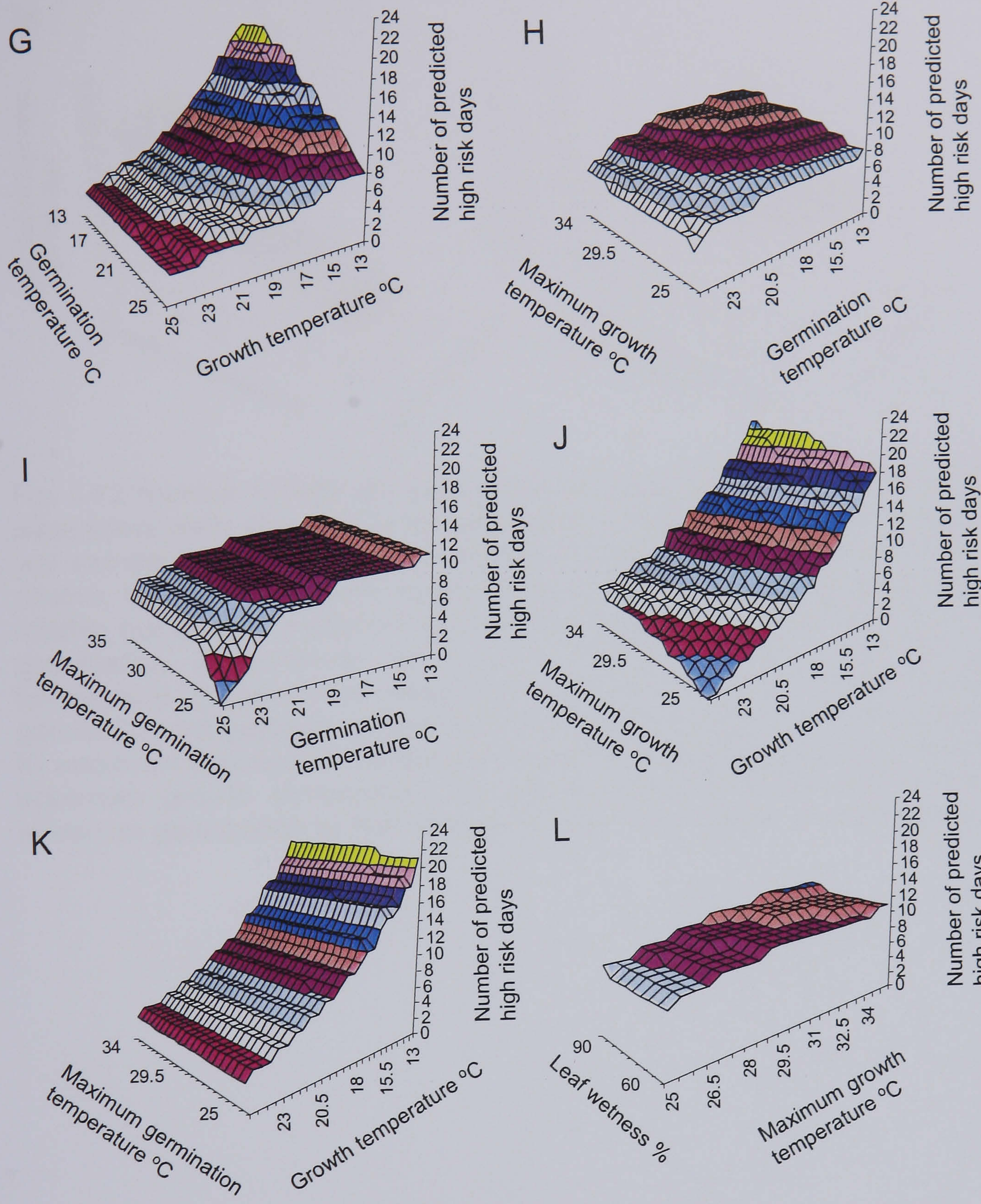

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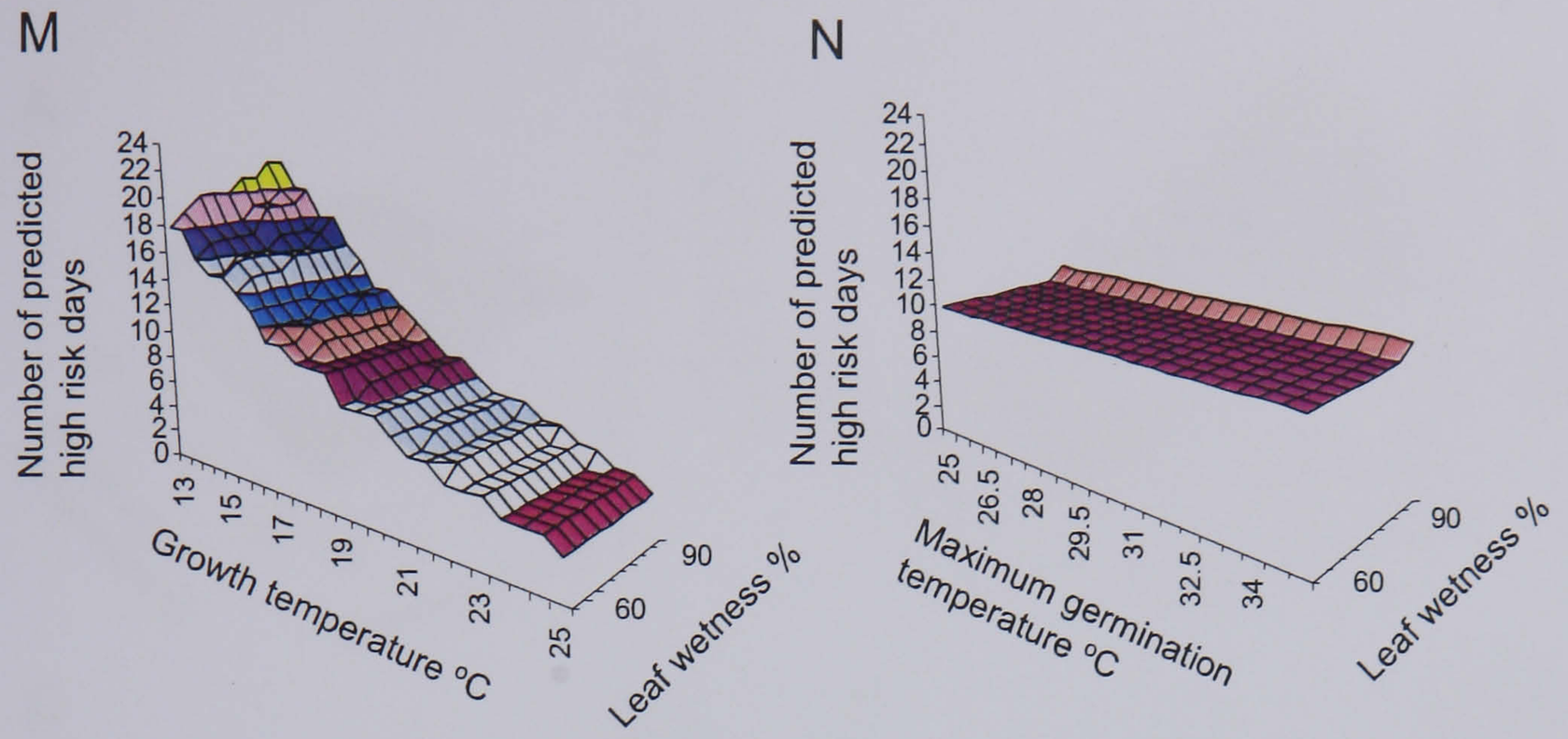
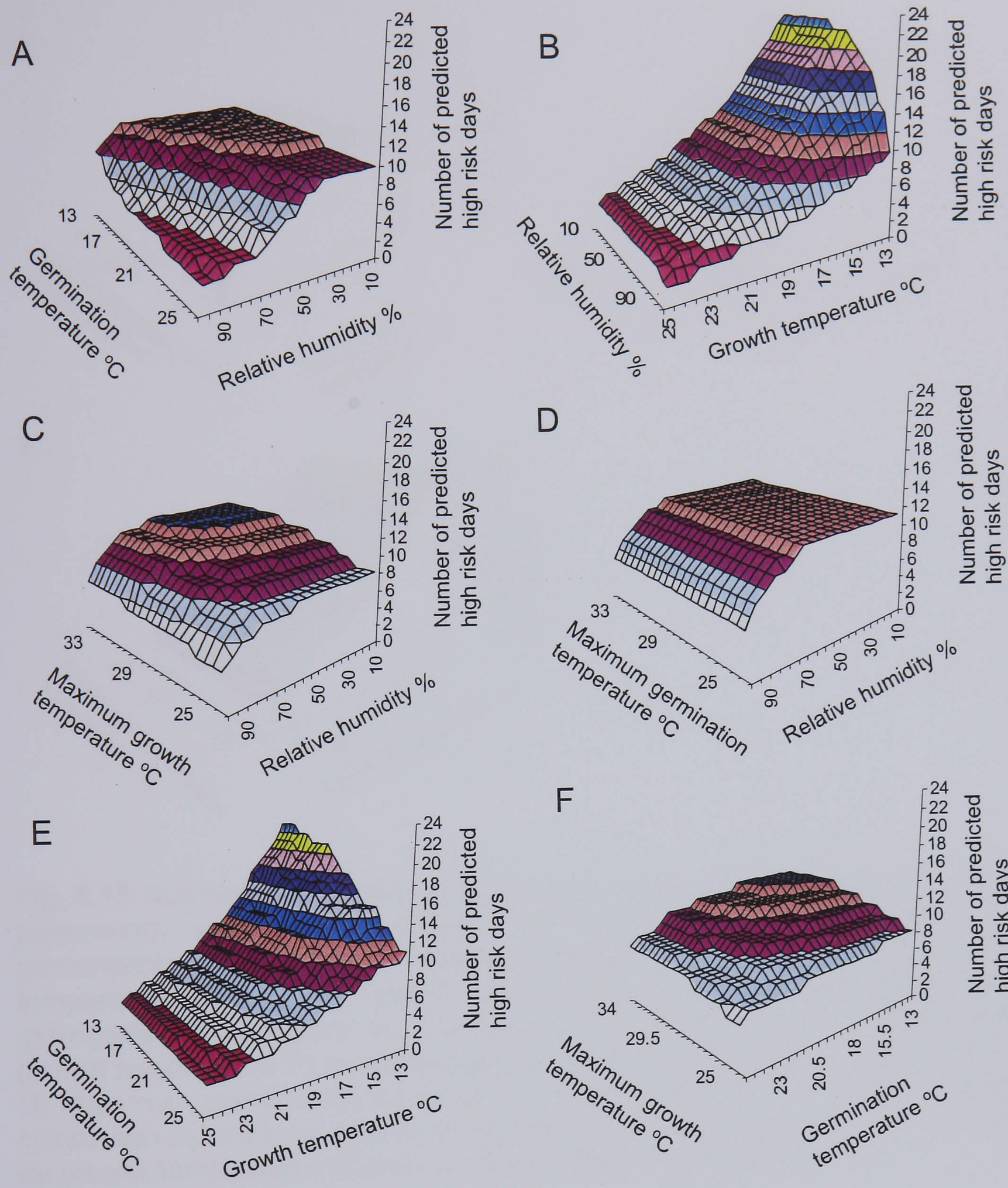


Fig. 4.12 Number of high risk days predicted when combinations of two different parameters were altered. For A) germination temperature by relative humidity B) leaf wetness by germination temperature C) leaf wetness by relative humidity D) relative humidity by growth temperature E) maximum growth temperature by relative humidity F) maximum germination temperature by relative humidity G) germination temperature by growth temperature H) maximum growth temperature by germination temperature I) maximum germination temperature by germination temperature J) maximum growth temperature by growth temperature K) maximum germination temperature by growth temperature L) leaf wetness by maximum growth temperature M) growth temperature by leaf wetness N) maximum germination by leaf wetness (Colour of no academic significance)



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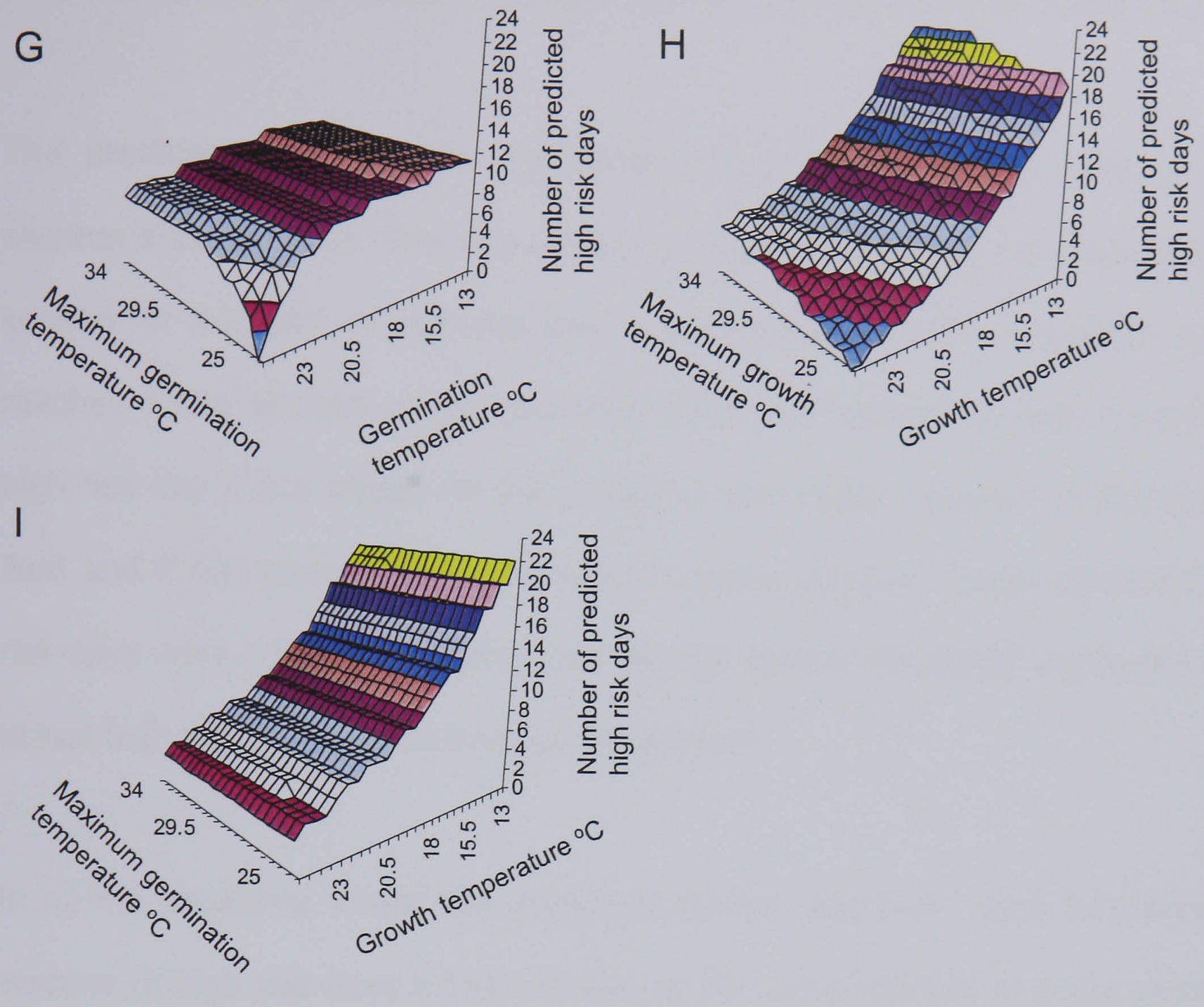

Fig. 4.13 Number of high risk days predicted when combinations of two different parameters were altered (Table 4.2) without leaf wetness data. For A) germination temperature by relative humidity B) Relative humidity by growth temperature C) maximum growth temperature by relative humidity D) maximum germination temperature by relative humidity E) germination temperature by growth temperature F) maximum growth temperature by germination temperature G) maximum germination temperature by germination temperature H) maximum growth temperature by growth temperature I) maximum germination temperature by growth temperature (Colour of no academic significance)

4.4.4 Comparison of predicted high risk periods with grower applications

The prediction system (new parameters) had data collected from on farm weather stations input. This was used to generate all the high risk days from one season for that site. A high risk day is generated when the prediction system reaches 100% disease cycle completed. When the prediction system predicts a high risk day it is a trigger for the grower to pay closer attention to that specific field and if necessary apply a fungicidal control product. These predicted high risk days were compared to the actual grower applied fungicidal applications (an actual high risk day as perceived by the grower).

In all the situations where the prediction system has been used to predict the number of high risk days it has resulted in the same number or fewer predicted high risk days than were perceived by the grower. For the two everbearer sites the grower applied 10 and 7 fungicidal applications where as the prediction system predicted 8 and 7 high risk days (Figs. 4.14 and 4.15). On both of the ever bearer sites the prediction system predicted fewer high risk days during the harvest period than perceived by the grower. On the first ever bearer site the grower applied 5 fungicide applications before the start of the harvest period and the prediction system also predicted 5 high risk days, but after the start of the harvest period the system predicted 3 applications compared to the 5 applied by the grower (Fig 4.14). For the second ever bearer site the grower applied 3 applications before and 4 after start of the harvest period compared to the

prediction system that predicted 4 before and 3 after the start of the harvest period (Fig 4.15).

For the three established sites the growers applied 3, 2 and 8 fungicidal applications where as the prediction system predicted 3, 2 and 5 high risk days (Figs. 4.16, 4.17 and 4.18). The first two established sites the number of applications (if not the date) applied by the grower and predicted by the system, before and after the start of the harvest period, were the same (Figs. 4.16 and 4.17). For the third established site the grower applied 6 applications before the start of the harvest period and 2 after whereas the prediction system predicted 3 before and 2 after (Fig. 4.18).

When the system was used with data relating to a field of propagation strawberry plants the system predicted 7 high risk days compared to the 14 fungicidal applications applied by the grower (Fig. 4.19). The predicted high risk days were more evenly spread through out the season where as the grower applied applications were not.

In all these situations the prediction system would have resulted in the grower applying the same number or few fungicide applications than if the prediction system had not been used. The fungicide use would have been reduced if the growers applied control products when there was a predicted high risk day. For ever bearer crops that have the longest harvest period it is particularly important

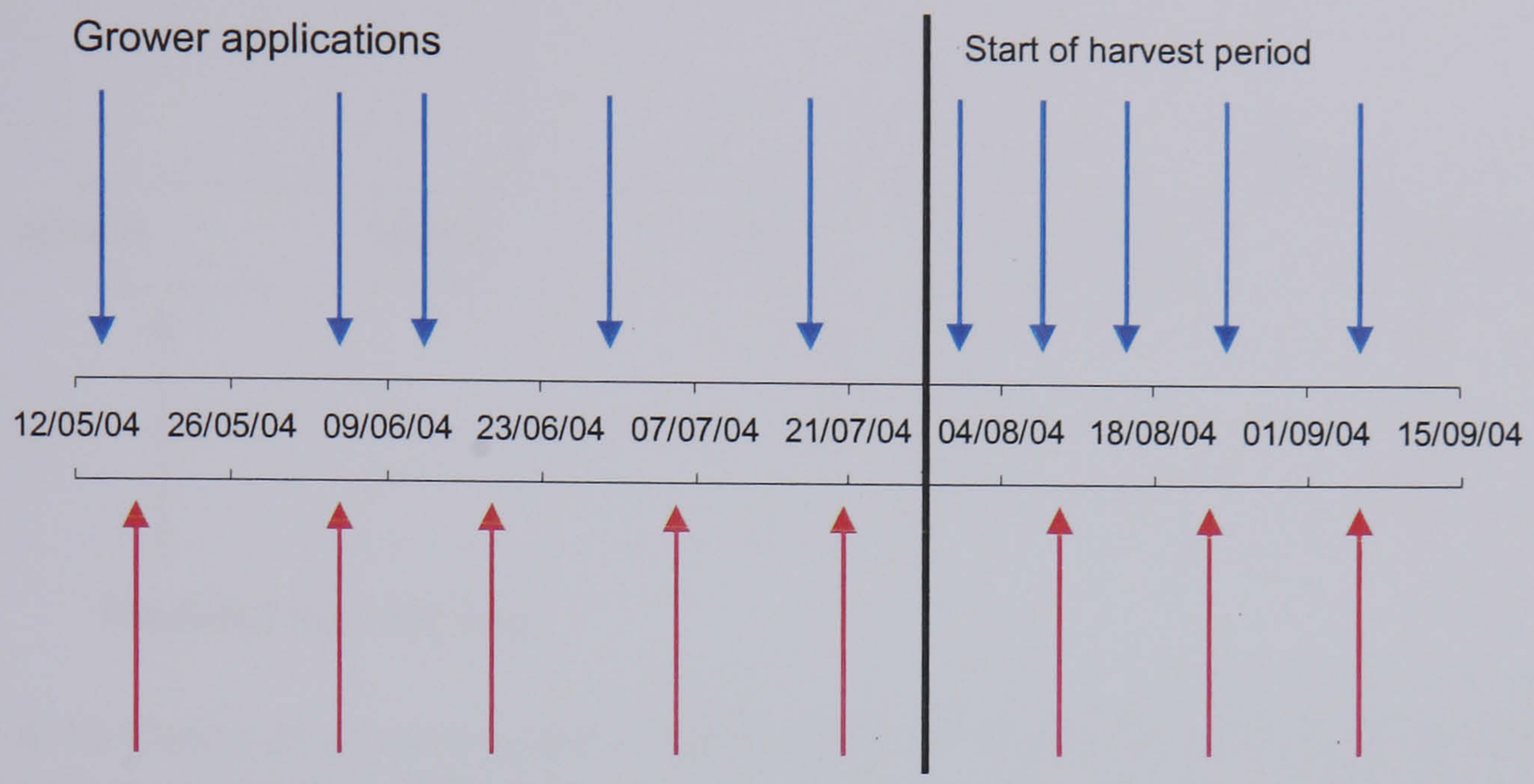

Fig. 4.14 Dates of grower-applied applications of fungicidal control product for *P. aphanis* compared to high risk days predicted by the prediction system for an ever bearer crop on a commercial holding near Wisbech 2004

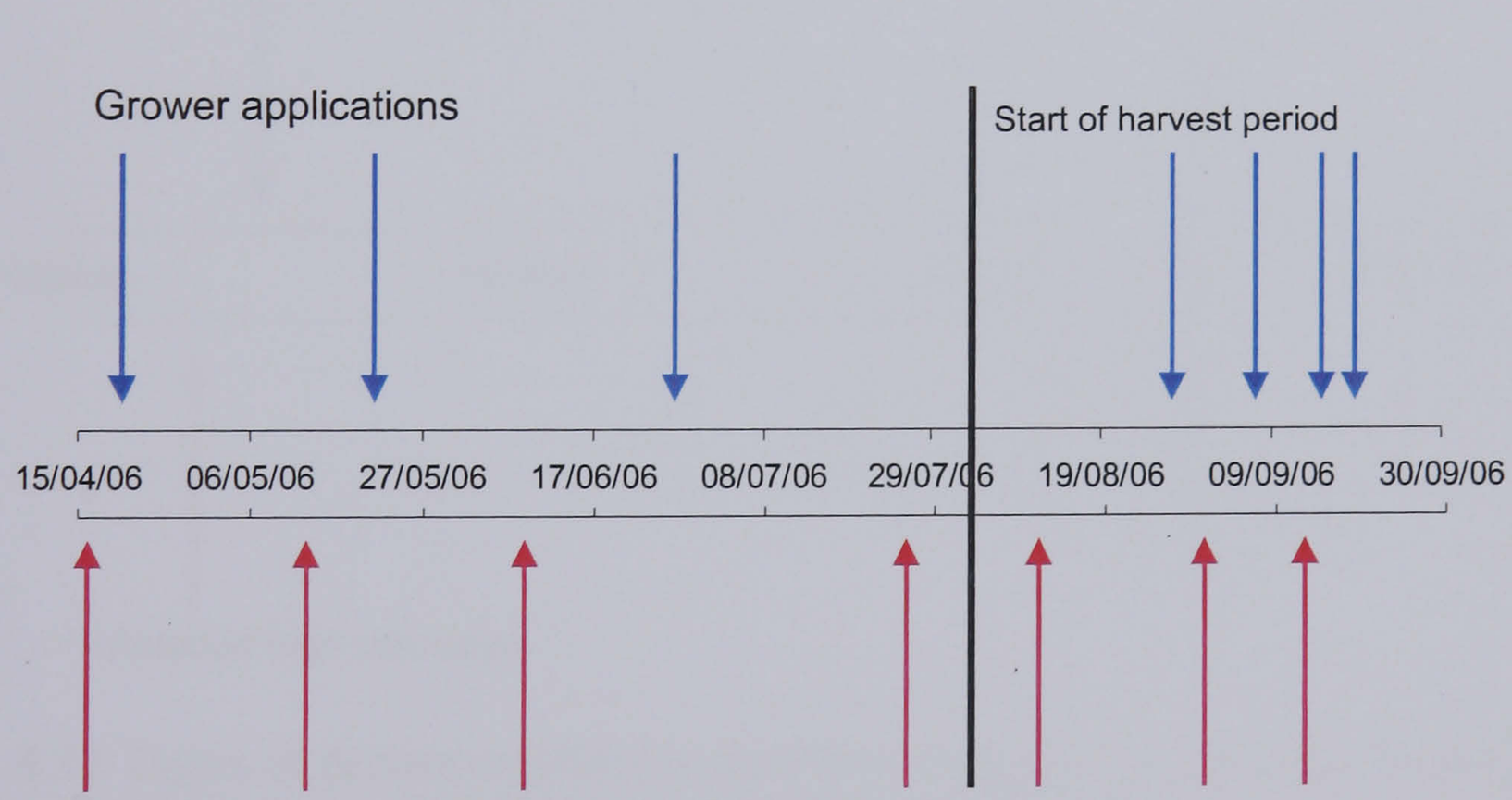

Fig. 4.15 Dates of grower-applied applications of fungicidal control product for *P. aphanis* compared to high risk days predicted by the prediction system for an ever bearer crop on a commercial holding near Wisbech 2006

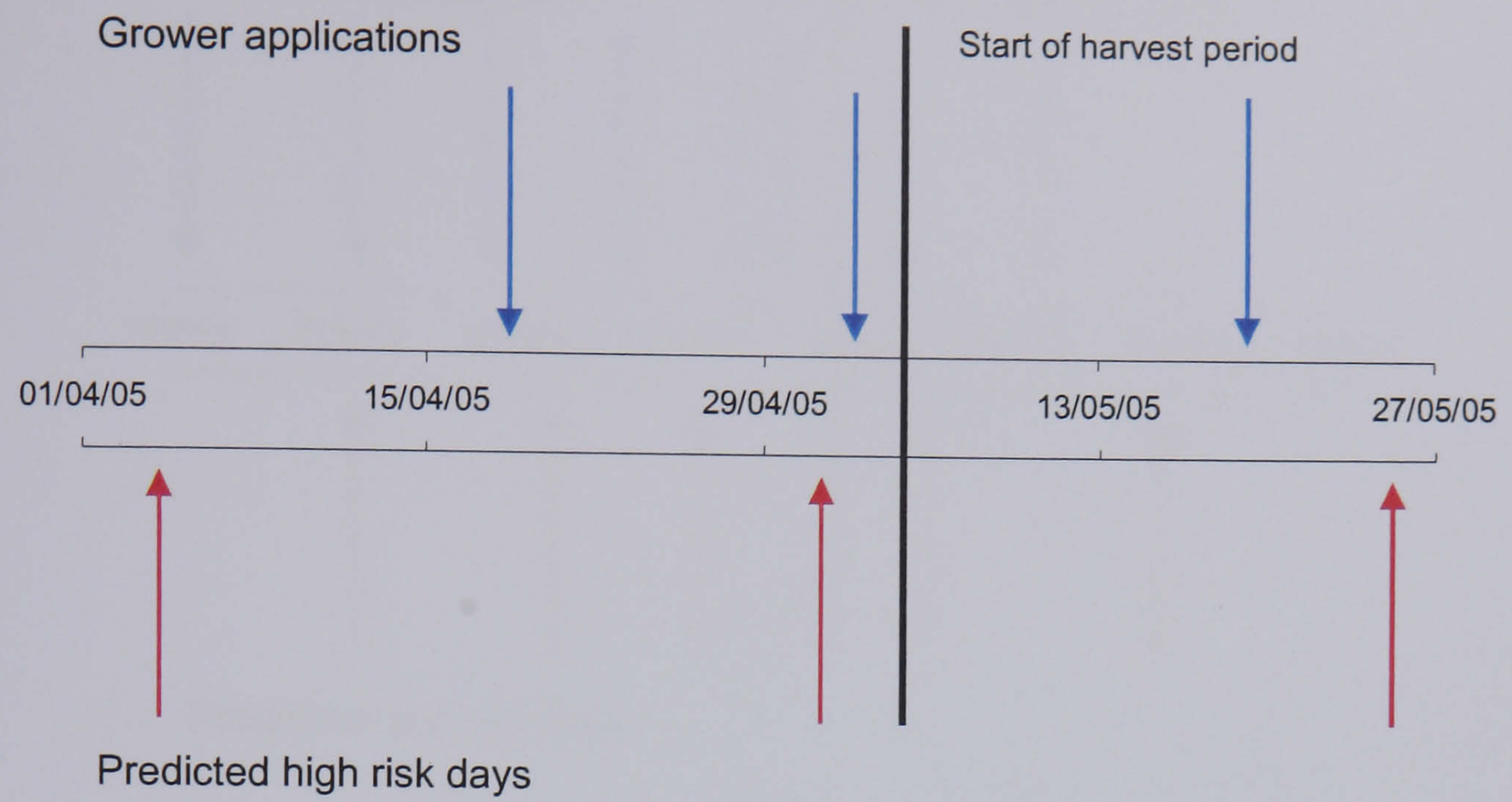

Fig. 4.16 Dates of grower-applied applications of fungicidal control product for *P. aphanis* compared to high risk days predicted by the prediction system for a 3rd season Elsanta crop on a commercial holding near Wisbech 2005

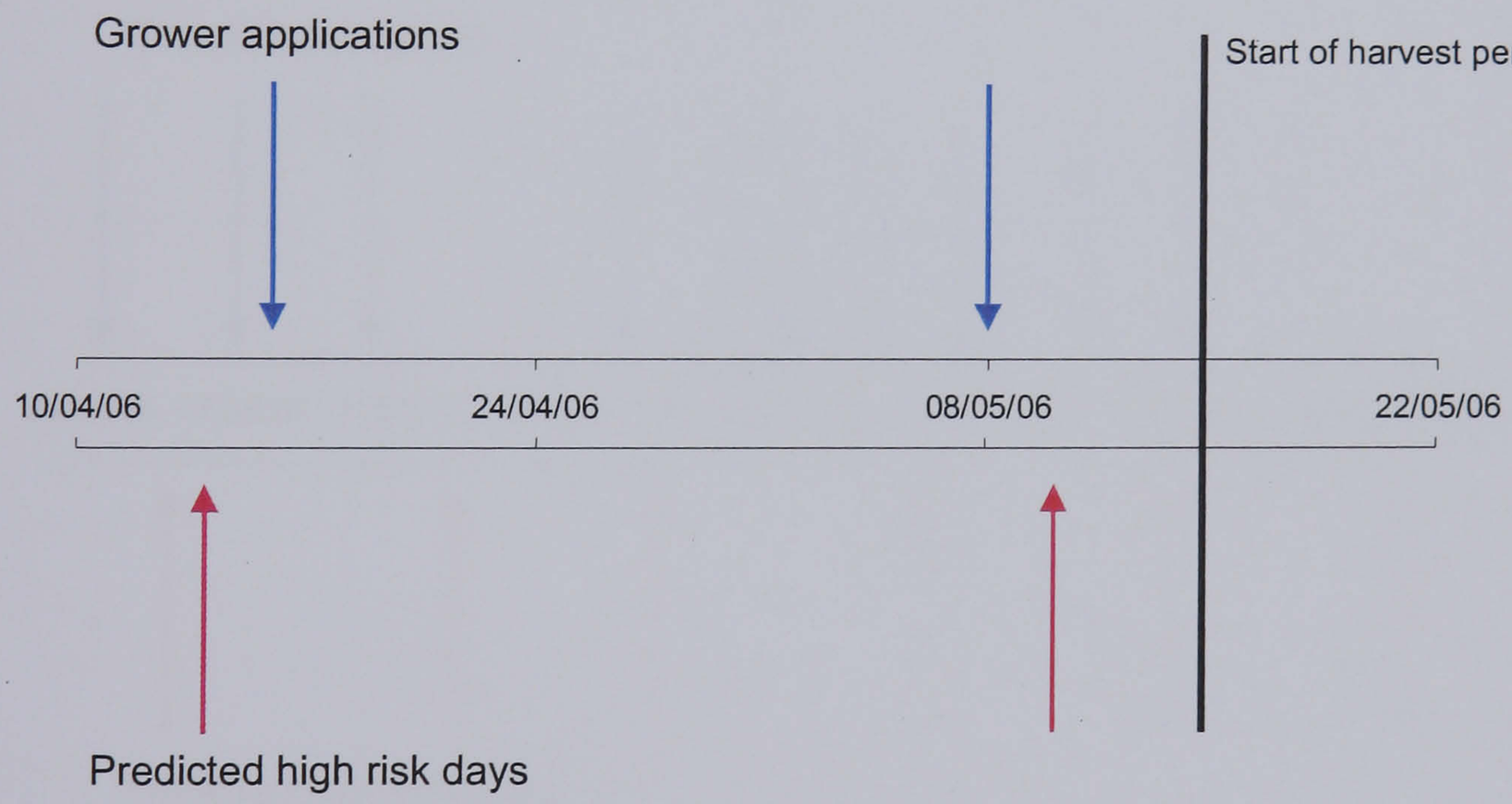

Fig. 4.17 Dates of grower-applied applications of fungicidal control product for *P. aphanis* compared to high risk days predicted by the prediction system for a 3rd season Elsanta crop on a commercial holding near Wisbech 2006

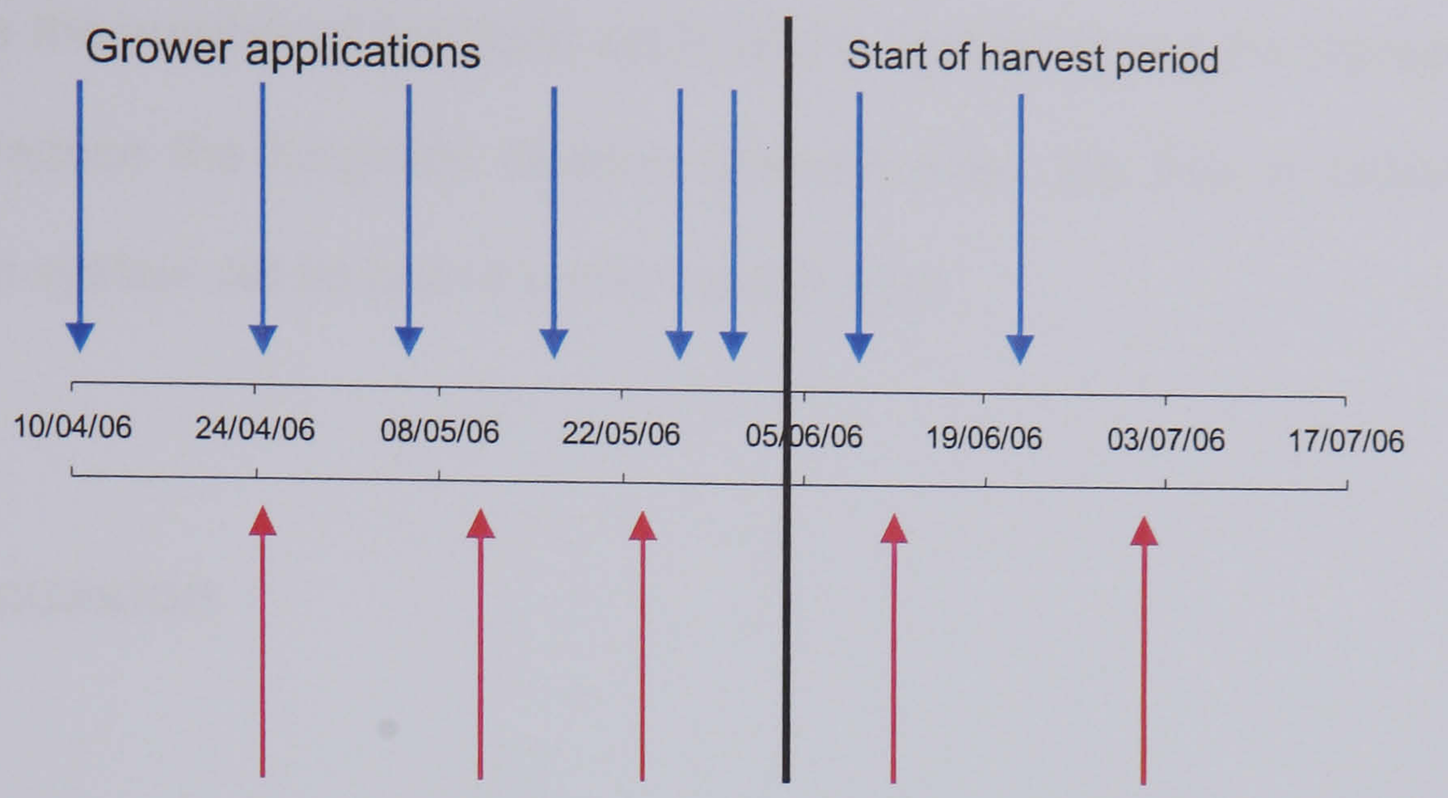

Fig. 4.18 Dates of grower-applied applications of fungicidal control product for *P. aphanis* compared to high risk days predicted by the prediction system for a 2nd season Elsanta crop on a commercial holding near Colchester 2006

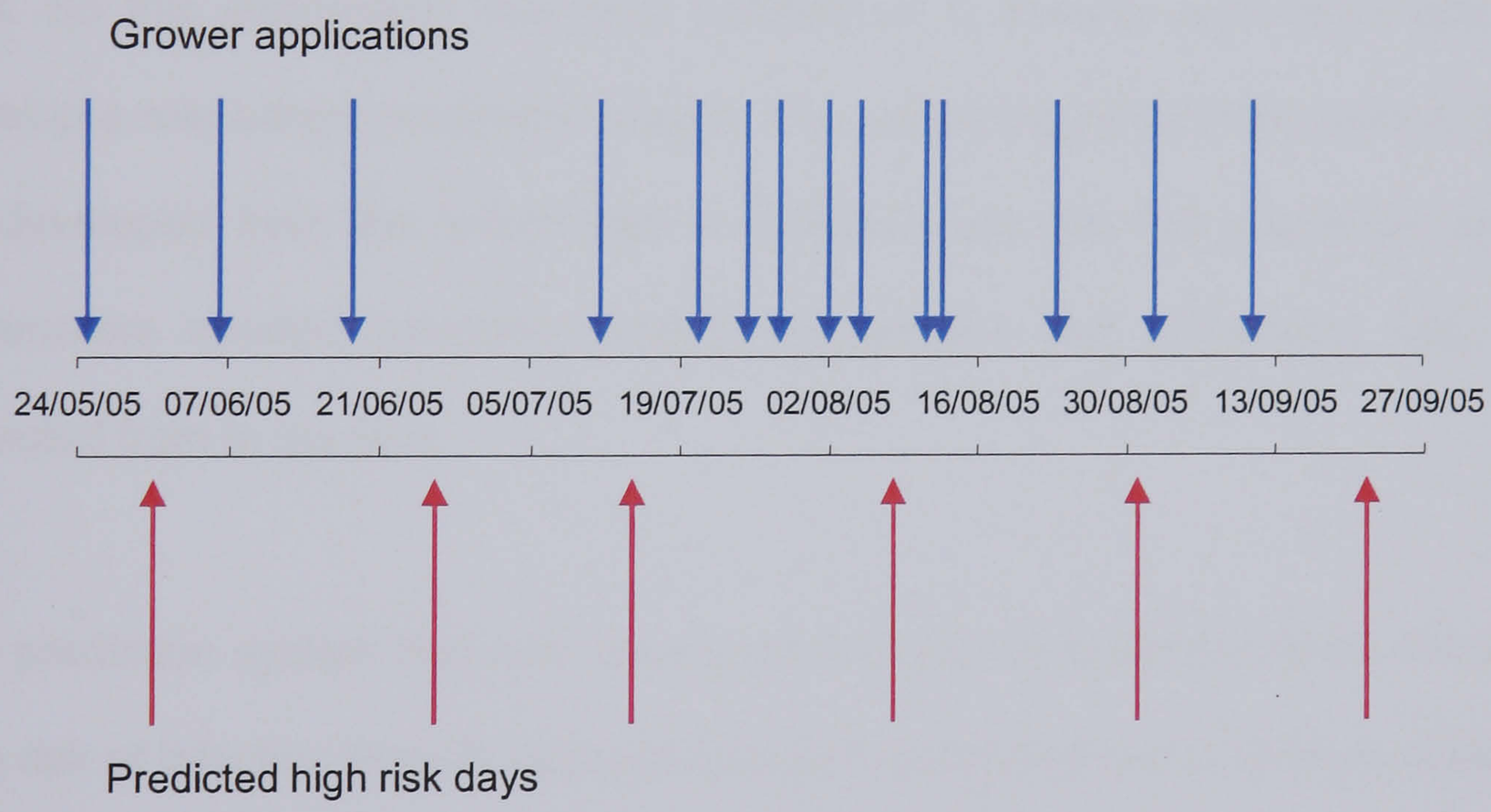

Fig. 4.19 Dates of grower-applied applications of fungicidal control product for *P. aphanis* compared to high risk days predicted by the prediction system for a propagation field of Elsanta plants near Kings Lynn 2005

to reduce the number of fungicide applications applied during the harvest period and so reduce the fungicide residues present when the fruit is picked, a the prediction system did on both the ever bearer sites.

4.5 Discussion

The literature yielded substantial information on the conditions that were suitable for the germination, growth and development of *P. aphanis* infections, as set out in the first objective of this chapter. The experiments reported in the literature, that this information came from were conducted in the laboratory and not in the field. So this information was only suitable as a starting point form which to develop a rule based prediction system. The underlying rules of the system could be developed from the information in the literature but the prediction system parameters needed extensive testing, comparison and refinement with data collected from in the field.

The prediction system that was developed is designed to predict when there is a high risk of infection from *P. aphanis* inoculum generated from infection within the field. The prediction system does not model the growth and development of infection by *P. aphanis* and does not model the growth and development of new leaves on the strawberry plant. Instead it identifies when there have been a suitable number of hours for any inoculum (spores) to develop into new sporulating colonies. Hence, the over all level of inoculum in a field will not

increase in the time between a spore landing on the leaf surface and it developing in to a mature colony. The lesion will expand and grow but it will not be producing more inoculum until the lesion has reached maturity. The grower needs to be able to apply fungicides as the lesion reaches maturity. The fungicide should be applied just before more inoculum is being produced. If a fungicide application is applied before the infection has reached maturity the grower is not gaining maximum benefit. The application has been applied too soon. The level of infection within the crop would not have been any greater if the grower had waited to apply the application until the infection was just reaching maturity. The application is applied before the infection levels increase. The application is acting as a 'curative' application reducing the amount of inoculum before it has acted as a source of infection for the rest of the field. So the growers are able to space their fungicide applications further apart and therefore use less fungicide applications to control *P. aphanis* infections in the field.

An initial set of parameters for the prediction system was developed from the literature review and field observations (Table 4.3). When used in the prediction system these parameters resulted in predicted high risk days that looked sensible. When the prediction system was run with actual field data the first predicted high risk periods were compared to the actual development of *P. aphanis* infection (Figs. 4.1, 4.2, 4.3 and 4.4). The predicted high risk days were similar to the dates that the infection actual developed on but were not close enough for use by the grower to plan fungicide applications. These observations

were used to alter the parameters so that new parameters were developed (Table 4.4).

The prediction system parameters were revised so that the predicted high risk days coincided with the days when the actual infection was seen in the field. In-addition to changing the number of hours of suitable conditions before the first high risk day was predicted for established sites after the initial parameters were compared to field data collected as part of this work (second aim for this chapter). Infection takes longer to develop on new sites compared to established sites (Chapter 3). Infection can overwinter as mycelium on established sites (Peries, 1961, Smith *et al.*, 1988). Some of the initial inoculum could be present as mycelium, so therefore would take less time to reach maturity (Table 1.4). The new parameters were used to compare the first predicted high risk day with the actual development of *P. aphanis* symptoms. For both established sites the development of actual symptoms as observed in the field happened just as the prediction system predicted a high risk day (Figs. 4.5 and 4.6). For the newly planted sites the development of visible symptoms of *P. aphanis* infection corresponded to the second predicted high risk day (Figs. 4.7 and 4.8). This could be due to infection being present in comparatively small amounts. The initial inoculum needs to develop (initial lag phase when there is the greatest multiplication of the pathogen numbers), before there is enough infection to be visible to the naked eye (Lucas, 1998, Zadocks and Schein, 1979).

For the prediction system to be useful for the growers it needed to result in the same amount or fewer applications of fungicides than are being applied currently. The growers generally achieve good control of *P. aphanis* using the current number of fungicide applications. When the predicted applications of fungicides (predicted high risk days) were compared with grower applied fungicides the prediction system resulted in the same number or fewer applications (Figs. 4.14, 4.15, 4.16, 4.17, 4.18 and 4.19). Often the grower applied two or more fungicide applications in close succession. Often it was these closely spaced applications that the prediction system eliminated. Where the system resulted in the same number of applications the timing of the application predicted by the system could well provide more efficient control. The system predicted fewer high risk days during the harvesting period which would result in reduced residues in the harvested fruit (Figs 4.14 and 4.15). Many growers apply more fungicide applications than the growers that provided their spray records for this work (personal communications). These growers would have the potential to reduce their fungicide use considerably, if they were to implement the prediction system.

The prediction system is most sensitive to changes in the growth temperature (this is the variable that acts over the largest time in the prediction system), when it is the only variable being altered or when it is one of a pair of variables being altered, with or without leaf wetness data (Figs. 4.9, 4.12 and 4.13). Leaf wetness and maximum germination temperature were the least sensitive variables when altered individually (Figs. 4.10 and 4.11). The least sensitive variables were often

masked by being paired with a more sensitive variable when two variables were altered at the same time (Figs 4.12 and 4.13), except when two less sensitive variables were paired together (leaf wetness by maximum growth temperature or maximum germination temperature by leaf wetness) (Fig. 4.12). Leaf wetness is the variable the growers would be least likely to be able to monitor from their on site weather stations. As leaf wetness is the least sensitive variable it might be possible for these growers to still benefit from the prediction system.

4.5.1 Comparison with previously published models/prediction systems

A rule based prediction system has been developed (Dent, 1995, Norton and Mumford, 1993) for *P. aphanis* infections. The prediction system is specific for *P. aphanis* infections of strawberry plants (Travis and Latin, 1991, Van Maanen and Xu, 2003) and the rules that govern the system have been developed by an expert. The prediction system is based on 'IF-THEN' rules and is not based on comparatively complex formulae such as those developed for grape powdery mildew (Chellemi and Marois, 1991, Sall, 1980). The prediction system is also based on a range of parameters that have been developed together to combine to form the finished system. Unlike other work that has been carried out where each individual parameter is modelled then all the models will be combined at the end, e.g. powdery mildew on rose (Xu, 1999a, Xu, 1999b), apple (Xu, 1996, Xu, 1999c), clematis (Xu and Robinson, 2001) and hawthorn (Xu and Robinson, 2000).

The rule based prediction system presented here highlights high risk days within the cropping season when the field of strawberry plants will be at higher risk of infection, so prompting the grower to make a decision on whether or not to apply a fungicide control for *P. aphanis*. This is similar to the Blitecast system for potato blight (Krause *et al.*, 1975, Taylor, 2000) which also predicted the initial development of infection and then the subsequent intervals between applications. Whereas models have been developed that only predict end of season disease pressures for powdery mildew infections of sugar beet (Asher and Williams, 1991) and jujube (Sinha, 2005).

4.5.2 Further development

The prediction system fits well with the field based data that has been collected over the last three years from several commercial sites on several different types of strawberry crop (including everbearers, which have the longest cropping time and so greatest time for *P. aphanis* infection to establish in the crop). It now needs to be tested in the field. Growers located in the main UK strawberry growing regions need to apply control products for *P. aphanis* when prompted to by the prediction system, to see if they are able to control fungal development using just applications recommended by the prediction system. If this is successful the prediction system can then be implemented by more strawberry growers.

In addition further work has been carried out on the role that chasmothecia (Belanger *et al.*, 2002) play in the overwintering of the fungus (Farooq *et al.*, 2007). When this work is completed the findings will need to be studied for possible implications that might effect the prediction system and if necessary changes will be made to the prediction of the first high risk period each season.

4.6 Conclusions

The published literature has been searched for conditions which are suitable for the growth and development of *P. aphanis* infections. These conditions were used as the basis for a rule based prediction system for high risk periods of infection by *P. aphanis*. These initial rules were then modified in light of field based experiments to identify where the optimum conditions obtained from laboratory based experiment did not match the observed data obtained from the field. The prediction system was modified so that it was able to predict the first development of *P. aphanis* symptoms in the field. The prediction system (using the new parameters) was used to predict the dates that fungicides applications for control of *P. aphanis* should be applied. These dates were compared to the actual dates that growers applied fungicides. The prediction system predicted fewer or the same number of fungicide applications as were applied by the grower. All the objectives as set out in section 4.2.2 have been met. The prediction system developed here predicts high risk periods for infection by *P.*

aphanis but field based tests are required before it can be made more widely available for all strawberry growers to use.

Chapter 5 - Development of control methods to form part of an integrated control strategy for *P. aphanis*

5.1 Introduction

Integrated pest management (IPM) is 'an approach to control insects and other crop pests that combines various physical, chemical, and biological methods in an attempt to reduce reliance on chemical pesticides, and hence minimize pollution and harmful residues in the product' (Anon., 2004b). IPM is also often referred to as integrated control which is 'the use of chemical, biological, cultural and legislative methods in a complementary way to control pests and pathogens' (Anon., 2005c).

Much of the early progress towards an integrated approach to crop heath management was made by entomologists attempting to manage insect pests (Lucas, 1998). This was necessary because of the rapid development of resistance to chemical control methods, an awareness of possible environmental hazards and the fact that many of the insecticides used affected non target insects. Implementation of integrated control for fungal plant pathogens has progressed much more slowly, probably because there were fewer problems with the use of fungicides compared to insecticides. The use of host genetic resistance to pathogens was partially successful (but not durable), there were

fewer options for directly acting predators and traditional practices such as crop rotation already contained an element of integrated control (Lucas, 1998).

Recently there has been an effort to reduce the amount of fungicides used. This is primarily due to a desire to cut costs, pressure to reduce environmental impacts as consumers become more aware of the impact food production can have and a drive for production of residue free food (Arslan *et al.*, 2006, Dent, 1995, Lucas, 1998). An integrated approach to disease control does not aim to remove all pesticide inputs, just to reduce the amounts used.

This can be achieved, in part by substituting other products as alternatives, where appropriate, such as low-toxicity compounds (organic or inorganic salts) (Arslan *et al.*, 2006). For example, phosphate solutions have been used to control powdery mildew of mango (Nofal and Haggag, 2006, Reuveni and Reuveni, 1995), sodium bicarbonate has been shown to inhibit cucumber powdery mildew (Homma *et al.*, 1981) and powdery mildew of rose (Horst *et al.*, 1992) while potassium bicarbonate has an effect on powdery mildew of sweet red pepper and cucurbit (Fallik *et al.*, 1997, McGrath and Shishkoff, 1999). Milk based foliar sprays have also been used successfully to control powdery mildew of pumpkin (Ferrandino and Smith, 2007). Organic or inorganic salts need contact with the pathogen to have an effect, they are not systemic and they have no lasting effect so can require more frequent applications than fungicides. Not all of these products provided the same level of season long control achievable

by using traditional fungicides, but in an integrated control program the low-toxicity compounds can be used to complement fungicides rather than replace them completely.

An integrated disease control program does not aim to eradicate all infection but instead to manage levels below economically determined thresholds if the level of symptoms are below the threshold level the crop is not treated but if the level of symptoms are equal to or above, the threshold level control products are applied (Fletcher, 1984). Such an approach can provide acceptable disease control. For example, comparable disease control was achieved when using a disease threshold to trigger fungicide applications rather than using a preventative application schedule for the control of powdery mildew of grapevine (Oliva *et al.*, 1999) and summer squash (McGrath and Staniszewska, 1996).

Reducing the rate at which the disease develops can be an important part of an integrated control program. If the infection builds up more slowly the disease thresholds will be reached later in the season. This could be achieved by using cultivars that are disease resistant (Dent, 1995, Fletcher, 1984, McGrath and Staniszewska, 1996). Disease free plants could be planted (Fletcher, 1984) or new plants could be treated (with fungicides or other suitable product) before they are planted. *Colletotrichum acutatum* (anthracnose crown rot) of strawberry has been treated by dipping the plants in a fungicide solution when transplanting (De los Santos *et al.*, 2002).

Disease forecasting can also be linked with integrated control. Disease forecasting aims to predict whether or not disease will actually occur (Lucas, 1998). This can be used to better time the fungicidal control products that a grower will apply so that they are only applied when the crop is at risk of (disease) infection, therefore reducing the number of applications compared to a preventative application schedule, as when using the Adem^{TM} model developed for apple powdery mildew (Berrie and Xu, 2003).

5.1.1 Why do strawberry growers need integrated control?

Strawberry growers are under increasing pressure to reduce the levels of residues in their fruit from the retailers. Some retailers are requesting fruit with near zero detectable residues. One retailer wants products to be free of herbicides and insecticides by the end of 2008 and free of fungicides by 2012 (personal communication, Richard Harnden, Berry Gardens). In addition the numbers of active ingredients available to growers are getting less. The few active ingredients the growers have are being over used so increasing the risk of fungicide resistance developing.

5.2 Aim + objectives

5.2.1 Aim

Identification of more efficient control methods for *P. aphanis* (4th aim, page 27)

5.2.2 Objectives

1. Quantify the level of disease resistance in strawberry cultivars available to strawberry growers

2. Identify new products for the control of *P. aphanis*

3. Develop method to reduce initial inoculum in newly planted sites

5.3 Methods

5.3.1 Cultivar screening

Seven cultivars were compared for their relative susceptibility to strawberry powdery mildew. The cultivars were selected so that there was a range of resistances to *P. aphanis* based upon consultation with growers (Table 5.1). Plants were arranged in a randomised block design of four replicates, within

tunnel B of the Mereworth site 2004 (Fig. 3.5). Plots consisted of 20 plants (2 rows × 10 plants), which were separated by 4 plants of cv. Florence (2 rows × 2 plants). The plants were not treated with fungicides active against powdery mildew. Fruit was removed when fully ripened. The plants were scored weekly between 17.04.04 (tunnel covered 16.04.04) and 13.07.04 for *P. aphanis* using the MAFF strawberry powdery mildew Key No. 8.1.1 (Appendix 4). Area under the disease progress curve (AUDPC) was used to quantify the magnitude of epidemics and these quantities were compared by ANOVA to identify differences between varieties (Genstat, 8th Edition, VSN International Ltd).

5.3.2 New *P. aphanis* control products

Treatments were applied to tunnel C Mereworth site 2004 (Fig 3.5) planted with cultivar Elsanta. Treatments were arranged in a randomised block design of 4 replicates. Each plot consisted of 40 plants (20 × 2 rows) and 2 plants separated each plot. Disease assessments were carried out on 10 plants chosen at random from each plot. The newest leaf on each plant was tagged on the 06.08.04. The leaves were then scored weekly through out the experiment for percentage of adaxial leaf surface covered with red blotches. The products (Table 5.2) were first applied on the 07.08.04 with a Hardi Backpack Sprayer BP 20 calibrated in accordance with NPTC recommendations. The potassium bicarbonate was applied for a second time 14 days after the first application (21.08.04). Fortress (Quinoxyfen) was a new product at the time of this work; it

has a different mode of action from the other products available to strawberry growers for control of *P. aphanis*. Area under the disease progress curve (AUDPC) was used quantify the magnitude of epidemics and these quantities were compared by ANOVA to identify differences between varieties (Genstat, 8th Edition, VSN International Ltd).

5.3.3 Dipping treatments prior to planting

Treatments for the control of *P. aphanis* on new planting stocks were compared (Table 5.3). Elsanta plants supplied by R.W. Walpole and Partners were removed from cold store and allowed to defrost. These were divided in to batches and then dipped for 1 minute in a single treatment. The plants were then allowed to drain before being put into plastic bags and were planted the following day (07.06.06) at the Colchester site. They were planted into 3 rows of raised troughs (at the south side of the tunnel). Treatments were arranged in a randomised block design of 3 replicates. Plots contained 84 plants (42 x 2). Plants were first scored on the 16.06.06 and for the last time on the 12.07.06 for presence or absence leaf cupping, mycelium and or red blotching. They were scored a total of 4 times at weekly intervals. Area under the disease progress curve (AUDPC) was used quantify the magnitude of epidemics and these quantities were compared by ANOVA to identify differences between varieties (Genstat, 8th Edition, VSN International Ltd).

Table 5.1 Resistance ratings for the cultivars tested, based upon consultation with growers

Cultivar	Ever bearer	Resistance to powdery mildew
Bolero	Yes	Moderate
Elsanta	No	Susceptible
Everest	Yes	Resistant
Florence	No	Moderate
Rosie	No	Susceptible
Royal Sovereign	No	Very susceptible
Symphony	No	Susceptible

Table 5.2 *P. aphanis* control products applied

Trade name	Active Ingredient	Dilution Rate	Application Rate
Untreated	Non applicable	Not applicable	Not applicable
Fortress	Quinoxyfen	0.3l/ha	200-400 l/ha
Systhane	Myclobutanil	0.45l/ha	200-500 l/ha
Bicarbonate (K50) + SW7	Potassium bicarbonate + Plant nutrient	0.02kg/l 0.6ml/l	300 l/ha

Table 5.3 Products and dilution rates for dipping trial

Product	Active Ingredient	Dilution
Untreated	Not applicable	Not applicable
Water	Not applicable	Not applicable
Bicarbonate (K50) +SW7	Potassium bicarbonate + Plant nutrient	10ml/l 0.6ml/l
Systhane	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

5.4 Results

5.4.1 Cultivar screening

All seven cultivars had low levels of symptoms within 3 days of the tunnel being covered (Fig. 5.1). The low levels of symptoms remained stable until the 18.05.04 when the severity of symptoms on Royal Sovereign started to increase. The amount of symptoms continued to increase until the final sample date on the 13.07.04. The amount of symptoms on the cultivars Bolero, Symphony, Rosie and Elsanta started to increase after the 08.06.04 and increased until the 23.06.04. The disease level then remained constant until the final sample date, 13.07.04. The remaining two cultivars, Everest and Florence had a constant low level (max 4.5%) of symptoms through out the experiment. Based on ANOVA of AUFPC the seven cultivars could be classified into 3 groups according to their resistance to *P. aphanis* infection, very susceptible (Royal Sovereign), susceptible (Bolero, Elsanta, Rosie & Symphony) and moderately resistant (Everest & Florence) (Fig 5.1).

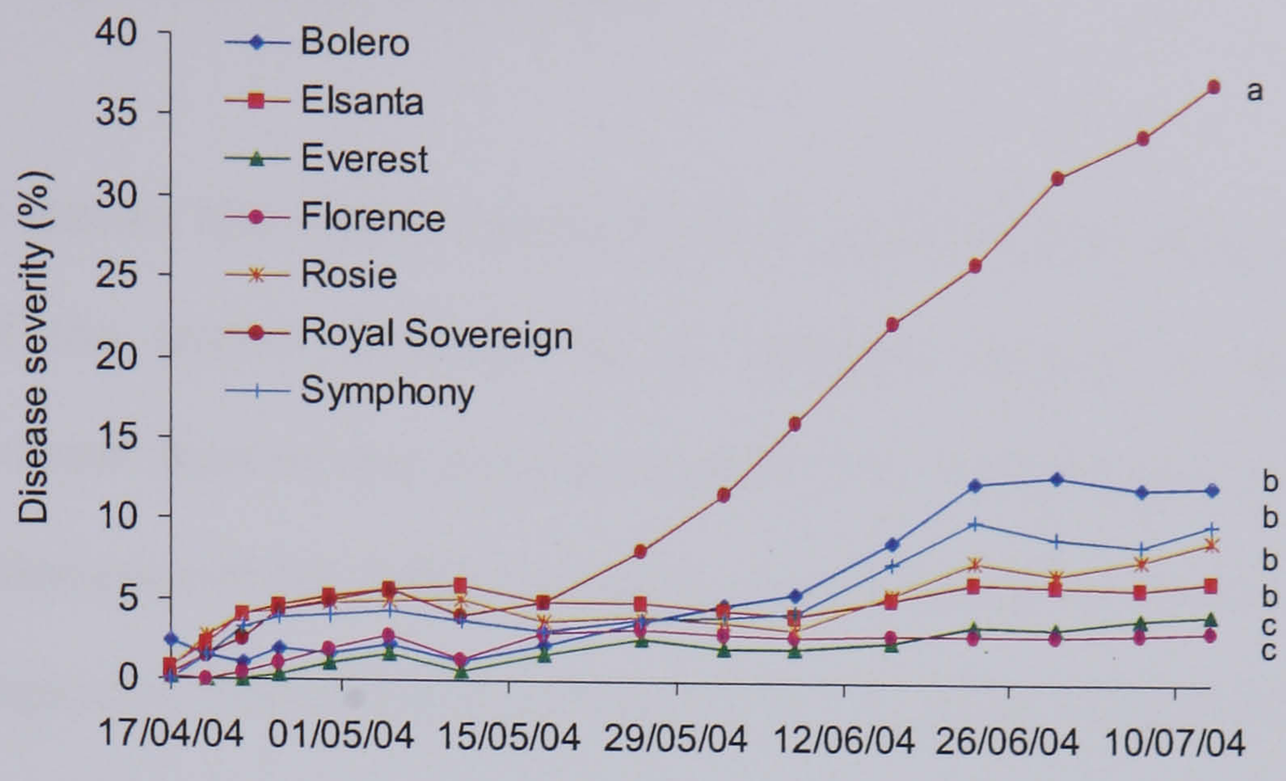

Fig. 5.1 Infection levels expressed as disease severity (%) scored with the MAFF strawberry powdery mildew key 8.1.1 for seven strawberry cultivars. Lower case letters indicate significant statistical differences ($p<0.05$) between epidemic magnitudes measured by AUDPC on the cultivars Genstat, 8th Edition, VSN International Ltd

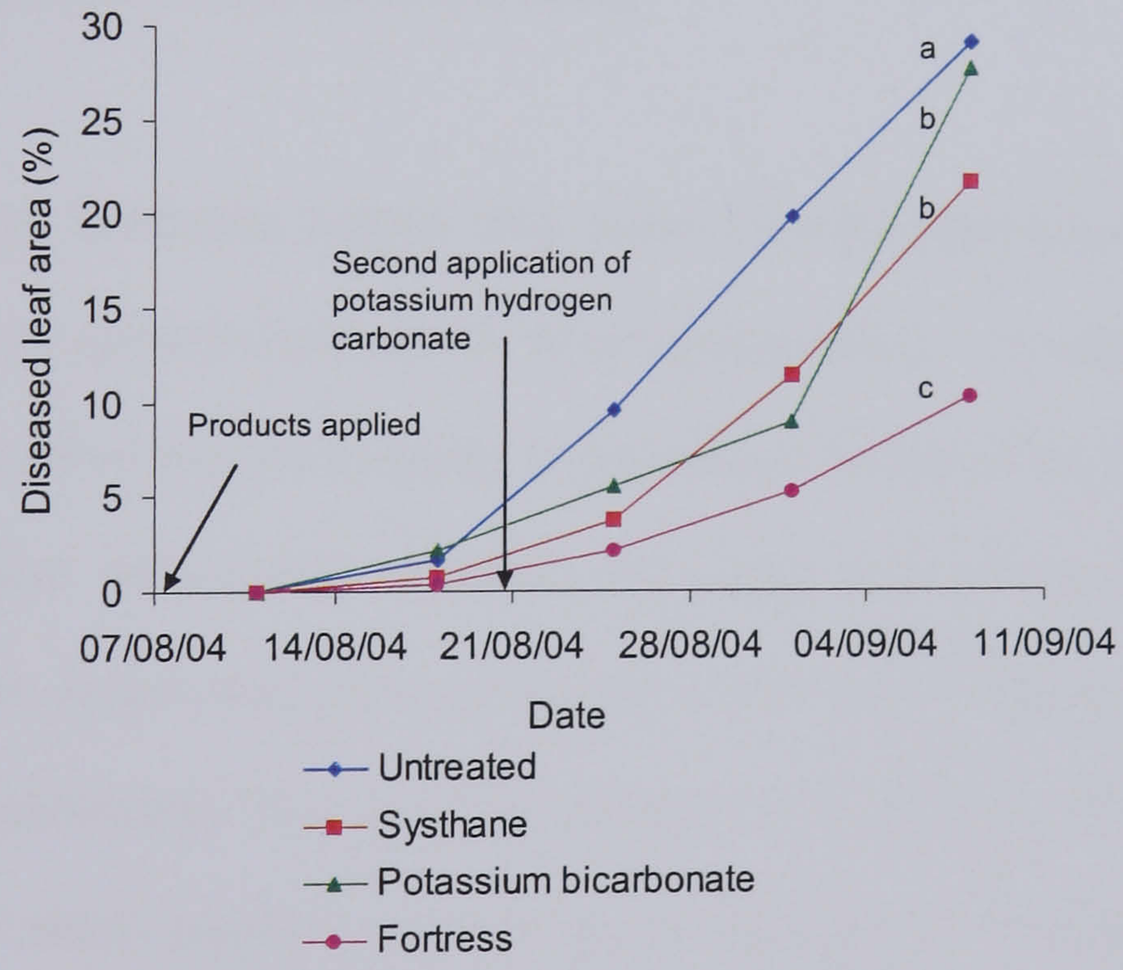

Fig. 5.2 Percentage disease leaf area (red blotches) for untreated plants and three control products for *P. aphanis*. Lower case letters indicate significant statistical differences ($p<0.05$) between epidemic magnitudes measured by AUDPC Genstat, 8th Edition, VSN International Ltd

5.4.2 New *P. aphanis* control products

None of the leaves had visible symptoms of *P. aphanis* when they were tagged. All three of the control products for *P. aphanis* resulted in disease levels significantly lower than on the untreated plants (Fig. 5.2). Potassium bicarbonate resulted in disease control levels that were comparable to Systhane (the industry standard fungicide). The levels of symptoms on the plants treated with potassium bicarbonate were starting to increase quickly two weeks after the second application of potassium bicarbonate. Fortress resulted in significantly better disease control than either Systhane or potassium bicarbonate (Fig. 5.2).

5.4.3 Dipping treatments prior to planting

Dipping plants in Systhane before they were planted significantly delayed the development of *P. aphanis* symptoms, when compared to untreated plants or the plants that were either dipped in water or potassium bicarbonate (Fig. 5.3). There were no statistical differences between the dates that *P. aphanis* symptoms developed on the plants that were untreated or had been dipped in either water or potassium bicarbonate (Fig. 5.3). Symptoms of *P. aphanis* developed 9 days after the plants were planted. On the plants dipped in Systhane, symptoms developed at least 14 days after the plants were planted, a delay of 5 days compared to other treatments.

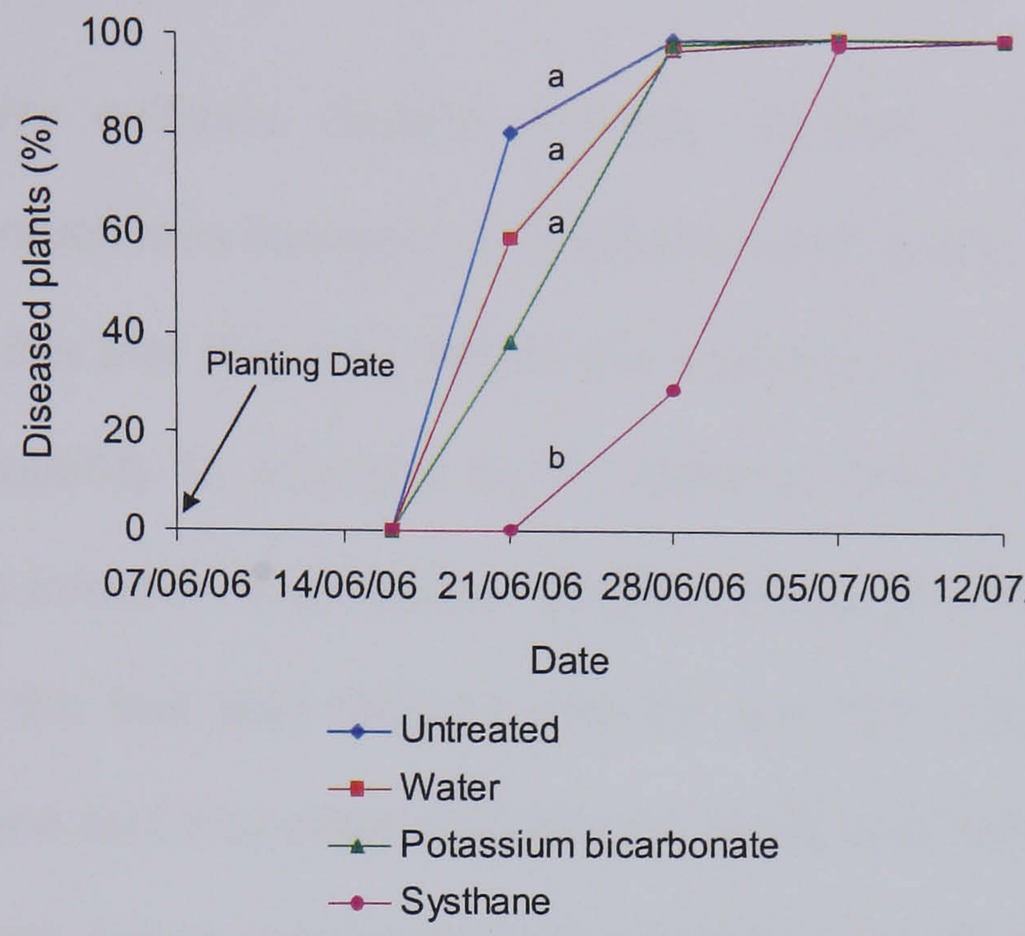

Fig. 5.3 Percentage of plants with symptoms of *P. aphanis* infection after plants were dipped and planted. Lower case letters indicate significant statistical differences ($p<0.05$) between epidemic magnitudes measured by AUDPC Genstat, 8th Edition, VSN International Ltd

5.5 Discussion

Newer strawberry cultivars (Bolero, Elsanta, Everest, Florence, Rosie and Symphony) are much less susceptible to infection than Royal Sovereign the older cultivar used in this trial (Fig 5.1). Within the newer cultivars there were differing levels of susceptibility to infection by *P. aphanis*. Everest and Florence had significantly less infection than Bolero, Elsanta, Rosie and Symphony (Fig. 5.1). The majority of the fruit sold (80%) in the UK are from Elsanta plants (Anon., 2005a) despite the fact that other cultivars are much less susceptible to infection by *P. aphanis* and hence require lower fungicide inputs. Elsanta is the retailers preferred berry due to shape and colour. If the growers were able to use these cultivars they could reduce the amount of fungicides they had to use to produce fruit of a suitable quality. However retailers specify the varieties that are suitable and they will not accept fruit from other varieties (personal communication, Richard Harnden, Berry gardens). Previously resistant varieties have lost their resistance when exposed to *P. aphanis* infections in the field (Davik and Honne, 2005). Both additive and non-additive variance components are important in the inheritance of *P. aphanis* resistance (Davik and Honne, 2005, Nelson *et al.*, 1995). Resistance to *P. aphanis* may not be durable but when combined with fungicidal applications the total amount of fungicide applied can be reduced (Lucas, 1998). Enabling growers to grow less susceptible cultivars would not necessarily be a lasting solution with which to control *P. aphanis* but could form part of a developing integrated control strategy.

Even if the retailers would buy the fruit from less susceptible cultivars the growers would still have some *P. aphanis* infection to control. To do this the grower will still need to apply fungicidal control products as part of an integrated approach to control *P. aphanis* (Arslan *et al.*, 2006, McGrath and Staniszewska, 1996, Oliva *et al.*, 1999). Strawberry growers have a limited range of products with which to control *P. aphanis* infections (Table 1.6). The industry 'standard' product, Systhane (Myclobutanil) provided effective control of *P. aphanis*, significantly reducing the amount of symptoms when compared to untreated plants (Fig. 5.2). While Fortress (Quinoxyfen), which was a new product to the strawberry industry, with a different mode of action that was not available to the strawberry grower, at the time of this work (Anon., 2005d, Anon., 2007), provided significantly better control than that achieved with Systhane (Fig. 5.2). When new effective products become available for control of *P. aphanis* their mode of action must be protected to stop resistance developing. The use of natural substances (organic or inorganic salts) can reduce the number of applications of fungicides that need to be used (Arslan *et al.*, 2006, Nofal and Haggag, 2006). Control of *P. aphanis* was achieved using potassium bicarbonate mixed with a silicon based plant nutrient (Fig. 5.2). Potassium bicarbonate however only works by contact with the fungus (personal communication, Harriet Duncalfe, Wisbech) so two applications of this product were needed to be comparable to the one application of Systhane, which is classed as a systemic, protectant and curative fungicide (Whitehead, 2007), suggesting that some fungicide applications in an integrated

control program, could be replaced with applications of potassium bicarbonate. This work was carried out on sites and in years where the overall *P. aphanis* levels were considered to be moderate or low. Potassium bicarbonate still needs to be tested on a site with high disease levels.

The initial inoculum for strawberry powdery mildew epidemics in newly planted field is introduced via infected planting stock (Chapter 3). Due to crop architecture and the shape of the strawberry leaf complete leaf coverage by fungicides is difficult to achieve. However the grower needs to achieve good control of the initial inoculum before the symptoms become widespread in the site, as this is the stage that there is the greatest rate of disease development (Lucas, 1998, Zadocks and Schein, 1979). Dipping of plants in fungicides has been used to control crown rot in strawberries (De los Santos *et al.*, 2002). In the experiment reported here dipping in Systhane was found to slow the development of *P. aphanis* (Fig. 5.3). Dipping the plants in either water or potassium bicarbonate did not result in significantly less disease than was present on the untreated plants (Fig. 5.3). Dipping in Systhane could be a possible method by which strawberry growers in the future could control the initial inoculum that is being planted into new fields and therefore delay the start of the epidemic. Currently however no products have approval for dipping of strawberry plants to control *P. aphanis*.

5.6 Conclusions

Strawberry growers are under pressure form the retail industry to produce fruit with as few fungicide residues as possible, and even to produce fruit with residues below the legally set levels. Retailers also instruct growers about the strawberry cultivars they are to grow. To be able to reduce fungicide residues strawberry growers need to develop an integrated control strategy for *P. aphanis*. To do this growers need to have the freedom to chose which cultivars they grow as the current industry standard cultivar, Elsanta is one of the more susceptible cultivars to *P. aphanis* infection. Once growers have chosen the best cultivar for the situation (yield, quality of fruit and level of disease susceptibility) they need to control the initial inoculum so that the level of disease does not build quickly. Growers could achieve this (when planting new plants) by dipping them in a suitably approved fungicide, when one is available. Once the crop is established in the field the grower needs to use the correct fungicidal control product for that specific situation. This could be the use of a new fungicide, a fungicide that is well established in the market or a natural 'bio-fungicide' such as potassium bicarbonate. What ever the control product is it needs to be applied so that it will return the optimum benefit therefore application needs to coincide with periods of greatest risk, as for example identified by the prediction system developed by this work (Chapter 4).

Chapter 6 - Overall discussion and conclusions

Three symptoms (leaf cupping, visible mycelium and red blotches) have been linked to infection by *P. aphanis*. The symptoms form a progression that growers can use to determine the level of infection within their crop. The first symptom of infection is leaf cupping, which is followed by the presence of mycelium on either leaf surface which finally leads to red blotches before the leaf dies (chapter 2). From this it has been possible to develop two new scoring methodologies that are more suited to current strawberry production practices. The first method developed can be used to identify if there is infection within a field. This can be used by growers to help identify when they should be applying control products for *P. aphanis*. This method takes into account all the symptoms linked with an infection by *P. aphanis* unlike the previous method that was available (MAFF Strawberry Powdery Mildew Key 8.1.1) which only used leaf cupping and the percentage of the leaf surface covered with red blotches, so should enable the grower to identify infection soon and therefore enable application of fungicides sooner. The method detailed for use by the grower measures incidence of disease rather than severity and so can be recorded with much less error (Parker *et al.*, 1997).

The second method is more suited to experimental work where fungicides or other control products are being evaluated as it gives more detailed information on disease development and any control of disease development. The method

however does rely on visual measurements being made by the assessor. There can be great variation between two different assessors as well as variation in the assessments made by the same assessor (Parker *et al.*, 1995). In the case of the work presented here all assessments were made by the same assessor. If this method was to be used more widely it would be important for the same assessor to score all the plants on a single occasion (Parker *et al.*, 1997). Work has been carried out to evaluate image capture methods for the scoring of disease severity (Corkidi *et al.*, 2006, Moya *et al.*, 2005). Moya *et al.* (2005) compared visual analysis, digital photography and the use of a scanner to estimate disease severity of powdery mildew on squash leaves. Corkidi *et a.l* (2006) developed an accurate image-analysis method to measure the severity of anthracnose of mango fruit. All of the image capture methods detailed used destructive sampling methods. The leaves or fruit had to be collected and presented in such a way that they were suitable for image capture. These image capture methods would not be suitable for repeat sampling of the same leaf in the field, as required by the method detailed here. In addition infection by *P. aphanis* causes the leaf to cup upwards so making non destructive (where the leaf can be held flat) image capture not viable at this time. Scoring of *P. aphanis* infection will have to be undertaken by skilled assessors as the image capture methods are not currently suitable for use with strawberry leaves. All symptoms of *P. aphanis* need to be considered when scoring an infection rather than just cupping and red blotching as in the MAFF Strawberry Powdery Mildew Key 8.1.1.

When this work was started there was minimal information on how *P. aphanis* over wintered, whether it was as chasmothecia or mycelium (Gourley, 1979, Peries, 1961, Rashid Khan, 1960, Salmon, 1900). The growers believed that the initial inoculum was wind borne, rather than being present on the plants when they were first planted or having over wintering in the field. This work has shown that infection develops throughout the tunnels at the start of the season when conditions first become suitable, temperature > 15°C and relative humidity > 70% (most often when the tunnels are first covered at the start of the season) (chapter 3). This happens both in newly planted tunnels where the plants had just come out of cold store and in established sites where the plants over wintered within the site. If the infection had been air borne the first plants with symptoms of *P. aphanis* would have been clustered around the entrances to the tunnels (Fletcher, 1984). Then from these initial infections inoculum would spread along the tunnel in waves of infection. The initial infections did not do this. *P. aphanis* developed through out the tunnels soon after they were first covered. Hop powdery mildew overwinters as chasmothecia (Liyanage and Royle, 1976) whereas grape (Pearson and Gartel, 1985, Sall and Wrysinski, 1982, Van der Spuy and Matthee, 1977), apple (Xu, 1996, Xu, 1999c), rose (Price, 1970) and hawthorn (Khairi and Preece, 1978) powdery mildew overwinter as mycelium within buds. Peries (1961) found that it was possible for *P. aphanis* to over winter as mycelium on green strawberry leaves. Recently chasmothecia have been observed on commercial strawberry plants over wintering in the field (Farooq *et al.*, 2007, Hall *et al.*, 2007). The growers managed their tunnels believing that the

P. aphanis inoculum was wind borne and did not overwinter in the tunnels where as they should be managing their tunnels with the aim of controlling the inoculum already on the plants.

Growers are under pressure from the retailers to reduce the amount of fungicide residues there are in the fruit that they produced. The UK growers closely involved with this work were applying between 3 and 14 fungicide applications to a crop each season; other growers were sometimes applying more than 14 fungicide applications (chapter 4). Research carried out in the Netherlands showed that 50 pesticides were applied to strawberries in a season, of which just less than half were fungicides (Van Drooge *et al.*, 2001). This number of applications poses a health risk to the operator as well increasing the possibility of finding residues in the fruit. A reduction in fungicide applications could be achieved by implementing several methods. These could be combined to produce an integrated, sustainable control program for *P. aphanis*.

It is possible to reduce the initial inoculum that is planted into a site by dipping the new plants in a fungicide. Systhane was used in this work to prove the principle that dipping plants can have an effect by increasing the lag phase at the start of the season. Development of infection was slowed by up to 5 days when the plants were dipped in Systhane compared to when they were dipped in products that were not a fungicide or not dipped at all (chapter 5). Fosetyl-aluminium has approval (until December 2007) to be used as a root dip of

strawberry plants against red core (Appendix 7 - PSD document 5230). As PSD has approved products to be used as dips it should be possible for approval to be sought for dipping plants to reduce the initial *P. aphanis* inoculum. As long as the operators are provided with suitable personal protective equipment (PPE) and the residue is disposed of legally dipping of plants should not pose a problem for the regulatory authority. Reduction of initial inoculum at the start of the season could be achieved in an established crop by the use of winter time sprays (unpublished work). Dipping of plants reduced the initial inoculum so slowing the initial development of the epidemic.

When the grower has to apply a fungicidal control product they can apply an organic or inorganic salt such as potassium bicarbonate which can provide control comparable to that achieved with Systhane (the industry standard product) (chapter 5). The grower has to change the way that they monitor and apply products when they switch to products such as potassium bicarbonate. They generally have no systemic activity. They only act by contact, so good spray coverage needs to be achieved and the crop needs to be monitored more closely than if a systemic fungicide had been applied, for subsequent development of infection. Potassium bicarbonate can also cause crop phytotoxicity so a test plot should be sprayed when a grower is using the product for the first time (Whitehead, 2007). Use of potassium bicarbonate provides control comparable to Systhane.

When they are available, the grower could apply new control products, such as Fortress, which had a novel chemistry that was not already available to the grower at the time of this work. Fortress provided control that was significantly better than that achieved with Systhane (chapter 5). New products (and older products) must be applied in a responsible way so that the new mode of action that has been introduced does not get overused, so resistance will not develop in the pathogen against the mode of action. A reduced sensitivity to Myclobutanil (Systhane) has been reported in grape powdery mildew from vineyards where DMI fungicides were used frequently (Ouimette and Gubler, 1990). New fungicides can provide control that is significantly better that that which can be achieved using current control products.

A rule based prediction system has been developed that identifies how many hours of suitable conditions (germination - 6 hours temperature >15.5°C and <30°C, relative humidity >60%, leaf wetness >95% and growth - 138 hours temperature >18°C and <30°C, only 78 hours if first infection cycle of a season in an established field) (chapter 4) there have been for the growth of a *P. aphanis* infection. Then when there have been enough hours of suitable conditions for a 'new' infection of *P. aphanis* to have reached maturity and the infection is just about to produce more inoculum the system predicts a high risk period so telling the grower to apply an application of a fungicidal control product. This so far has resulted in the same number or fewer applications being predicted when compared to the actual number of fungicide applications applied by the grower

(chapter 4). Growers are often reluctant to use new models or rule based prediction systems when they have been developed (Parker, 2001, Vallavieille-Pope *et al.*, 2000). Parker and Sinclair (2001) identified eleven reasons why (in their case) decision support systems (DSS) were not widely used by growers or agronomists (Table 6.1). The prediction system detailed here was developed in such a way that it would be relevant to as many growers as possible and would be able to cope with changes in variety grown and changes in production protocols. The system has been developed for use by the growers and as such has been designed to be accessible to the grower and robust.

It would also be possible for the growers to reduce the amount of fungicide that they used to control *P. aphanis* infections if they were able to choose which variety of strawberry they were to grow (chapter 5) rather than have the retailers determine which varieties can be grown. Currently Elsanta is the most commonly grown main crop variety. There are other varieties that have significantly better resistance to infection by *P. aphanis* than Elsanta does. However the growers are not able to grow these varieties as the retailers want the growers to grow the varieties that they believe the consumers want to buy. Using the current varieties that have at least partial resistance would not be a long term answer to reduce fungicide use unless there was continual breeding program to develop new resistant varieties. The Norwegian variety Korona was resistant to infection by *P. aphanis* when it was first developed in 1983. Korona is still the main variety grown in Norway but is now susceptible to severe attacks by *P. aphanis* unless

Table 6.1 Eleven reasons why DSS have not been widely used (Parker and Sinclair, 2001) and where appropriate solutions offered to those problems by the rule based prediction system developed as part of this work

Limited computer ownership and use on farms	This is no longer relevant as the vast majority of farms now have access to computers
Too great a time commitment	It is planned that the system will start up automatically when the computer is turned on and then give the grower a recommendation automatically each morning
Inappropriate use of model	Many DSS were in-fact research models repackaged for use by the grower, where as the rule based prediction system detail here has been developed from the start for use by the growers
Infeasible data requirements	The rule based prediction system needs detailed on farm, in tunnel weather data, it is not suitable for use by growers with out an on farm met station, if the system is not provided with accurate information it will not produce an accurate prediction
Poor integration between systems	the rule based prediction system has been developed in such a way that the output can be interpreted with some flexibility so that it can fit in with other on farm pressures
Lack of confidence in results	Validation is important in the development of models, DSS or rule based prediction systems; the system still needs further testing and validation by selected growers. The validation of the system is very important as one bizarre prediction could cause the grower to lose confidence and so stop using it
Absence of support for users	Once the system has been developed the HDC will distribute the model to it's members and will be responsible for any support
Perceived threat to the advisor	The system is not meant to replace the advisor. It is meant to complement the advice given by the advisors
No ability to tailor systems	The system predicts a high risk period and then immediately starts to predict the next high risk period but the system has the option for the grower to input the actual date of the application if it is different from the predicted high risk period
Poor user interface design	Currently the rule based prediction system does not have a user interface. This needs to be designed carefully so it will be easy for the grower to use
No updating of material	The system has been deliberately designed so that the variety of strawberry grown or the specific control product applied will not have a bearing on the system

treated with fungicides (Davik and Honne, 2005). Davik and Honne (2005) conclude that is feasible to develop new varieties with resistance to *P. aphanis* but constant breeding will be needed as resistance to powdery mildews in other crops has been non durable. Wild varieties of strawberry could be used as new sources of genetic material that could provide improved disease resistance. It could be possible to retrieve fruit quality that is comparable to current standard within three generations of the backcross being performed (Hancock *et al.*, 2002). Resistance to *P. aphanis* infection appears to be controlled by both additive and non additive components. There is no simple inheritance of resistance so breeding needs to be for partial resistance (Nelson *et al.*, 1995). It is possible to reduce the level of infection by growing varieties that are less susceptible to infection by *P. aphanis* than the most popular current variety, Elsanta.

This work has identified which symptoms are linked to a *P. aphanis* infection and the order that the three symptoms develop. This has lead to two new scoring methods that will enable growers and scientist to identify and quantify the levels of infection that are present in commercial fields as well as on experimental plots. The initial source of inoculum has been identified for newly planted and established fields, so enabling growers to alter their management practices at the start of the season, which were directed at keeping the inoculum out of the tunnels, when it was already present in the tunnels. A rule based prediction system has been developed which highlights periods where the crop is at highest

risk of infection by *P. aphanis* infection, therefore allowing the growers to target applications of fungicides when they will result in the most benefit. In addition work has been carried out to highlight better used of fungicides. Dipping plants prior to planting has been shown to have the potential to slow the speed at which initial inoculum develops in newly planted sites. Potassium bicarbonate (a natural product) has been shown to be as effective as the industry standard product and when one is available a new product has been shown to provide better control than the current industry standard product. This work has also demonstrated that if the growers were allowed to grow different cultivars they would be able to reduce the levels of *P. aphanis* that builds up in their crops. The strawberry growing season and the time that each of the integrated control methods could be used each season is shown in Fig 6.1.

6.1 Future work

The prediction system is showing potential at the moment, but it needs extensive field testing. The system should be tested on commercial sites from the main strawberry growing regions of the UK (Scotland, Herefordshire, East Anglia and Kent). A comparison should be made between the first part of a field managed by the grower, applying applications as they normally would and the second part of the field where the fungicide applications are applied as and when the prediction system predicts a high risk period. The level of control achieved and the number of fungicide applications would be compared to determine the overall reduction in

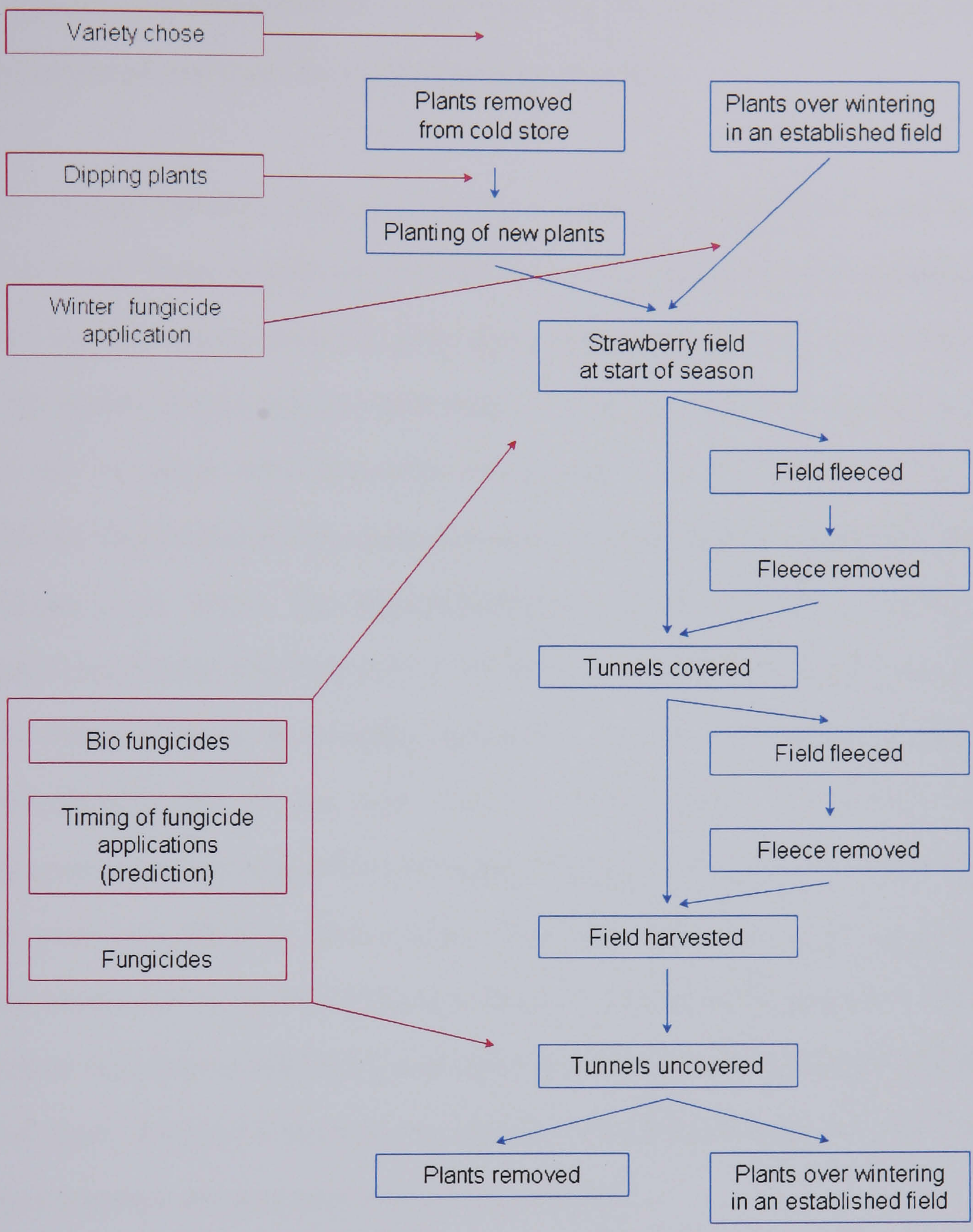

Fig. 6.1 Strawberry growing season and timing of integrated control measures

fungicides used (unpublished). Then a user interface needs to be developed for the system so that it can be distributed to the growers.

The precise method(s) that are used by *P. aphanis* to overwinter need to be determined. There is little leaf material left on the plants from the propagators once they have been graded. Further study needs to be carried out to determine if the source of inoculum on these plants is either mycelium on the leaves that are left, mycelium within the crown of the plant or chasmothecia on the leaf material. Chasmothecia have been observed on established overwintering plants (Farooq *et al.*, 2007). The initial infections on leaves need to be studied to determine whether they form from a conidium or an ascospore. When searching the literature there is minimal information on the role that *P. aphanis* chasmothecia play in the over wintering of the fungus. The growers and agronomists do not believe that the chasmothecia are common and therefore do not make any effort to control them. Initial work has shown that germinated ascospores can be found on leaves at the start of the season (Hall *et al.*, 2007). Further experiments need to be undertaken to determine if the chasmothecia that have been observed in the field are mature at the start of the season and if they could therefore act as a source of primary inoculum.

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Appendix 1 - HDC final report project SF62

Grower summary

SF 62

The epidemiology and control of strawberry powdery mildew under protection

Annual report 2006

Project title:	The epidemiology and control of strawberry powdery mildew under protection
Project number:	SF 62
Project leaders:	Dr Steven Parker Central Science Laboratory Sand Hutton York YO41 1LZ Dr Avice Hall Bioscience, CP Snow Building University of Hertfordshire College Lane Hatfield Hertfordshire AL10 9AB
Key Workers	Mr Jolyon Dodgson Same address as Dr Hall
Report:	Annual report, 2006
Previous reports:	Annual report, 2004 Annual report, 2005
Location:	Central Science Laboratory
Project co-ordinator:	Mrs Harriet Duncalfe Maltmas Farm Fridaybridge Wisbech Cambridgeshire PE14 0HS
Date commenced:	1 June 2003
Expected completion date:	30 November 2006
Key words:	Strawberry, powdery mildew, disease control, fungicide, plant stimulant, epidemiology, disease pattern, inoculum, resistance, *Podosphaera aphanis, Sphaerotheca macularis,* integrated disease management, bicarbonate, corbel, flexity, fortress, signum, stroby, systhane, boscalid, fenpropimorph, kresoxim-methyl, metrafenone, myclobutanil, potassium hydrogen carbonate, pyraclostrobin, quinoxifen, sulphur

The results and conclusions in this report are based on a series of experiments 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.

SF 62: Grower Summary

The epidemiology and control of strawberry powdery mildew under protection

Headline

- Powdery mildew over-winters in established fields and provides a potential source of inoculum in the following season
- Powdery mildew is planted in to new fields on the planting stock
- In these experiments, powdery mildew symptoms have not been detectable by visual inspection until shortly after tunnels are covered
- Disease development is slower at the start of the season on new fields compared to established sites. This could be due to the form the inoculum is in.
- Powdery mildew mycelium is present on leaves that are cupping and that have red blotches even if it is not visible to the naked eye
- Fortress (Quinoxyfen) provides long lasting protection as long as there is no visible infection when it is applied.
- Potassium hydrogen carbonate (bicarbonate) provides control comparable to Systhane (Myclobutanil). It however is not systemic and only works by contact, so only works when there is some established infection
- Growers should continue to combat the development of fungicide resistance (see SF 62 2004 annual report).
- If retailers would accept different varieties growers could reduce the amount of fungicide used to control powdery mildew.

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 project will improve understanding of strawberry powdery mildew and use this knowledge to suggest control strategies, which will integrate agronomic and chemical control methods to suppress disease to tolerable levels.

The expected deliverables from this work include:

- Improved knowledge of the efficacy of fungicides against strawberry powdery mildew, which are approved for use on crops grown under protection.
- Clear guidelines on the design of chemical control schedules that are effective, and which reduce selection pressure for fungicide insensitivity in the pathogen population.
- An evaluation of the value of plant stimulants and 'natural products' to reduce disease severity.
- Identification of agronomic management practices that can reduce disease pressure.
- The identification of high risk environmental conditions that favour the spread and development of disease.

Summary of the project and main conclusions

Preliminary Review of the Literature

A powdery mildew on strawberries was reported at the start of the last century. The causal pathogen has variously 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 powdery mildew on strawberry is *Podosphaera aphanis* and that this is distinct from 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 that the pathogen can survive. Laboratory experiments have been used to estimate the time for completion of important life-cycle phases. These estimates can provide a useful basis for planning the investigation of disease progress in the field experiments within the current project.

Inoculum and primary disease spread

Disease developed through out the two plots of newly planted strawberry plants that were score. The infection was not clustered at one end or the other end of the plots. The infection was randomly distributed through out the plots. This result confirms last years work that infection in newly planted fields comes from the planting stock. The infection takes longer to develop on newly planted plants suggesting that it could be overwintering as either cleistothecia or conidia. Where as the infection overwintering in established fields could well be as mycelium. Which would take a shorter time to develop than cleistothecia or conidia would.

Dipping plants to control initial disease development

Infection already present on newly planted plants develops once they have been planted. Due to the shape of even the young strawberry leaf it is very hard for the grower to achieve good coverage with fungicidal products once the plant has been planted. This experiment showed that it is possible to delay the onset of symptoms of strawberry powdery mildew by at least 7 days (in an high pressure mildew environment) when the plants were dipped in a chemical control product before planting, compared to plants that were not dipped or were dipped in either water or bicarbonate. At the moment there are no products approved by PSD for the dipping of strawberry plants to control powdery mildew. PSD has however approved a product for the dipping of strawberry plants to control red core. So it might be possible to get approval to dip for powdery mildew.

Inoculum levels linked to cupping and red blotches

Infection by strawberry powdery mildew causes a progression of symptoms (cupping leaves, mycelium on the leaves, red blotches on the leaves and finally mycelium on the fruit). The only symptom that can be linked with powdery mildew, with any certainty by visual assessment is mycelium. This work showed that there was significantly more infection on leaves that were cupped or that had red blotches than there was on the flat healthy appearing leaves. Also there was significantly more infection on the lower leaf surface than there was on the upper leaf surface. This means that growers should start to control powdery mildew when they first see leaf cupping and they should go on controlling powdery mildew when the only symptom left is red blotching. It also highlights the fact that the majority of the infection will be on the lower leaf surfaces. So growers need to try and get good spray coverage of the lower leaf surface.

Prediction of high risk periods

The prediction system developed in SF62 2005 has been further refined so that it can better predict high risk periods. When the disease development data (that was collected as part of the epidemiological studies) is run through the prediction system it now predicts a high risk period when the infection first starts to develop. The prediction system needs to be tested with further data sets before being trialled by growers in the field.

Financial benefits

In the short-term

- Improved control of strawberry powdery mildew. The recommendations provided at the end of the project will aim to assist growers to design fungicide schedules that are more dose efficient.
- Whilst repeated applications of potassium hydrogen carbonate can offer similar levels of powdery mildew control as conventional fungicides, its approval as a commodity substance is beneficial to the aim of minimising residues and reliance on agrochemicals.

In the medium-term

- An effective armoury of fungicide products to manage disease. Improved stewardship of important active ingredients will reduce selection pressure on the pathogen population, so that options for control are not eroded.
- Better targeting of fungicide application when they will be most cost effective.

Action points for growers

- Growers should avoid repeated applications of fungicides with the same Mode of Action (MOA). Consecutive and frequent applications of products from the same MOA group increase the likelihood that the pathogen will develop fungicide insensitivity. Information about The MOAs of fungicides approved for use on strawberries are available in the previous (2004) Annual Report or online via the LIAISON subscription service (http://liaison.csl.gov.uk), which is updated daily.
- 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.
- Growers should consider using applications of potassium bicarbonate within 3 days of covering tunnels (or removing fleece) in order to suppress disease spread. An early application of Fortress (Quinoxyfen) applied after the bicarbonate will provide protection.
- Strawberry powdery mildew is not airborne.
- 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 control powdery mildew infection when these symptoms are present even though there is no visible mycelium.
- 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 powdery mildew on strawberry 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.

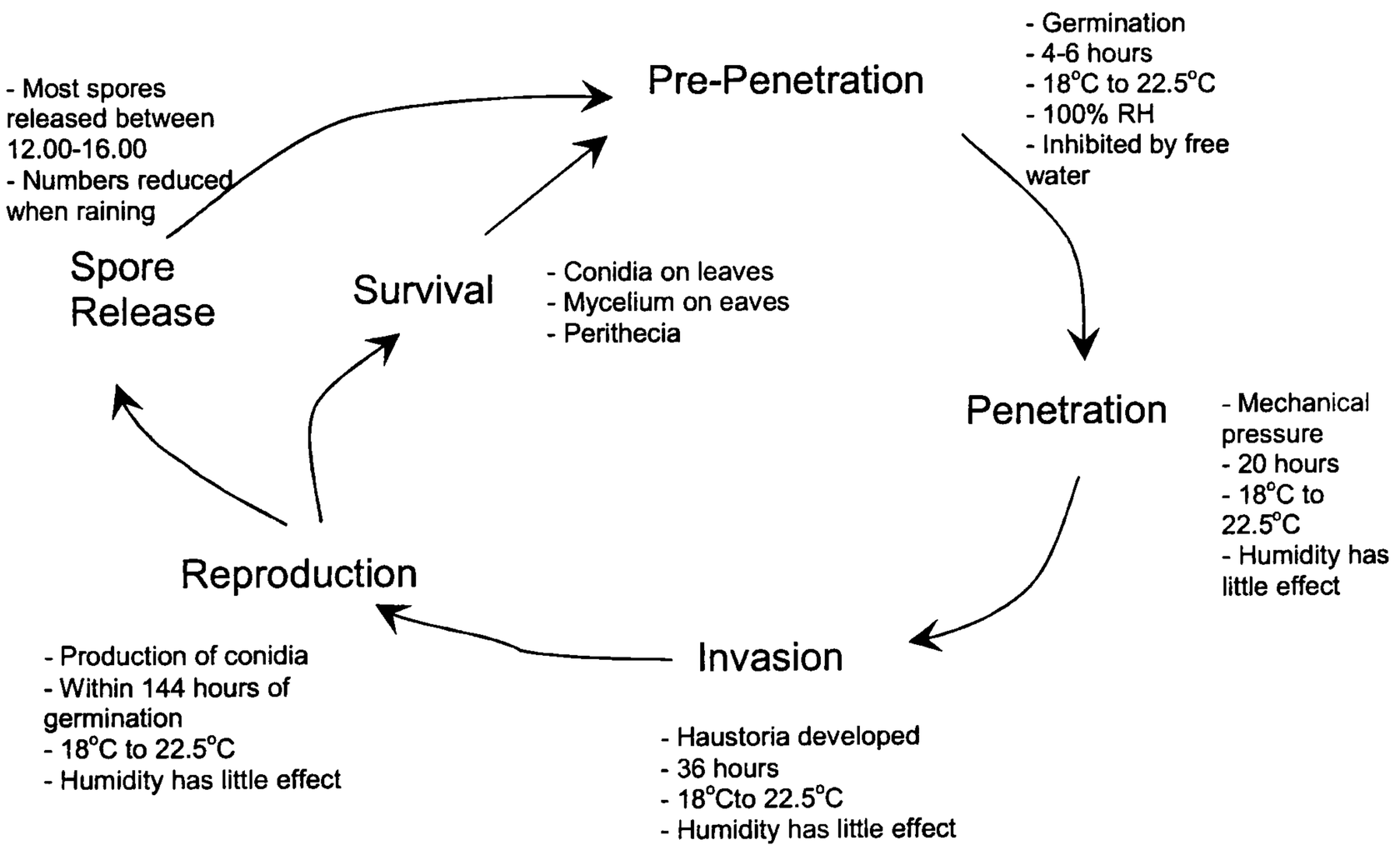

Fig. 1. Life cycle of strawberry powdery mildew *Podosphaera aphanis* (syn. *Sphaerotheca macularis*).

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 planned 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 germination of conidia (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).

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

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

[1] Blanco *et al,* (2004), [2] Jhooty and Mckeen (1964a), [3] Jhooty and Mckeen (1965), [4] Miller *et al,* (2003) and [5] Peries (1962a)
* Radial growth is slow at 15°C but maturity is reached in the same time as at 18°C.

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 (≈1.5m) from their source and vertically from within 3 feet (≈1.0m, Peries, 1962a). Relationships between environmental conditions, incidence of powdery mildew in strawberry and concentrations of *P. aphanis* (syn. *S. macularis*) conidia in the air have been described recently for US conditions (Blanco *et al.*, 2004).

Peries (1962b) tested the germination and growth of *P. aphanis* (syn. *S. macularis)* on several different varieties of strawberry plants. He found that some varieties were more susceptible than others, but none of them were resistant. He found that the least susceptible varieties had higher 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 sites

A field site was established on a commercial holding near Colchester, Essex (Grid reference: TM 068 305). The site consisted of 1 Spanish tunnel (30m × 7m, covered with normal plastic sheeting). The site contained second season Elsanta in peat filled troughs. The plants were grown as glasshouse plants in their first season then transferred (in their troughs) to the

Spanish tunnels for a second season. The plants were transferred to the Spanish tunnel in the autumn of 2005. The plants were managed commercially when they were in the glasshouse. Plants were bare root waiting bed plants supplied by Peter Wensak (Holland). The tunnel consisted of 7 raised beds. 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 the 12th April 2006. The tunnel was vented and irrigated according to normal farm pratices.

A second field site was established on a commercial holding near Wisbech, Cambridgeshire (Grid reference: TF 459 037). 3 sites were used for experimental work on the holding. The 1st site consisted of a Spanish tunnel (150m × 6m, covered with normal plastic sheeting). The site contained third season Elsanta in the ground. The site was planted in 2004 with plants supplied by Stefan Kraege. The site had been managed commercially in the previous 2 seasons. 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 the tunnels were covered 2006. The fleece was removed from the tunnel on the 02 May 2006. The tunnel were vented and irrigated according to commercial practice.

The 2nd site consisted parts of two Spanish tunnels (each 10.5m × 7.5m, covered with normal plastic sheeting). The site contained one tunnel of first season Elsanta and one of first season ever bearer in the ground. The ever bearer tunnel was planted in the 1st 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 ever bearer tunnel was fleeced the 1st week of March 2006 and the fleece was removed the 3rd week of April 2006. Both tunnels were covered 3rd July 2006. The tunnel were vented and irrigated according to commercial practice.

The 3rd site consisted of part of a Spanish tunnel (43m × 4.5m, covered with normal plastic sheeting). The site contained Everest in peat filled troughs. The site was planted with plants supplied by Edward Vinson Limited. 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. Plants were separated by 16cm within rows and the distance between rows was 17cm. Within rows, plants were off set by 8cm. Rows of troughs were separated by 0.9m. The tunnel was fleeced in mid March 2006 and the fleece was removed mid June 2006. The tunnel was covered in the 3rd week in July 2006. The tunnels were vented and irrigated according to commercial practice.

The field sites were used for the experiments described below.

Materials and Methods

Inoculum and primary disease spread

All the plants at the 2nd Wisbech site 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 analyzes 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

Cultivar Elsanta was used for this experiment. Four treatments were compared (Table 3). The plants were removed from cold store and allowed to defrost. They were then dipped for 1 minute. The plants were then allowed to drain before being put back in plastic bags to be planted the following day. They were planted on 07th June 2006 at the Colchester site. They were planted into 3 rows of raised troughs (at the south side of the tunnel) (see materials and methods/field sites for site description). Each treatment was planted in 3 plots each. Plots contained 84 plants (42 x 2) and plots were arranged randomly. Plants were first scored for presence or absence leaf cupping, mycelium and red blotching on the 16 June and then weekly from the 21 June until the 12 July 2006.

Table 3. Products and dilution rates for dipping trial.

Product	Active Ingredient	Dilution
Untreated	Not applicable	Not applicable
Water	Not applicable	Not applicable
Bicarbonate (K50)	Potassium hydrogen carbonate	10ml/l
+SW7	Plant nutrient	0.6ml/l
Systhane	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:

$$Area = \sum_{i}^{n-1} \frac{1}{2}\left[\left(S_i + S_{i+1}\right)\left(t_{i+1} - t_i\right)\right.$$

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 that were flat, that were cupping and that had red blotches (with no mycelium visible to the naked eye) were collected from 2 fields at the Wisbech site. In field A there was visible mycelium present and in the other field, B there was no visible mycelium present. From field A leaves were collected on the 08th, 22nd and 30th August 2006 and from field B on the 30th August and the 05th September 2006. At all but the first sample date (08th August) leaves were collected that were flat, that were cupping or that had red blotches (red blotches were not present on the 08th August). Leaves were frozen in liquid nitrogen and stored at -70°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). Individual leaflets were removed from the stain and washed (to remove excess stain). 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 the high powered microscope (magnification x100). 2 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 SF62 annual report 2005 recommended 2 less applications than were actually applied in an ever bearer crop (over five months the grower applied 10 treatments and the system recommended 8 treatments). This year the prediction system has been refined using the conditions identified in SF62 2005 as a starting point.
The disease development data, collected over the last 3 years, 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 carried out. They however did not produce results due to extremely low disease levels which resulted from abnormal weather conditions experienced this season. During the early and mid parts of the season the weather was extremely hot and so not conducive to disease development. The growers who farm on the Wisbech site reported extremely low disease levels on the rest of the farm right up until the start of September. This could be in part due them implementing the recommendations made in the first and second year reports produced by project SF62.
In all the experiments reported here there were no differences between the treated plots and the untreated controls. The methods will be summarised.

Disease control products. 10 products were used. 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 weekly. The experiment was run twice on the 1st Wisbech site and once on the Colchester site.
Development of control program. Ever bearers in the 3rd Wisbech site where treated over the course of the season for powdery mildew. There were 4 treatments; 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 08 June 2006 and the last products were applied on the 23 August 2006. At the last visit to the site on the 05 October 2006 there were no signs of infection. Leaves were collected on 05 September. They were stored and scored as detailed under the method for 'Inoculum levels linked to Cupping and red blotches'. There was no mycelium present.
Control of over wintering infection. A range of currently available control products were applied in December 05 and April 06 (just before the tunnel was fleeced) to part of the 1st site at Wisbech. Some plots just had the control products applied in December 05, some just had the control products applied in April 06 and some had products applied in December 05 and April 06. The plots were scored for symptoms of strawberry powdery mildew weekly 9 times after the fleece was removed, starting on the 2nd 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 ever bearer had symptoms of powdery mildew. Within 617 hours>15°C 44% of the Elsanta and 64% of the ever bearer had symptoms. Incidence grew until both tunnels had 100% diseased plants (Figure 2).

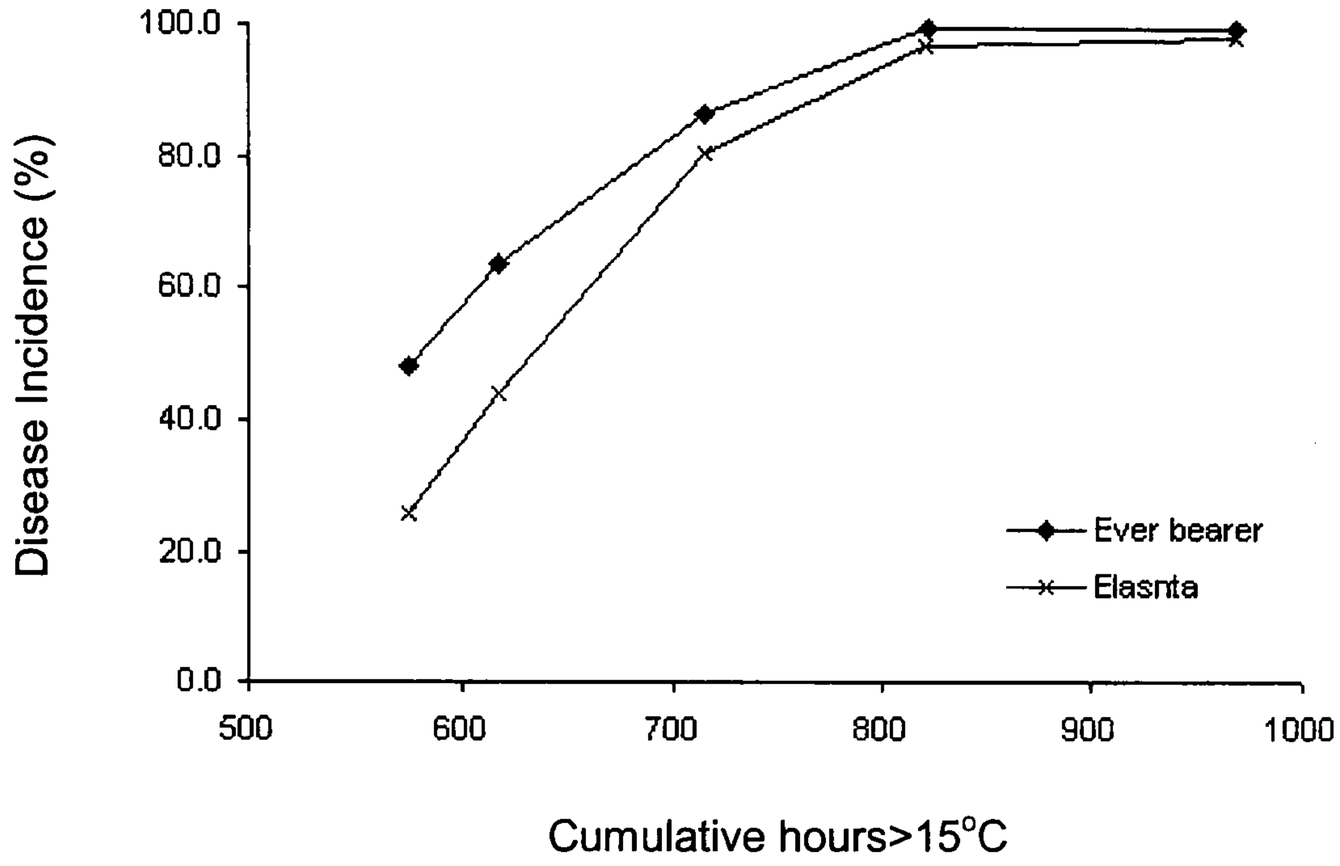

Figure 2. Growth in disease incidence (percent plants with symptoms), after tunnels were covered on an established site at Wisbech, Cambridgeshire.

The diseased plants in both the Elsanta and ever bearer were distributed through out the tunnel, 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.

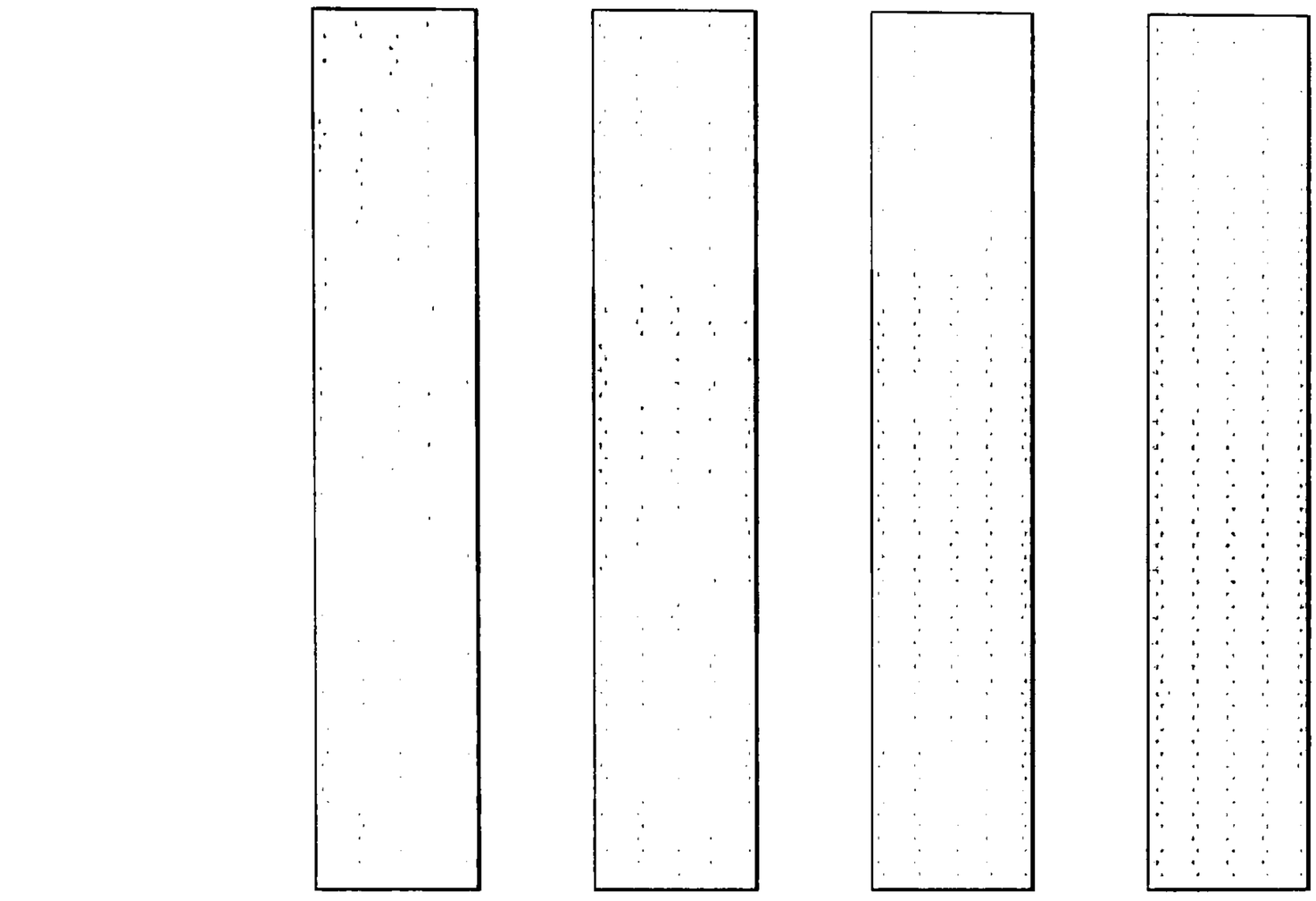

Figure 3. Pattern of increase for Elsanta plants with mildew symptoms.

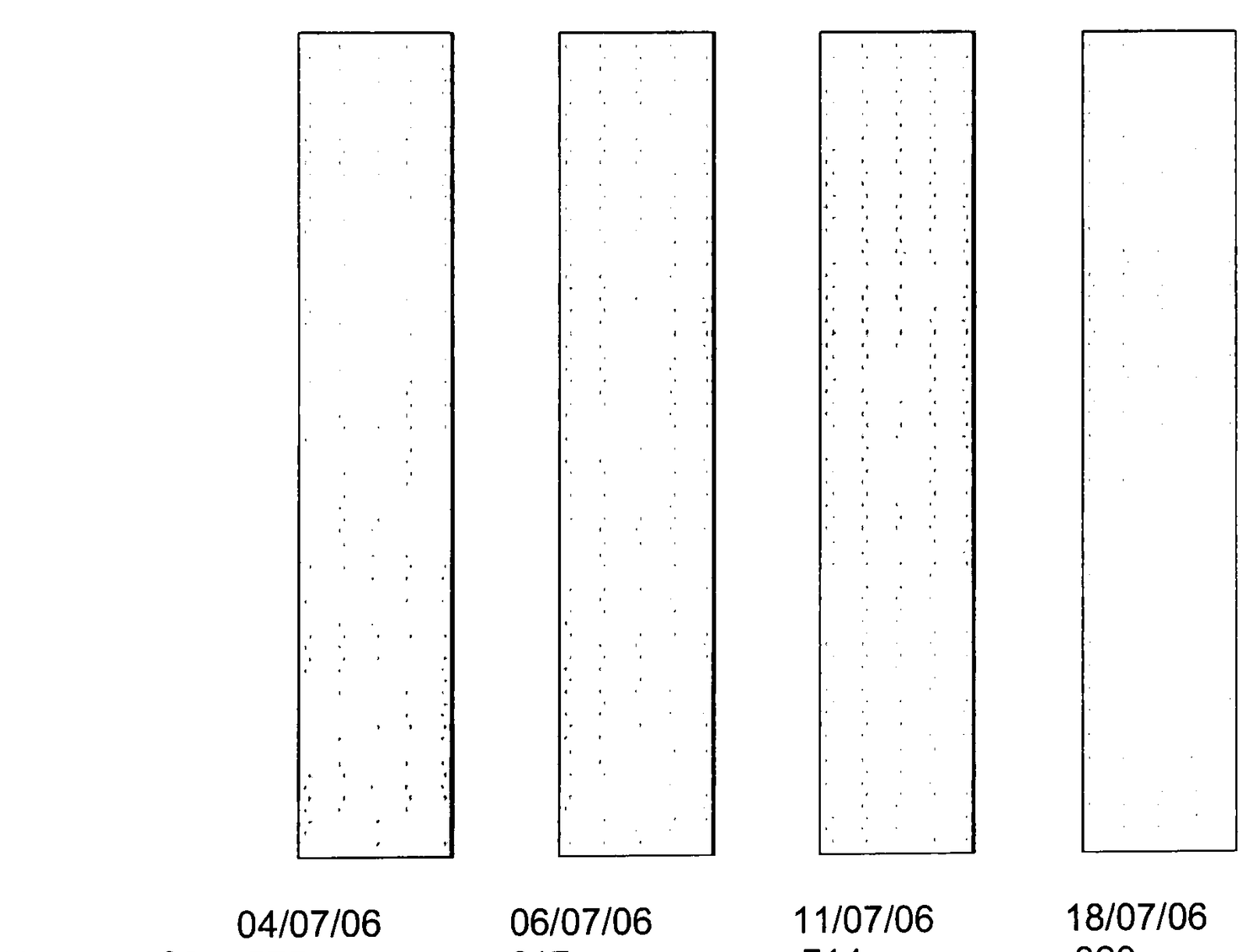

Figure 4. Pattern of increase for ever bearer plants with mildew.

Dipping plants to control initial disease development

The results of this experiment are summarised in figure 5. There were no differences between the untreated plants, the plants dipped in water or the plants dipped in bicarbonate. The onset of symptoms was significantly slowed by dipping the plants in Systhane. This experiment was carried out in an environment which was conducive for the development of powdery mildew infection. No other powdery mildew control products applied to this experiment.

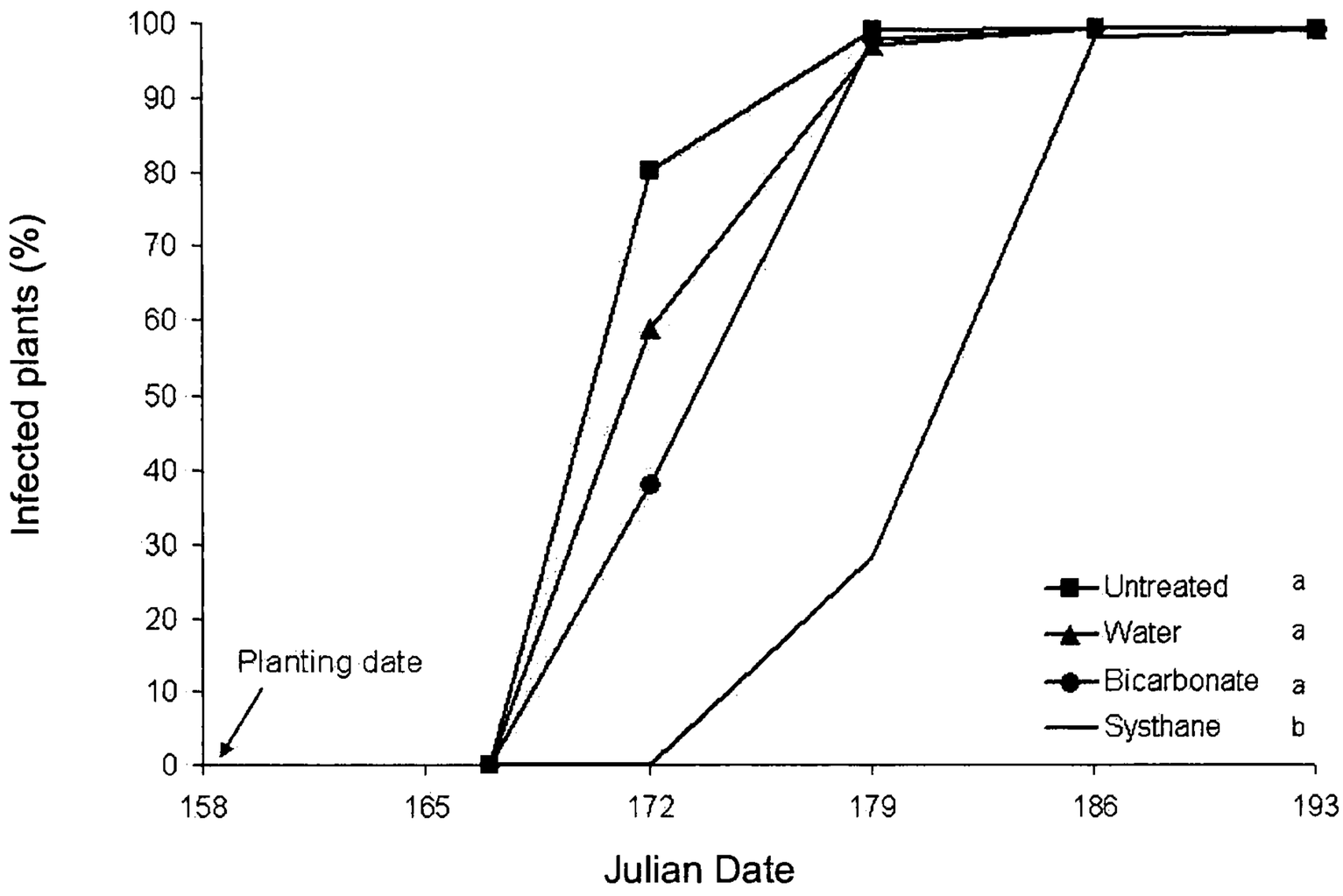

Figure 5. Percent of plants with symptoms of powdery mildew after plants were dipped and planted. Lower case letters next to the product name in the key indicate significant differences in the AUDPC at the 5% level.

Inoculum levels linked to cupping and red blotches

Figure 6 summarizes the data from field A and figure 7 summarizes the data from field B, showing how much mycelium is present if the leaf was either flat, cupping or had red blotches. The data has been presented as the number of colonies per square centimetre for the upper and lower leaf surfaces, and as the percentage of the leaf surface covered by mycelium for the upper and lower leaf surfaces. The data was analyzed for statistical differences at the 5% level using the Mann-Whitney *U* Test in SPSS for windows 11.5.0, SPSS Inc.

For both sites there were more colonies on the leaves the later in the year the sample was collected. For both sites and all samples there were more colonies on the leaves that were cupping or had red blotches than there were on the flat leaves. There was more infection on the lower surface of the leaves than the upper surface. In field B where there was no visible infection there was virtually no mycelium on the upper leaf surface.

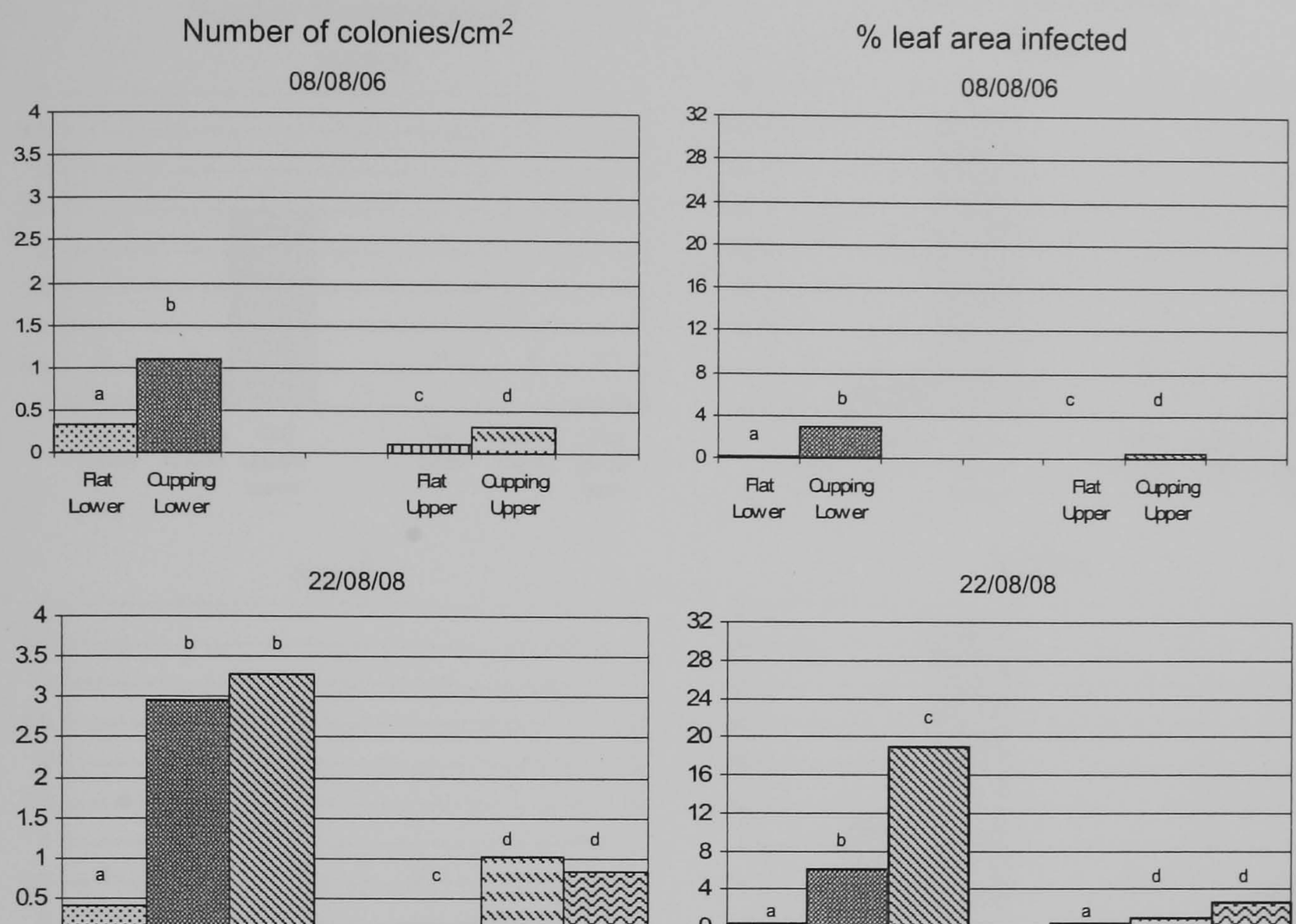

Figure 6. Number of colonies per square centimetre and the percentage leaf area covered by mycelium for 3 sampling dates from field A. Lower case letters indicate significant differences at the 5% level as indicated by the Mann-Whitney *U* statistical test.

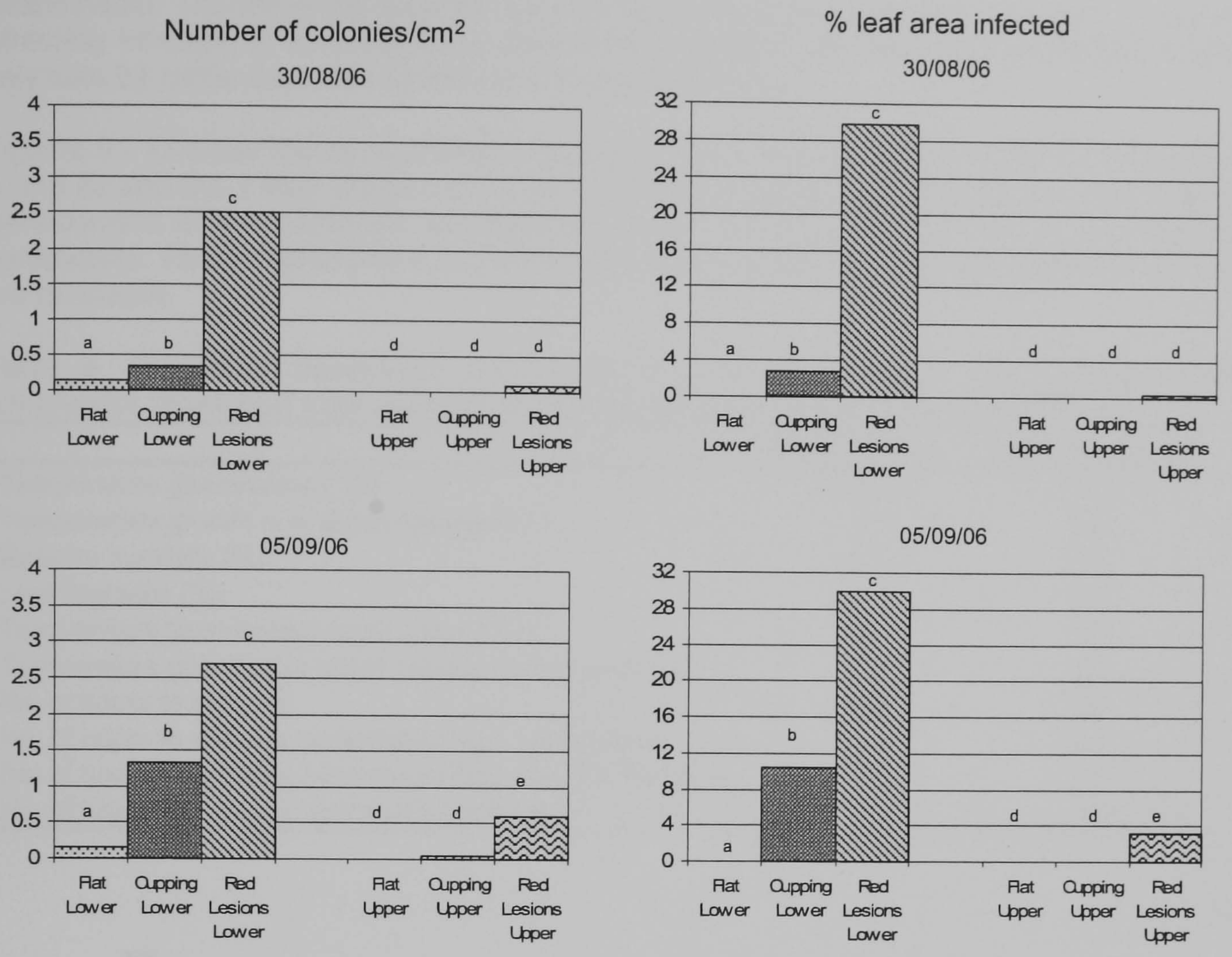

Figure 7. Number of colonies per square centimetre and the percentage leaf area covered by mycelium for 2 sampling dates from field B. Lower case letters indicate significant differences at the 5% level as indicated by the Mann-Whitney *U* statistical test.

Prediction of high risk periods

The literature was searched for previous work that determined the range of conditions under which strawberry powdery mildew developed. The previous work is summarized in table 2. These values were obtained under laboratory conditions. All other conditions apart from the one being studied would have been kept constant. These experiments did not concentrate on interactions between the different values. Table 1 details previous work carried out under optimum conditions, in the laboratory, in to the time it takes for a strawberry powdery mildew spore to germinate. The range of conditions (including the optimum) detailed in tables 1 and 2 were used as the starting point for the prediction system. Especially the time frame for spore development detailed in table 1 was used to create the bare bones of the prediction system. The system was created using default values obtained from previous work. These default values were then modified using observations and grower input obtained in the first 2 years of SF62. The parameters developed previously as part of SF62 2005 have been further modified after further analysis of data collected from all 3 years of SF62. The parameters developed previously and the new parameters established after this years work are presented in table 4. The (lower) temperature values for growth and germination have been changed slightly between SF62 2005 and this report. The main alteration to the prediction system is to distinguish between plants have over wintered in the field or in the cold store, as previous results from SF62 2004 and 2005 have shown inoculum over winters in established fields. Previous work (Smith *et al.*, 1988) has shown that strawberry powdery mildew can over winter as mycelium. This means that the initial infection would not take as long to reach maturity, and start to produce more inoculum, as would an infection starting from a spore or

cleistothecia. Therefore the time for the first high risk period has been reduced. If the over wintering infection in an established field is as mycelium with haustoria developed it would only take 84 rather than 144 for infection to reach maturity.

Figures 8 - 12 show the development of infection in 3 established sites (Kent 04, Wisbech 05 A and B) and two newly planted sites (Kent 05 and Wisbech 06). The figures also show the development of the pathogen as predicted by the prediction system for the old and new parameters. This is represented as the percent of the fungal life cycle completed (until spores are released).

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	*na*
No. of hours to maturity *established* field 1st infection	*na*	84
No. of hours to maturity *established* field after 1st infection	*na*	144
No. of hours to maturity *new* field all infections	*na*	144

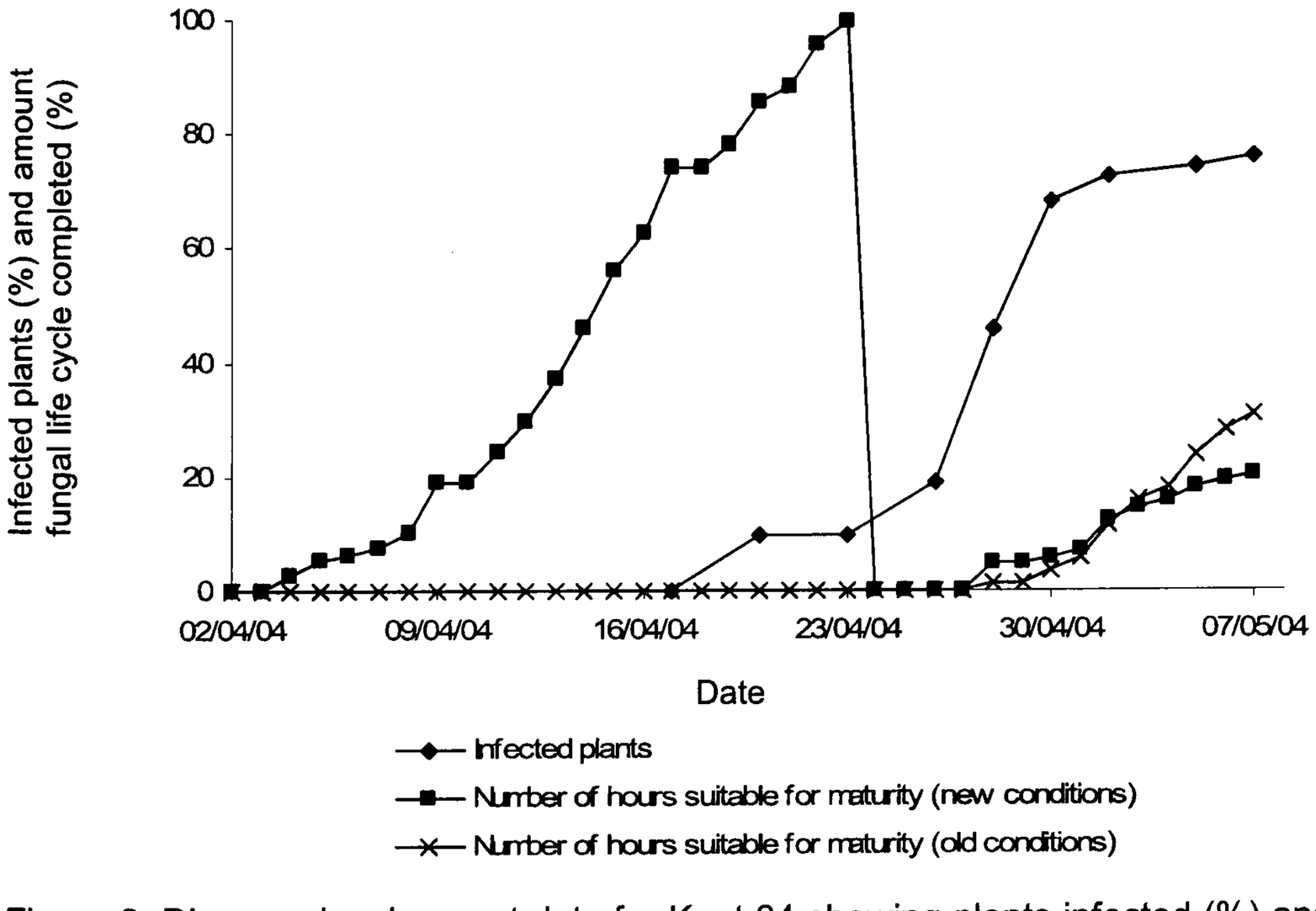

Figure 8. Disease development data for Kent 04 showing plants infected (%) and the amount of the fungal life cycle completed before spore release (%) for the old and new parameters.

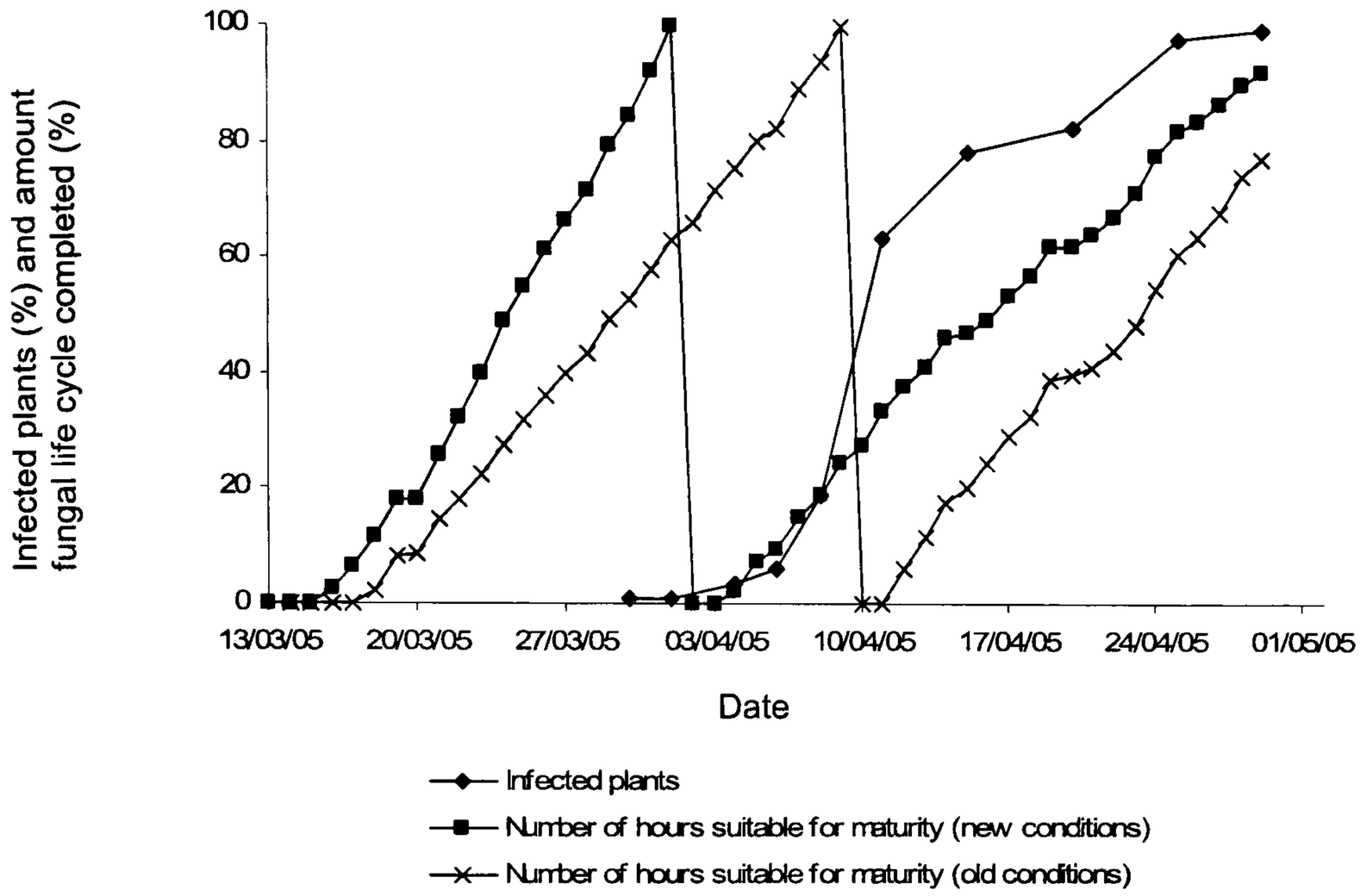

Figure 9. Disease development data for Wisbech 05 A showing plants infected (%) and the amount of the fungal life cycle completed before spore release (%) for the old and new parameters.

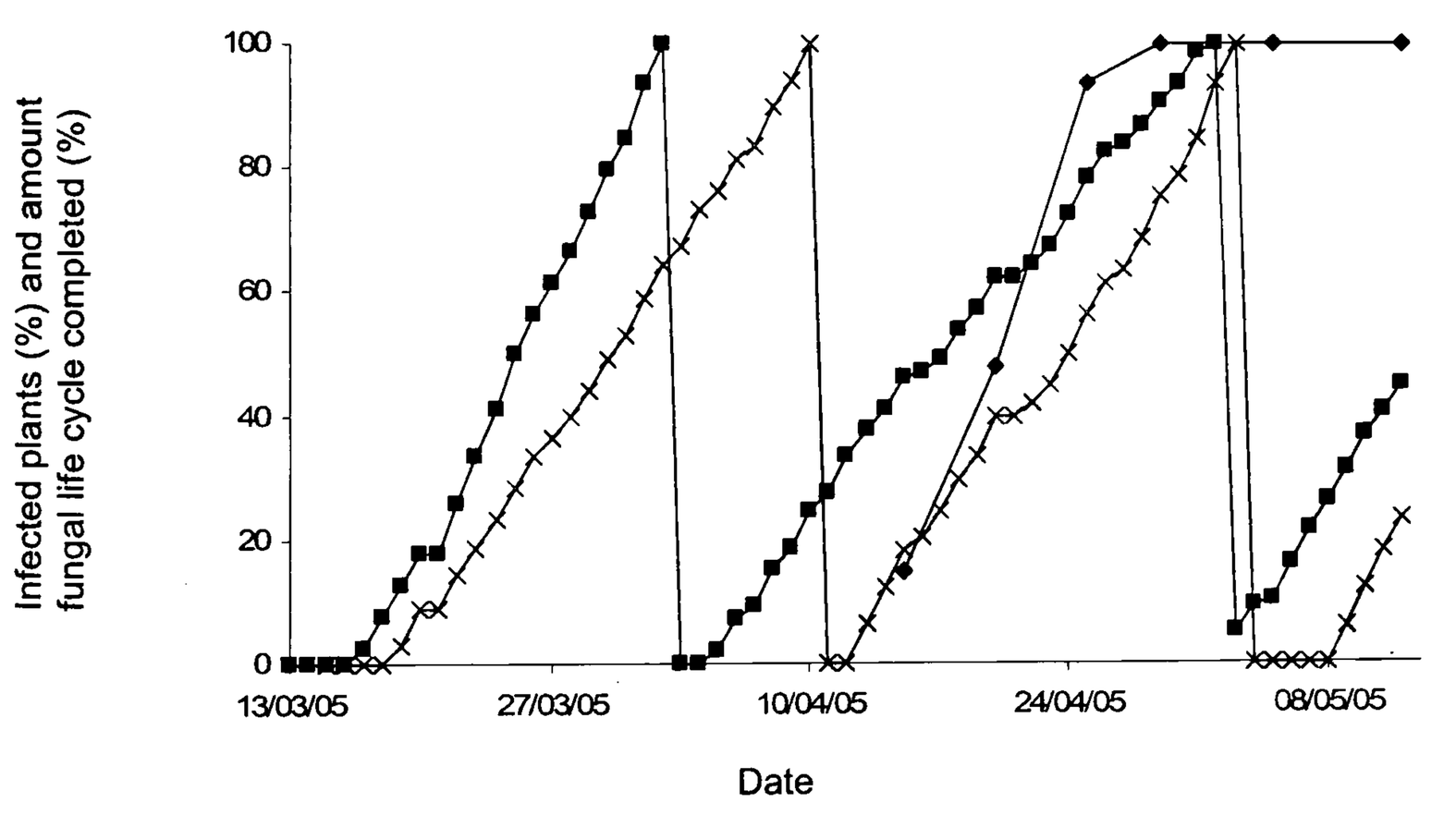

Figure 10. Disease development data for Wisbech 05 B showing plants infected (%) and the amount of the fungal life cycle completed before spore release (%) for the old and new parameters.

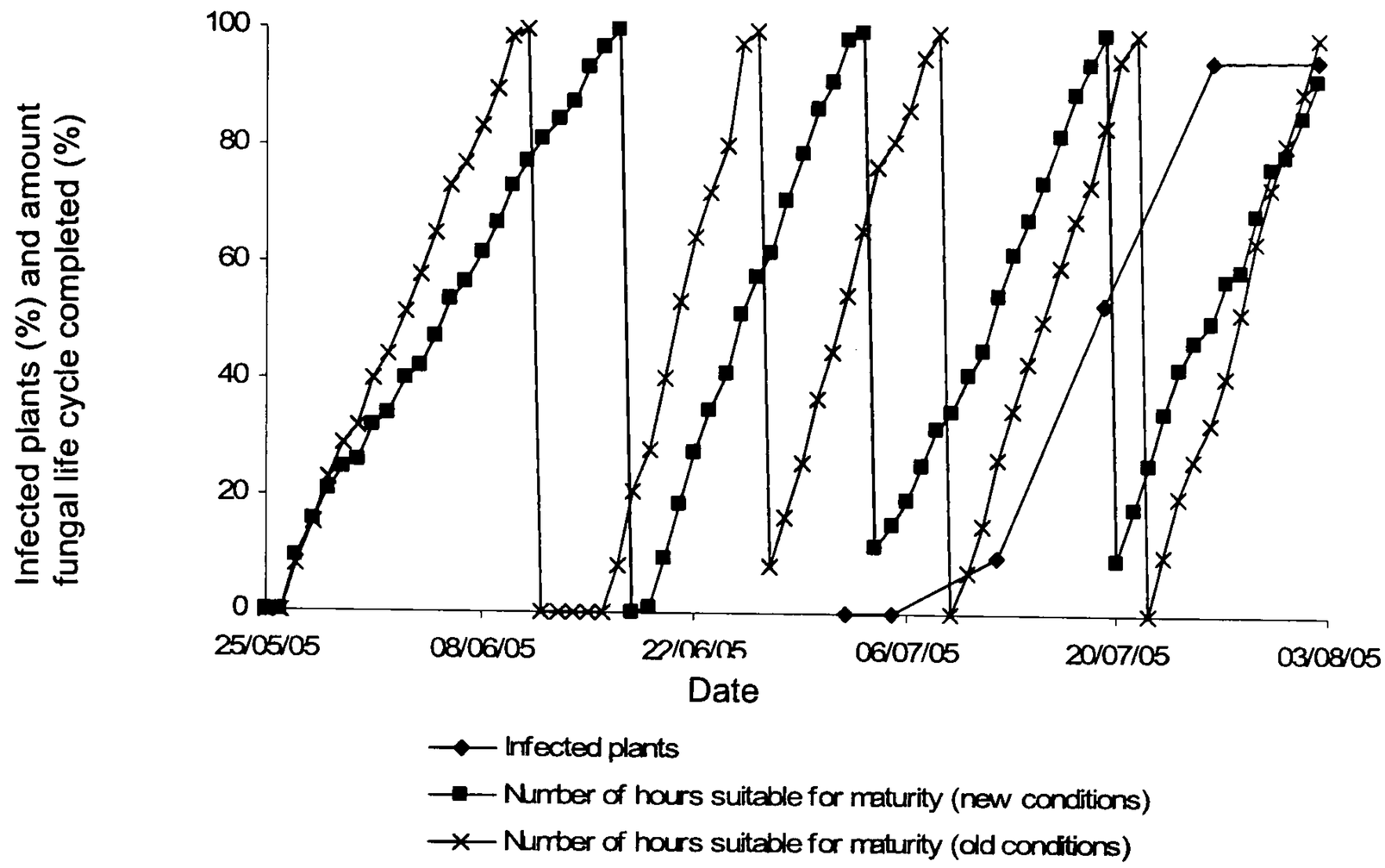

Figure 11. Disease development data for Kent 05 showing plants infected (%) and the amount of the fungal life cycle completed before spore release (%) for the old and new parameters.

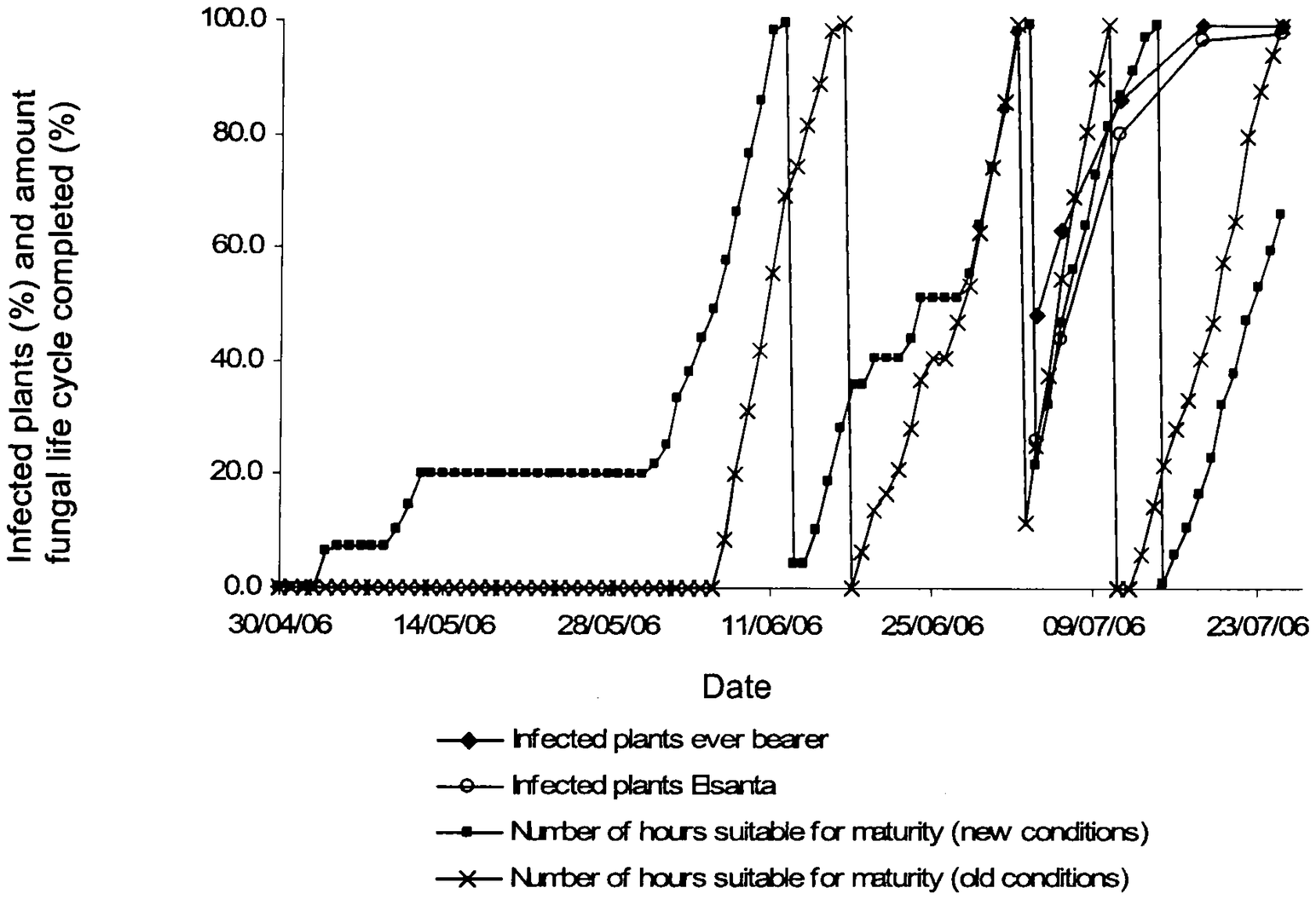

Figure 12. Disease development data for Wisbech 06 showing plants infected (%) and the amount of the fungal life cycle completed before spore release (%) for the old and new parameters.

Discussion

Inoculum and primary disease spread

The plants that were infected were distributed through out the tunnel. They were not clustered towards either end of the plots that were scored. This confirms the result from 2005 that the source of inoculum in a newly planted field is the planting material. The infection develops when the conditions are suitable for growth of the fungus. It is likely that the inoculum on plants is coming from propagators as either conidia or cleistothecia. Both of which would need time to germinate and infect the host plant before be able to produce more spores and then act as a source of inoculum to uninfected plants. There was time for two generations of powdery mildew to reach maturity before the tunnels were covered (figure 12). The first generation would be the development of inoculum that over wintered in the cold store on the plants and the second generation would have been the inoculum spreading to the plants that were next to the plants infected with the overwintering infection.

Infection is much easier to control when there is a small amount of inoculum than when there is a lot of infected material and therefore a lot of inoculum. It is much better to control infection before disease levels have built up. Inoculum should be controlled from the outset. The best way to control the initial inoculum in a new is field to control it before it has started to develop. As strawberry leaves are flat the under surface is very hard to treat with sprayer applied control products. Growers have the opportunity to ensure that all plant surfaces come into contact with the control product by dipping their plant in the control product before they are planted.

Dipping plants to control initial disease development

Dipping of plants to control powdery mildew is currently not approved by PSD. PSD has approved another product to be used as a dip for the control of red core. This experiment was carried out to prove the principle that dipping of strawberry plants can reduce or remove the inoculum on the new strawberry plants. If the initial inoculum is reduced the build of disease will be slowed. The untreated plants developed visible signs of infection between 9 to 14 days after the plants were planted. The plants treated with Systhane developed visible signs of infection between 14 to 21 days after the plants were planted. The plants were grown in a high pressure mildew environment without any other treatments for the control powdery mildew applied. If the plants were to be grown in a commercial situation with other products applied to control the development of powdery mildew infection the development of disease could be slowed even more.

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 start to cup as infection first develops which progresses to visible mycelium on the leaves (first on the lower side then the upper side), red blotches then form on the leaves and finally if the infection is not treated mycelium can form on the fruit. This is a well established progression of symptoms. However the only symptom that can be linked to strawberry powdery mildew with certainty is visible mycelium. Leaf cupping can be caused by water stress and red blotching is a general stress response of strawberry plants.

The results presented here confirm the above assumptions. Cupping leaves and leaves with red blotches both have more mycelium present the flat leaves. Where mycelium is present on flat leaves it is in very small amounts. At each subsequent sample date more infection was present than at the previous sample date. The increase in colony size from cupping leaves to leaves with red blotches is comparably greater then the increase in the number of colonies from cupping leaves to leaves with red blotches. Showing that while there is new infection of

cupping leaves the increase in infection is mainly due to growth of colonies. There was significantly more infection on the under side of the leaves than the upper side. Depending on the conditions infection can be very advanced before there are any visible signs of mycelium on the upper leaf surface. In field A mycelium was visible on the upper leaf surface (of some leaves) but in field B there was no visible mycelium present. The leaves from field B have a lager percentage of their lower surface covered with mycelium than the leaves from field A.

These results show that growers need to apply their control treatments in such a way that they are able to control the infection on the lower leaf surface, as infection develops there before it develops on the upper leaf surface. Growers should be starting disease control once cupping leaves have started to appear and they should continue disease control even after red blotches are the only visible symptom.

Prediction of high risk periods

The parameters that are used by the prediction system to trigger a prediction of a high risk period have been modified. The original parameters came from a review of the available literature. These values were then altered in light of the initial test runs of the prediction system.

The original (old) parameters were run in the prediction system and the high risk periods were compared to know disease development periods. The predicted high risk periods did not match the actual disease increases observed in the field. The infection in the field increased before the prediction system had predicted there should have been a high risk period. The parameters were modified (new parameter) so that the prediction system predicted a high risk period just as the disease levels were starting to increase.

The new parameters at this stage predict when the first disease will develop in the season when the environmental data from the 1st January (for that year) is run through the prediction system. If the site is already established the first high risk period will develop sooner than if the site was newly planted as results suggest that inoculum that overwinters in the field is as mycelium where as inoculum in a newly planted field could well be as cleistothecia or conidia which would take longer to each maturity than established mycelium would.

Integrated control of strawberry powdery mildew

Conclusions of 3 years work

In order for the grower to achieve better control of strawberry powdery mildew all the results from the 3 years of project SF 62 need to be combined together to make an inter-grated control method.

Results from all three years work have shown that the source of inoculum is from within the field rather than being an external source. Inoculum most likely overwinters as mycelium in established fields and could overwinter as either cleistothecia or conidia on plants in cold store. The inoculum is not wind borne. Therefore growers do not need to try and keep the inoculum out of their tunnels at the start of the season. It is already there. They should apply control products early in the season. Probably as soon at the tunnels are covered or the fleece has been removed. In SF62 2004 Fortress (quinoxifen) was the most effective fungicide tested. It is a protectant fungicide with a long effect and long harvest interval (14 days); it has no effect on established infection so it is best used at the start of the season before any infection has built up so it can offer long protection with out the grower having to worry about its harvest interval. An application of Corbel (fenpropimorph) which acts as a systemic eradicant would reduce or remove any overwintering inoculum so enabling the application of Fortress to have the greatest effect.

When growers are first planting a new field they have a good opportunity to get excellent coverage of the plant by the fungicide. If they were to dip the plant in the fungicide rather than try to apply the treatment as a foliar application after the plant was planted. SF62 2006 has shown that a dipping treatment can be effective at delaying the build up of initial inoculum. Systhane was used in this experiment and it delayed the onset of disease by over 7 days. Systhane does not currently have a PSD approval for use as a dipping treatment. This experiment was done to prove the principle that growers could control disease with a dipping treatment.

Once the initial inoculum has been reduced the grower needs to be aware of powdery mildew inoculum building up. As the initial inoculum has been reduced powdery mildew will build up more slowly but it will still build up. SF62 2006 showed that mycelium can be associated with cupping leaves so when a grower is crop walking they should be aware of cupping as the first symptom and be prepared to control the unseen inoculum that is associated with cupping leaves. This mycelium will however be on the lower leaf surface so growers should aim to get good spray coverage on the under side of the leaf.

Growers need to use an integrated control program to control powdery mildew through out the season. This should include the sustainable use of fungicides as well as bio control products. Strawberry growers have a limited range of active ingredients with which to control powdery mildew. So strategies have to be used that will combat the development of fungicide resistance in the powdery mildew fungus. When choosing a fungicide growers need to be aware of its mode of action as well as whether it has a protectant, curative or anti-sporulant etc. activity. If two fungicides have the same mode of action they should not be used consecutively. Modes of action should all ways be mixed up so that a spray program does not have the same mode of action too often or too close to each other. The mode of action represents where the fungicide stops the fungus from 'working'. Each fungicide attacks a specific part of the fungus and stops it from functioning. If the same specific part is attacked too often it is possible that the fungus will develop resistance to that mode of action. SF62 2004 gave advice on resistance management strategies. Where there is approval two fungicides with different modes of action can be tank mixed to achieve better control than a single fungicide would give.

Bio control products can also be integrated in to a spray program to help conserve modes of action. SF62 2004 showed that bicarbonate (potassium hydrogen carbonate) was as effective as Systhane at controlling established infection. Bicarbonate only works by contact, so needs to be applied so that as much as possible of the leaf surface is covered. Bicarbonate does not have a harvest interval so is an ideal product to use when the crop is being harvested, however if the grower has not used bicarbonate before they should spray a test plot, as too much bicarbonate can lead to scorched leaves. Bicarbonate does not have any lasting effect. It just kills the infection that it contacts so the grower needs to be vigilant after using bicarbonate to the next signs of disease. Sulphur is another product that can be used in an integrated spray program to reduce the pressures on the available modes of action. There are other bio control products that might be suitable for use by strawberry growers. They should be used after consulting an independent agronomist.

SF62 is also developing a system to predict periods of high risk of infection by powdery mildew. The system is still under development at the moment but when completed it should let the growers know when there is a high risk period. At the moment it appears that the growers could be applying too many control products. This increases the risk of resistance developing, retailers finding fungicide residues and wastes grower's money. The system predicts the minimum time between applications of control products that it would take any remaining inoculum to reach maturity and start producing more spores to infect more plants.

In this time the amount of infection would be stationary while the infective potential of the infection would be increasing. The application of the control product needs to be applied just before the infective potential reaches a maximum and translates into more infection.

SF62 2004 trialled several different varieties of strawberry plant and found that there were significant differences in the resistance they displayed to infection by powdery mildew. The grower tends to be forced to grow a certain variety by the retailer. The retailer should be made aware that the variety grown could have a significant effect on how many fungicides are applied. If the retailers are serious about reduction of residues in the fruit they sell they should enable the grower to use all means to reduce fungicide use. This would include giving the grower a chose of variety.

Acknowledgements

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Appendix 2 - Published papers

Overwintering *Podosphaera aphanis* as main source of inoculum in a second year Elsanta strawberry crop under Spanish tunnels and relative resistance of seven varieties

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ABSTRACT

The spatial development of disease within a new planting of strawberries showed that *Podosphaera aphanis,* the cause of strawberry powdery mildew, can over winter within the crop. Latent infections were present in approximately 10% of the plants and covering the tunnels provided suitable conditions for symptom expression. Seven cultivars, untreated by fungicides, were compared for the development of powdery mildew. All of them developed symptoms of the disease. However, even in this environment, that favoured powdery mildew, Everest and Florence had less than 5% disease symptoms. This reduction in disease pressure may offer opportunities to reduce the amount of fungicide used.

INTRODUCTION

Spanish tunnels are now used commonly in UK strawberry production, to improve crop yields and extend the cropping season. Covers are used to force the fruit at the start of the season, to reduce infection of the crop by *Botrytis cinerea* and to provide protection from rain damage. A field of strawberries can be harvested for two or three years. Usually the first harvest is taken 60 days after planting, the plants then produce the second year main crop and can be kept for a third year when they are forced to produce an early crop.

Strawberry powdery mildew is a threat to the economic sustainability of crops grown under protection. Temperatures over 13^0C are required for sporulation (Peries, 1962) while the ideal temperature for growth is 20°C (Jhooty & McKeen, 1965). 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 limits on the products used, harvest intervals and allowable residues. In addition, growers rely on a limited range of fungicide active ingredients, placing enormous selection pressure on the pathogen population.

The source of inoculum that initiates primary infections in Spanish tunnels is unknown. British weather conditions are not favourable for pathogen growth over the winter months; but are not severe enough to kill it. The aim of the work described here is to improve understanding of how disease pressure develops during strawberry production. This knowledge will be used to develop crop management strategies that suppress inoculum pressures for the entire production cycle. In particular this report details work to discover the:

- Origin of initial crop infection,
- Amount of disease control achievable by deployment of varietal resistance.

METHODS

Seven cultivars (Bolero, Elsanta, Everest, Florence, Rosie, Royal Sovereign and Symphony) were grown in a section of a commercially managed site, located near Mereworth, Kent. Cold stored bare root plants were planted the previous summer and arranged in a randomised block design of four replicates. Plots consisted of 20 plants (2 rows × 10 plants). Blocks were separated by 2 plants of cv Elsanta. These plants were scored for *P. aphanis* infection using the MAFF Strawberry Powdery Mildew Key 8.1.1. (1976), developed for use on Royal Sovereign. It uses presence or absence of leaf cupping and the amount of red blotching on the leaves to quantify disease severity.

One Spanish tunnel was planted the previous summer with 2248 Elsanta plants. These were scored for the presence or absence of red blotches on the upper surface of the leaves. There were four beds with two rows in each bed. Each row was 281 plants long. Spacing between the plants was 30cm; each row was separated by 15cm. 10 assessments were made between the 17 April and 11 May, 2004. The Spanish tunnels were covered on the 16 April, 2004. They were vented and irrigated according to normal commercial practice. Disease patterns were mapped and analyzed using ArcGis (ESRI Corporation, Redland California, USA), a geostatistical software system. The pattern analysis was done by the method of Average Nearest Neighbour Distance. This method measures the distance between each diseased plant and its nearest diseased neighbour. All the distances are then averaged. An expected distance is also calculated based on a hypothetical random pattern of the same incidence of diseased plants, covering the same area. A Nearest Neighbour Index is expressed as the ratio of the observed distance divided by the distance expected for the random pattern. If the index is less than 1 the pattern exhibits clustering and tends toward dispersion if the index is more than 1.

RESULTS

All seven varieties grown in the trial showed low levels of disease (leaf cupping) within 13 cumulative hours of temperature $\geq 15^{o}C$ after covering the tunnel (Figure 1). Disease levels on the variety Royal Sovereign started to increase after 265 thermal hours $\geq 15^{o}C$ and continued to increase until the end of the experiment (17 July 2004, reaching 37% of plants diseased). Disease on Bolero, Elsanta, Rosie and Symphony started to increase after 531 thermal hours $\geq 15^{o}C$, levelling off after a further 243 thermal hours at $\geq 15^{o}C$ (at 6-12%). The level of disease on the more resistant varieties, Everest and Florence, remained at a constant low level (3-4%). The seven varieties that were grown in this trial could be classified into three categories (very susceptible, susceptible and moderately resistant) using area under disease progress curve (AUDPC) at a 95% confidence level (Figure 1).

Only four plants had any symptoms of powdery mildew when the tunnel of Elsanta was covered. Within 4 days 10% of the plants had symptoms (Figure. 2) and disease incidence grew logistically ($P<0.001$, $R^2=99.6$; Figure 2). At the first assessment the plants with powdery mildew symptoms were well separated and the spatial pattern was fully dispersed (Figure 3.). Within three days the number of infected plants had risen to 214 (9.5%) and the distribution showed clear signs of clustering. The likelihood of the pattern arising due to random chance was <10%. Midway through the assessments (assessment 5, 28 April) 1037 plants are infected (46%), the clustering of disease was very pronounced and unlikely to arise

from random chance (<1%). By the eighth assessment (5 May), 1677 plants had symptoms (75%) and the disease pattern was more uniform due to coalescence of the clusters.

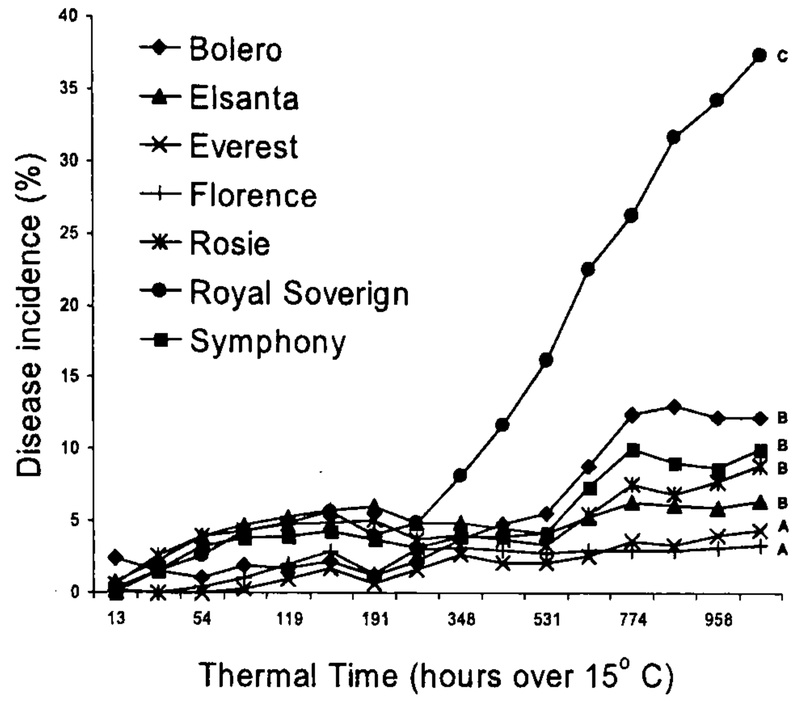

Figure 1. Disease progress on seven cultivars measured against thermal time. Letters indicate significant differences for comparison of AUDPC at the 95% confidence level.

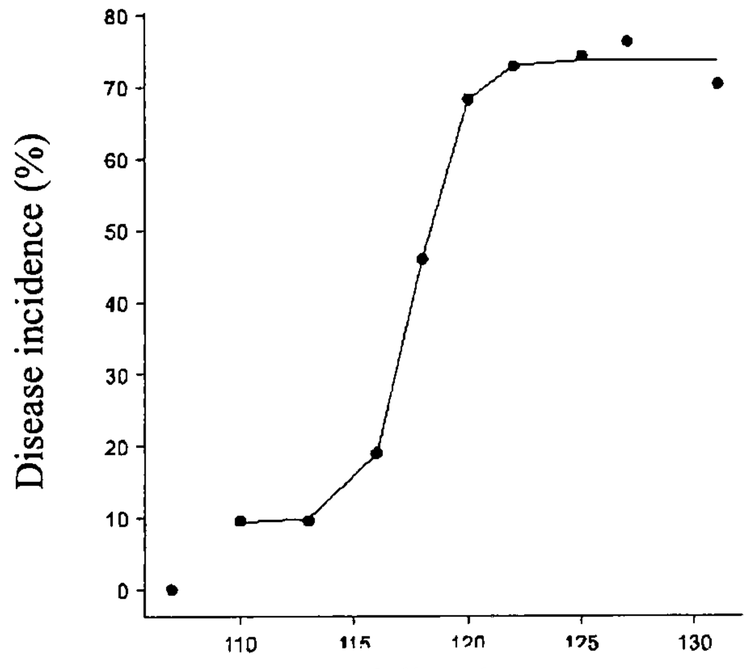

Figure 2. Growth in disease incidence (percent plants with symptoms) after the tunnel was covered. The logistic curve is fitted to all points except the first observation, which was made on the day after the covers were put up.

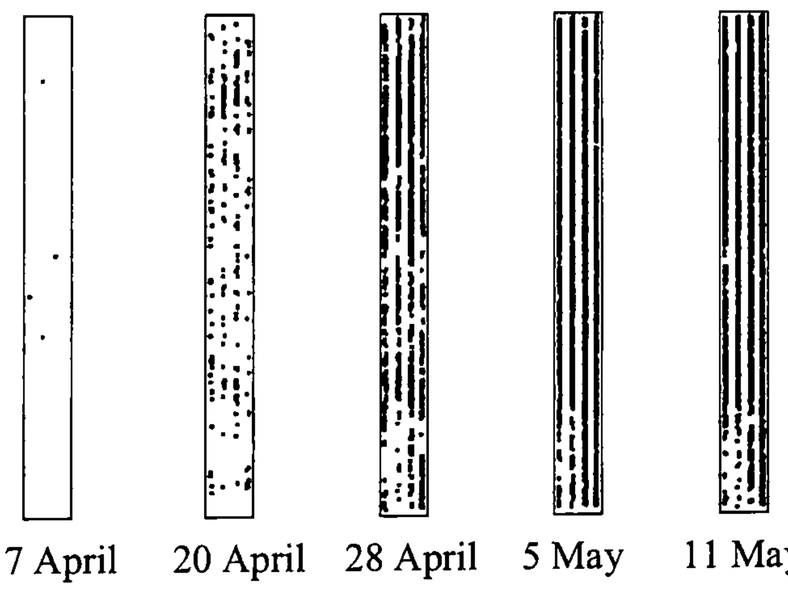

Figure 3. Location of individual plants that showed symptoms of *P. aphanis* within a tunnel over a period of 25 days (the figure is oriented north south as shown on the page).

DISCUSSION

Low levels of powdery mildew were visible soon after the tunnels had been covered in the experiment comparing varietal resistance. All seven varieties developed symptoms of the disease. However, even in this high disease pressure environment, 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 offers opportunities to reduce the amount of fungicide applied, especially when the environment is suboptimal for disease development. Currently, however, growers do not have a wide choice of varieties which are acceptable to wholesalers and supermarkets and that have good resistance to powdery mildew.

Measuring the spatial development of disease within a second year commercial scale planting showed that inoculum can over winter within a crop of strawberry plants. Latent infections of mildew were present in approximately 10% of the plants and covering the tunnels provided suitable conditions for symptom expression. Provisional tests using a molecular diagnostic tool indicate that powdery mildew can be present in commercial planting stocks. There was time for an epidemic to develop on the planting stock the previous summer between the plants being planted in late July and the end of the season when the condition would not be favourable for the disease. This would suggest that the inoculum managed to overwinter successfully on about 10% of the plants.

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Aspects of Applied Biology 83, 2007
Crop Protection in Southern Britain

System to predict high risk periods for *Podosphaera aphanis* infection of strawberries grown in polythene tunnels

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Summary

Strawberry powdery mildew, *Podosphaera aphanis* is the main fungal pathogen of strawberries grown in the UK. Conditions favourable for the growth of *P. aphanis* have been created by the use of polythene tunnels. Strawberry crops are harvested every 3 or 4 days when they are producing fruit. Growers therefore have a small window of opportunity to treat powdery mildew if they are to comply with harvest intervals following applications of fungicides. This paper details the preliminary development of a rule-based prediction system to highlight when strawberry crops are at greatest risk from *P. aphanis*. This will allow growers to time treatments more effectively and remove unnecessary applications from spray schedules.

Key words: Powdery mildew, *Podosphaera aphanis*, strawberry, prediction, risk

Introduction

Strawberries were traditionally grown in open fields, which left them exposed to the vagaries of the British weather. This resulted in a short, 6 week season from late May to early July (Anon., 2005*b*). There were extensive losses due to infection of the fruit by *Botrytis cinerea* and the remaining fruit was often damaged by heavy rains (Fletcher, 2006). Growers started to use polythene tunnels in 1993 to protect fruit from rain damage and to prevent infection by *B. cinerea* (Fletcher, 2006). The use of polythene tunnels has also extended the strawberry production season from 5 or 6 weeks in June and July to a 5 month season from May to the end of September (Anon., 2005*a*). Polythene tunnels have improved conditions for the production of strawberries, but created an environment favourable for growth and development of strawberry powdery mildew, *P. aphanis*.

UK strawberry breeders concentrate on fruit quality and appearance rather than disease resistance when selecting cultivars, and the main UK retailers specify the varieties they prefer, which are mostly susceptible to *P. aphanis*. As a consequence, there is little, if any, scope for disease control by using resistant varieties. Growers could change their tunnel management practices to provide some control of *P. aphanis,* but this could also result in detrimental effects on fruit production. Management of commercial crops is therefore dependant upon the appropriate use of fungicides.

Generally, fungicide applications are scheduled prophylactically for strawberry crops, though additional treatments are sometimes applied in response to the appearance of disease symptoms. Each field of strawberries produces commercially viable fruit for a three-week harvest period. During harvest, fruit is collected every three or four days, so fungicide treatment during this period is difficult because prescribed harvest intervals are observed. Currently only four products (bupirimate, myclobutanil, sulpur, potassium hydrogen carbonate) offer a harvest interval of less than 3 days (Anon., 2007, Whitehead, 2006). The continued profitability of the strawberry industry therefore depends on the employment of disease management strategies that optimise the use of fungicides, so that disease control is achieved consistently, whilst slowing, as far as is feasible, the development of fungicide resistance.

The work reported here aims to develop a prediction system, based on tunnel conditions, which will highlight when a field is at greatest risk from *P. aphanis*. This should allow more cost-effective treatment and the achievement of commercially acceptable levels of disease control.

Materials and Methods

An extensive review of the literature provided the basis to specify the conditions that favour and inhibit disease progress. Disease development (as a percentage of plants infected) was recorded at several sites at the start of the season. All the plants in each plot were scored for symptoms of *P. aphanis* infection at regular intervals until the number of plants with *P. aphanis* symptoms neared 100%. The temperature, relative humidity and leaf wetness were recorded in tunnels at each site by TinyTag data loggers. Spray programmes used by growers were obtained.

Results

The literature review yielded a large amount of information regarding the range of conditions under which *P. aphanis* can grow and infect strawberry plants. The germination of conidia is limited by the temperature, relative humidity and the leaf wetness, whereas the rate of mycelial growth and sporulation is only limited by the temperature (Amsalem *et al.*, 2006; Blanco *et al.*, 2004; Jhooty & McKeen, 1964; Jhooty & McKeen, 1965; Miller, *et al.*, 2003; Peries, 1962*a*). The development time for fungal infection by *P. aphanis* is 144 hours from conidial germination to visible symptoms. Once established, infections generate further inoculum after 84 h of suitable conditions (Peries, 1962*b*).

From the literature review, information about the development time of *P. aphanis* and field observations, it was possible to develop a provisional set of conditions (Table 1) for a rule-based prediction system, to identify when strawberry plants would be at greatest risk of infection by *P. aphanis*. The life cycle of *P. aphanis* is divided into two parts, germination of the conidia and then growth of the fungus including sporulation. The system calculates the length of time of conditions suitable for a conidium to germinate, reach maturity and generate new inoculum. The output is presented as a percent of the total hours completed for a conidium to reach maturity.

Table 1. *Parameters from literature and initial field observations (old parameters) and adjusted values after analysis of disease development data (new parameters) for the prediction system*

	Old parameters*	New parameters
Temperature germination (°C)	17.5	15.5
Temperature growth and spore release (°C)	16	18
Relative humidity germination (%)	60	60
Leaf Wetness germination (%)	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	*na*
No. of hours to maturity *established* field 1st infection	*na*	84
No. of hours to maturity *established* field after 1st infection	*na*	144
No. of hours to maturity *new* field all infections	*na*	144

*Amsalem, *et al.*, 2006, Blanco, *et al.*, 2004, Jhooty and McKeen, 1964, 1965, Miller, *et al.*, 2003, Peries, 1962a

The conditions within commercially managed (*i.e.* without control for *P. aphanis*) tunnels were recorded. This data was input into the prediction system and the first predicted high risk periods were compared with the actual development of the first symptoms within each tunnel. The predicted high-risk periods were close, but not exactly the same, as the actual periods of disease development. In light of this the parameters of the prediction system were modified slightly (Table 1) and the environmental data was input in to the prediction system (new parameters) again. This resulted in the predicted high-risk periods and the dates when disease actually developed being closer. The results from two established sites in two seasons (where plants over- wintered in the ground) are presented in Fig. 1.

The conditions measured (from 1 January) in a commercially managed tunnel were input in to the prediction system for a whole season and the predicted high risk periods were compared with the actual applications that the grower made to control *P. aphanis* (Fig. 2). The grower applied 10 applications while the prediction system recommended only 8 applications.

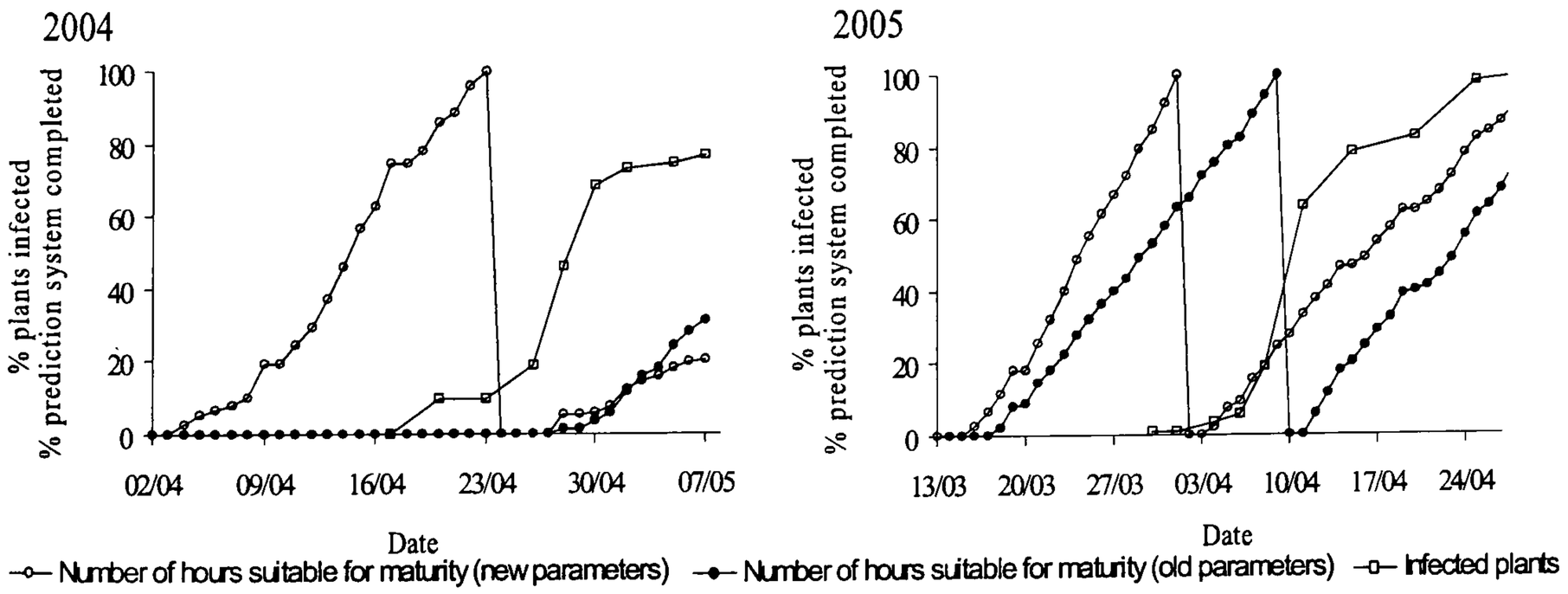

Fig. 1. Disease development data for A) Kent 04 and B) Wisbech 05 showing plants infected (%) and the output from the prediction system (100% equals high risk) for the old and new parameters.

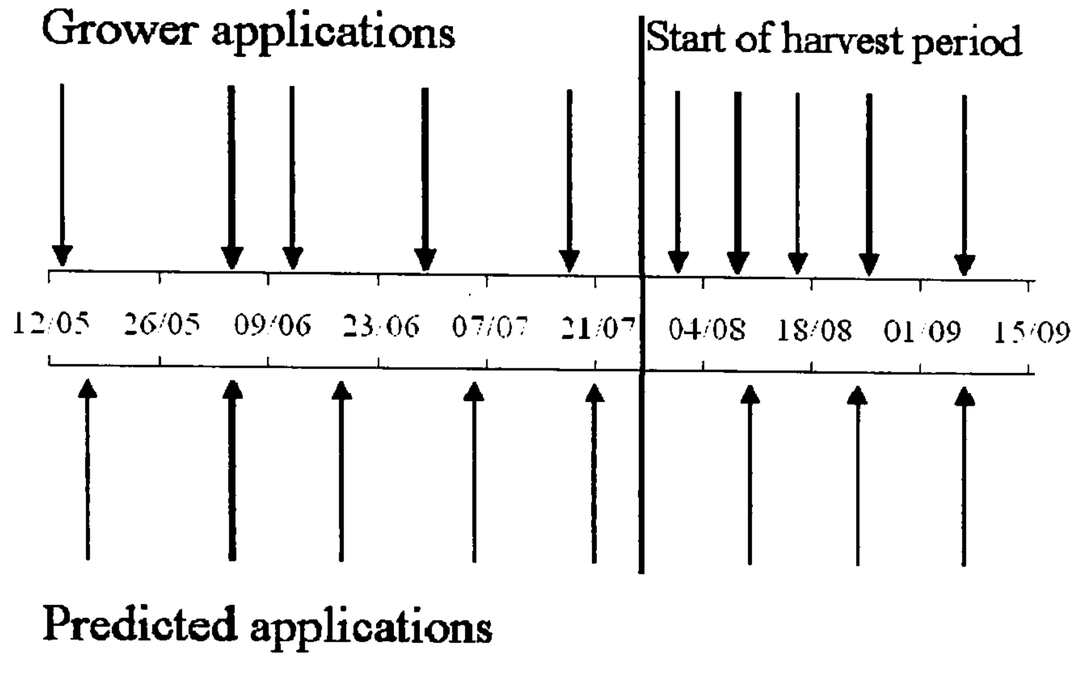

Fig. 2. Dates of grower-applied applications of fungicidal control product for *P. aphanis* compared to application dates recommended by the prediction system.

Discussion

The prediction system (new parameters) is able to predict the first high risk period of the season (Fig. 1) as the infection is starting to build up after over-wintering in the field (Dodgson *et al.*, 2005). Epidemic progress can be slowed substantially by controlling the initial inoculum, but requires the grower to achieve timely treatment and good spray coverage. The prediction system (new parameters) was able to forecast high-risk periods. These are close to, but not exactly the same as applications made by the grower (Fig. 2). The prediction system highlighted 5 high risk periods, in each instance, timed several days before the grower applied a control product. This is understandable, because the growers applied treatments when symptoms were visible, whereas the prediction system predicts high-risk periods before the symptoms are visible. The use of a control product is much more efficient before symptoms are visible. During fruit production, the grower applied a further 5 applications, whereas when the system recommended only 3. The prediction system detailed here uses environmental data from within the tunnels which is supplied by on-farm meteorological stations. The high risk periods highlighted by the system are therefore field specific. Some prediction systems are based on data from a network of meteorological stations but this would not result in farm-specific predictions.

The system will be deployed as an on-farm tool for application at the field scale. The prediction system is still under development and requires field-testing to confirm that applications applied in response to the predicted high risk periods will control strawberry powdery mildew to a commercially acceptable level.

Acknowledgements

This work was funded by the Soft Fruit Panel of the Horticultural Development Council. The authors are grateful to John Clark (Hugh Lowe Farms), Harriet and Henry Duncalfe (Maltmas Farm) for providing and managing the field sites.

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Aspects of Applied Biology 83, 2007
Crop Protection in Southern Britain

Examination of the morphology of *Podosphaera aphanis* cleistothecia and their role in over wintering of the fungus

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Summary

Strawberry powdery mildew (*P. aphanis*) has been shown to over winter within established strawberry fields. This is contrary to what the growers believed, who thought that the source of primary inoculum in each season was wind borne. The work reported here has shown that cleistothecia are present in comparatively high numbers at the end of the summer season. The numbers of leaves with cleistothecia on them is reduced as the winter progresses but the sizes of the cleistothecia that remain increase and a higher proportion have an ascus present. This suggests that cleistothecia mature through out the winter.

Key words: Strawberry powdery mildew, *Podosphaera aphanis*, cleistothecia, over winter, primary inoculum

Introduction

Strawberry powdery mildew, (*P. aphanis*, formerly *Sphaerotheca macularis*), is a fungal pathogen which infects the leaves, flowers and fruit of strawberry plants. This can dramatically reduce the crop yield and fruit quality (Mass, 1998). *P. aphanis* is an obligate biotroph (Belanger, *et al.*, 2002), so requires living plant material on which to grow. When conditions are not suitable for growth of the pathogen it needs a strategy to survive, and cleistothecia are formed. *P. aphanis* (referred to as *S. macularis*) cleistothecia are clustered or scattered, 60-125µm in diameter, dark brown to black, smooth and with numerous hyphal appendages from the lower half, and each contains one ascus (Mukerji, 1968).

Cleistothecia can provide a route for the fungus to survive across strawberry production seasons (Gourley, 1979). *P. aphanis* cleistothecia have been observed in the field (UK) on strawberry plants (identified as *S. humuli*) (Peries, 1961; Salmon, 1900). Howard & Albregts (1982) reported seeing cleistothecia in the field in Florida. Peries (1962) witnessed cleistothecia under one set of conditions in a glass house, in specially built

chambers covered with muslin giving 75–90% reduction in light intensity. There is also evidence that *P. aphanis* can survive as mycelia on over wintering strawberry leaves (Peries, 1961; Smith *et al.*, 1988). This is contrary to what the growers believed. This meant that, while growers and crop walkers were aware of cleistothecia being present in their fields at the end of the season, they did not regard them as a risk of inoculum for the next season.

P. aphanis infection progresses through a range of symptoms on the leaves and fruit. A healthy strawberry leaf is flat and green. Infected leaves then start to cup upwards exposing the underside of the leaf, mycelia become visible on the lower leaf surface followed by the upper surface. Red blotches then form on the leaf (visible on the lower and upper surfaces) as the quantity of visible mycelia reduces (Blanco, *et al.*, 2004; Mass, 1998; Salmon, 1900). The leaf cupping symptom persists throughout the infection.

P. aphanis infections have been shown to overwinter in UK strawberry fields (Dodgson, *et al.*, 2005), with infected plants showing symptoms soon after the tunnels were covered. Before that work, growers and agronomists believed that *P. aphanis* inoculum was wind borne at the start of the season (personal communication). Therefore there had not been any work to identify the development and maturation of cleistothecia that were visible at the end of the season nor had their role in disease carry over been investigated (personal communication). The purpose of the work reported here is to follow the maturation of the cleistothecia through the winter.

Materials and Methods

Leaves were collected each month from a commercially managed field planted with strawberry cv. Elsanta that showed extensive mycelial growth at the end of the commercial season (late August and September 2006). The site was located on Maltmas Farm, Wisbech, Cambridgeshire (Grid reference: TF 459 037). All samples were collected from the same part of the field.

Samples were stored in a refrigerator and scored within 2 days of being collected. After examination, leaves with cleistothecia were separated and kept for further examination. The dates of collection and development of symptoms on the leaves were noted. Individual cleistothecia were removed from the leaves by mounted needle and placed in a drop of fungal mounting fluid (equal parts of glycerine, lactic acid and sterile distilled water) on a slide. This was covered with a cover slip. The width and length of the cleistothecia were measured using x100 magnification on a Vickers microscope. The slide was then removed from the microscope and pressure applied to the cover slip to split the cleistothecia. The slide was replaced on the microscope and the presence or absence of an ascus containing ascospores was recorded. Digital photographs were taken at each stage using a 'Premiere' digital microscope eyepiece, model MA88 produced by KEY Scientific Products, Texas supplied by Cosmos Biomedical Ltd, Derbyshire.

Results

Fig. 1. summarises the percentage of each sample that had cleistothecia present when they were collected. The percentage of leaves with cleistothecia decreased as the winter progressed.

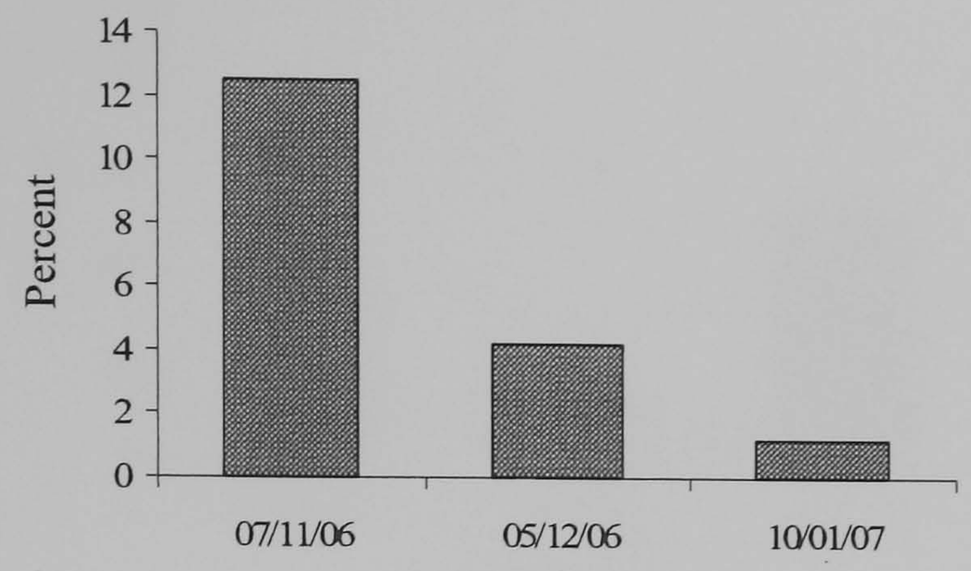

Fig. 1. Percentage of leaves at each sample date that had cleistothecia present.

200 cleistothecia were measured from the sample collected on the 7 November 2006, 150, from the sample on 15 December 2006, and 50 from the sample collected on 10 January 2007. The cleistothecia have one axis slightly longer than the other. The mean width of the cleistothecia was larger on 5 December 2006 and 10 January 2007 than it was on the 7 November 2006. The mean length of the cleistothecia was larger on each sample date than the previous one (Mann-Whitney U test at $P > 0.05$, SPSS for windows 11.5.0, SPSS Inc.).

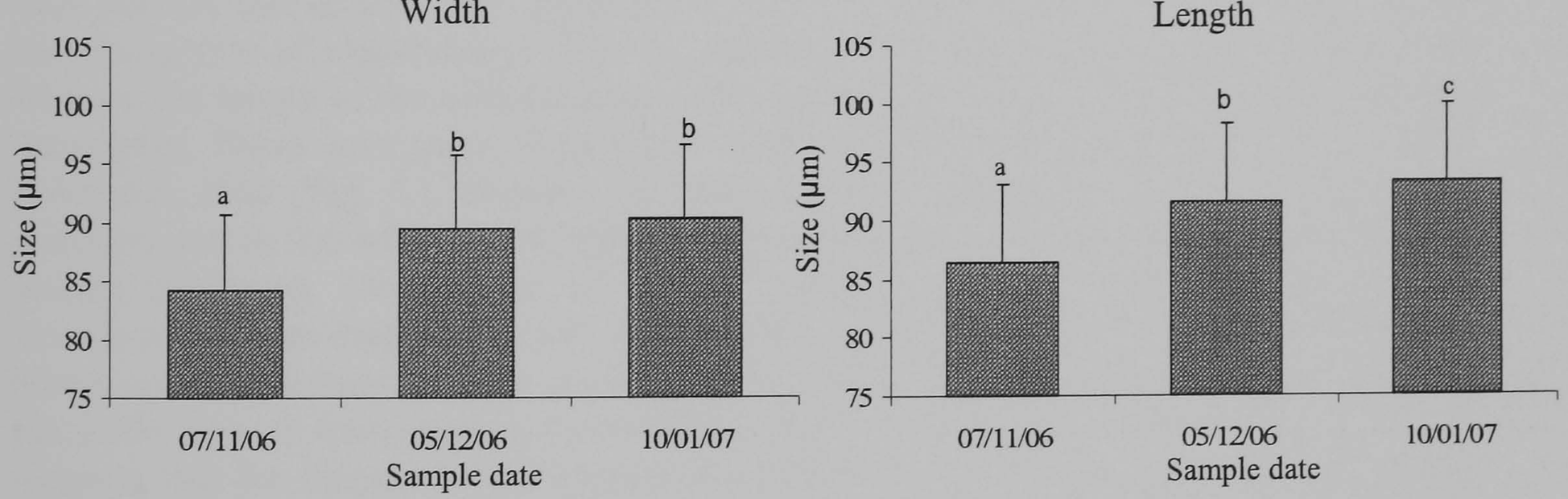

Fig. 2. Mean width and length of cleistothecia at each sample date. Lower case letters indicate statistical differences $P = 0.05$. Bars indicate standard errors, 7 November 2006 199 df., 5 December 2006 149 df. and 10 January 2007 49 df.

The percentage of cleistothecia containing an ascus, from each sample date is presented in Fig. 3. At present there is no explanation for the low number of full cleistothecia on the 5 December 2006. A higher percentage of the cleistothecia present on the 10 January 2007 contain an ascus than on the 7 November 2006.

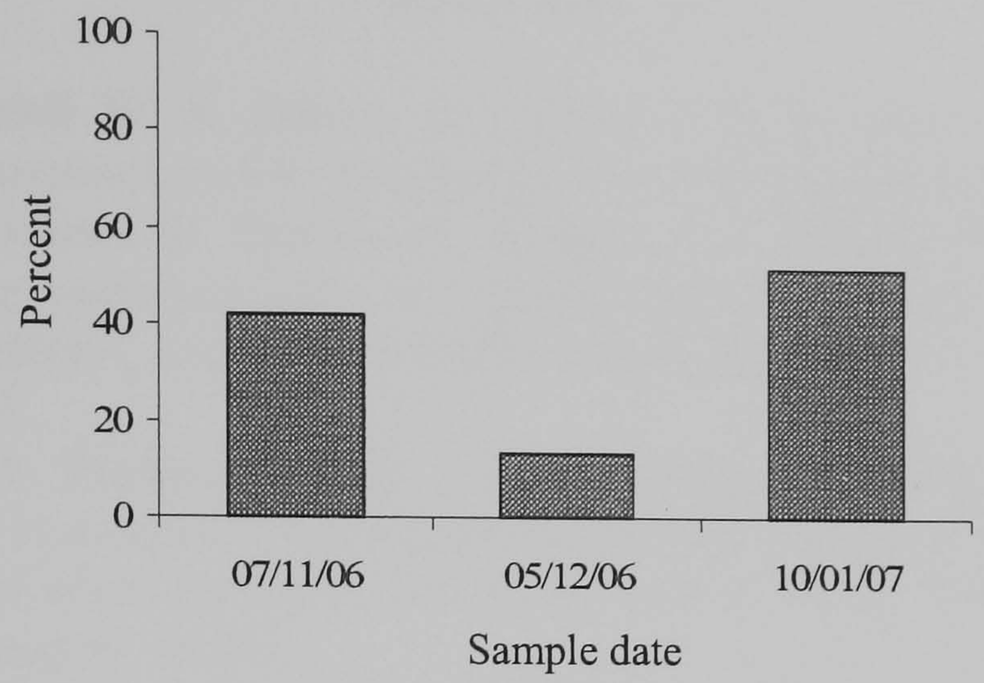

Fig. 3. Percentage of cleistothecia at each sample date that contained an ascus.

Discussion

Maturation can be assessed by the overall increase in size of the cleistothecia and by the increasing percentage of cleistothecia containing an ascus. At present the conditions necessary for maturation of cleistothecia for this species are unknown but maturation is probably governed by temperature (Toscano-Underwood, *et al.*, 2003) and moisture (Liu, *et al.*, 2007). In the autumn (Fig. 1.) there were more leaves with cleistothecia on them than later in the season. The progress of cleistothecial maturation can be seen in the increasing size of cleistothecia (Fig. 2.). Increase in width seems to stop in December whereas the length of the cleistothecia is still increasing in January indicating continuing maturation. There were more cleistothecia containing asci on 10 January 2007 than on 7 November 2006 (Fig. 3.). However the role of these cleistothecia in the survival of the pathogen and in the initiation of infection in the spring could be an important element of disease prediction. Dodgson *et al.* (2005) suggests strongly that initial infections in tunnels come from disease that has over wintered in the field and not from conidiospores blown into the tunnels. Other studies (unpublished) showed that dipping plants in a fungicide prior to transplanting increased the time to first appearance of symptoms. This suggests that the disease is surviving on the plants taken from the propagator's field for transplanting by the growers. The work reported here suggests that this survival might be as cleistothecia. In order to follow this process, further samples must be taken and scored in February 07, March 07 and April 07 and the process of infection by ascospores under field conditions must be studied.

Acknowledgments

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Overwintering *Podosphaera aphanis* as Main Source of Inoculum in a Second Year Elsanta Strawberry Crop under Spanish Tunnels and Relative Resistance of Seven Varieties

Central Science Laboratory

Background

Spanish tunnels provide favourable conditions for the crop production, but also for Podosphaera aphanis – the cause of strawberry powdery mildew. Growers are under pressure to produce high quality fruit while reducing fungicide use.
Effective targeting of fungicides requires understanding of the epidemiology of powdery mildew. Efforts to reduce dependence on chemical control would also be supported by exploiting disease resistant cultivars, which provide fruit quality acceptable to retailers.

Objectives

- Improve quantification of epidemics
- Identify the source of initial inoculum
- Evaluate the value of cultivar resistance

Symptoms

Under ideal conditions it takes 6 days for an infection to cause a lesion visible to the naked eye. However, due to the range of symptoms quantification of disease progress is difficult.

Initially, infected leaves become cupped. Mycelium then becomes visible

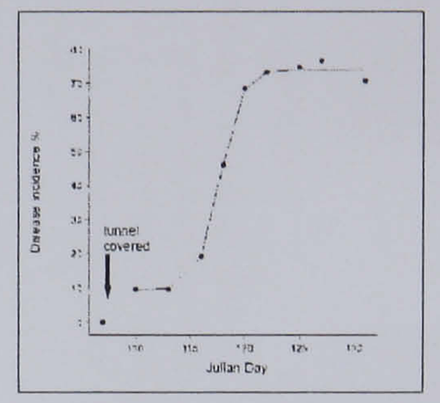
Mycelium becomes more sparse as symptoms progress to red blotching

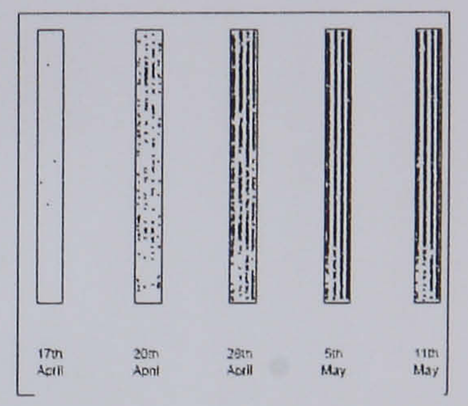
And premature senscence

Severe infection can result in diseased fruit

We are aiming to to improve existing disease assessment keys, so that epidemics can be tracked more accurately.

Initial inoculum





Three days after the tunnel was covered 10% of the plants were showing symptoms. Disease incidence then grew logistically over time($P<0.001$, $R2=99.6$)

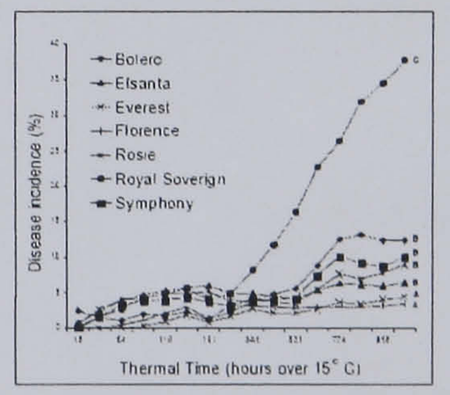
Plants showing symptoms were well separated. Within three days clusters of diseased plants were scattered throughout the tunnel.

Our results indicate that:

- *P. aphanis* can over winter within the crop
- Latent infections were present in 10% of the plants when the crop was covered
- The clustered pattern of these initial infections is not consistent with establishment by wind-borne inoculum

Relative resistance of seven varieties

All cultivars showed leaf cupping soon after the tunnel was covered. Disease levels increased most rapidly on Royal Sovereign and most slowly on Everest and Florence. The cultivars could be classified in to three resistance categories (very susceptible, susceptible and moderately resistant) on the basis of the Area Under the Disease Progress Curve.



Letters indicate significant differences for AUDPC at the 95% confidence level

These results indicate that:

- None of the cultivars are immune to strawberry powdery mildew
- Cultivar choice effects magnitude of disease control problems
- Growers do not have enough choice over which varieties to grow

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BSPP presidential meeting 2005

Control of *Podosphaera aphanis* in Response to Disease Risk

Central Science Laboratory

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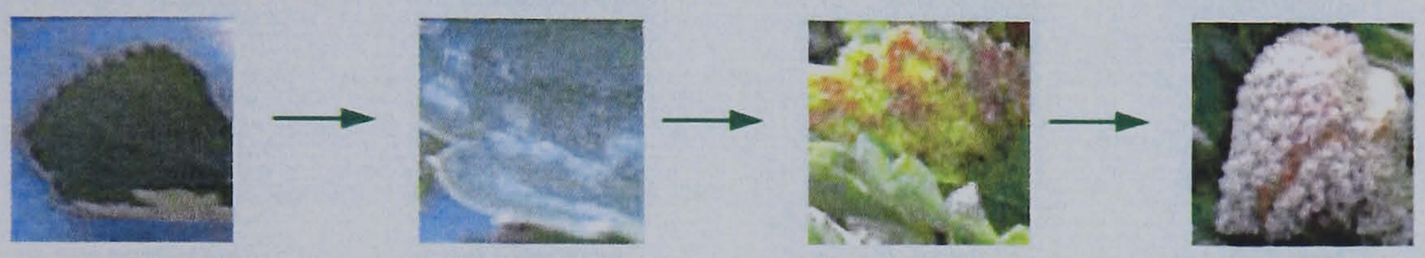
Background

Spanish tunnels are used to extend the season and boost the yield and quality of strawberries. However, this system also favours development of powdery mildew, caused by *Podosphaera aphanis*. To combat this disease, crops are treated with fungicides on 10 or more occasions during the season. Limits on the pesticide residues permitted in fruit are becoming increasingly stringent, making this approach to disease control untenable

The purpose of this work is to devise non-chemical strategies to reduce disease pressure and optimise the timing and dose of fungicide treatments that are justified by the risk.

Better disease assessment methodologies are needed

P. Aphanis causes a succession of symptoms;



Leaf cupping → **Mycelium** → **Red Blotches** → **Mycelium on fruit**

- Current disease assessment keys are inadequate for measuring progression
- Other stress inducing factors elicit symptoms similar to *P. aphanis* infection
- Frameworks for analysing *P. aphanis* epidemics are poorly developed
- This work is developing new methodologies to deliver reliable quantitative measures of disease as outlined below

Cultivar resistance can help

We have shown that;

- No commercial cultivars are immune to *P. aphanis*
- Symptoms develop more slowly on moderately resistant cultivars
- Cultivars preferred by supermarkets are susceptible

Better fungicide advice is needed

We are aiming to provide;

- Quantitative measures of fungicide efficacy for eradicant and protectant performance
- Advice on anti resistance strategies
- Evaluation of new chemistry and alternative products

Treatments should be applied in response to risk

- Disease risk varies enormously through the season
- Previous work in controlled environments has quantified key growth and development ranges for temperature, relative humidity & leaf wetness (Peries 1962 a & b)
- Our experiments in crops grown under commercial conditions support the findings of that work
- We are trying to devise a simple rule based system, based on environmental triggers, to identify the disease risk thresholds
- A prototype system indicates that treatment frequency and timings used in commercial practice do not align with estimated risk periods
- The effectiveness of the system will be verified in experiments that mirror commercial practice as closely as possible

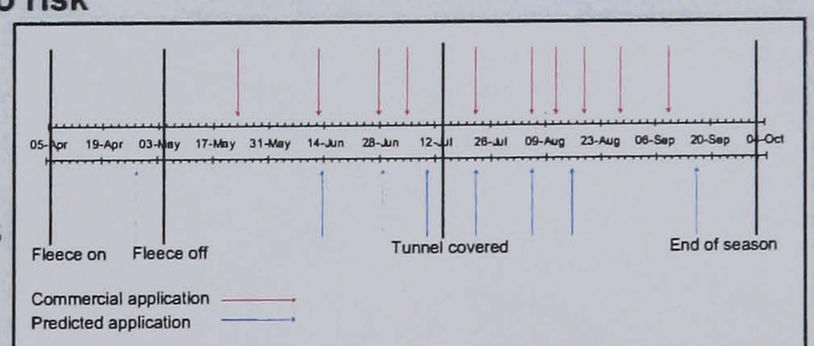


Figure 1. Application dates for mildewicides applied in 2004 and application timed according to the occurrence of conditions favourable to disease spread

Acknowledgements
The project is funded by the HDC (project no. SF62). The authors would like to thank Mr. and Mrs. H Duncalfe, Maltmes Farm and Mr. S. Turner, Agritech for all their help

Reference Peries O.S. 1962a *Annals of Applied Biology* 50, 211-224
Peries O.S. 1962b *Annals of Applied Biology 50, 225-233*

System to predict high risk periods for *Podosphaera aphanis* infection of strawberries grown in polythene tunnels

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Introduction

- Strawberries were traditionally grown in open fields
- There were extensive losses due to *Botrytis cinerea* and rain damage
- Growers started to use polythene tunnels in 1993 to reduce these loses
- Growers use tunnel based measurement of environmental conditions for tunnel management
- Polythene tunnels also create an environment more favourable for epidemics of *P. aphanis*
- Generally, fungicides are scheduled prophylactically for strawberry crops

Aim

Development of a prediction system based on tunnel conditions, which will highlight when a field is at greatest risk from *P. aphanis* and therefore allow more cost-effective treatment.

Method

- A literature review was carried out
- Disease development was recorded
- The temperature, relative humidity and leaf wetness were recorded in the tunnels

Results

- The fungal life cycle is divided into two phases, germination and then growth of the fungus
- The system calculates the number of hours of favourable conditions (new parameters), for conidial development (Table 1)
- The output is presented as a percentage of the total number of hours that a conidium takes to reach maturity

Table 1. Parameters from literature and initial field observations (old parameters) and adjusted values after analysis of disease development data (new parameters) for prediction system

	Old parameters[1]	New parameters
Temperature germination (°C)	17.5	15.5
Temperature growth and spore release (°C)	16	18
Relative humidity germination (%)	60	60
Leaf Wetness germination (%)	95	95
Temperature germination upper value (°C)	30	30
Temperature growth and spore release upper value (°C)	30	30
No. of hours[2] to maturity	144	*na*
No. of hours[2] to maturity *established* field 1st infection	*na*	84
No. of hours[2] to maturity *established* field after 1st infection	*na*	144
No. of hours[2] to maturity *new* field all infections	*Na*	144

[1]Amsalem, *et al*., 2006, Blanco, *et al*., 2004, Jhooty and McKeen, 1964, 1965, Miller, *et al*., 2003, Peries, 1962a
[2]Number of hours of suitable conditions (temperature, relative humidity and leaf wetness)

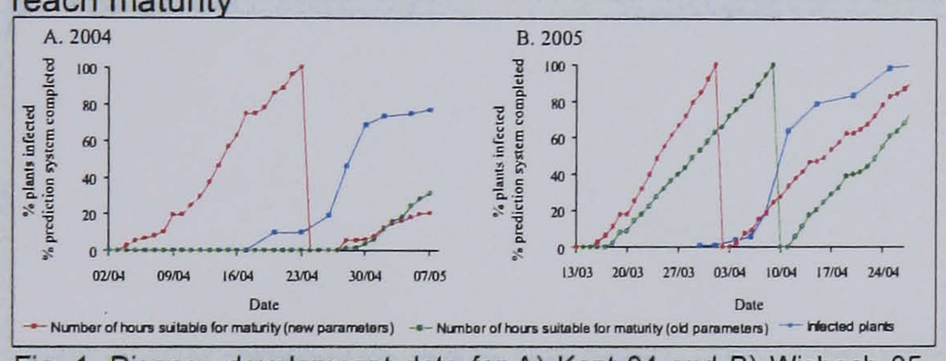

Fig. 1. Disease development data for A) Kent 04 and B) Wisbech 05 showing plants infected (%) and the output from the prediction system (100% equals high risk) for the old and new parameters

- The parameters from the literature were refined by comparing the predicted high risk periods with the actual development of the first symptoms in a tunnel (Fig.1)
- The parameters of the prediction system were then modified slightly (Table 1) and the environmental data was input in to the prediction system (new parameters) (Fig. 1)
- The predicted high-risk periods and the dates when disease actually developed were closer, when using the new parameters rather than the old parameters (Fig. 1)
- The new parameters predict the on set of the epidemic in the lag phase before symptoms are visible
- The predicted high risk periods were compared with the actual applications the grower made (Fig. 2)
- The grower applied 10 applications while the prediction system would have recommended 8 applications

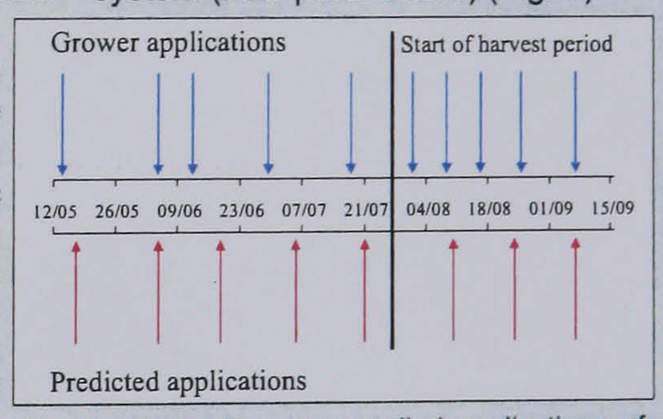

Fig. 2. Dates of grower applied applications of fungicidal control product for *P. aphanis* and application dates as recommended by the prediction system (new parameters)

Discussion

- System is based on environmental measurements in the tunnel
- Therefore high risk periods are field specific
- Tunnel based measurements are of benefit compared with a system based on data from a network of meteorological stations
- The system enables growers to apply fungicidal control products before visible symptoms appear
- System needs to be further tested in the field to confirm that predicted applications would result in commercially acceptable control

Acknowledgements

This work was funded by the Soft Fruit Panel of the Horticultural Development Council. The authors are grateful to John Clark (Hugh Lowe Farms), Harriet and Henry Duncalfe (Maltmas Farm) for providing and managing the field sites.

References

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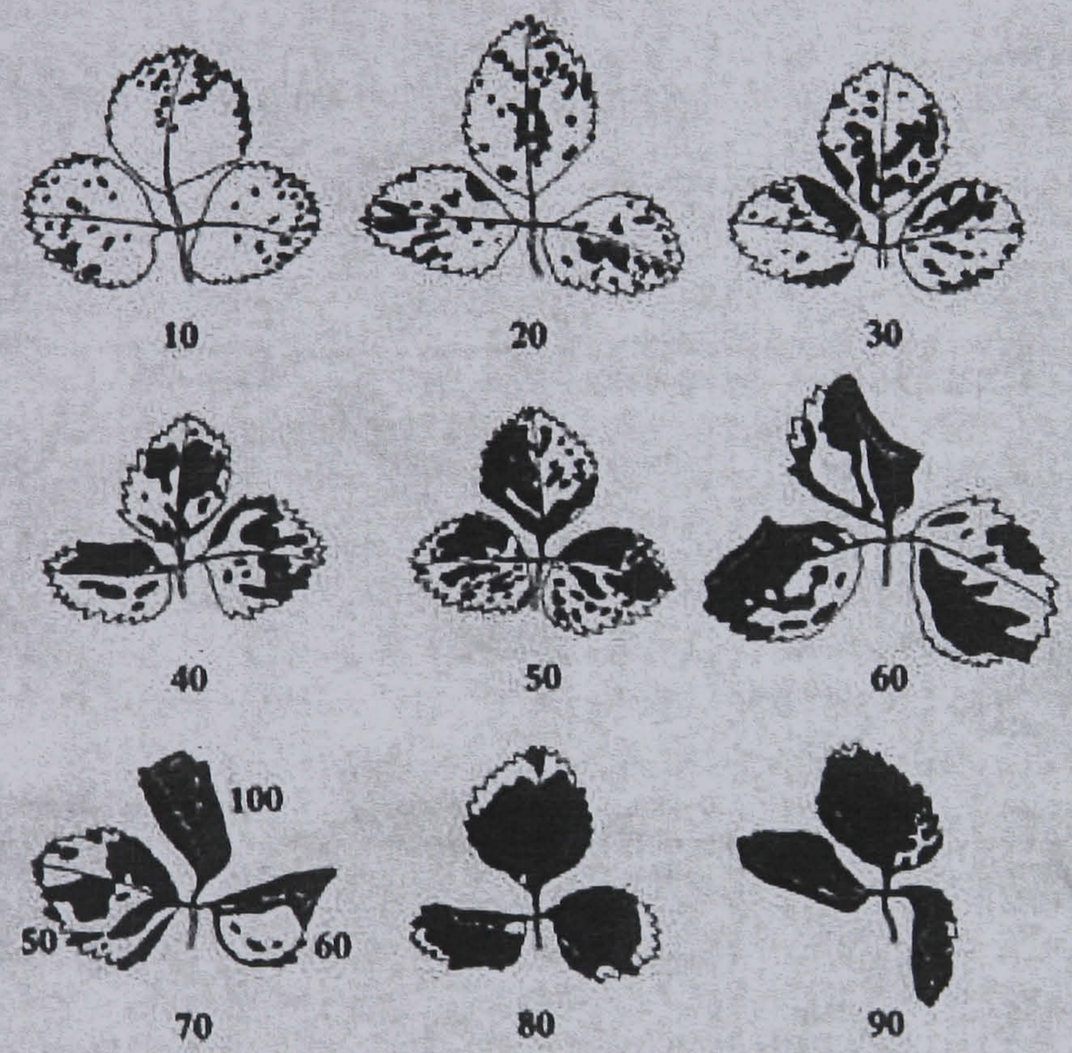
AGRICULTURAL DEVELOPMENT AND ADVISORY SERVICE

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

Sphaerotheca alchemillae Grev.



Percentage of leaf area affected

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

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

Powdery mildew (%)

0 Leaves fully extended, flat and green.

5 Slight curling noticeable, mildew found with difficulty.

10 Leaves with small red-purple spots. Curling slight. Mildew visible on lower surface.

20 Red blotches tending to be confluent. Some browning. Curling obvious from a distance.

30 More blotches confluent, with browning becoming more severe. Splitting in centre of larger lesions and curling severe.

40 Confluent red and brown blotches. Splitting in centre of larger lesions. Curling now approaching rolling. Leaf becoming brittle.

50 Half of leaf area affected and apparently dead.

60 Some yellowing in addition to reddening and browning may be present.

70 Severe distortion of at least one leaflet.

80 Much of leaf affected. Distortion of all leaflets.

90 Small marginal areas only remain green.

100 Whole leaf red or brown. Severe distortion and very brittle.

Notes:

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

PLANT PATHOLOGY LABORATORY
HARPENDEN, HERTS

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

Appendix 4 - MAFF strawberry powdery mildew key 8.1.1

Appendix 5 - MRL data for strawberries

Product	Notes	Level applies until	MRL
Abamectin		Applying until 20 January 2007	0.1
Abamectin		Applying from 21 January 2007	0.1
Acephate			0.02*
Acetamiprid			
Acibenzolar-S-methyl			0.02*
Aldicarb			0.05*
Aldrin & dieldrin			0.01*
Aminopyralid			
Amitraz		Applying until 9 January 2007	0.05*
Amitraz including the metabolites containing the 2,4-dimethylaniline moiety expressed as amitraz		Applying from 10 January 2007	0.05*
Amitrole			0.01*
Aramite			0.01*
Atrazine		Applying until 20 January 2007	0.1*
Atrazine		Applying from 21 January 2007	0.05*
Azimsulfuron			0.02*
Azinphos-ethyl		Applying until 20 January 2007	0.05*
Azinphos-ethyl		Applying from 21 January 2007	0.02*
Azinphos-methyl			0.5
Azocyclotin and Cyhexatin			0.05*
Azoxystrobin		Applying until 9 May 2006	2
Azoxystrobin		Applying from 10 May 2006	2
Barban			0.05*
Benalaxyl			0.05*
Benfuracarb			0.05*
Benomyl/carbendazim/thiophanatemethyl (sum expressed as carbendazim)		Applying until 14 September 2006	0.1*
Benomyl/carbendazim/thiophanatemethyl (sum expressed as carbendazim)		Applying from 15 September 2006	0.1*
Benomyl and carbendazim (sum expressed as carbendazim)	Directive 2006/60 replaces the previous column	Applying from 15 September 2006	0.1*
Bentazone			0.1*
Benthiavalicarb-isopropyl			
Bifenthrin		Applying until 9 May 2006	0.5

Bifenthrin	Applying 10 May 2006	from	0.5
Binapacryl			0.05*
Bitertanol			0.05*
Boscalid			3
Bromophos- ethyl			0.05*
Bromopropylate			0.05*
Bromoxynil including its esters expressed as bromoxynil	Applying 21 April 2007	from	0.05*
Camphechlor (Toxaphene)			0.1*
Captafol			0.02*
Captan	Applying 11 May 2007	from	3[(1)]
Carbaryl	Applying 29 December 2006	until	1
Carbaryl	Applying 30 December 2006	from	0.05*
Carbendazim	See benomyl		
Carbofuran	Applying 26 July 2006	until	0.1*
Carbofuran (sum of carbofuran and 3-hydroxy-carbofuran expressed as carbofuran)	Applying 27 July 2006	from	0.02*
Carbon disulphide			
Carbon tetrachloride			
Carbosulfan			0.05*
Carfentrazone-ethyl	Applying 3 December 2006	until	
Carfentrazone-ethyl (determined as cerfentrazone and expressed as carfentrazone-ethyl)	Applying 4 December 2006	from	0.01*
Chlorbenside			0.01*
Chlorbufam			0.05*
Chlordane			0.01*
Chlorfenapyr			0.05*
Chlorfenson			0.01*
Chlorfenvinphos			0.05
Chlorfenvinphos	Applying 21 January 2008	from	0.02*
Chlormequat			0.05*
Chlorobenzilate			0.02*
Chlorothalonil			3
Chloroxuron			0.05*
Chlorpropham (chlorpropham and 3-chloroaniline, expressed as chlorpropham)	Applying 21 April 2007	from	0.05*
Chlorpyrifos			0.2
Chlorpyrifos- methyl			0.5
Chlozolinate			0.05*
Cinidon-ethyl			0.05*
Clofentezine			2
Clothianidin			

Cyazofamid	Applying until 8 December 2006	0.01*
Cyazofamid	Applying from 9 December 2006	0.01*
Cyclanilide		0.05*
Cyflufenamid		
Cyfluthrin	Applying until 20 January 2007	0.02*
Cyfluthrin	Applying from 21 January 2007	0.02*
Cyhalofop butyl		0.02*
Cymoxanil		5
Cypermethrin		0.05*
Cyprodinil		5
Cyromazine	Applying until 9 May 2006	0.05*
Cyromazine	Applying from 10 May 2006	0.05*
2,4-D		0.05*
2,4-DB		0.05*
Daminozide		0.02*
DDT		0.05*
DE-126		0.5
Deltamethrin	Applying until 29 December 2006	0.05*
Deltamethrin (cis-deltamethrin)	Applying from 30 December 2006	0.2
Desmedipham	Applying from 21 January 2008	0.05*
Diallate		0.05*
Diazinon		0.02*
1,2 - Dibromoethane		0.01*
Dichlofluanid		10
Dichlorprop		0.05*
Dichlorvos	Applying up to 10 May 2007	0.1
Dichlorvos	Applying from 11 May 2007	0.01*
Dicofol		0.02*
1,1-Dichloro- 2,2- bis- (4-ethyl-phenyl-) ethane		0.01*
1,2-Dichloroethane		0.01*
Dimethenamid-P including other mixtures of constituent isomers (sum of isomers)	Applying from 21 April 2007	0.01*
Dimethoate		0.02*
Dimethomorph		
Dinoseb		0.05*
Dinoterb		0.05*
Dioxathion		0.05*
Diphenylamine		0.05*
Diquat	Applying until 26 July 2006	0.05*
Diquat	Applying from 27 July 2006	0.05*

Disulfoton			0.02*
DNOC			0.05*
Endosulfan		Applying until 29 December 2006	0.05*
Endosulfan		Applying from 30 December 2006	0.05*
Endrin			0.01*
Ethephon		Applying until 20 January 2007	0.05*
Ethephon		Applying from 21 January 2007	0.05*
Ethion		Applying up to 10 May 2007	0.1
Ethion		Applying from 11 May 2007	0.01*
Ethofumesate		Applying until 26 April 2006	0.05*
Ethofumesate (sum of ethofumesate and the metabolite 2,3-dihydro-3,3-dimethyl-2-oxo-benzofuran-5-yl methane sulphonate expressed as ethofumesate)		Applying from 27 April 2006	0.05*
Ethoxysulfuron			0.05*
Ethylene Oxide			0.1*
Famoxadone			0.02*
Fenamidone		Applying until 3 December 2006	
Fenamidone		Applying from 4 December 2006	0.02*
Fenamiphos			0.02*
Fenarimol			0.3
Fenbutatin Oxide		Applying until 8 December 2006	1
Fenbutatin oxide		Applying from 9 December 2006	1
Fenchlorphos			0.01*
Fenhexamid		Applying until 8 December 2006	5
Fenhexamid		Applying from 9 December 2006	5
Fenitrothion		Applying until 29 December 2006	0.5
Fenitrothion		Applying from 30 December 2006	0.01*
Fenpropathrin			2
Fenpropimorph		Applying until 20 January 2007	1
Fenpropimorph		Applying from 21 January 2007	1
Fenthion		Applying from 21 January 2007	0.01*
Fentin			0.05*
Fentin acetate			0.05*
Fentin hydroxide			0.05*
Fenvalerate and Esfenvalerate	Sum of RR and SS isomers		0.02*
	Sum of RS and SR isomers		0.02*
Flazasulfuron		Applying from 21 April 2007	0.01*

Florasulam		0.01*
Flucythrinate		0.05*
Fludioxonil		2
Flufenacet	Applying from 24 February 2007	0.05*
Flumioxazine		0.05*
Fluopicolide		
Fluoxastrobin		
Flupyrsulfuron-methyl		0.02*
Fluroxypyr and its esters expressed as furoxypyr		0.05*
Flurtamone	Applying from 21 April 2007	0.02*
Folpet	Applying from 11 May 2007	3[(1)]
Foramsulfuron		0.01*
Formothion		0.02*
Fosthiazate	Applying from 24 February 2007	0.02*
Furathiocarb		0.05*
Glyphosate	Applying until 20 January 2007	0.1*
Glyphosate	Applying from 21 January 2007	0.1*
Heptachlor		0.01*
Hexachlorobenzene (HCB)		0.01*
Hexachlorocyclohexane (isomers other than gamma)		0.01*
Hexaconazole		0.2
Imazalil		0.02*
Imazamox		0.05*
Iodosulfuron-methyl sodium (iodosulfuron-methyl including salts, expressed as iodosulfuron-methyl)	Applying from 24 February 2007	0.02*
Ioxynil, including its esters expressed as ioxynil	Applying from 21 April 2007	0.05*
Iprodione	Applying until 23 February 2007	10
Iprodione	Applying from 24 February 2007	15
Iprovalicarb		0.05*
Isoproturon		0.05*
Isoxaflutole (sum of isoxaflutole, RPA 202248A and RPA 203328, expressed as isoxaflutole [(56)]	Applying from 4 December 2006	0.05*
Kresoxim-methyl	Applying until 9 May 2006	1
Kresoxim-methyl	Applying from 10 May 2006	1
Lambda-cyhalothrin	Applying until 26 April 2006	0.5
Lambda-cyhalothrin	Applying from 27 April 2006	0.5
Lindane		0.01*
Linuron	Applying until 8/12/2006	0.05*
Linuron	Applying from 9/12/2006	0.05*

Malathion		0.5
Maleic- hydrazide		0.2*
Maneb Mancozeb Metiram Propineb Zineb		2
Mecoprop (sum of mecoprop-p and mecoprop expresssed as mecoprop)	Applying from 4 December 2006	0.05*
Mecarbam		0.05*
Mepanipyrim		2
Mepanipyrim and its metabolite (2-anilino-4-(2-hydroxy-propyl)-6-methyl-pyrimidine expressed as mepanipyrim	Applying from 21 April 2007	2
Mercury compounds		0.01*
Mesosulphuron-methyl		
Mesotrione (Sum of mesotrione and MNBA (4-methyl-sulfonyl-2-nitrobenzoic acid), expressed as mesotrione)	Applying from 24 February 2007	0.05*
Metalaxyl		0.5
Metalaxyl including other mixtures of constituent isomers including metalaxyl-m (sum of isomers)	Applying until 9 May 2006	0.5
Metalaxyl including other mixtures of constituent isomers including metalaxyl-m (sum of isomers)	Applying from 10 May 2006	0.5
Methacrifos		0.05*
Methamidophos	Applying until 20 January 2007	0.01*
Methamidophos	Applying from 21 January 2007	0.01*
Methidathion	Applying until 29 December 2006	0.02*
Methidathion	Applying from 30 December 2006	0.02*
Methomyl thiodicarb (sum expressed as methomyl)	Applying until 20 January 2007	0.05*
Methomyl thiodicarb (sum expressed as methomyl)	Applying from 21 January 2007	0.05*
Methoxychlor		0.01*
Methyl bromide		0.05*
Metsulfuron-methyl		0.05*
Mevinphos		0.1
Molinate	Applying from 24 February 2007	0.05*
Monolinuron		0.05*
Myclobutanil	Applying until 20 January 2007	1
Myclobutanil	Applying from 21 January 2007	1
Nitrofen		0.01*
Oxadiargyl		0.01*
Oxamyl	Applying from 30 December 2007	0.01*
Oxasulfuron		0.05*
Oxydemeton-methyl		0.02*
Paraquat	Applying until 20 January 2007	0.05*
Paraquat	Applying from 21 January 2007	0.02*
Parathion		0.05*
Parathion methyl		0.02*

Penconazole		0.05*
Pendimethalin		0.05*
Permethrin		0.05*
Phenmedipham	Applying from 21 January 2008	0.1*
Phorate		0.05*
Phosalone		1
Picolinafen		0.05*
Picoxystrobin	Applying from 24 February 2007	0.05*
Pirimiphos-methyl		0.05*
Prochloraz		0.05*
Procymidone		5
Profenophos		0.05*
Prohexadione and its salts expressed as prohexadione		0.05*
Propham		0.05*
Propiconazole	Applying until 23 February 2007	0.05*
Propiconazole	Applying from 24 February 2007	0.05*
Propoxur		0.05*
Propoxycarbazone, its salts and 2-hydroxypropoxypropoxycarbazone, calculated as propoxycarbazone	Applying from 21 April 2007	0.02*
Propyzamide	Applying until 3 December 2006	0.02*
Propyzamide	Applying from 4 December 2006	0.02*
Prosulfuron		0.02*
Pymetrozine	Applying until 8 Decemberl 2006	0.5
Pymetrozine	Applying from 9 December 2006	0.5
Pyraclostrobin		0.5
Pyraclostrobin	Applying from 21 April 2007	0.5
Pyraflufen-ethyl		0.02*
Pyrazophos		0.05*
Pyridate		0.05*
Quinalphos		0.05*
Quinoxyfen		0.3
Quinoxyfen	Applying from 21 April 2007	0.3
Quintozene		0.02*
Resmethrin		0.1*
Silthiofam	Applying from 24 February 2007	0.05*
Spinosad		0.3
Spiromesifen		1
Spiroxamine		0.05*
Sulfosulfuron		0.05*
Tecnazene		0.05*
TEPP		0.01*
Thiabendazole	Applying until 26 April 2006	0.05*

Thiabendazole	Applying from 27 April 2006 until 20 January 2007	0.05*
Thiabendazole	Applying from 21 January 2007	0.05*
Thiacloprid		0.5
Thifensulfuron methyl		0.05*
Thiophanate-methyl	Applying from 15 September 2006	0.1*
Triadimefon and Triadimenol	Applying until 8 December 2006	0.5
Triadimefon and Triadimenol	Applying from 9 December 2006	0.5
Triasulfuron		0.05*
Triazophos	Applying until 20 January 2007	0.02*
Triazophos	Applying from 21 January 2007	0.01*
Tridemorph		0.05*
Trifloxystrobin	Applying from 4 December 2006 until 20 January 2007	0.02*
Trifloxystrobin	Applying from 21 January 2007	0.5*
Triforine		0.05*
Trimethylsulfonium cation resulting from the use of glyphpsate	Applying from 21 January 2007	0.05*
2,4,5-T		0.05*
Vinclozolin		5
Zoxamide	Applying from 21 April 2007	0.02*

* Level at or about the limit of determination.
[1] Sum of captan and folpet

Appendix 6 - Copies of PA1 and PA6 certificates, selected risk assessment and COSHH forms

OAKLANDS COLLEGE

College Certificate Awarded to

Jolyon Dodgson

attended a

PA1 Pesticide

Course on:

Monday 1st March 2004

Curriculum Manager
Land Based Industries

OAKLANDS COLLEGE

College Certificate Awarded to

Jolyon Dodgson

attended a

PA6Pesticide

Course on

Tuesday 6th March 2004

Roger Rowson

Curriculum Manager
Land Based Industries

FACULTY OF HEALTH AND HUMAN SCIENCES
DEPT OF BIOSCIENCES

Initial Risk Assessment

Ref No

Date

Review

Title of activity	Applying Fungicides to Strawberry plants
Location of activity	Bradbourne Glass House
Brief description of activity	Fungicide will be applied to strawberry plants using a petrol sprayer.
Personnel involved	Tolga Dodgion

List the hazards that will be encountered in the activity designated above using the information overleaf and calculate the risk classification where **RISK = Likelihood x Severity**

Hazard	Likelihood Score	Severity Score	Risk Score & Classification
Fungicide could contaminate operator.	2	4	8

Risk Score	Risk Classification	Action
1-2	Trivial	NO FURTHER ACTION REQUIRED
4-6	Tolerable	No additional controls required. Maintain current controls. Monitor.
8-16	Moderate	Reduce risk if cost effective. Implement new controls over a set period.
24-36	Substantial	DO NOT START activity. If work in progress, take urgent action.
48-64	Intolerable	Activity MUST STOP and not be started unless risk is reduced. Activity prohibited if no reduction in risk.

If risk classification is moderate (score of 8 and above) a full risk assessment will be required.

Types of hazard likely to be encountered in the Dept of Biosciences:

Animal allergens	Glass	Sharps
Biological agents	Hand tools	Slips/trips/falls
Chemical compounds	Ionising radiation	Stress
Compressed/liquefied gases	Laboratory/office equipment	Vacuum systems
Display screen equipment	Ladders	Vehicles
Electricity	Manual handling	Workshop machinery
Farm machinery	Non-ionising radiation	Others - please list
Fieldwork	Pressure systems	
Fire	Repetitive handling	

Definition of terms used:

Factor	Risk Classification	Likelihood	Severity
1	Trivial	Harm will not occur or is very unlikely to occur	No injury or disease Minor damage Group 1 organisms
2	Tolerable	Harm could occur but is unlikely to occur	Minor injury or disease Minor damage. Harmful/irritant compounds Group 2 organisms Manual handling less than guideline weights
4	Moderate	Harm possible	Moderate injury (over 3 days) Moderate damage to building/plant Corrosive/toxic/flammable compounds Group 3 organisms Manual handling at guideline weights
6	Substantial	Harm likely to occur	Serious injury or disease Serious damage to building or plant Suspect carcinogens Manual handling up to twice guideline weights
8	Intolerable	Harm will occur or is very likely to occur	Likely fatality Serious structural damage Plant damaged beyond repair Very toxic compounds Human carcinogens Group 4 organisms

Is a full risk assessment required?[1] (Yes) / No

Please print and sign your name below when the initial risk assessment is complete.

Assessor Tolga Bedyloo Tolga Bedyloo **Date** 20.07.04.

Status[1] Undergraduate/MSc student/PhD student / (Staff)

Supervisor Alice M Hall. A.M. Hall **Date** 22.07.04

Head of Department[2] **Date**

Technical Manager[2] **Date**

---

[1] Please delete

[2] Authority may be devolved to Head of Division/Departmental Safety Advisor as appropriate

/—

COSHH Risk Assessment - Faculty of Natural Science

Ref No

Date

Review

Title of Experiment/Procedure Applying Fungicides to Strawberry plants.

Location Buckland Farm glasshouse

Personnel Involved Jolyon Cobson

1. Brief description of activity or process.

Fungicides will be applied to strawberry plants growing in a glasshouse, using a pressure sprayer.

2. Risk Assessment of Substances Used

Substance Used, (and conc if appropriate)	Hazards	Route of Entry	Risk/ Severity
– Strobe WG (Kresoxim-methyl)	Harmful Dangerous for the environment	Inhalation Contact with/ skin Ingestion Improper disposal	2 2 2 2
– Systhane (Myclobutanil)	Harmful	Inhalation Contact with/ skin Ingestion	2 2 2
	Dangerous for the Environment	Improper. disposal	2
– Corbel (Fenpropimorph)	Irritant to eyes	Contact with/ eyes	2

Risk is calculated on a score of 1-8 as listed on the Initial Risk Assessment Form (Severity)

Types of Hazard likely to be encountered are:

Harmful	Oxidising	Biohazard
Irritant	Flammable	Terratogenic
Corrosive	Explosive	Mutagenic

3. Information sources *(eg Databases, Suppliers Hazard Warning Sheet, HSE Guideline)*
The UK Pesticide Guide 2004

4. Are less hazardous substances available? No

If so, why not use them?

5. Control Measures to be used

Is good laboratory practice sufficient? (Yes) No

If not, list additional measures required. and find pesticide use procedure.

6. Required checks and their frequency on the adequacy and maintenance of control measures during the course of the activity:
Yearly

7. Disposal procedures, during and at the end of the acitivity:
Tank washing to be disposed of in accordance with established pesticide use, unused concentrate to be returned to supplier.

8. Emergency procedures

If any of the above substances or procedures are likely to pose a specific hazard in an emergency, then identify below the action to be taken.

Spillage/Uncontrolled release
Sand to be used to contain spillage - then disposed of following established pesticide procedures.

Fire
Until pesticides stored on site will be made aware of the emergency

First Aid
First aid kit including eye wash bottle

Assessor Tolson Rodgers **Date** 20.04.07.

Supervisor Alice M Hall **Date** 28.07.04

Head of Department _____ **Date** _____

Technical Manager _____ **Date** _____

FACULTY OF HEALTH AND HUMAN SCIENCES
DEPT OF BIOSCIENCES

Initial Risk Assessment

Ref No

Date

Review

Title of activity	Dipping plantlets in fungicides and potassium bicarbonate
Location of activity	Maltman Farm, Wisbech
Brief description of activity	Dipping plantlets into a container containing dilute fungicides or potassium bicarbonate. Quinoxyfen (Fortress) ~~Myclobutanil~~ (Systhane)
Personnel involved	Alison Rodgers

List the hazards that will be encountered in the activity designated above using the information overleaf and calculate the risk classification where RISK = Likelihood x Severity

Hazard	Likelihood Score	Severity Score	Risk Score & Classification
fungicide could contaminate operator.	2	4	8

Risk Score	Risk Classification	Action
1-2	Trivial	NO FURTHER ACTION REQUIRED
4-6	Tolerable	No additional controls required. Maintain current controls. Monitor.
8-16	Moderate	Reduce risk if cost effective. Implement new controls over a set period.
24-36	Substantial	DO NOT START activity. If work in progress, take urgent action.
48-64	Intolerable	Activity MUST STOP and not be started unless risk is reduced. Activity prohibited if no reduction in risk.

If risk classification is moderate (score of 8 and above) a full risk assessment will be required.

Types of hazard likely to be encountered in the Dept of Biosciences:

Animal allergens
Biological agents
Chemical compounds
Compressed/liquefied gases
Display screen equipment
Electricity
Farm machinery
Fieldwork
Fire
Glass
Hand tools
Ionising radiation
Laboratory/office equipment
Ladders
Manual handling
Non-ionising radiation
Pressure systems
Repetitive handling
Sharps
Slips/trips/falls
Stress
Vacuum systems
Vehicles
Workshop machinery
Others - please list

Definition of terms used:

Factor	Risk Classification	Likelihood	Severity
1	Trivial	Harm will not occur or is very unlikely to occur	No injury or disease Minor damage Group 1 organisms
2	Tolerable	Harm could occur but is unlikely to occur	Minor injury or disease Minor damage. Harmful/irritant compounds Group 2 organisms Manual handling less than guideline weights
4	Moderate	Harm possible	Moderate injury (over 3 days) Moderate damage to building/plant Corrosive/toxic/flammable compounds Group 3 organisms Manual handling at guideline weights
6	Substantial	Harm likely to occur	Serious injury or disease Serious damage to building or plant Suspect carcinogens Manual handling up to twice guideline weights
8	Intolerable	Harm will occur or is very likely to occur	Likely fatality Serious structural damage Plant damaged beyond repair Very toxic compounds Human carcinogens Group 4 organisms

Is a full risk assessment required?[1] (Yes) / No

Please print and sign your name below when the initial risk assessment is complete.

Assessor Tolga Bulgin **Date** 22-11-04

Status[1] Undergraduate/MSc student/PhD student, (Staff)

Supervisor Anne Millar **Date** 22.11.04

Head of Department[2] **Date** 24/11/04

Technical Manager[2] **Date** 22/11/04

D S A.

[1] Please delete
[2] Authority may be devolved to Head of Division/Departmental Safety Advisor as appropriate

COSHH Risk Assessment - Faculty of Natural Science

Ref No

Date

Review

Title of Experiment/Procedure Dipping plantlets in fungicides and potassium bicarbonate

Location Mallows Farm Wisbech

Personnel Involved

Jolyon Medgon (Staff).

1. Brief description of activity or process.

Dipping plantlets into a container, containing dilute fungicide or potassium bicarbonate, while wearing full protective outfits.

2. Risk Assessment of Substances Used

Substance Used, (and conc if appropriate)	Hazards	Route of Entry	Risk/ Severity
Quinoxyfen (Fortress)	- Irritant to eyes, skin, respiratory system - Sensitization to skin - Damaging to aquatic env.	skin, mouth nose	2
Myclobutanil (Systhane)	- Keep away from food and drink - Damaging to aquatic env.	skin, mouth nose	2
Potassium bicarbonate	- Irritant to respiratory system when concentrated.	none	2

Risk is calculated on a score of 1-8 as listed on the Initial Risk Assessment Form (Severity)

Types of Hazard likely to be encountered are:

Harmful	Oxidising	Biohazard
Irritant	Flammable	Terratogenic
Corrosive	Explosive	Mutagenic

3. Information sources *(eg Databases, Suppliers Hazard Warning Sheet, HSE Guideline)*

The UK pesticide guide 2004

4. Are less hazardous substances available? No

If so, why not use them?

5. Control Measures to be used

Is good laboratory practice sufficient? (Yes) No

If not, list additional measures required. including good pesticide usage guidelines. - regulations subs to be worn

6. Required checks and their frequency on the adequacy and maintenance of control measures during the course of the activity: overall + hood. check face mask, use new ~~filter~~ each time procedure is carried out.

7. Disposal procedures, during and at the end of the acitivity:

Normal disposal of pesticides will take place. - eg usual

8. Emergency procedures Mallam harm procedures will be followed.

If any of the above substances or procedures are likely to pose a specific hazard in an emergency, then identify below the action to be taken.

Spillage/Uncontrolled release

use an absorbant substance - inform harm + Dir. safety.

Fire

Comply with pesticide regulations

First Aid

Call an ambulance - inform Hse of substance.

Assessor Julian Padgham **Date** 21.11.04

Supervisor Anne M Hall **Date** 21.11.04

Head of Department L. Boggis **Date** 22/11/04

Technical Manager D. S. A. **Date** 22/11/04

FACULTY OF HEALTH AND HUMAN SCIENCES
SCHOOL OF LIFE SCIENCES

Initial Risk Assessment

Ref No

Date

Review

Title of activity	Clearing (Bleaching) strawberry leaves Then staining leaves with trypan blue.
Location of activity	Lab at University of Hertfordshire
Brief description of activity	Strawberry leaves will be placed in bleach, once they are clear the leaves will be placed in trypan blue.
Personnel involved	Tolga Hodgson + Summer student,

List the hazards that will be encountered in the activity designated above using the information overleaf and calculate the risk classification where **RISK = Likelihood x Severity**

Hazard	Likelihood Score	Severity Score	Risk Score & Classification
Bleach could contaminate worker	2	4	8
Trypan blue could contaminate worker	2	4	8

Risk Score	Risk Classification	Action
1-2	Trivial	NO FURTHER ACTION REQUIRED
4-6	Tolerable	No additional controls required. Maintain current controls. Monitor.
8-16	Moderate	Reduce risk if cost effective. Implement new controls over a set period.
24-36	Substantial	DO NOT START activity. If work in progress, take urgent action.
48-64	Intolerable	Activity **MUST STOP** and not be started unless risk is reduced. Activity prohibited if no reduction in risk.

If risk classification is moderate (score of 8 and above) a full risk assessment will be required.

Types of hazard likely to be encountered in the School of Life Sciences:

Animal allergens	Glass	Sharps
Biological agents	Hand tools	Slips/trips/falls
Chemical compounds	Ionising radiation	Stress
Compressed/liquefied gases	Laboratory/office equipment	Vacuum systems
Display screen equipment	Ladders	Vehicles
Electricity	Manual handling	Workshop machinery
Farm machinery	Non-ionising radiation	Others - please list
Fieldwork	Pressure systems	
Fire	Repetitive handling	

Definition of terms used:

Factor	Risk Classification	Likelihood	Severity
1	Trivial	Harm will not occur or is very unlikely to occur	No injury or disease Minor damage Group 1 organisms
2	Tolerable	Harm could occur but is unlikely to occur	Minor injury or disease Minor damage. Harmful/irritant compounds Group 2 organisms Manual handling less than guideline weights
4	Moderate	Harm possible	Moderate injury (over 3 days) Moderate damage to building/plant Corrosive/toxic/flammable compounds Group 3 organisms Manual handling at guideline weights
6	Substantial	Harm likely to occur	Serious injury or disease Serious damage to building or plant Suspect carcinogens Manual handling up to twice guideline weights
8	Intolerable	Harm will occur or is very likely to occur	Likely fatality Serious structural damage Plant damaged beyond repair Very toxic compounds Human carcinogens Group 4 organisms

Is a full risk assessment required?[1] (Yes) / No

Please print and sign your name below when the initial risk assessment is complete.

Assessor Tolga Polyn Zi Ke **Date** 20.07.06

Status[1] **Undergraduate / MSc student / PhD student / (Staff)**

Supervisor Alice M. Hall **Date** 24.07.06.

Head of Department[2] **Date**

Technical Manager[2] DSA D Wilson **Date** 20/7/06

[1] Please delete

[2] Authority may be devolved to Head of Division/Departmental Safety Advisor as appropriate

1

COSHH Risk Assessment - Faculty of Natural Science

Ref No

Date

Review

Title of Experiment/Procedure Clearing and staining strawberry leaves

Location Lab at university of Hertfordshire

Personnel Involved

Tolga Bodgan + Summer Student,

1. Brief description of activity or process.

Strawberry leaves will be placed in bleach, once they are clear the leaves will be placed in stain (trypan blue).

2. Risk Assessment of Substances Used

Substance Used, (and conc if appropriate)	**Hazards**	**Route of Entry**	**Risk/ Severity**
Bleach	Irritation	Skin (contact)	2
		Eyes (contact)	8
		Ingestion	2
Trypan Blue	Irritation	skin (contact)	2
		eyes (contact)	2
		Ingestion	8

Risk is calculated on a score of 1-8 as listed on the Initial Risk Assessment Form (Severity)

Types of Hazard likely to be encountered are:

Harmful	Oxidising	Biohazard
Irritant	Flammable	Terratogenic
Corrosive	Explosive	Mutagenic

3. Information sources *(eg Databases, Suppliers Hazard Warning Sheet, HSE Guideline)*

Data Sheet, Warning information

4. Are less hazardous substances available?

If so, why not use them? No.

5. Control Measures to be used

Is good laboratory practice sufficient? (Yes)/No — Lab Coats, Gloves Eye Protection

If not, list additional measures required.

6. Required checks and their frequency on the adequacy and maintenance of control measures during the course of the activity:

Weekly

7. Disposal procedures, during and at the end of the acitivity:

Conc.d Trypan Blue → Yellow Toxicban. Dilute solution → sink. ≥ bleach 5%.

8. Emergency procedures

If any of the above substances or procedures are likely to pose a specific hazard in an emergency, then identify below the action to be taken.

Spillage/Uncontrolled release Mop up ≥ tissues + 5% bleach

Fire As appropriate to environment

First Aid Seek medical advice
Wash affected area ≥ H_2O.

Assessor Taiyo Taylor **Date** 20.07.06

Supervisor Anne Mthall **Date** 24.07.06

Head of Department _____ **Date** _____

Technical Manager D.S.A. **Date** 20/7/06

Appendix 7 - PSD document 5230

FOOD AND ENVIRONMENT PROTECTION ACT 1985
CONTROL OF PESTICIDES REGULATIONS 1986

Notice is hereby given that in exercise of the powers conferred by Regulation 5 of the Control of Pesticides Regulations 1986 (SI 1986/1510) (as amended) and of all other powers enabling them in that behalf, the Secretary of State, and the Scottish Ministers (as regards Scotland) and the National Assembly for Wales and the Secretary of State (acting jointly as regards Wales) have given

Level and scope:		full approval
	a)	expiring on 31 December 2006 for the advertisement, sale, supply and use by the approval holder or their agents.
	b)	expiring on 31 December 2007 for the storage by any person and for the advertisement, sale, supply and use by persons other than the approval holder or their agents.
Product name:		'Fosal' with MAPP Number 13086
Formulation:		being a water dispersible granule containing 80% w/w fosetyl-aluminium to
Approval holder:		Agriguard Ltd., Unit 3, Block B, Tally House, Broomfield Business Park, Malahide, Co. Dublin, Ireland.

Subject to the conditions set out below:

Date of expiry:	a)	31 December 2006 (unless earlier decisions are made or further prescribed extensions are granted)
	b)	31 December 2007 (unless earlier decisions are made or further prescribed extensions are granted)

Sale and Supply

Label: Product to be sold or supplied with label text as detailed in Annex A to Pesticides Safety Directorate's letter dated 30 August 2006.

Container: Cardboard carton with integral barrier comprised of a polyethylene lined paper/aluminium bag containing up to 10 kg product

Use:

Field of use: **ONLY AS A HORTICULTURAL FUNGICIDE**

Crops/situations:	*Maximum individual dose*:	*Maximum number of treatments* (per year unless otherwise stated):	*Latest time of application*:	*Other specific restrictions:*
Hardy nursery stock	10 g/m^2	-	-	-
Glasshouse grown pot plants	5 g/m^2	-	-	-
Strawberry (a) Foliar spray	3.75 kg/hectare	One	-	This product must only be applied between harvest and 31 December
(b) Root dip	-	One	Pre-planting	The maximum concentration must not exceed 3.75 g product /litre of water

Crops/situations:	*Maximum individual dose*:	*Maximum number of treatments* (per year unless otherwise stated):	*Latest time of application*:	*Other specific restrictions:*
Hop (a) Basal spray	830 g/hectare	Two	14 days before harvest	A minimum interval of 14 days must be observed between treatments
(b) Foliar spray	-	Six	14 days before harvest	A minimum interval of 10 days must be observed between treatments The maximum concentration must not exceed 200g product / 100 litres of water
Protected Lettuce	-	One per batch of compost	pre-sowing	The maximum concentration must not exceed 900 g product per m^3 of compost
Broad bean	1.68 kg/hectare	Two per crop	17 days before harvest	-

Crops/situations:	*Maximum individual dose*:	*Maximum number of treatments* (per year unless otherwise stated):	*Latest time of application*:	*Other specific restrictions:*
Apple (a) Foliar spray	-	Two per crop	4 weeks before harvest	The maximum concentration must not exceed 250 g product /100 litres of water
(b) Bark paste	-	One	5 months before harvest	A minimum of four weeks must be observed between treatments. The maximum concentration must not exceed 1 kg product/ litre water

Operator protection:

(1) Engineering control of operator exposure must be used where reasonably practicable in addition to the following personal protective equipment:

Operators must wear suitable protective clothing (coveralls), rubber gloves and face protection (faceshield) when handling or applying the product as a paste.

(2) However, engineering controls may replace personal protective equipment if a COSHH assessment shows they provide an equal or higher standard of protection.

Adverse Effects:

Approval holders are under an on-going obligation to submit immediately any new information on the potentially dangerous effects of a product or of residues of an active substance contained in a product, on human or animal health, ground water or the environment.

Signed by: wendy woodfine
Signing time: Wednesday, August 30 2006, 14:42:18 GMT
Location: PSD York
Reason to sign: For The Pesticides Safety Directorate

PSD Digital Signature

Date of issue 30 August 2006.

EXPLANATORY NOTES

1. This Notice of Approval is number 2845 of 2006.

2. Application Reference Number: COP 2006/00988.