Project title:
Extending and exploiting new knowledge of strawberry powdery mildew

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SF 62a

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

CONTENTS

GROWER SUMMARY

Headline

- A rule-based system has been developed to optimise the timing of treatments to control strawberry 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 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 the chemical residues permitted. In addition, growers rely on a relatively limited armoury of fungicide active ingredients, placing enormous selection pressure on the pathogen population.

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

The expected deliverables from SF 62a include:

- Quantify the dose efficiency of key fungicides approved for controlling strawberry powdery mildew.
- Improved knowledge of the effect of venting practice on the environment within tunnels, interpreted with reference to the optimum conditions for fruit production, and for the growth and development of strawberry powdery mildew.
- Development of a rule based prediction system tested under commercial conditions.

Summary of the project and main conclusions

The results are summarised under the key objectives of the work.

Quantify the dose efficiency of key fungicides approved (or proposed) for controlling strawberry powdery mildew

Five fungicides were tested for the control of powdery mildew infection at quarter label rate and at full label rate. However the amount of infection measured in the field reduced throughout the time the experiment was being carried out even on the untreated plots. This was due to the weather conditions within the site at that time in the 2007 season. This meant that it was not possible to draw significant conclusions from this piece of the work.

Define good tunnel management practices

Ideally, tunnels should be managed so that their environmental conditions remain optimal for fruit production for as much of the day as possible, whilst also avoiding conditions that are within the optimal range for powdery mildew development. This project demonstrated that this goal can be achieved on commercial scales. Temperature and relative humidity within and outside tunnels were measured at two sites. One site was managed in accordance with normal farm practice. On the other site, the grower paid special attention to venting. The external conditions on both sites were broadly similar, with few significant differences. The internal temperatures were also similar on both sites. However, the internal relative humidity was significantly lower on the site that had been managed with special attention to venting.

A venting plan should be produced each night (or early next morning) for the next day, by reference to the temperature forecast. The venting plan should aim to keep internal temperatures in the range 18-25 ^{o}C range during the day and to reduce the internal relative humidity to less than 70% during the day. Weather forecasts that predict air temperatures of 12-15^{o}C or greater, should act as a trigger to plan tunnel venting.

Implement and refine prediction risk warning scheme

Published literature was reviewed to investigate the extent of understanding about the relationships between environmental conditions and growth and development of strawberry powdery mildew. This provided considerable detail, which mostly originated from laboratory tests. The information was used as the basis for developing a rule based prediction system, which identifies the occurrence of high-risk infection periods for strawberry powdery mildew.

The initial rules, defined from the laboratory-based experiments, were modified after field-based experiments that tested whether predictions of infection risk matched observations of disease progress in commercial crops.

These modifications had the objective of improving the prediction of initial development of strawberry powdery mildew symptoms in the field. Using historical records collected from commercial crops, the revised system was used to identify high-risk dates (and hence treatment timings) for strawberry powdery mildew, and these were compared to the dates that growers applied fungicides.

- The system predicted the same requirement for, or fewer applications than applied by the growers.
- Two growers also tested the system under commercial conditions.
- The control achieved was comparable to that achieved by the grower’s normal management strategies, with less chemical products applications.

Financial benefits

In the short-term

- Improved venting can reduce relative humidity, resulting in slower germination of strawberry powdery mildew spores. It also helps maintain temperatures closer to the optimum range for fruit production. These benefits combine to reduce the need for fungicide treatment and to improve fruit quality.

In the medium-term

- The rule based system will allow fungicide timings to be targeted when they are likely to be most cost effective.

Action points for growers

- Appropriate venting is important for optimising crop development and growth and, as a component of integrated disease management, for suppression of powdery mildew.
- Venting decisions should be based upon weather forecasts and adjusted in response to observations in the tunnels.
- Growers should consider obtaining an on farm weather station, which can at least measure internal temperature and relative humidity, ideally from several fields.

- The rule-based prediction system will act as a guide for identifying high-risk infection periods, but growers should combine this with crop walking to check for disease symptoms.

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, 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 & 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.

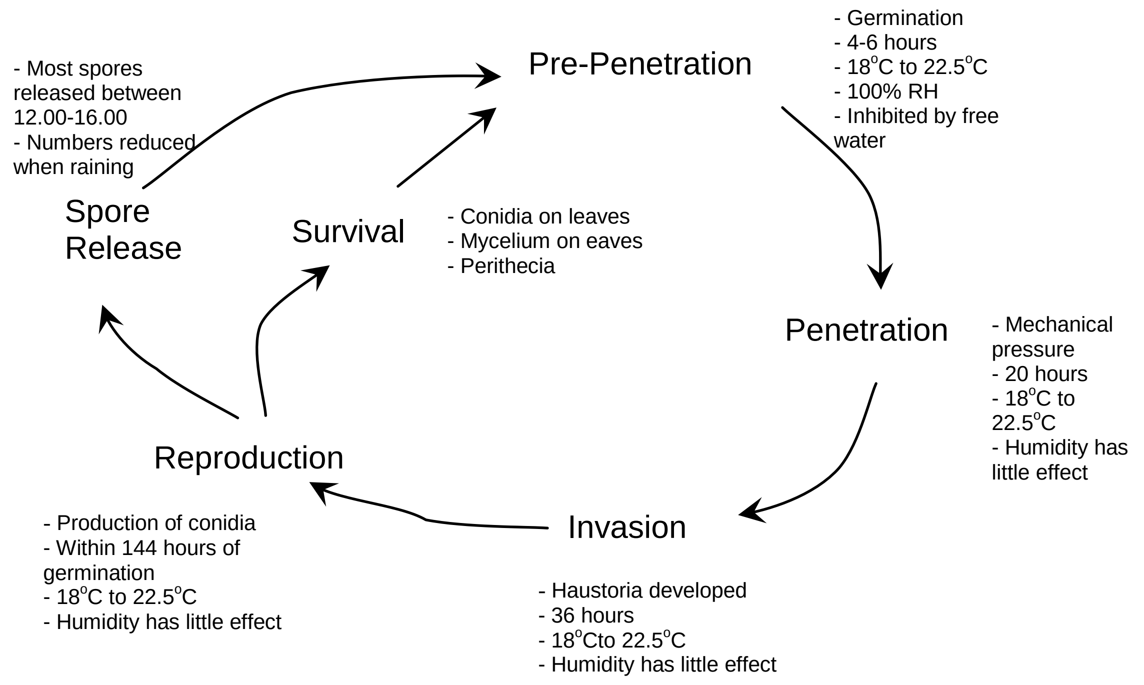


Fig. 1. Life cycle of strawberry powdery mildew *Podosphaera aphanis* (syn. *Sphaerotheca macularis*).

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 planning the investigation of disease progress in the field experiments, or as an aid to formulating disease management strategies.

Table 1. Time for development of major stages in fungal infection. Compiled from work by Peries (1961).

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

Peries (1961) reported the optimum temperature for germination of the conidia was within the range 18^{o}C - 22.5^{o}C and subsequent authors found 20^{o}C to be the optimum (Jhooty and McKeen, 1965, Miller *et al.*, 2003). Jhooty and McKeen (1965), found that the minimum and maximum temperatures for spore germination were 3^{o}C and 38^{o}C respectively. This is supported by Miller, *et al.* (2003), who showed germination of 8% of spores at 4^{o}C, and at greatly reduced frequency at 36^{o}C. Peries (1961) found that less than one percent of spores germinated at 2^{o}C and that they did not infect the plant unless the temperature was at least 5^{o}C. While some conidia will germinate at 10^{o}C and 30^{o}C these temperatures are not conducive for disease development. The amount of infection at 15^{o}C is consistently greater than at 25^{o}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, 1961, Jhooty and McKeen, 1965, Jhooty and McKeen, 1964b, Jhooty and McKeen, 1964a) and reduces when RH is below 95%. Peries (1961) found that, after germination, humidity does not affect the development of the fungus.

Whilst conidia need a high RH to germinate, exposure to free water can have a detrimental effect on disease progress (Peries, 1961). Even short periods of immersion in water inhibit germination of the majority of conidia (conditions are summarised in Table 2).

Table 2. Summary of conditions that affect 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, 1961). 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 released 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 (1961) tested the germination and growth of *P. aphanis* (syn. *S. macularis)* on several different varieties of strawberry. He found that some varieties were more susceptible than others, but none of them were fully resistant (immune). He found that the least susceptible varieties had higher levels of cutin acids and suggested that these are potentially fungitoxic. Cuticle penetration is achieved by mechanical pressure (Peries, 1961). 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, 1961, Rashid Khan, 1960, Salmon, 1900). During the experiments done by (Peries, 1961), perithecia were only witnessed under one set of conditions: 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). The terms ‘cleistothecium / cleistothecia and perithecia’ 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).

Rule based prediction systems

Many different types of model have been developed for the study of plant pathogens. For example:

- Analytical models which use explicit formulae to derive predicted values or distributions (written as algebraic expressions),
- Simulation models of the population dynamics of the pathogen and host, generally requiring less mathematical sophistication than the analytical models
- Expert systems which mimic the processes employed by a human expert
- 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 utilise a rule based approach, in the 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)

Sensitivity analysis

Sensitivity analysis is used to identify the parameters of a model (prediction system) that have the greatest effect on the output (Norton and Mumford, 1993, Sgrillo *et al.*, 2005). This is achieved by keeping some parameters constant while altering others and measuring the outputs, which can then be analysed for differences (Andrade-Piedra *et al.*, 2005, Berger *et al.*, 1995, Willocquet and Savary, 2004) and ranked for importance. Logically, the most sensitive parameters are those that cause a large change in output for a small change in their value.

Materials and Methods

Field sites

A field site was established on a commercial holding near Sevenoaks, Kent (Grid reference: TQ 626 535). The site consisted of 1 Spanish tunnel (48m × 6m, covered with normal plastic sheeting). The site contained second season Flamenco in raised beds. The plants were managed commercially in the first season and had severe powdery mildew symptoms (grower observation). The tunnel consisted of 4 raised beds. Plants were separated by 44cm within rows and the distance between rows was 30cm. The tunnel was covered for all the time that it was used as an experimental site and was vented and irrigated according to normal farm practices.

All other sites referred to in this report were commercial sites from which data (temperature, relative humidity, leaf wetness and spray schedules) were obtained directly from the growers.

Dose efficiency

After consultation with growers, six fungicides were selected for evaluation against powder mildew (Table 3). The fungicide active ingredients, bupirimate, boscalid + pyraclostrobin, myclobutanil and quinoxyfen are approved for use on strawberry powdery mildew. These fungicides have different modes of action, so could potentially be combined within a control program designed to minimise selection pressures for fungicide insensitivity. Pyraclostribin was included so that efficacies of boscalid and pyraclostrobin could be quantified separately. Cyprodinal + fludioxnil are the active ingredients of a new proprietary formulated product with activity against *Botrytis,* and was therefore included to determine any effect against powdery mildew.

Fungicides were applied at full and quarter label rates, and were compared to an untreated control (*i.e.,* zero rate). Treatments were arranged in a randomised block design of three replicates on the commercial holding near Sevenoaks. Plots consisted of 20 plants (2 rows x 10 plants). Treatments were applied using a Hardi Backpack Sprayer BP 20, calibrated in accordance with NPTC recommendations.

On each of ten plants, chosen randomly from each plot, three leaves were tagged and scored weekly for symptoms of powdery mildew. The first leaf (new, but fully emerged) was tagged and scored the day before the treatments were applied, the second leaf (newest emerged leaf when treatments were applied) was tagged at the same time, but not scored until one week after the treatments were applied. The third leaf (newest emerging leaf 1 week after treatments were applied) was not scored until the third week after the treatments were applied.

Table 3. Products tested for dose efficieny against strawberry powdery mildew

Chemical name	Trade name	Full rate	Quarter rate
Bupirimate	Nimrod	1.4l/ha 550l/ha	0.7l/ha 550l/ha
Boscalid + Pyraclostrobin	Signum	5ml/l 300l/ha	2.25ml/l 300l/ha
Cyprodinil + Fludioxnil	Switch	1kg/ha 550l/ha	0.5kg/ha 550l/ha
Myclobutanil	Systhane	0.45l/ha 500l/ha	0.225l/ha 500l/ha
Pyraclostrobin	Comet	1.25l/ha 200l/ha	0.63l/ha 200l/ha
Quinoxyfen	Fortress	1ml/l 300l/ha	0.5ml/l 300l/ha

Comparison of venting practice

Venting practice was compared in two commercially managed tunnels on separate sites: located in South Staffordshire and North Cambridgeshire. The grower managed the tunnels at the Staffordshire site as normal. At the Cambridgeshire site, tunnels were managed with particular attention to venting, which aimed to control the temperature close to the range ideal for fruit production. A venting plan was produced each night (or early next morning) for the next day, by reference to the temperature forecast. Temperature predictions of 12-15^{o}C acted as a trigger to plan tunnel venting. Venting was implemented each day in accordance with the plan and temperatures and relative humidity were measured within and outside the tunnels. Differences in the tunnel conditions were compared using ANOVA in SPSS for windows 11.5.0, SPSS Inc.

Rule based prediction system

Development of rule based prediction system

The literature was reviewed to examine the range of conditions known to affect 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. The parameters defined initially for use in the prediction system were developed from this comparison.

Comparison of predicted high-risk periods with first symptoms

Within commercially managed tunnels, the conditions (temperature, relative humidity and leaf wetness) and the dates that first symptoms of *P. aphanis* developed were collected as part of the work presented in previous reports for HDC project SF 62. Conditions measured within the commercially managed tunnels were inputted into the prototype system, which used the initial parameter estimates (Table 6). The first high-risk period predicted by the system was compared to the dates when the first symptoms of *P. aphanis* infection developed.

The predicted high-risk periods were close but not identical to the actual dates that symptoms developed. As a consequence, the initial parameters were revised, so that the first predicted high-risk period matched development of symptoms observed in the field. This revised version of the prediction system was used in subsequent evaluations.

Sensitivity analysis of prediction system parameters

Sensitivity analysis was used to determine which of the revised parameters had the greatest effect on the output (*i.e.,* the number of high-risk periods predicted). Environmental data collected from the field was inputted into the prediction system. 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. All the parameters were tested through the prediction with and without leaf wetness data present; because currently this measurement is likely to be unavailable to most growers.

Sensitivity analysis was also used to test the interactions between selected pairs of parameters (Table 5). The same values were used as when a single parameter was being tested (Table 4). At each increment of the first parameter of the pair, the second parameter was altered to obtain the number of predicted high risk-periods for each increment of the second parameter. Again each pair of parameters was tested with and without leaf wetness data.

Table 4. Prediction system parameters, range of values and increments used for sensitivity analysis**.**

	Range of values	Increment
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 5. 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)

Comparison of predicted high-risk periods with grower applications

Spray schedules were obtained for commercially managed fields along with the corresponding measurements from on-farm weather stations. Data from the weather stations (starting from 1 January) were inputted into the prediction system. The number of high-risk days predicted (equivalent to the fungicide applications required), and the dates that they occurred, were compared with the number and dates of treatments applied by the growers for control of *P. aphanis*.

Use of rule based prediction system in commercial sites

During the 2007 season, growers at the Cambridgeshire and Staffordshire sites, each managed powdery mildew in half of a field using their normal practice and the other half of the field according to the prediction system. In both cases, the crops were day neutral (everbearers). Predictions were made from 1 July, until the end of the season. The prediction system was informed by data collected from environmental sensors (temperature and relative humidity) located within the fields.

Results

Dose efficiency

Fungicides were applied to the site when there were visible symptoms of strawberry powdery mildew (cupping and mycelium). The levels of symptoms decreased after the treatment on all plots, including the untreated. The levels of symptoms did not increase again over the duration of the experiment. There were no statistical differences between any of the plots.

Comparison of venting practice

For both sites, the average temperatures outside the tunnels were in the range 16-18^{o}C during the day and 12-14^{o}C during the night. The average relative humidity outside the tunnels was in the range 70-75 during the day and 83-87% during the night. Despite the difference in location, there were few statistical differences between these external conditions at the two sites (Fig 1).

Inside the tunnels at both sites, the average temperatures were 18-21 and 13-15^{o}C during the day and night respectively (Fig.2). The average relative humidity inside the tunnels during the day and night respectively were 92-99 and 88-96% for the Staffordshire site, compared to 66-72 and 84-87% at the Cambridgeshire site.

These differences between sites in the relative humidity inside the tunnel were significant between the tunnels (Fig 2). At the Staffordshire site, relative humidity and daytime temperature were significantly greater inside than outside the tunnel (Fig 3). Whilst the daytime temperature was significantly hotter inside the tunnel than outside the tunnel at the Cambridgeshire site, relative humidity was the same inside and outside the tunnel during August and September (Fig 4).

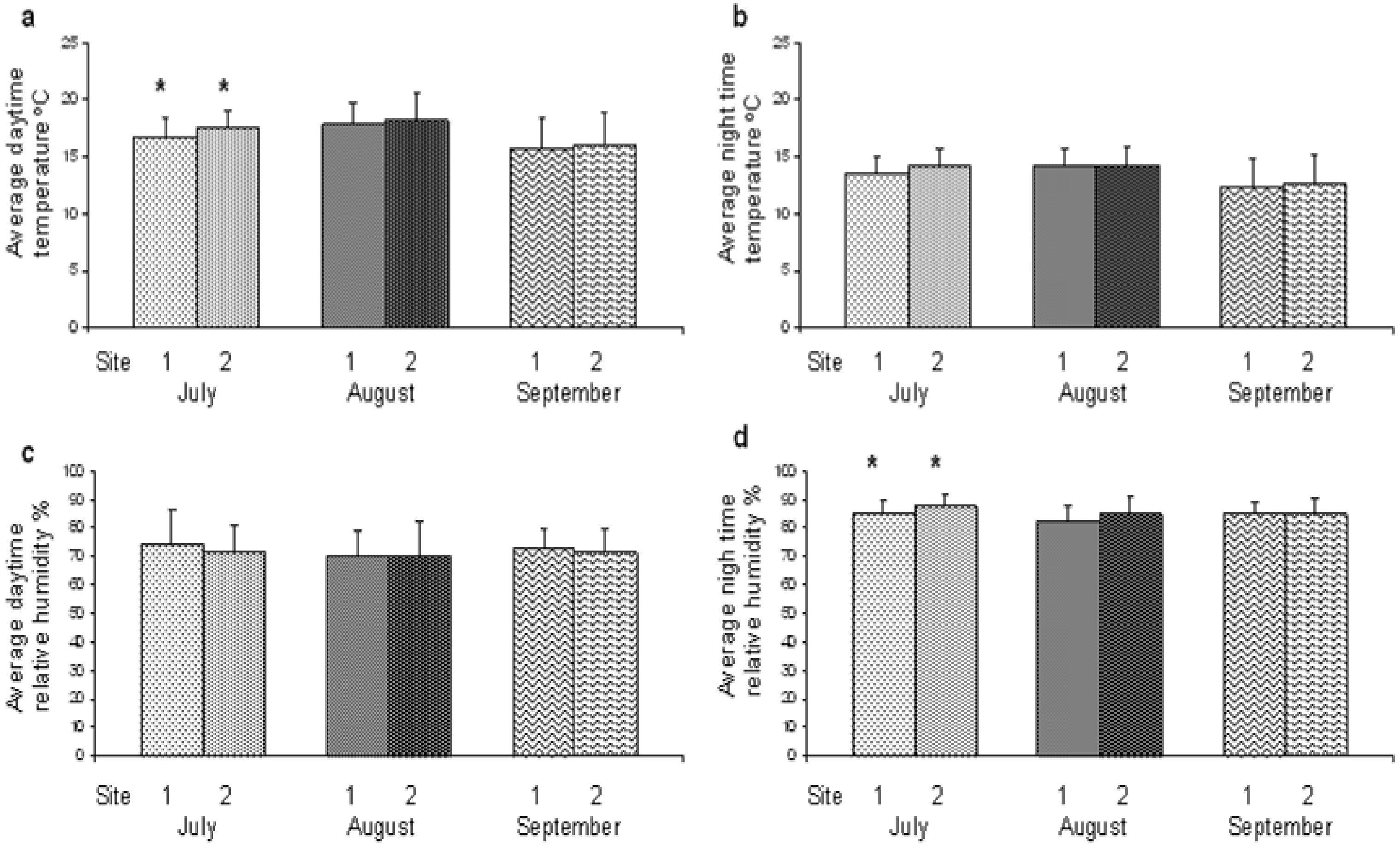


Fig. 1. For sites located in Staffordshire [1] and Cambridgeshire [2], comparison of average conditions outside tunnels, for July, August and September for a) daytime temperature b) night time temperature c) daytime relative humidity d) night time relative humidity. Significant differences ($p<0.05$ ANOVA) between the two sites in a given month are denoted by *.

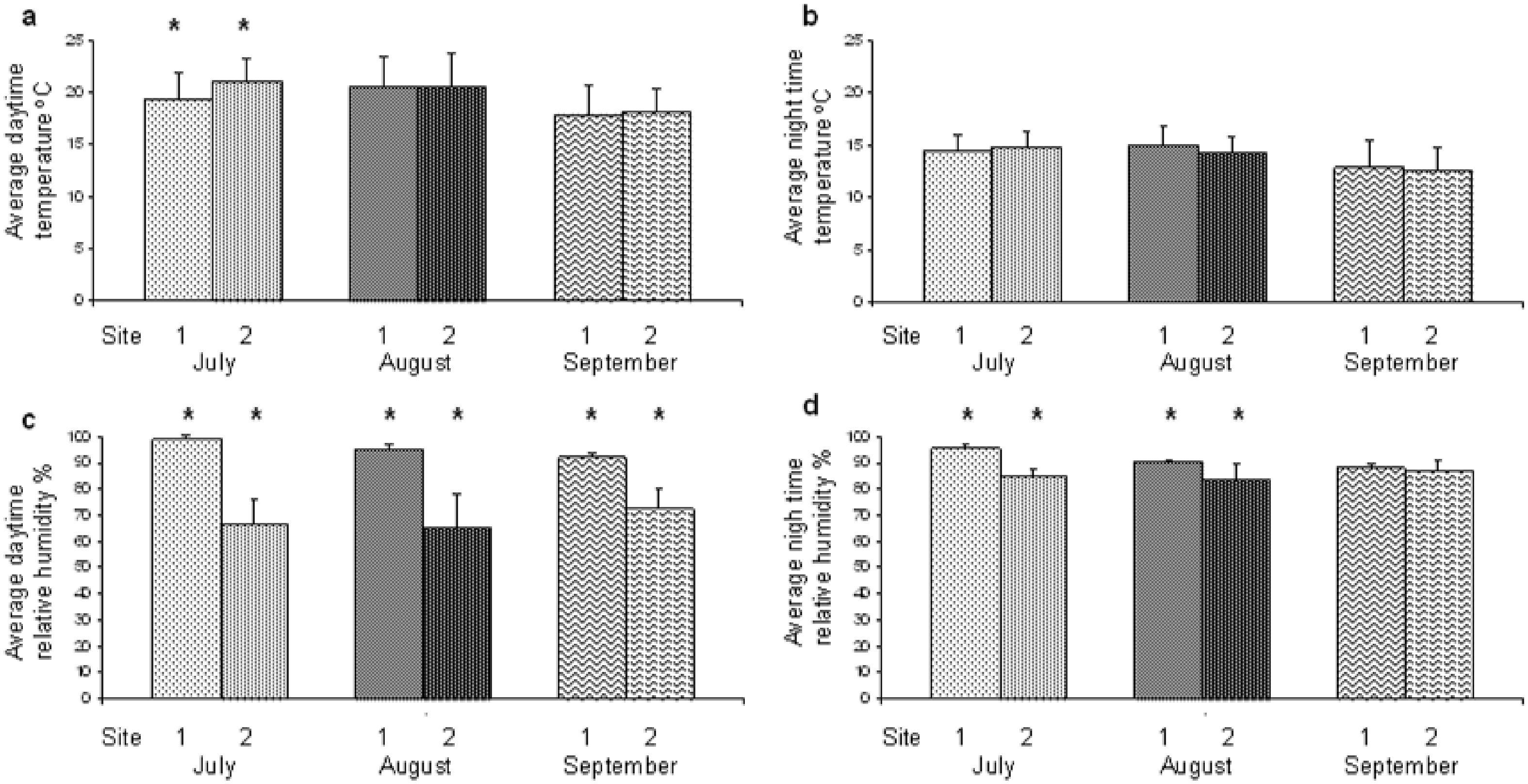


Fig. 2. For sites located in Staffordshire [1] and Cambridgeshire [2], comparison of average conditions inside tunnels, for July, August and September for a) daytime temperature b) night time temperature c) daytime relative humidity d) night time relative humidity. Significant differences ($p<0.05$ ANOVA) between the two sites in a given month are denoted by *.

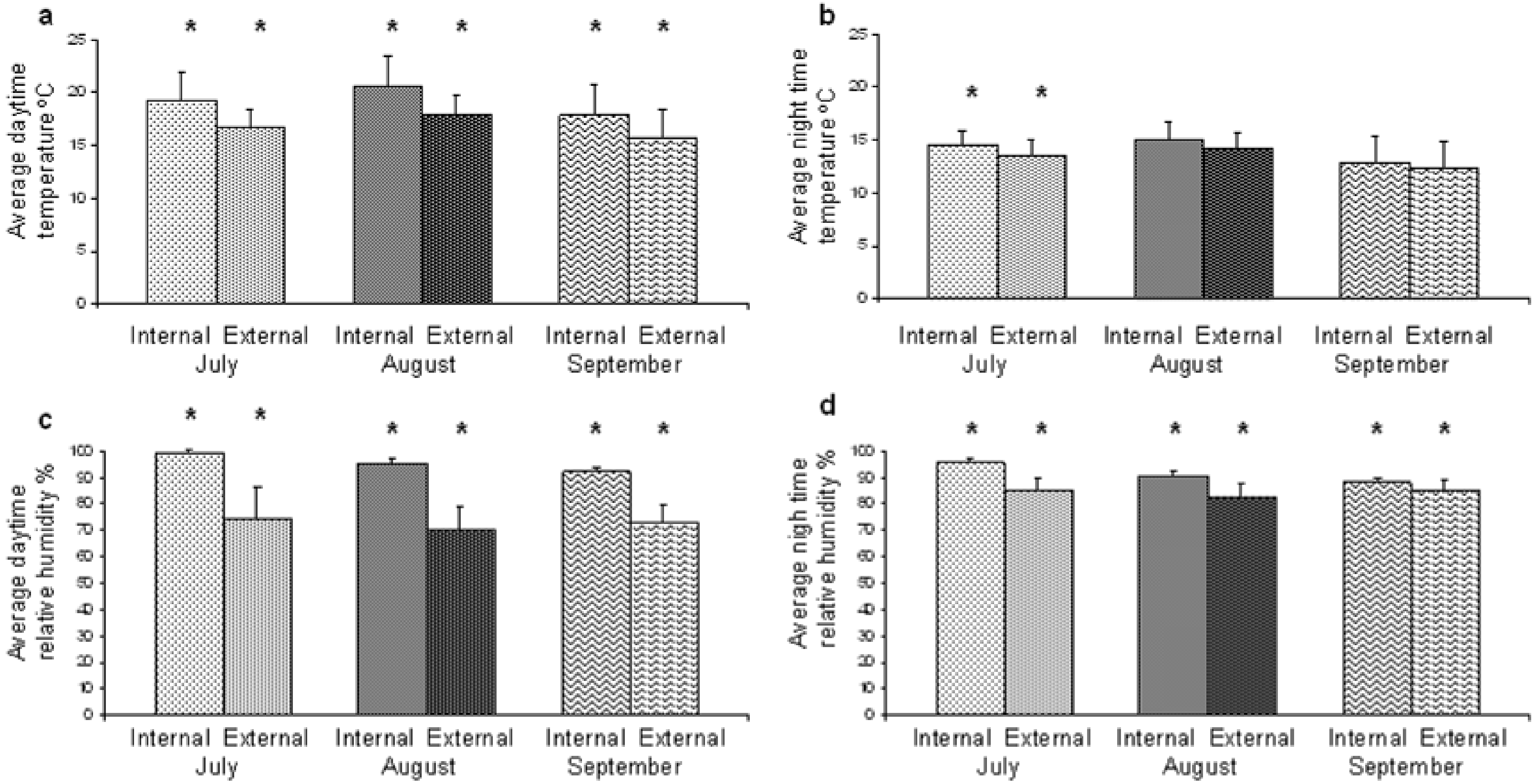


Fig. 3. Comparison of average conditions inside (internal) and outside (external) the tunnels at the Staffordshire site for July, August and September for a) daytime temperature b) night time temperature c) daytime relative humidity d) night time relative humidity. Significant differences (p<0.05 ANOVA) between the internal and external conditions for a given month are denoted by *.

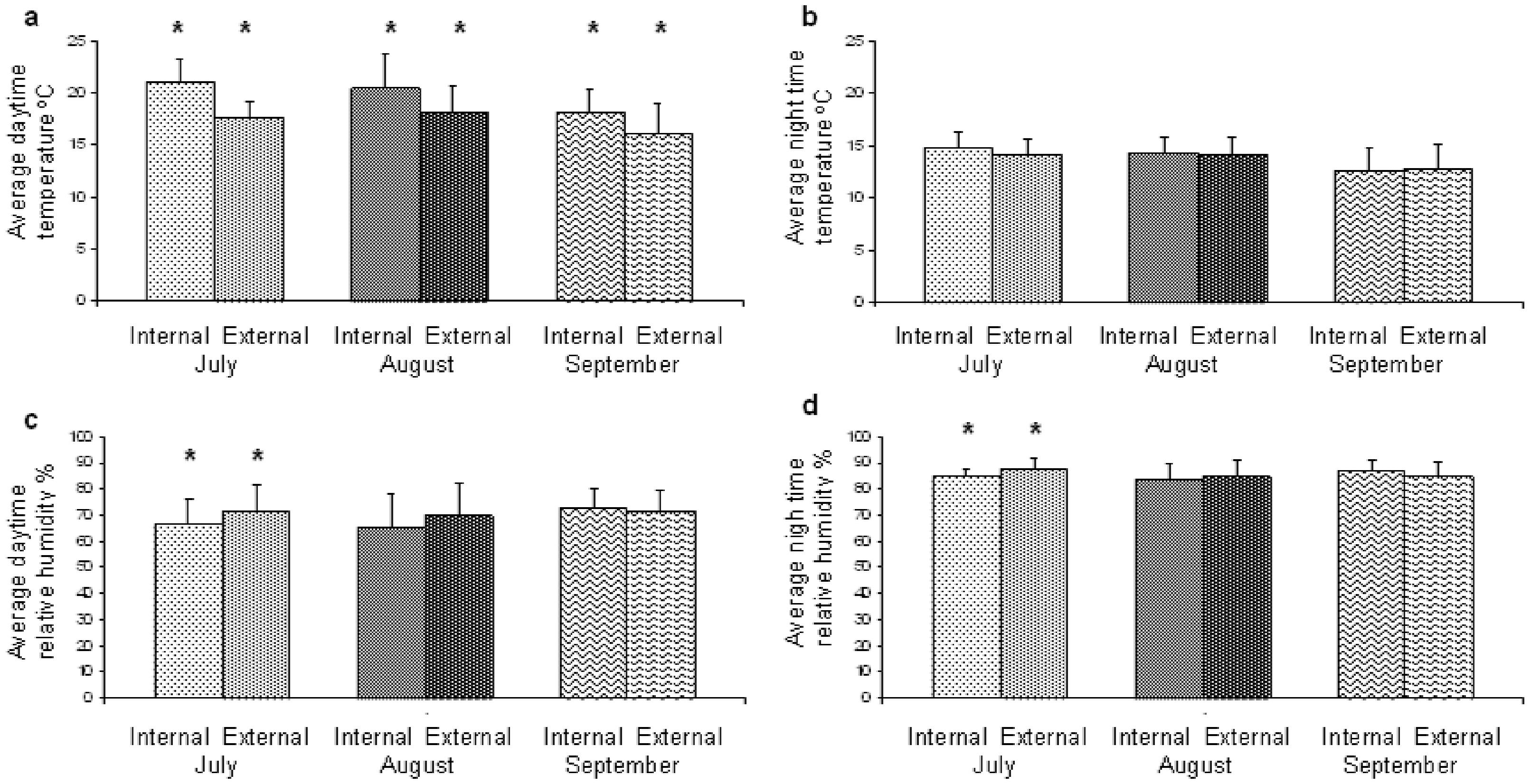


Fig. 4. Comparison of average conditions inside (internal) and outside (external) the tunnels at the Cambridgeshire site for July, August and September for a) daytime temperature b) night time temperature c) daytime relative humidity d) night time relative humidity. Significant differences ($p<0.05$ ANOVA) between the internal and external conditions for a given month are denoted by *.

Rule based prediction system

Development of rule based prediction system

The literature review provided a large amount of information about the range of conditions affecting the infection processes and subsequent growth and development of *P. aphanis* (Table 2). Germination of conidia is limited by temperature, relative humidity and leaf wetness, whereas the rate of mycelial growth and sporulation is primarily limited by temperature ((Amsalem *et al.*, 2006, Blanco *et al.*, 2004, Jhooty and McKeen, 1964b, Jhooty and McKeen, 1965, Miller *et al.*, 2003, Peries, 1962a). In the laboratory, development from conidial germination to visible symptoms of *P. aphanis* requires 144 hours of suitable conditions (*i.e.,* that range within the maxima and minimum environmental limits, see Tables 1 & 2). Infections established as viable mycelium are able to generate further inoculum after 84 hours of suitable conditions (Table 1; (Peries, 1962b).

Knowledge about the development time of *P. aphanis* gained from the literature review and field observations was used to inform the development and parameterization (Table 6) of rule-based prediction system, which identifies when strawberry plants are at greatest risk of infection by the pathogen.

The life cycle of *P. aphanis* can be divided into two parts:

- Germination of the conidia
- Growth of the fungus and sporulation.

Germination of a spore requires a total of 6 hours (Table 1) when temperature is within the range 17.5 - 30°C, relative humidity is greater than 60% and leaf wetness is less than 95% (Table 6). Growth and development of a germinated spore (up to and including spore release) requires a further 138 hours (Table 1) when the temperature is greater than 16°C and less than 30°C (Table 6). The prediction system uses these lifecycle time requirements as the basis for predicting crop infection risks. More specifically, the system calculates the time elapsed when conditions are suitable for germination, maturation and generation of new inoculum. When conditions are unsuitable for germination or growth of *P. aphanis i.e.,* outside the defined environmental thresholds, the disease cycle is stationary. The disease cycle continues from the point reached previously when environmental conditions are within the threshold bounds. Output of the prediction system is presented as percent completion of the total hours needed for a conidium to reach maturity *i.e,* percent completion of a full disease cycle.

Comparison of predicted high-risk periods with first symptoms

Environmental conditions within four commercially managed tunnels were recorded in the preceding HDC project SF 62. Two tunnels had established crops (Mereworth 04 and Wisbech A 05) and two were newly planted (Mereworth 05 and Wisbech 06). The environmental data collected at these sites were computed using the prediction system to identify the first high-risk days, and these were compared with appearance of first symptoms observed within each tunnel (Figs. 5, 6, 7 and 8). A predicted high-risk day is indicated in Figures 5-8 when the line showing the number of hours suitable for maturity reaches 100%.

For both of the established fields Mereworth 04 (Fig. 5) and Wisbech A 05 (Fig 6), the predicted high-risk days were later than the actual development of first symptoms: the first predicted high-risk days did not occur until after most plants had symptoms at Mereworth 04 (Fig. 5) and when incidence was approximately 40% at Wisbech A 05 (Fig. 6).

For both the newly planted fields, Mereworth 05 (Fig. 7) and Wisbech 06 (Fig. 8), the predicted high-risk days occurred before the first symptoms were visible in the tunnels. Two high-risk days were predicted for Mereworth 05 (Fig. 7), before the third predicted high-risk day coincided with the first development symptoms in the tunnel. For Wisbech 06 (Fig. 8) the first predicted high-risk day occurred before symptoms developed, and the second predicted high-risk period soon after they became visible.

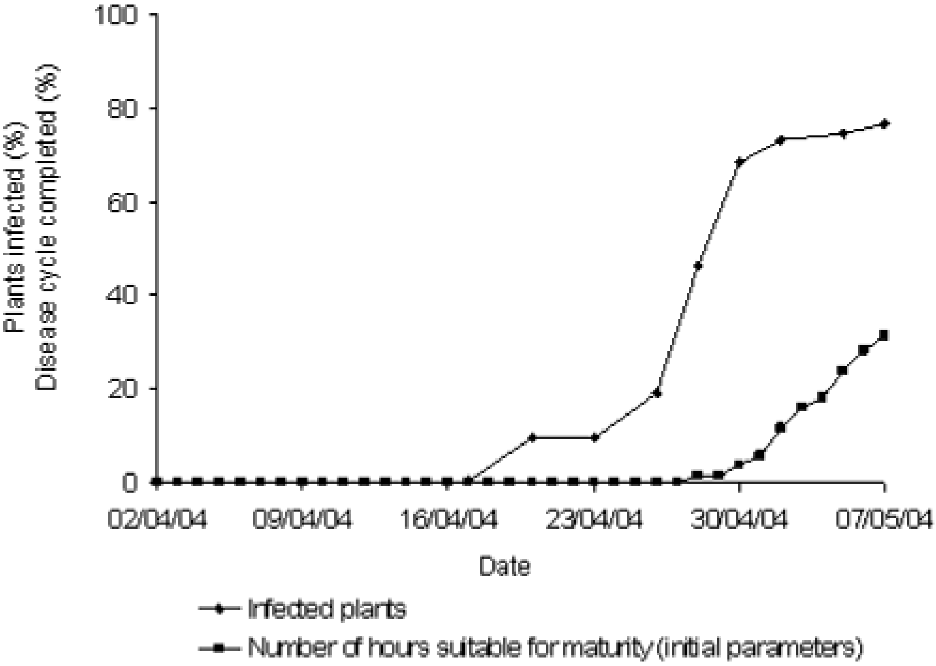


Fig. 5. Disease development data for Mereworth 04 (established field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed.

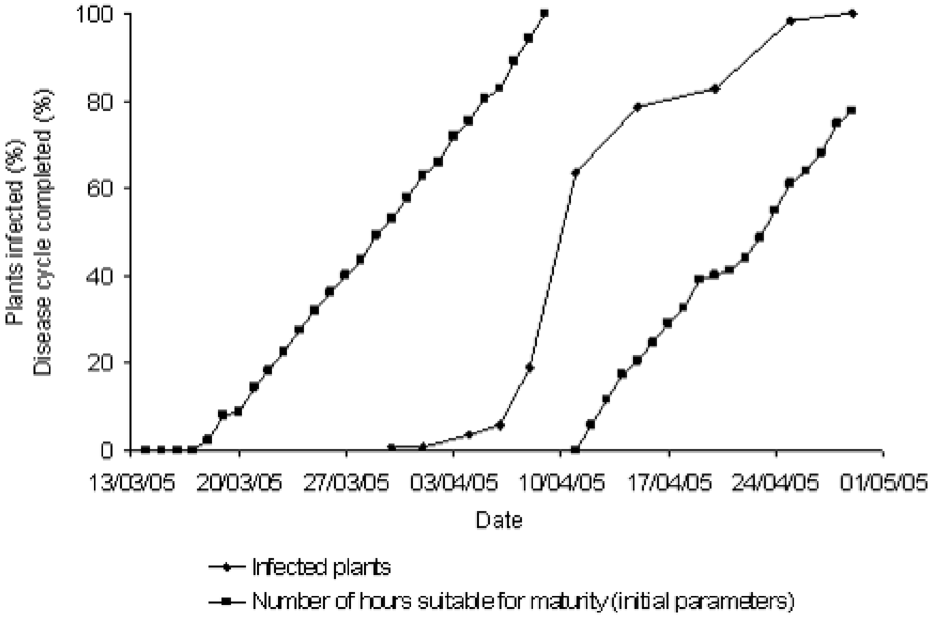


Fig. 6 Disease development data for Wisbech A 05 (established field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed.

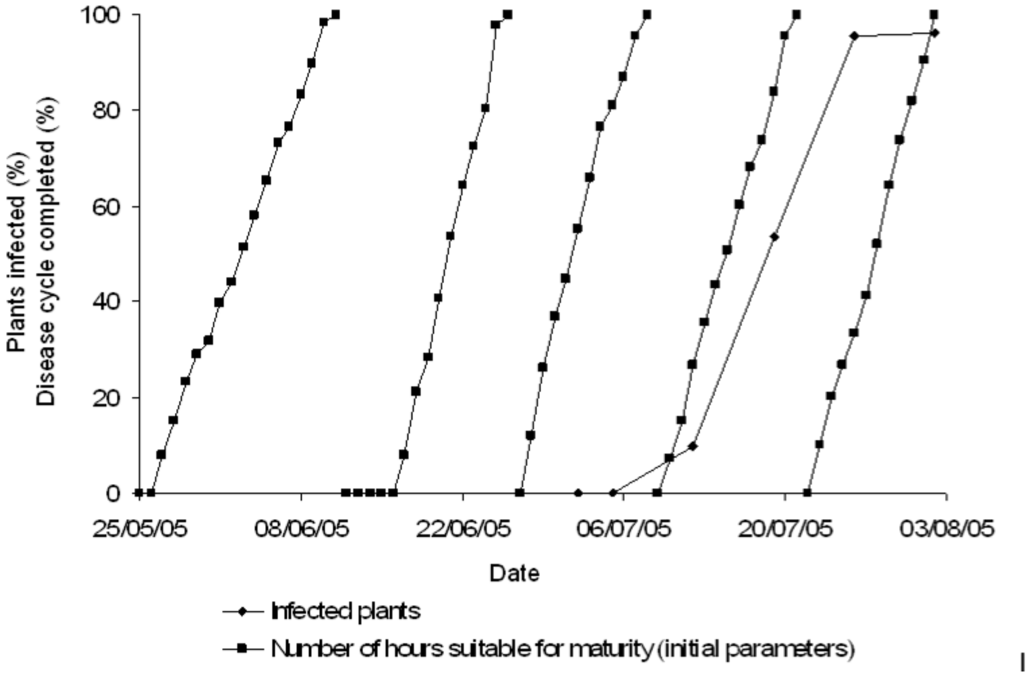


Fig. 7 Disease development data for Mereworth 05 (newly planted field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed.

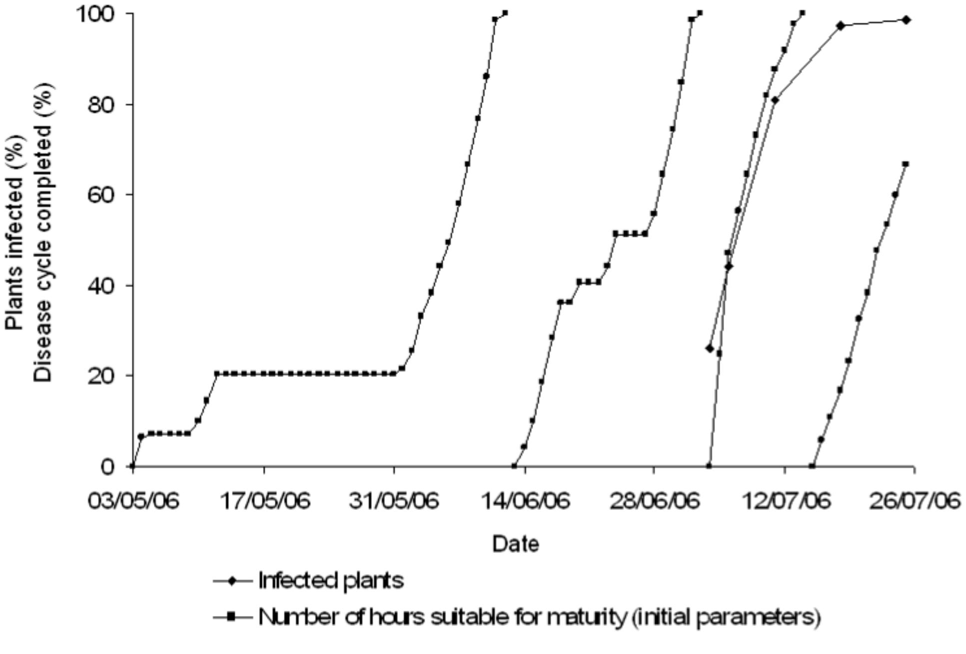


Fig. 8 Disease development data for Wisbech 06 (newly planted field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed.

Prediction system parameters were revised slightly, so that the first high-risk days predicted corresponded more closely with the appearance of first symptoms in the crops (new parameters, Table 6). Explicitly, to allow spore germination the revised prediction system requires a total of 6 hours (Table 1) when temperature is within the range 15.5 - 30°C, relative humidity is greater than 60% and leaf wetness is less than 95% (Table 6). In addition, to allow further growth of the germinated spore (up to and including spore release), requires temperature in the range 18 - 30°C for 78 hours when the first infection could arise from over-wintered mycelium (the first disease cycle). Otherwise, the same temperatures are required for 138 hours in newly established crops and in established crops after the first disease cycle (Tables 1 & 6).

Using the revised system, the predicted high-risk days were closer to the dates when symptoms first appeared in the crops (Figs. 9 - 12) than achieved using the original parameters (Figs. 5 - 8). The first predicted high-risk days for the established sites Mereworth 04 (Fig. 9) and Wisbech A 05 (Fig. 10) were within a few days of the appearance of symptoms in the crops. For the newly planted sites Mereworth 05 (Fig. 11) and Wisbech 06 (Fig. 12), the symptoms appeared in the field when the second high-risk day was predicted.

Table 6. Parameters from literature and initial field observations (initial parameters) and adjusted values after analysis of disease development data (revised parameters) for the prediction system

	Initial parameters[1]	Revised 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 (minimum) [%]	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 [hours]	*na*	6 + 78
No. of hours[2] to maturity germination and growth *established* field after 1st infection [hours]	*na*	6 + 138
No. of hours[2] to maturity germination and growth *new* field all infections [hours]	*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)

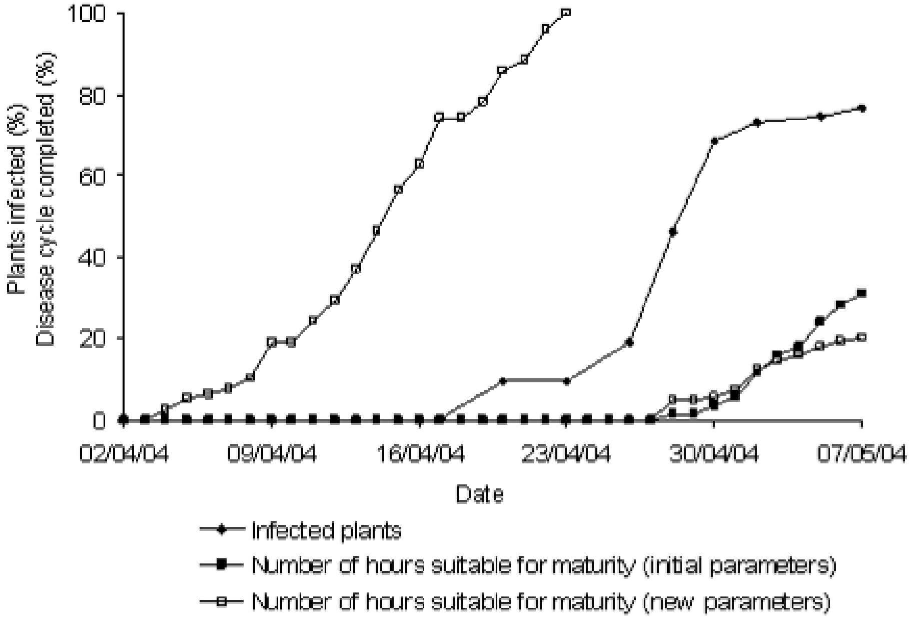


Fig. 9. Disease development data for Mereworth 04 (established field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed.

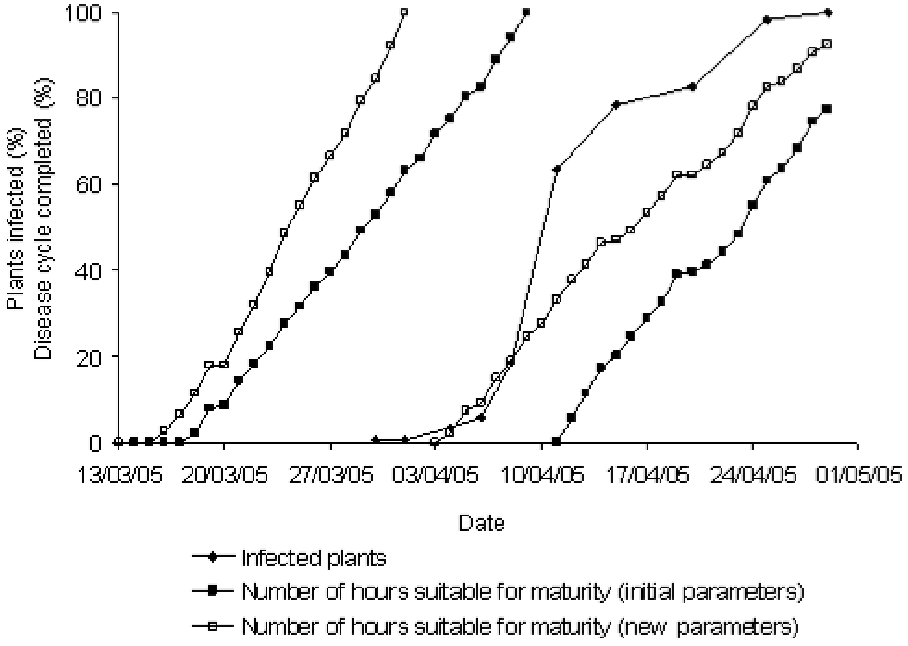


Fig. 10 Disease development data for Wisbech A 05 (established field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed.

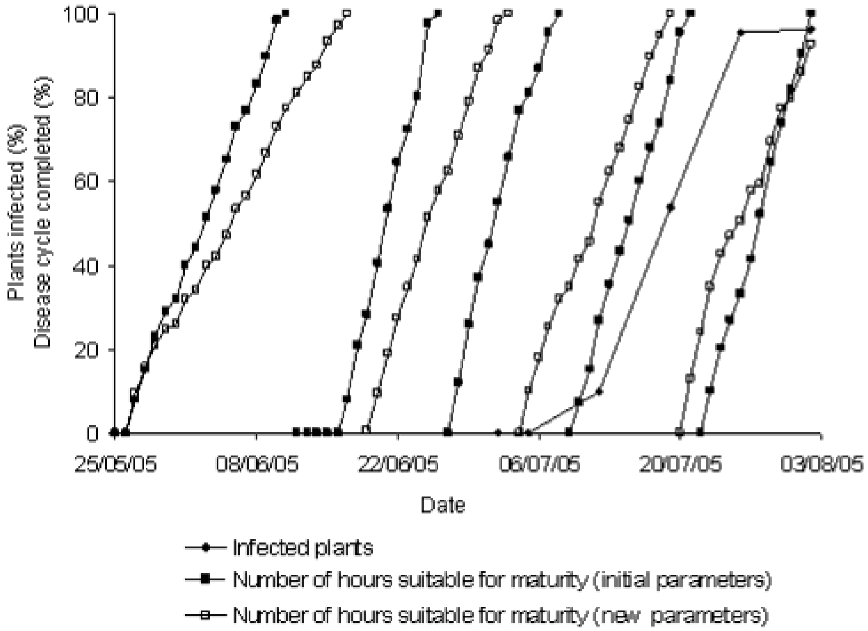


Fig. 11 Disease development data for Mereworth 05 (newly planted field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed.

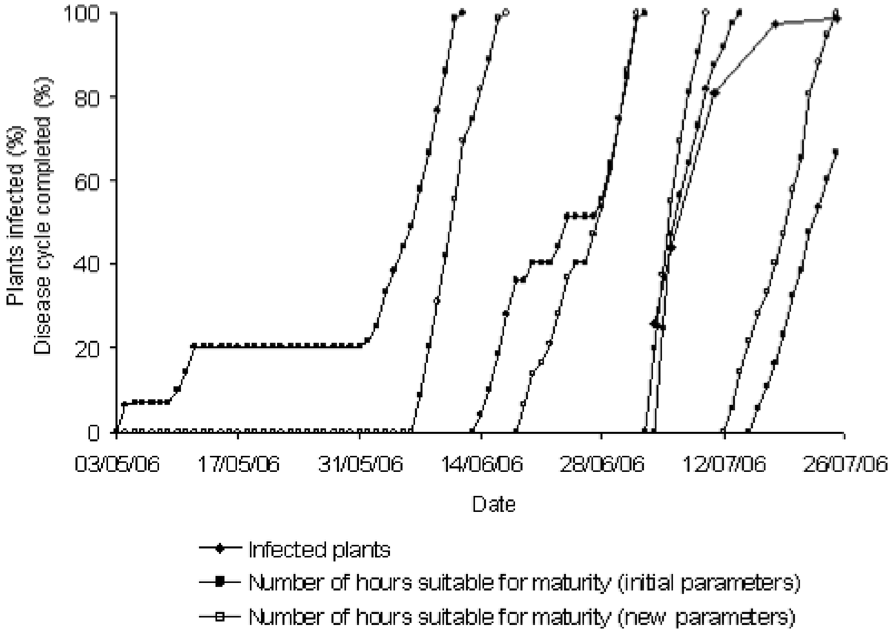


Fig. 12 Disease development data for Wisbech 06 (newly planted field) showing plants infected (%) and the predicted completion of a disease cycles (%). A new cycle is initiated as soon as the previous one is completed.

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 13, 14 and 15). Alteration of the growth temperature parameter was the only change that resulted in different output response across the entire range of values tested (Fig 13). When the other parameters were altered, the resulting output response lines were mainly flat. For parameters describing germination temperature and relative humidity, small changes in the slope of the output response line were found at the upper-end of the range of values tested (Figs 13 and 14). For maximum growth temperature there was a slight slope of the output response line at the lower-end of the range. No change in output response was found the range of the parameter values tested for maximum germination temperature (Fig 15).

Using leaf wetness data, the number of predicted high-risk days decreased from 11 to 5 when the germination temperature was increased across the range tested (Fig 13) and from 11 to 7 high-risk days without leaf wetness data. The number of predicted high-risk days decreased from 22 to 4 when the growth temperature was increased (Fig 13), and from 23 to 4 without leaf wetness. The number of high-risk days reduced from 11 to 4 (5 with out leaf wetness data) when the relative humidity was altered (Fig 14). When the leaf wetness was altered, only a small change in the number of high-risk days was found: an increase 10 to 11 (Fig 14). The number of predicted high-risk days did not change when the maximum germination temperature (with or with out leaf wetness data) was altered (Fig 15). The number of predicted high-risk days went from 8 to 12 when the maximum growth temperature (with or with out leaf wetness data) was altered (Fig 15).

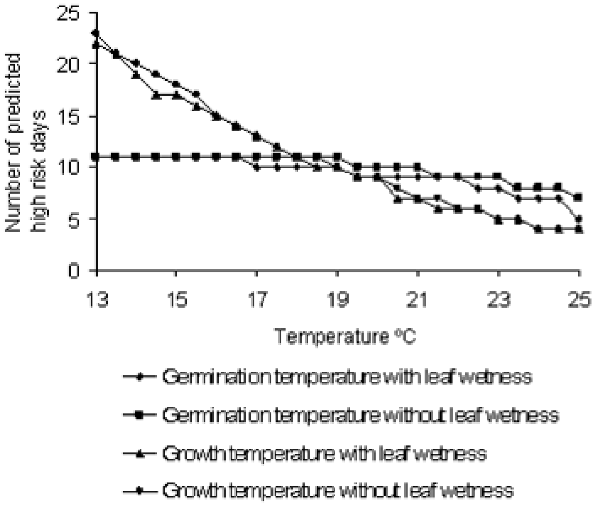


Fig. 13 Number of high risk days predicted by the prediction system when germination and growth temperatures were altered with and with out leaf wetness data

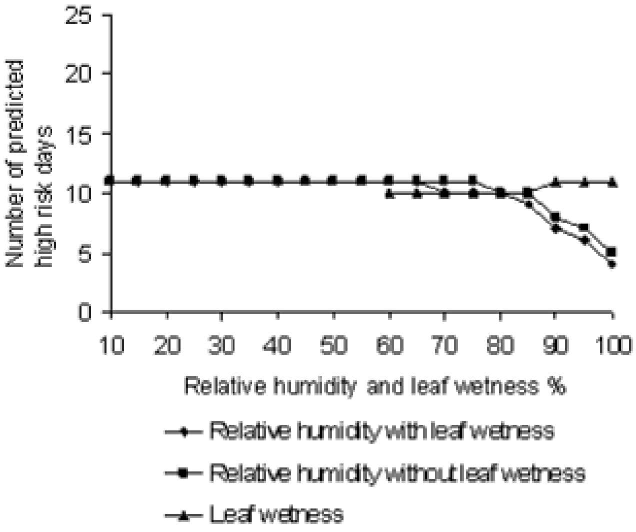


Fig. 14 Number of high risk days predicted by prediction system when the relative humidity was altered with and with out leaf wetness data and for when leaf wetness was altered

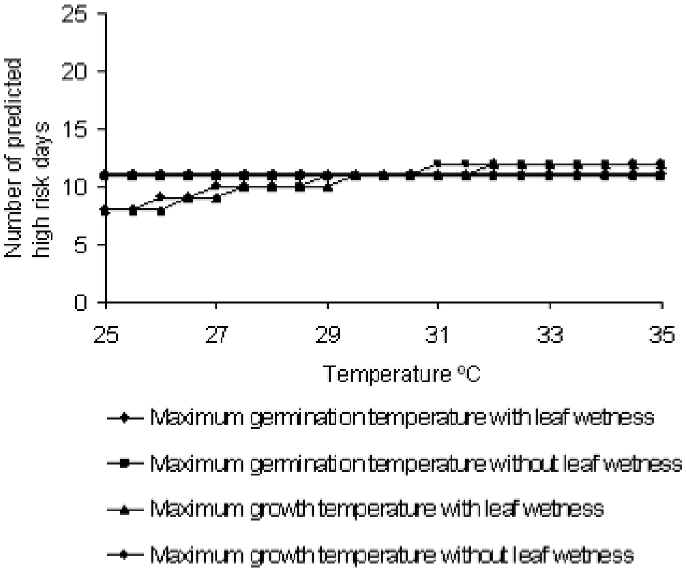
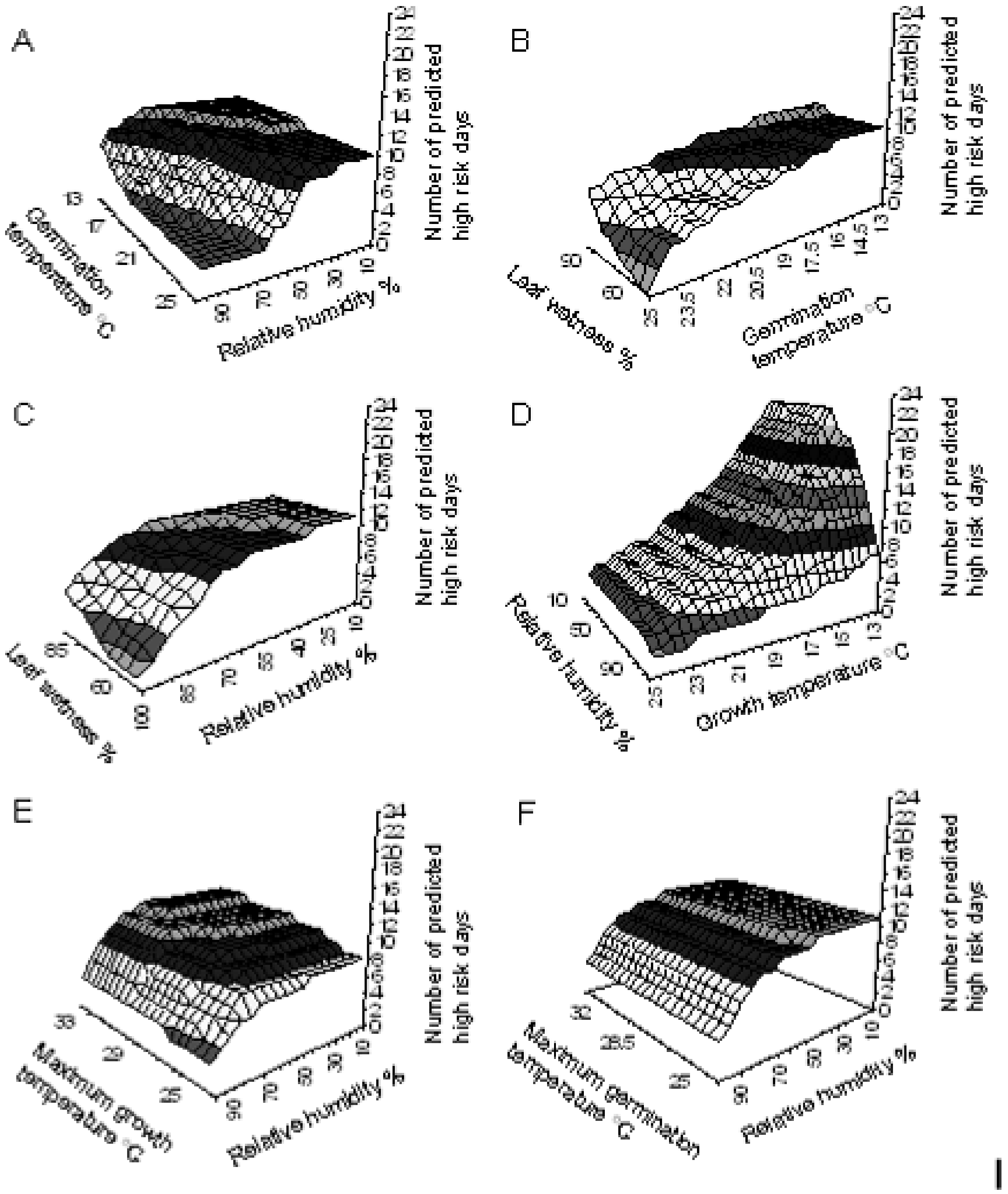


Fig. 15 Number of high risk days predicted by prediction system when maximum germination and growth temperatures were altered with and with out leaf wetness data

As with the alteration of a single parameter the greatest change in the output response was produced when the growth temperature parameter was one of the two parameters altered (Figs 16 and 17). When growth temperature was not one of the parameters, the response was generally flat (Figs 16 and 17).

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 for change in a germination parameter, and 22 to 23 high-risk days for a growth parameter was altered) as when one parameter was altered (Figs. 16 and 17). Greater variability was found in the minimum number of high-risk days predicted, with several combinations resulting in no high-risk days (Figs. 16 and 17). 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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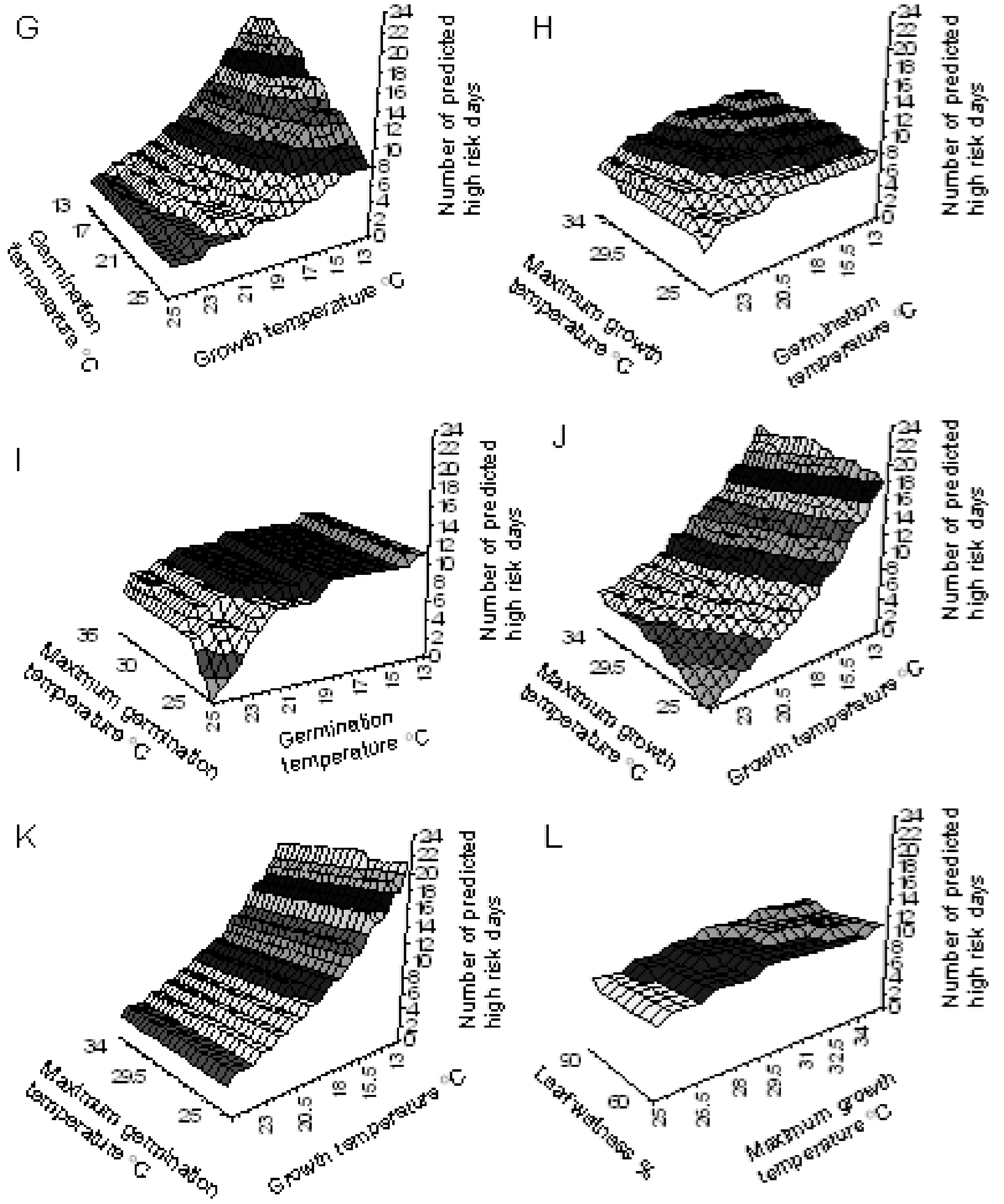

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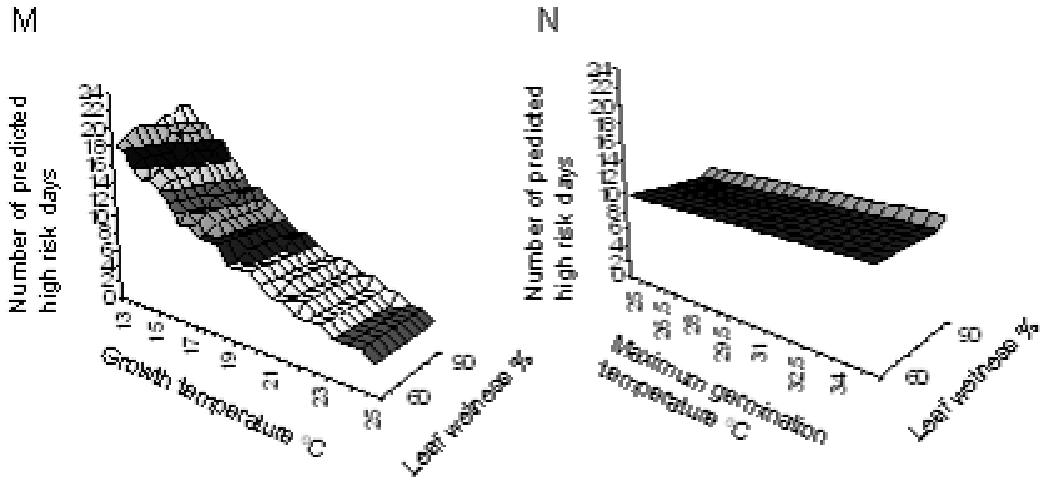
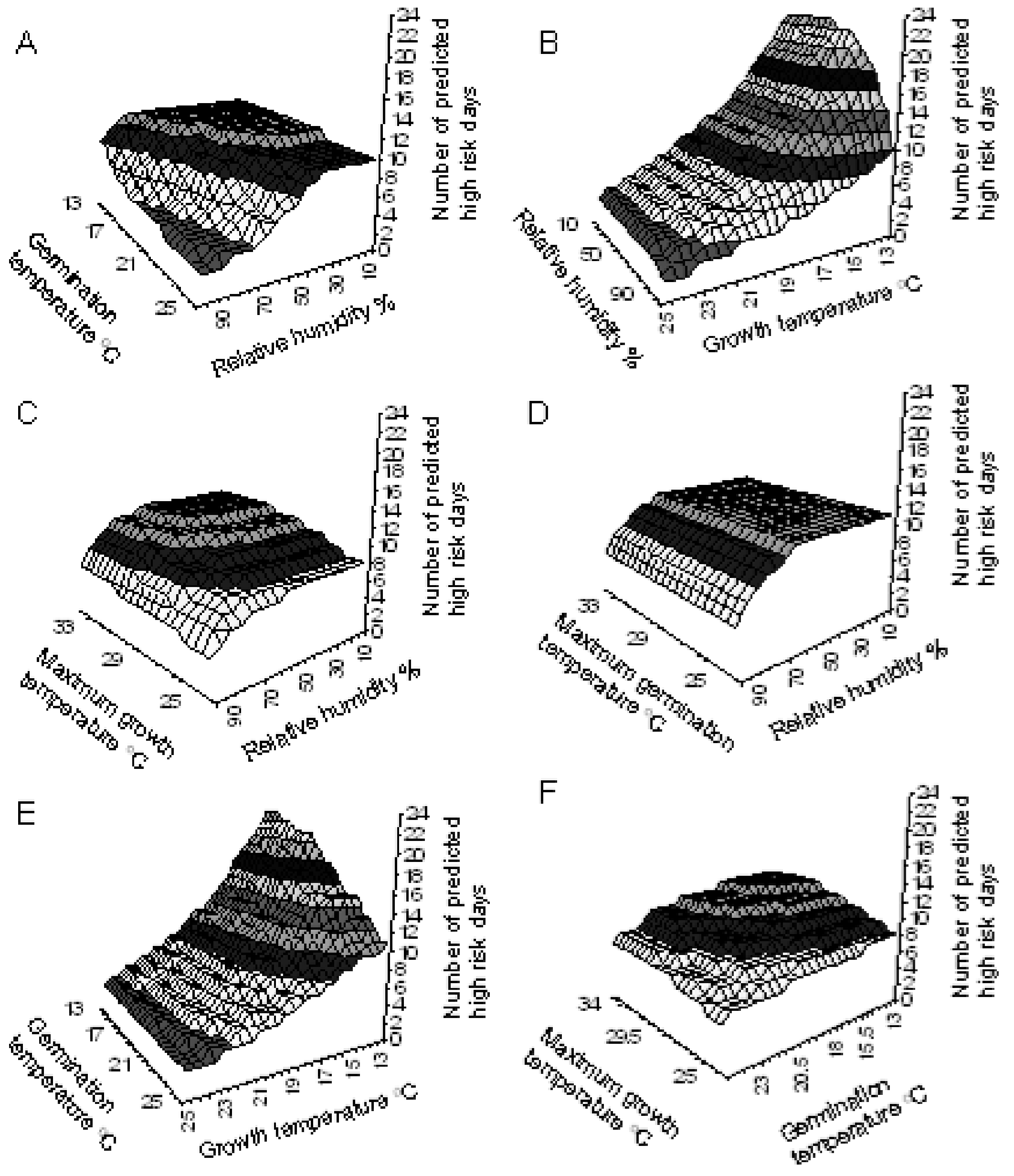


Fig. 16 Number of high-risk days predicted when combinations of two parameters were altered: (A) germination temperature by relative humidity (B) leaf wetness by germination temperature (C) leaf wetness by relative humidity (D) relative humidity by growth temperature (E) maximum growth temperature by 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.



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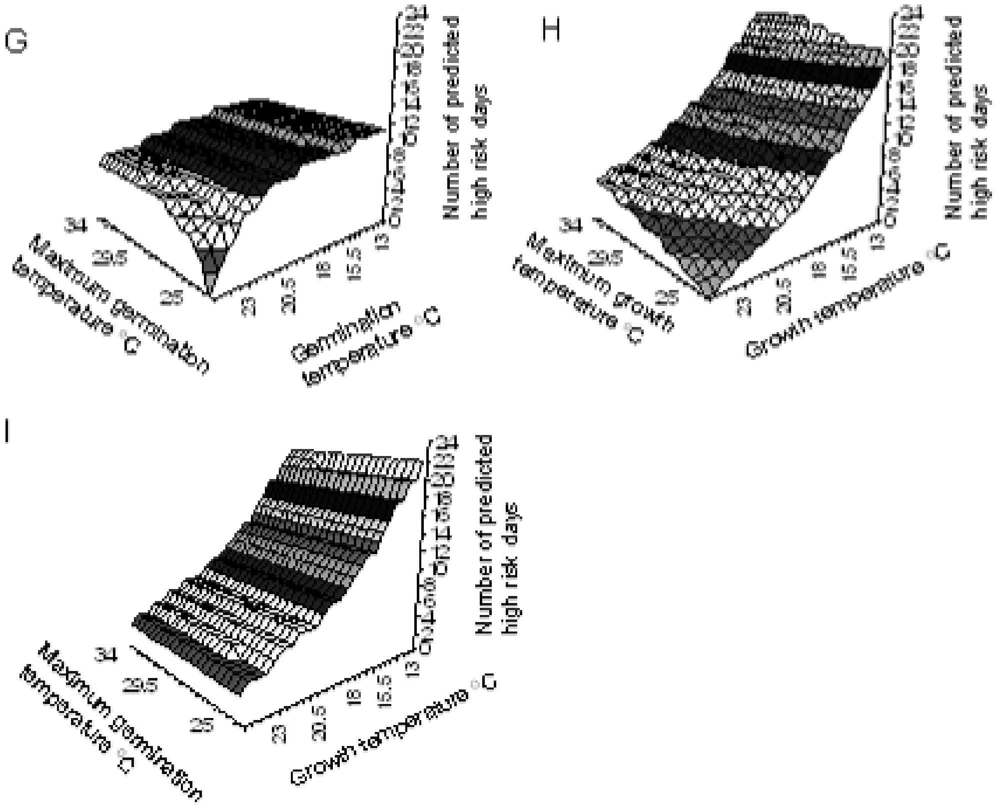

Fig. 17 Number of high-risk days predicted when combinations of two parameters were altered with out leaf wetness data: (A) germination temperature by relative humidity (B) Relative humidity by growth temperature (C) maximum growth temperature by relative humidity (D) maximum germination temperature 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.

Comparison of predicted high-risk periods with grower applications

Historical weather and crop management records were collected for six commercial crops. These data were used to test whether high-risk days predicted by the system corresponded with fungicide treatments in commercially managed crops.

A high-risk day is predicted after completion of a complete disease cycle (*i.e.*, from establishment of infection to sporulation*).* Therefore, the prediction of a high-risk day acts as a trigger for the grower to monitor the crop more closely and consider crop treatment, dependant upon previous applications. The predicted high-risk days were compared to the treatment records for the crops, which is equivalent to the risk perceived by the crop manager.

In all cases tested, the system predicted the same number or fewer high-risk days than perceived by the crop managers. For the two day-neutral (everbearer) crops, ten and seven treatments were applied, whereas the system predicted eight and seven high-risk days respectively (Figs. 18 and 19). For both of these crops, the system predicted fewer high-risk days during the harvest period than perceived by the crop managers. For one (everbearer) crop the grower applied five fungicide applications before the start of the harvest period, which agreed with the 5 high risk-days predicted. However, during the harvest period, the system predicted three high-risk days, whereas five treatments were applied to the crop (Fig. 18). For the other day-neutral (everbearer) crop, the grower applied three treatments before and four during the harvest period, compared respectively to four and three predicted high-risk periods (Fig 19).

Three, two and eight treatments were applied at the established sites, compared respectively to three, two and five predicted high-risk days (Figs. 20, 21 and 22). While the number of treatments and predicted high-risk days were the same for two of these sites, the treatments dates were not coincident with the predicted high-risk days (Figs. 20 and 21). For the third established site, twice as many treatments were applied than predicted to be necessary by the occurrence of high-risk days (Fig. 22). Similarly, when the system was tested with data collected for a strawberry propagation field, seven high-risk days were predicted, compared to the fourteen treatments applied to the crop (Fig. 23). In addition, the predicted high-risk days occurred roughly evenly across the season, whereas the actual treatments were less evenly spread, concentrated particularly around August.

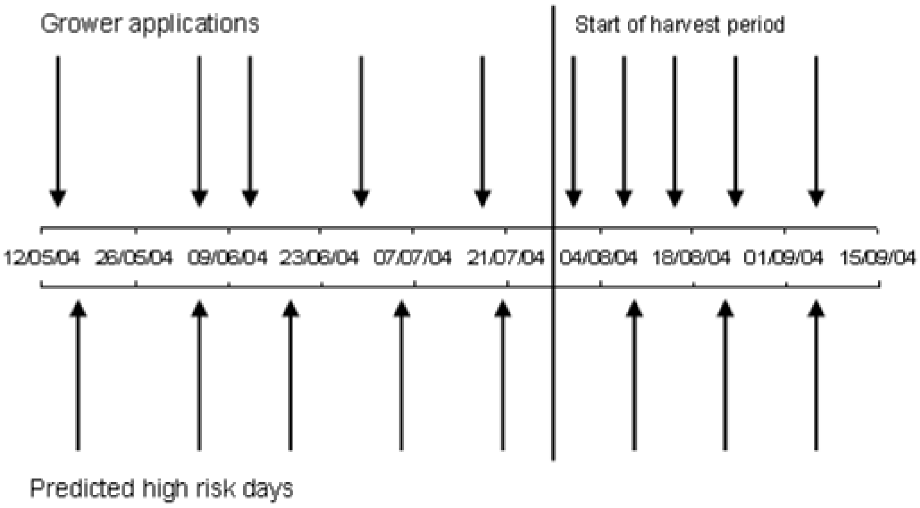

Fig. 18 Dates of grower-applied treatments to control *P. aphanis,* compared to high-risk days predicted by the warning-system for a day-neutral (everbearer) crop on a commercial holding near Wisbech 2004.

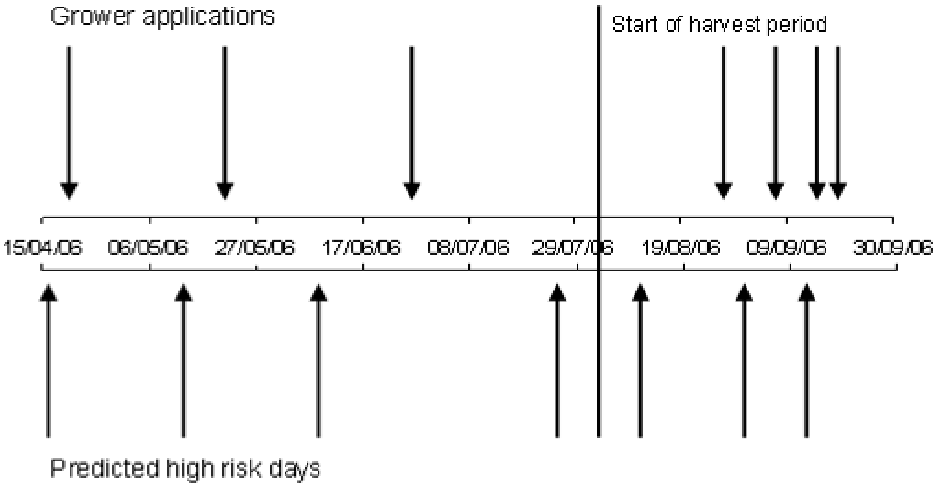

Fig. 19. Dates of grower-applied treatments to control *P. aphanis,* compared to high-risk days predicted by the warning-system for a day-neutral (everbearer) crop on a commercial holding near Wisbech 2006.

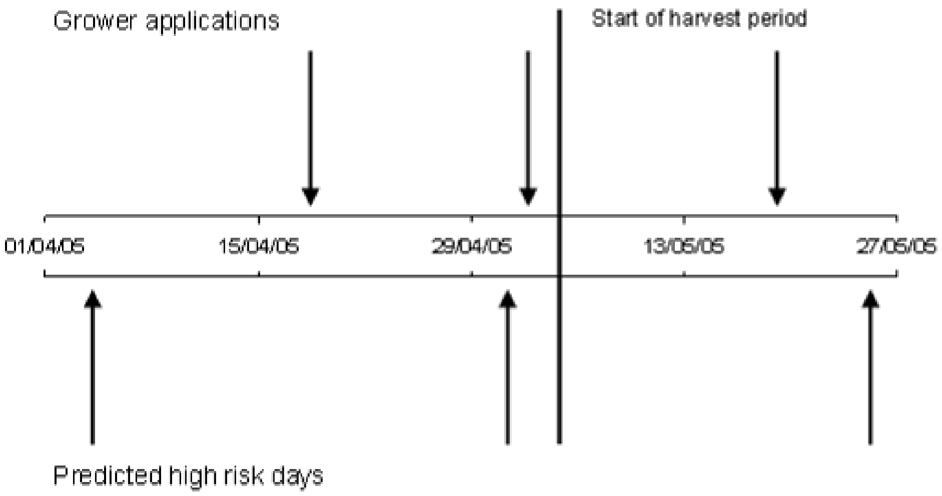

Fig. 20. Dates of grower-applied treatments to control *P. aphanis,* compared to high-risk days predicted by the warning-system for a third season Elsanta crop on a commercial holding near Wisbech 2005.

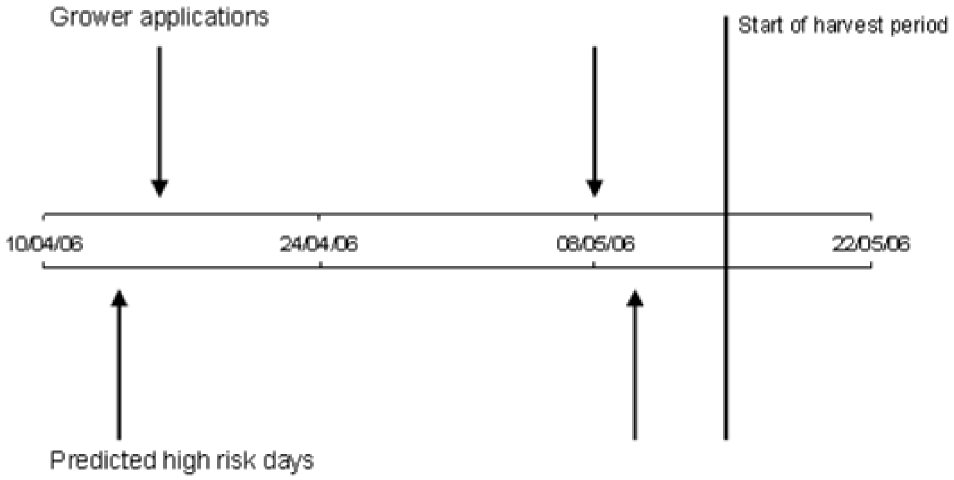

Fig. 21. Dates of grower-applied treatments to control *P. aphanis,* compared to high risk days predicted by the warning-system for a third season Elsanta crop on a commercial holding near Wisbech 2006.

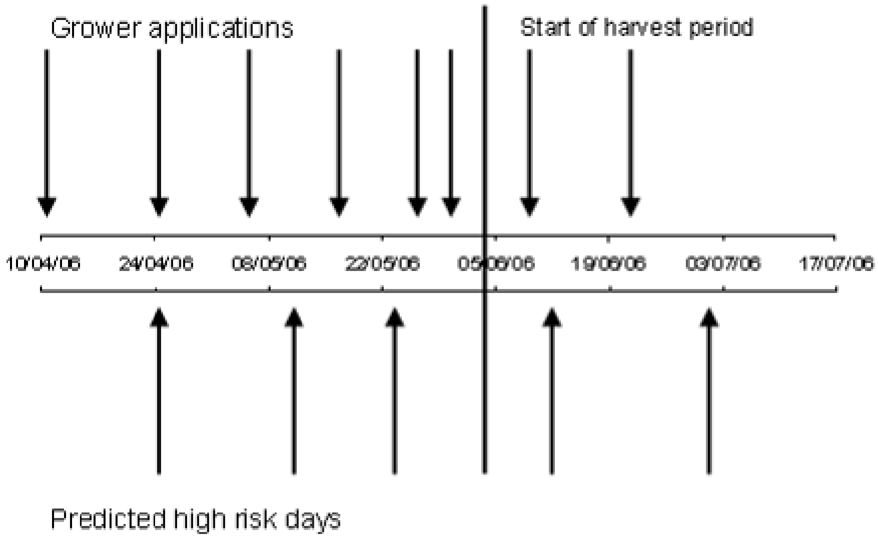

Fig. 22 Dates of grower-applied treatments to control *P. aphanis,* compared to high-risk days predicted by the warning-system for a second season Elsanta crop on a commercial holding near Colchester 2006.

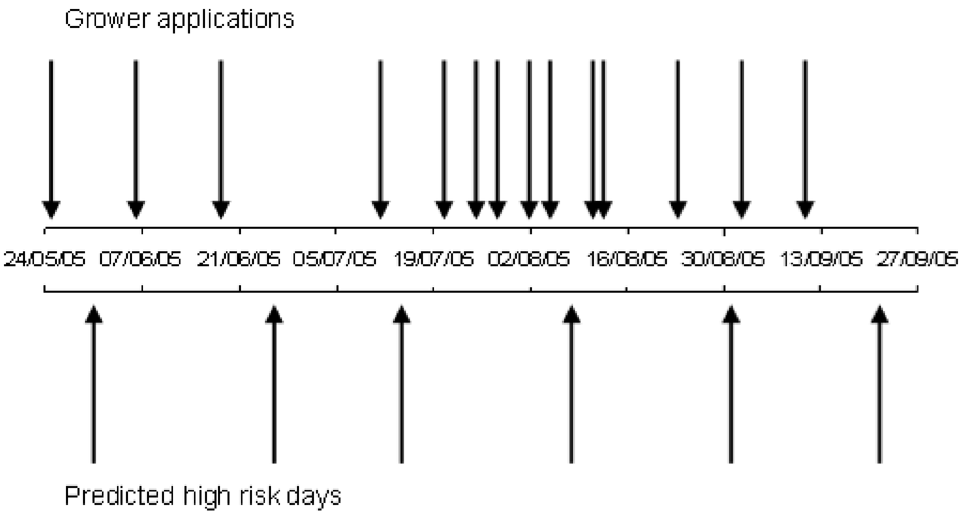

Fig. 23 Dates of grower-applied applications of fungicidal control product for *P. aphanis* compared to high risk days predicted by the prediction system for a propagation field of Elsanta plants near Kings Lynn 2005.

Use of rule based prediction system in commercial sites

At the Staffordshire site, the prediction system triggered in 4 fungicide applications to control powdery mildew, which was identical to the treatment timings used by the grower (Fig 24). In the two months prior to using the prediction system the grower applied 11 fungicide treatments.

The grower at the Cambridgeshire site applied 7 treatments, compared with 6 triggered by the prediction system (Fig. 25). The dates of application were broadly similar for these treatments. Before the prediction system was used 2 applications were made.

Growers at both sites reported that control of powdery mildew using the system was equivalent to achieved using their normal spray schedules, which resulted in negligible powdery mildew symptoms.

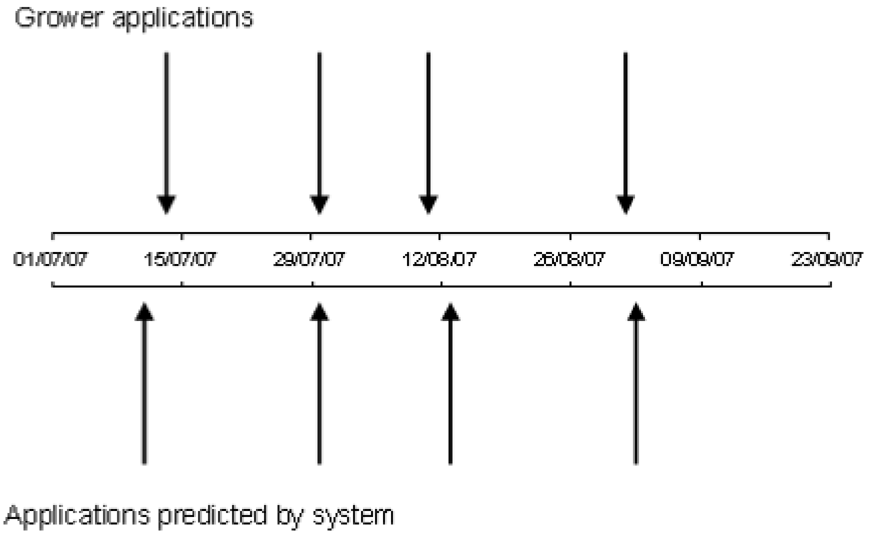

Fig. 24. Dates of grower-applied treatments to control *P. aphanis,* compared to applications triggered the warning-system for a commercial day-neutral (everbearer) crop at Staffordshire site, 2007.

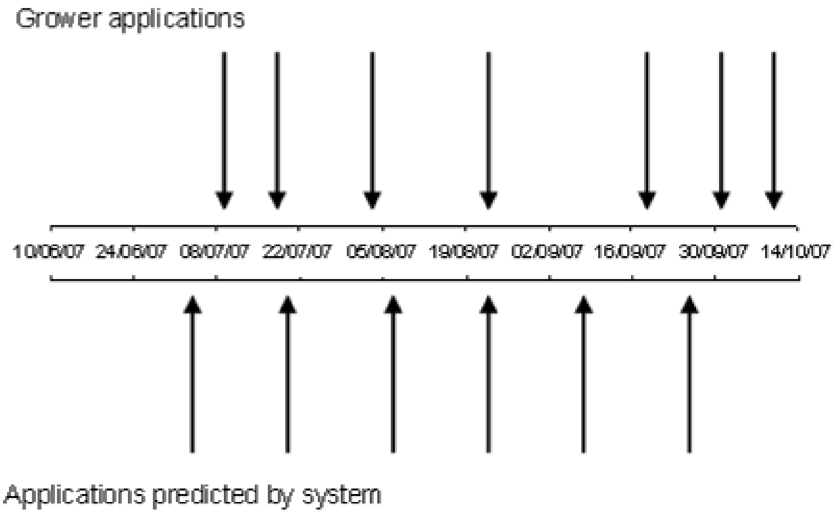

Fig. 24. Dates of grower-applied treatments to control *P. aphanis,* compared to applications triggered the warning-system for a commercial day-neutral (everbearer) crop at the Cambridgeshire site, 2007.

Discussion

Comparison of venting practice

Conditions outside the tunnels at both commercial sites were very similar, except for the daytime temperature in July and the night-time relative humidity in July (Fig. 1). It is therefore reasonable to assume that differences in the conditions inside the tunnels were attributable to differences in the venting practice at the sites. Temperatures inside the tunnels at both sites were similar, but the relative humidity was significantly higher in the site that was managed ‘normally’ (Fig 2). The internal relative humidity at the site managed with attention to venting practice ranged within 66-72% during the day. This is well below the optimum (≈ 95% RH) for germination of *P. aphanis* spores. In contrast, RH inside the tunnels at the site managed normally was in the range 92-99%.

The summer of 2007 was not particularly hot, so at both sites temperatures inside the tunnels sites stayed within the range 18-21^{o}C, which is ideal for fruit production. However, this range is also favourable for the growth and development of strawberry powdery mildew. Venting practice should aim to maintain temperatures close to the optimum for fruit production. The optimum temperature for fruit production is in the same range as the optimum for growth and development of strawberry powdery mildew but with good control of strawberry powdery mildew (as might be achieved using the rule based prediction system) the benefits for fruit quality achieved from the optimum temperatures would outweigh the disadvantages of possible greater infection by strawberry powdery mildew.

Rule based prediction system

The rules underlying the warning-system were developed using information from the literature, which was largely derived by laboratory experiments. The warning system therefore needed prediction system parameters required extensive testing, comparison and refinement using data collected from the field.

The warning-system was designed to predict the occurrence of infection risk from *P. aphanis,* due to inoculum generated within the crop. Therefore, the warning-system does not model the growth and development of *P. aphanis* (*i.e., epidemic severity) or* the crop. Instead it identifies when there have been a suitable number of hours for any inoculum (spores) to develop into new sporulating colonies. Implicitly, it is assumed that the overall level of inoculum in a field will not increase in the time between spores arriving at the leaf surface and developing into mature colonies: lesions expand and grow, but do not produce inoculum until sporulating structures have formed and reached maturity.

Infection is followed by a latent period when the fungus is growing in the plant, without visible symptoms. Many modern fungicides can control disease after infection, but only for a proportion of the latent period: an asymptomatic infection might be so advanced through the latent period that control can not be achieved at any fungicide dose. The idea behind the warning system is to provide a route to maximise the protectant and curative properties of fungicide treatments around the primary infection events. In order to achieve this the system identifies the earliest time that sporulation can occur within the crop, either from over-wintered inoculum, or latent infections, and subsequently lead to the expression of new symptoms. The crop manager can then use this as a guide for deciding the appropriate treatments. Since fungicides are most effective when used in protectant situations, treatments close to the warnings are likely to be the most beneficial. However, even if other priorities make treatment impracticable at the time of the warning, it remains useful for informing fungicide choice. For example, by indicating when a curative product and robust dose is required.

An initial set of parameters for the warning-system was developed from the literature review and field observations (Table 6). When tested against an historical dataset, these parameters resulted in predicted high-risk days that appeared broadly sensible. When the prediction system was run with actual field data the first predicted high risk when compared to the actual development of *P. aphanis* infection (Figs. 5, 6, 7 and 8). Whilst the predicted high-risk days were similar to the dates that symptoms were observed in the crops, they were not close enough for use by the grower to plan fungicide treatments. As a consequence, it was necessary to adjust the parameters (*cf.* calibrate the system). The need for this calibration was primarily due to the fact that the original parameter values were obtained from laboratory, rather than field based experiments.

The prediction system parameters were revised so that the predicted high-risk days coincided with the days when 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. Infection takes longer to develop on new sites compared to established sites. 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 2). 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. 9 and 10). For the newly planted sites the development of visible symptoms of *P. aphanis* infection corresponded to the second predicted high risk day (Figs. 11 and 12). 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. Growers generally achieve good control of *P. aphanis* using the current number of fungicide applications (personal communication). 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. 18, 19, 20, 21, 22 and 23). 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 18 and 19). 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. 13, 16 and 17). Leaf wetness and maximum germination temperature were the least sensitive variables when altered individually (Figs. 14 and 15). The least sensitive variables were often masked by being paired with a more sensitive variables when two variables were altered at the same time (Figs 16 and 17), except when two less sensitive variables were paired together (leaf wetness by maximum growth temperature or maximum germination temperature by leaf wetness) (Fig. 16). 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.

Use of rule based prediction system in commercial sites

Two growers used the prediction system over the fruit production phase of the season. This is the period when growers need to reduce fungicide use as much as possible to minimise the occurrence of residues in fruit. The system resulted in fewer, or the same number of fungicide applications, when compared to the treatments applied by the grower. Following introduction of the prediction system, there was a large reduction in the number of fungicides applied to the Staffordshire site, compared with the previous two months. The grower at the Cambridgeshire site had been involved closely in the work reported by HDC Project SF 62 and had implemented many of its recommendations, resulting in a reduction of fungicide use. The outcome of this test by growers was very similar to the results produced when predictions by the system were compared retrospectively to commercial fungicide schedules: on average, for 6 sites growers applied 7.3 applications compared to a predicted requirement for 4.8 applications (Figs. 18, 19, 20, 21, 22 and 23).

Comparison with previously published models/prediction systems

A rule based warning system (Dent, 1995, Norton and Mumford, 1993) specific for *P. aphanis* infections has been developed. The system is based on 'IF-THEN' rules, rather than comparatively complex formulae such as those developed for grape powdery mildew (Chellemi and Marois, 1991, Sall, 1980).

The rule based prediction system presented here highlights days within the season when the crop is at greatest risk of infection. This information can be used to inform treatment decisions. This approach 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. However, it differs markedly from prediction schemes that estimate end of season disease pressures for powdery mildew infections of sugar beet (Asher and Williams, 1991) and jujube (Sinha, 2005).

Growers are often reluctant to use new models or rule based prediction systems (Parker, 2001, Vallavieille-Pope *et al.*, 2000). Parker and Sinclair (2001) identified eleven reasons why (in their case) decision support systems (DSS) are not widely used by growers or agronomists (Table 8). The prediction system detailed here was developed to be practicable for use under commercial conditions, and to be flexible to changes in production protocols, including the varieties grown.

Table 7. 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

Barrier	Resolution
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 could distribute the model to its members and could help with arrangements for any further 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

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