ANNUAL REPORT

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Annual Report (2007)

Narcissus White Mould
Decision Support System

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

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Grower Summary

Headline

The results to date indicate that a model to predict white mould infection of narcissus crops has good potential to be used for predicting disease development in the field and for giving target dates for fungicide applications. This will enable growers to reduce the number of fungicide sprays applied and the associated costs, and give a better environmental assessment.

Background and expected deliverables

The control of pests and diseases is a major factor in meeting the exacting specifications for narcissus required by the export and multiple-retail sectors. There appears to have been an increase in epidemics of the fungal foliar disease white mould (caused by *Ramularia vallisumbrosae*) in UK crops over the past 10 years or so, resulting in significant loss of bulb and flower yields. Bulb growers control fungal diseases such as white mould by applying regular fungicide sprays from emergence until after flowering. In an earlier project, funded through the 'Horticulture LINK' programme, the HDC and ten companies, a white mould infection model, driven by temperature and leaf wetness duration, was formulated. This indicated the key dates at which fungicide sprays should be targetted for maximal control of infection. Those trials indicated that the number of sprays used could be halved by eliminating those otherwise applied on inappropriate dates.

The aim of the current project is to validate - to test, then confirm or modify - the infection model, and deliver it to the industry as a practical 'spray timing system'. Such a system would provide improved management of white mould, leading to enhanced yields of better quality bulbs and flowers, with lower costs and a smaller environmental impact.

Summary of the project and main conclusions

In 2007 commercial narcissus crops at Gweek and Castle Kayle, Cornwall, were monitored for the incidence and severity of white mould (*Ramularia vallisumbrosae*) in the absence of fungicide spray applications. Meteorological loggers were used to record temperature, leaf wetness, precipitation impact (PI) and other factors at each site. The previously developed white mould infection model was run weekly at each site, producing daily infection scores. At both sites high infection scores occurred in December and January, continuing into February

at Gweek but not at Castle Kayle. The persistent high scores at Gweek resulted in a white mould infection starting in February. At Castle Kayle, white mould was not seen until a month later, in mid-March, and this may have been due to leaf damage from high-impact rain, acting in spite of a lowered infection score. Given that there is a latent period for white mould symptoms to appear, the timing of potential damage fits well with the onset of observed white mould symptoms in the field.

Spore traps indicated that air-borne white mould spores were present in large numbers at Gweek over much of February and March, and after 3 April high numbers of spores were present continuously. White mould symptoms were first observed in the crop at Gweek on 15 February, with a marked increase in symptom appearance after 15 March. Given the latent incubation period associated with white mould, the results of the air sampling corresponded to the observations of white mould within the crop.

The results confirmed the potential value of using an infection model to forecast infective periods, and hence the optimal dates at which to apply fungicides. The forecasting system will be tested in 2008, using either a conventional spray programme or a spray programme driven by the white mould infection model.

Financial benefits

The earlier 'Horticulture LINK' project (BOF 41) was subject to independent scrutiny which concluded that very considerable financial saving could be made by using a fungicide spray programme that reduces the total number of fungicide sprays applied. The present project will help growers to apply these fewer fungicide sprays to crops at the best, most effective time to control foliar fungal diseases, thereby improving crop quality and reducing wastage.

Action points for growers

Until the white mould infection model and associated spray-timing system are fully available to the industry at the conclusion of this project, growers could apply white mould fungicides following prolonged wet periods when temperatures are 5 to 10°C, particularly following crop damage (such as that caused by frost, heavy rain or flower picking).

Science Section

Introduction

The reports of the previous project (BOF 56) outlined the rationale behind the investigation, describing the monitoring of white mould in commercial Cornish narcissus crops that had not been treated with fungicides. This enabled the 'natural' development of the disease to be recorded, so that these (observed) data could be compared with white mould development as predicted using a white mould infection model developed in an earlier 'Horticulture LINK' project (BOF 41). Comparing the predictions with what actually occurred enables the accuracy, or otherwise, of the model to be ascertained. Previous results indicated that the model showed potential for use in forecasting white mould. There was also a relationship between trap-plant infection and the occurrence of higher infection scores. The results confirmed that crop damage (for example, due to frost or hail) was also important if infection were to take place, though the extent of the damage needed has still to be defined.

In studies of this type the natural year-to-year differences in weather, crop performance and other factors mean it is necessary to base any conclusions on data collected over a number of years and crops, and the present report describes further trials carried out in 2007. The eventual aim is to determine the optimal dates for spraying commercial crops with fungicides, and in 2008 the effects of a 'model' spray programme will be compared with those of the growers' normal, conventional spray programme. This will also give the opportunity to work out practical methods for using the model – a 'spray timing system' - and delivering it to growers.

Materials and methods

Sites for crop monitoring

In autumn 2006, four Cornish narcissus crops were selected for monitoring (Table 1). The crops used were considered typical commercial crops of the region. In each crop an area *ca.* 0.2ha in extent was clearly marked with corner posts and other markers, and it was agreed with the growers that no fungicide sprays would be applied during this year in these designated areas. In all other respects, it was agreed that each crop would be farmed according to its grower's normal commercial practices. The central 0.1ha of each designated area was further demarcated for monitoring and observation purposes, leaving the surrounding area as a buffer zone for protection from any spray drift from adjacent crops. The project was concentrated at the Gweek and Castle Kayle sites, the sites at Mawnan and Gulval being kept as 'reserves' should white mould symptoms fail to appear at the first sites.

Table 1. Sites used in white mould project in 2007.

Owner's name and address	*Site name*	*Cultivar*	*Crop year*
Winchester Growers Ltd Varfell Farm Long Rock Penzance Cornwall TR20 8AQ	Castle Kayle	'Golden Ducat' and 'Standard Value'	3
	Gulval	'Cheerfulness'	3
Maurice Crouch (Growers) Ltd Lands Vue Blackwater Truro Cornwall TR4 8JA	Gweek	'Dutch Master' and 'Golden Ducat'	3
	Mawnan	'California' and 'Hollywood'	1

Weather data

A meteorological data logger ('Smaartlog'; Aardware Design Ltd., Kingston, UK) was set up in the centre of the monitoring area at Gweek and Castle Kayle in December 2006, prior to crop emergence. The loggers, powered by battery and solar panel and downloadable *via* a modem and digital cell telephone, were provided with sensors recording soil and air temperature, relative humidity (RH), surface wetness, rainfall and precipitation impact (PI) at 30-minute intervals. The PI sensor ranks impacts into 14 levels ('bins') from the lowest impact energy (1) to the highest (14).

Crop and disease monitoring

The allocated areas of crops were checked at weekly intervals from December onwards, and the date of first appearance of white mould symptoms was recorded (the symptoms were described in the previous report). Following the appearance of first symptoms, disease levels were assessed weekly for the incidence and severity of the disease. The central, 0.1ha area of each was walked in a standard fashion in an X-pattern, starting from a marked corner, and on crossing ridges a 0.5m-long sub-sample was delimited with a ruler at the intercept to give 50, 0.5m-long sub-samples for assessment over the 0.1ha area.

The incidence and severity of white mould were scored in each of the sub-samples according to the scale shown in Table 2; overall incidence and severity scores were then calculated by summing the scores for all 50 sub-samples. The crop growth stage and (later in the season) the percentage of foliage that was senescent or dead were also noted.

Table 2. White mould incidence and severity scales.

Score	*Incidence*	*Score*	*Severity*
0	None	0	None
1	1 or 2 leaves affected	1	Single lesions
2	>2 but <10 leaves affected	2	Single lesions or occasionally >1 lesion per leaf
3	>10 leaves but <50% leaves affected	3	Generally 2 or more lesions per leaf
4	>50% but <100% leaves affected	4	Lesions coalescing to form larger damaged areas
5	All leaves affected	5	Extensive leaf die-back

Trap-plant production and use

In August 2006 narcissus bulbs (cv 'Carlton', grade 12-14cm circumference) were allocated from a stock grown at Warwick HRI, The Kirton Research Centre (KRC), Lincolnshire for the production of trap-plants. To provide comparability with the commercial crops being monitored, the bulbs were not given the usual hot-water treatment nor any fungicide applications from lifting in June-July.

The bulbs were stored at 17°C until early-October, when they were planted in a standard fashion, five bulbs per 20cm-diameter, 4L-capacity plant-pot, using a peat growing medium. After planting the pots were placed outdoors at KRC, covered with fleece to protect the plants from extreme weather, and kept watered as required. In December, the plant-pots were transported to Camborne, Cornwall and grown-on outdoors until required.

Between 7 February and 2 May 2007, pot-grown trap-plants were placed adjacent to crop foliage near the centre of the plot at Gweek for exposure periods of *ca*. 5 days. For each exposure period, three plant-pots were used. In a change from previous years' protocol, only undamaged trap-plants were used. Following collection from the field site the trap-plants were placed in a frost-protected glasshouse at Duchy College, Rosewarne, Camborne, Cornwall and grown-on. Further control pots, not exposed in the field, were moved straight to the glasshouse (three pots per week). For an initial 48-hour period the pots were placed in high humidity provided by a humidifier running under a polythene-film cover within the glasshouse, after which they were moved to the body of the glasshouse. The three replicate pots in each set were arranged in the glasshouse in three blocks, all pots being spaced well apart from one another to reduce the likelihood of cross-infection. Pots were kept well watered, bottom-watering into saucers to avoid spreading infection. The trap-plants were examined for disease lesions at weekly intervals for 12 weeks, recording incidence and severity scores (Table 2).

Spore trapping studies

After shoot emergence, and continuing until the end of the growing season, a sticky-tape spore trap was set up at the Gweek site. This was a Hirst-type Burkard 24-h volumetric trap (Burkard Manufacturing Co. Ltd., Rickmansworth, UK; see BOF 56 Final Report for references). The sampler consists of a metal body with a rectangular inlet slit (14mm-high and 2mm-wide) through which air is sampled at *ca.* 10L/min using a battery-operated pump. The air-flow is controlled by individually calibrated orifices mounted behind the trap. Inside each sampler, spores are impacted onto a slide coated with silicone, the glass slide being attached to a 24-h clock which moves the slide progressively. At weekly intervals the recording tape was replaced and the exposed tape sent to Warwick HRI, University of Warwick, Wellesbourne for examination. Spores deposited on the slide at different points during the 24-h period were examined, initially under a light microscope (at x400 magnification) and later using a previously developed polyclonal antibody (PAb) (see BOF 56 Final Report).

During the 2007 season a second air sampler (MTIST) was used at Gweek to determine the presence or absence of white mould spores in the air. This sampler impacts air-borne particulates onto microtitre strips which can be processed using enzyme-linked immunosorbant assay (ELISA) which has the advantage of being a faster technique for white mould detection and quantification in air samples. A detailed description of the MTIST device can be found in Kennedy *et al*. (2000). In the out-door version of the sampler air is drawn through a manifold consisting of a plastic tube with a right-angle bend placed over the sampler inlet. The manifold samples air through a 9cm-diameter vertical circular inlet and

directs it into the sampler body which is held horizontally. For field use the sampler (including manifold) is mounted on a wind-vane so that the manifold inlet faces into the wind. Within the sampler the airflow is channelled through 32 trumpet-shaped nozzles each directed at the base of a microtitre well. The sampler contains four microtitre strips (catalogue no. 469957, Nunc Immunodiagnostics, Life Technologies Ltd, Paisley, Scotland, UK) each containing eight wells. Two types of well-coating preparations were used on each of two strips: 0.05% sodium azide and silicone, which following melting was mixed with hexane. One hundred µl of each single coating solution was applied to each well of 60 microtitre strips (catalogue no. 469957, Nunc Immunodiagnostics, as above). This process was repeated for both the coating preparations. After treatment the coated microtitre well strips were secured within ELISA multiframes (Catalogue No. 9503060, Life Technologies Ltd, as above) and incubated at 20°C for 1 hour, after which any unbound material was removed by inverting the microtitre strips and tapping them downwards onto absorbent towelling. An inverted binocular microscope (Nikon model TMS) was used to check that the well coatings had been applied evenly. Prior to field exposure the microstrips were stored at 4°C in a sealed container. Air flow through the sampler was estimated in still air by measuring the air speed at different points across the inlet manifold using a hot-film anemometer (air velocity transducer model number 8460, TSI Incorporated, St Paul, MN, USA) and integrating over the area of the inlet. In the tests reported here, the volume flow rate through the device was measured at 57L/min. The MTIST sampler was operated daily for 12h-periods (06:00–18:00h), since previous studies had shown that white mould spores were present in air samples during daylight hours (BOF 56 Final Report).

Detection of white mould in air samples using ELISA

Field-exposed microtitre strips were blocked with 200µl of 1% casein buffer (1%w/v casein PBS) and incubated at 37°C for 45min. Residual blocking buffer was removed and wells were washed four times for one minute each with 200µl PBS, 0.05% Tween 20 and 0.1% casein (PBSTwC), after which wells 1-4 of each strip received 100µl of white mould PAb, with the remaining wells 5-8 each receiving 100µl of PBSTwC. Following incubation in a Wellwarm shaker incubater (30°C) for 45min as above, wells were washed three times for one minute each with 200µl PBSTwC. A DAKO duet amplification system (catalogue no. K0492; DAKO Ltd, Angel Drive, Ely, Cambridge, UK) was used to amplify the signal generated by bound tissue culture supernatant antibodies. Wells were washed as described above and 100µl of 3,3',5,5'-tetramethylbenzidene substrate (catalogue no. T-3405 and P-4922; Sigma, Poole, Dorset, UK) was added to each well. The reaction was stopped by adding 25µl 20% 1M sulphuric acid solution to each well. Absorbance was determined at 450nm with a Biohit BP800 ELISA plate reader (Alpha Laboratories, 40 Parham Drive, Eastleigh, Hampshire, UK).

Quantification of airborne inoculum of R. vallisumbrosae using immuno-fluorescence

R. *vallisumbrosae* spores trapped on the spore tape of the 7-day spore trap were immunolabelled with PAb and an anti-species fluoroscein conjugate using the appropriate antibody dilution (Kennedy *et al*., 1999) and counted using a fluorescence microscope.

Forecasting infection

A white mould infection model that relates the number of disease lesions to temperature and durations of leaf wetness periods (see Final Report for BOF 41) showed that the critical weather conditions favouring white mould infection were temperatures between 5 and 10°C combined with leaf wetness durations of 12 to 24h. The infection model was therefore run using the weather data obtained from loggers in the field crops, enabling a comparison to be made between the predicted infection score and the observed, actual levels of white mould symptoms. A correspondance of predicted and observed levels would validate the model, while dissimilar results would indicate that the model is inappropriate or needs to be refined. For interpretation, the infection score was averaged for 24-hour periods each starting at 00:00h.

As well as temperature and leaf wetness duration, some additional criteria were used experimentally to determine their relationship to white mould infection, namely (1) the occurence of frost (a screen temperature below -1°C) and (2) the occurrence of >2 impact events in 'bin' 7 or higher of the PI sensor (bin 7 being designated as the threshold for potential leaf damage). These criteria were derived from observations on drop-impaction energy studies conducted in 'Horticulture LINK' project BOF 41.

During 2007 the model was programmed using the computer modelling software 'Matlab'. This was necessary to speed up the processing of meteological data. Improved accuracy and ease of processing was required as 'spray timing' experiments were to be conducted subsequently using the white mould infection model. Comparisons were made using the Matlab version of the model and that compiled using Microsoft Excel, which had been used in years one and two of the project, and it was shown that both versions of the software produced essentially similar results.

Results

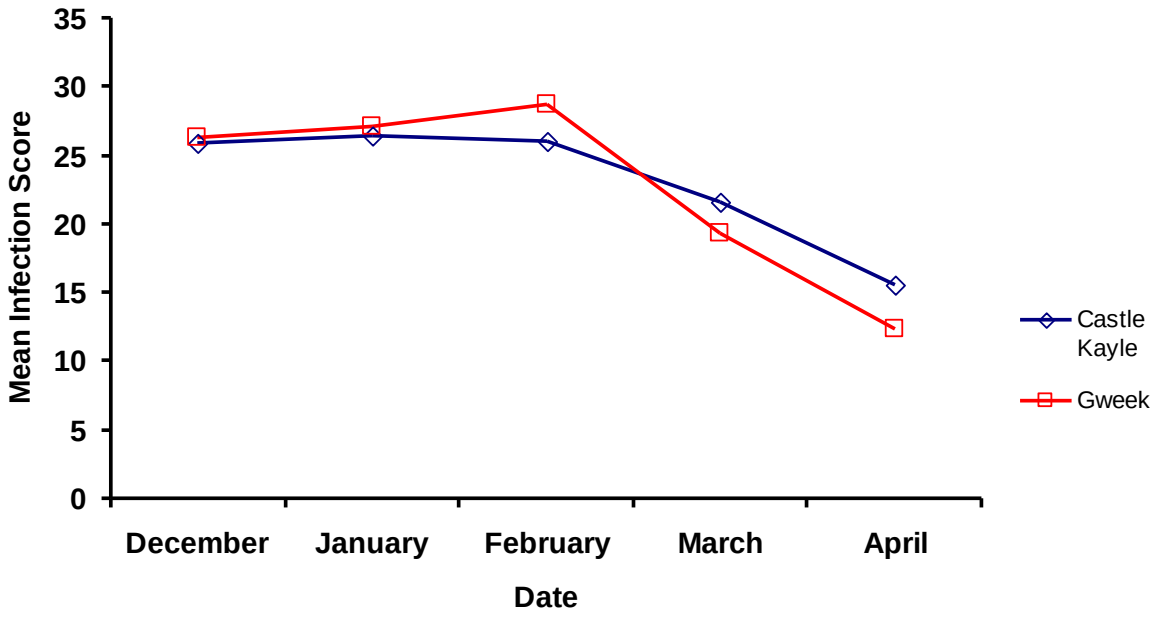
White mould forecasts and observations

The white mould infection model was run weekly for the Gweek and Castle Kayle sites using the air temperature and leaf wetness duration data recorded by data loggers positioned in the crops. The model produced the daily infection scores shown in Figure 2. During December and January, the pattern of infection scores was similar at both sites, while in February scores were higher at Gweek and, in March and April, at Castle Kayle. On Figure 2 the infection scores are presented in red for days on which there was a high incidence of potentially leaf damaging events, i.e. frost or high-impact precipitation. At Gweek only two leaf damage events were observed, namely frost on 22 December 2006 and 21 March 2007. In contrast, at Castle Kayle several damage events were recorded during February, March and April as a result of high-impact readings from the PI sensor.

For easier interpretation, the daily infection scores from Figure 2 are presented as monthly averages in Figure 1.

This figure shows more clearly the somewhat higher infection scores at Gweek in February but at Castle Kayle in March and April. While the white mould model produces an infection score, it cannot determine when the crop should be sprayed with fungicide; for this a suitable threshold score has to be agreed, and in this study an average infection scores of approximately 25 appeared to denote a line between higher and lower white mould risk, although higher daily individual scores were observed at both sites.

Figure 1. Mean monthly infection scores predicted using the white mould model at Gweek and Castle Kayle in 2007.



The observed incidence and severity of white mould infection are shown in Figure 3. There was a clearly distinct pattern of infection at the two sites. At Gweek white mould first appeared in mid-February, subsequently increasing steadily until mid-March, when disease incidence and severity increased rapidly and all 50 sample areas were affected by the disease. At Castle Kayle white mould was first seen in mid-March, increasing relatively slowly over the next two months. It is suggested that the later onset of white mould at Castle Kayle may have been due a lessening of infective conditions (lower infection score) there at the beginning of February, compared with Gweek where high infection scores persisted. From late-February onwards only Castle Kayle experienced several damaging high-impact rain events, which could have led to a late, but steadily increasing, white mould infection. Given that there is a latent period for white mould symptoms to appear, the timing of potential damage at Castle Kayle fits well with the onset of white mould symptoms.

Figure 2. White mould infection scores in 2007 at Gweek (above) and Castle Kayle (below). The red bars indicate the simultaneous occurrence of potentially leaf-damaging events (frost or high-impact precipitation).

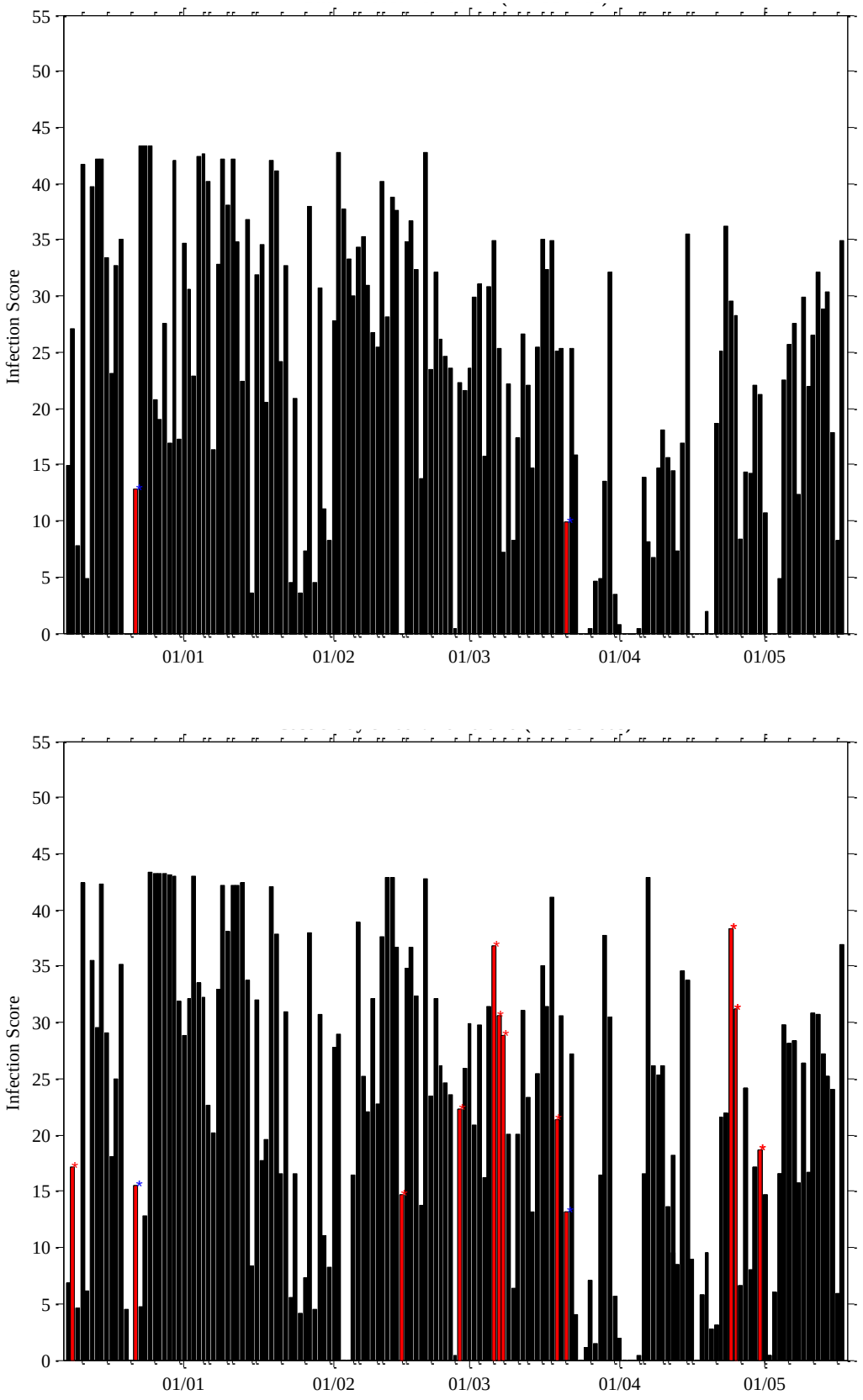
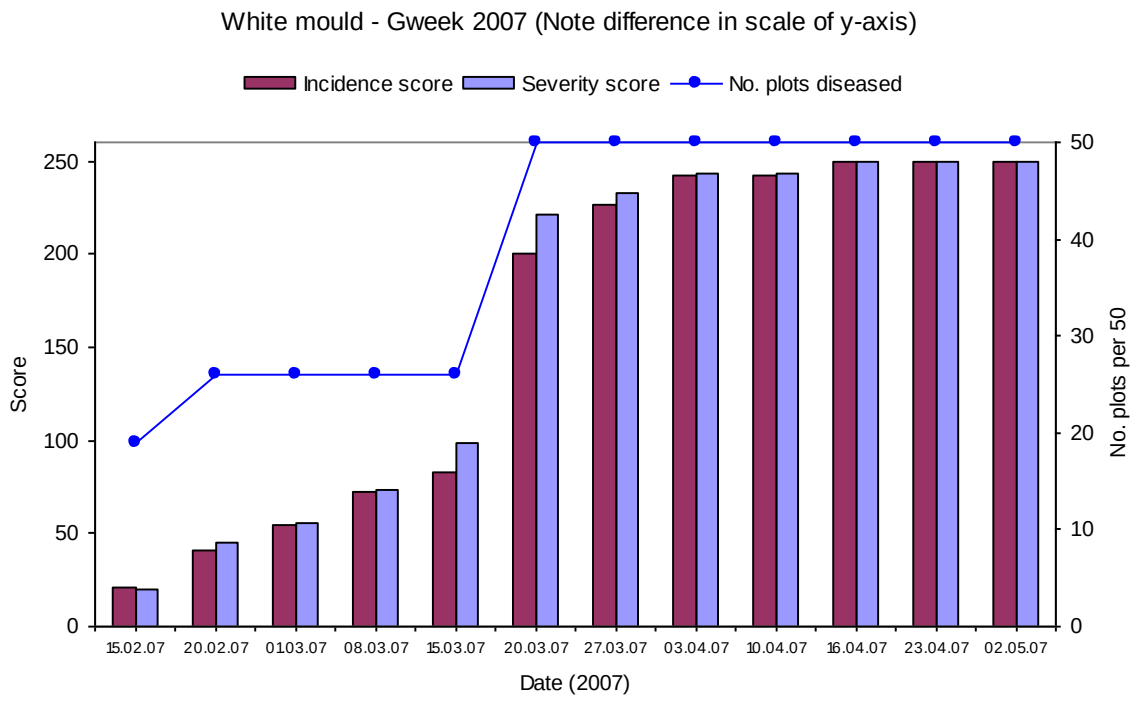
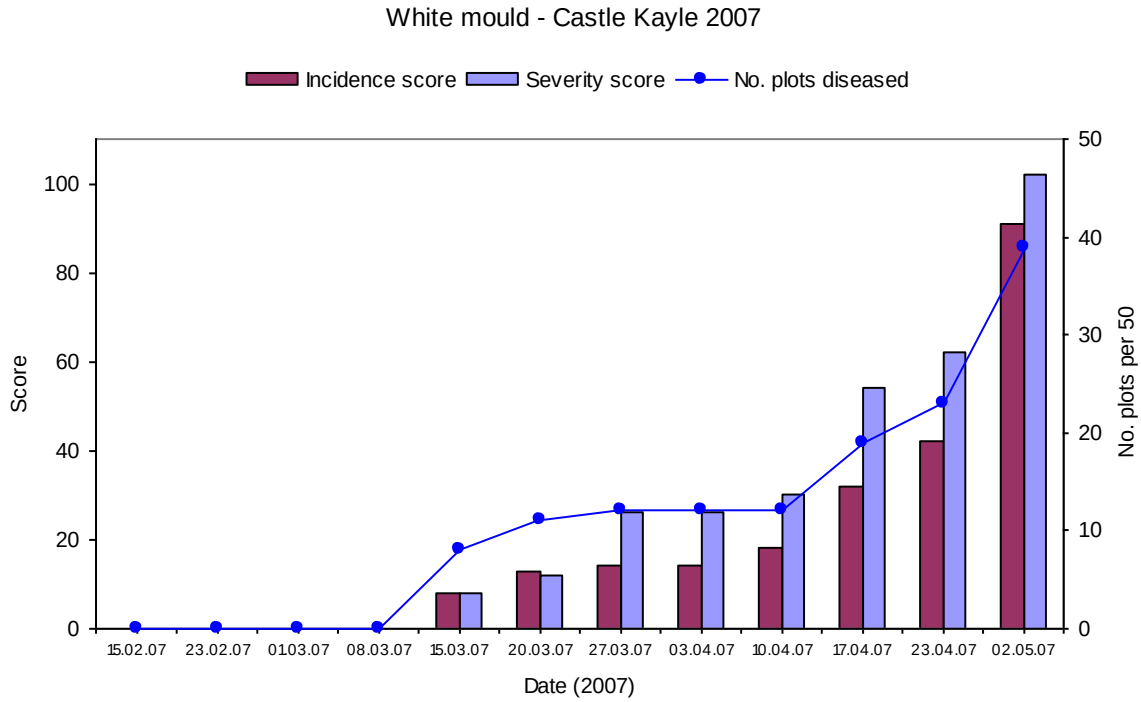


Figure 3. White mould monitoring in 2007 at Gweek and Castle Kayle. Note that the left-hand scale is different for the two sites.



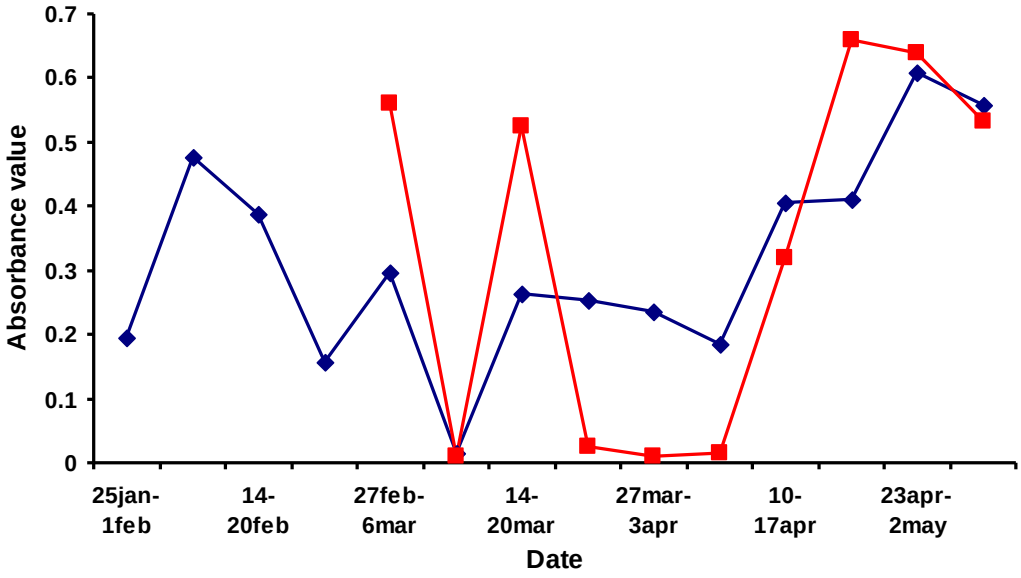


Spore trapping studies

The ELISA technique described above was applied to air samples collected using the MTIST spore trap at Gweek where white mould was already present and no fungicide sprays had been applied. Figure 4 shows the absorbance values of wells which had been exposed for 7-day periods in the crop. The sampling wells were coated with either silicone or sodium azide to improve the collection and retention of air-borne particles within the trap. It was not possible to determine if the antibody was reacting only to scolecospores or phragmospores or to both spore types. However, the results from the Burkard 7-day spore sampler indicated that both spore types were present in the air surrounding the crop. During the early part of the observation periods all four microtitre strips (each containing 8 wells) were coated with silicone alone. From the beginning of March onwards two strips were coated with sodium azide and the remaing two receiving a coating of silicone alone.

The results indicated that white mould spores were present in large numbers at Gweek in the air from 1–7 February, 20–27 February, 1–14 March and after 3 April. After 3 April high numbers of air-borne spores of white mould were present continuously, with high weekly absorbance values indicating very high numbers of white mould spores in the sample wells (Figure 4). White mould symptoms were first observed in the crop at Gweek on 15 February with a marked increase in symptom appearance after 15 March. Given the latent incubation period associated with white mould, the results of the air sampling correspond to the observations of white mould within the crop. The results from microtitre wells coated with silicone were not markedly different from those coated with sodium azide, indicating that well coating did not affect significantly the ELISA assay. However, uncoated wells were not used during the trapping period, so it is unclear if well coating affected the collection and retention of white mould spores within the air sampler.

Figure 4. Absorbance values in wells containing particulates from the air after staining with a white mould sensitive antibody, using either silicone- (blue line) or sodium azide- (red line) coated wells.



The seven-day Burkard spore trap was operated between 14 March and 14 April at Gweek. The results from this trap were used to confirm the presence of both scolecospores and phragmospores of white mould in air samples. Low daily numbers of both spore types were found in air samples collected during this period. The results from 15 to 16 March are shown in Table 3. Scolecospores were only found during day-time periods, confirming the results obtained at Kirton in 2006. The results also confirmed the pattern of inoculum availability seen using the MTIST trap.

Table 3. Numbers of white mould scolecospores and phragmospores present on 15–16 March 2007 at Gweek.

TIME (H)	Scolecospores	Phragmospores
01:00	1	0
03:00	0	1
04:00	0	1
06:00	0	3
07:00	2	0
08:00	3	1
10:00	1	0
11:00	1	0
15:00	0	1
20:00	1	0
TOTAL	9	7

Trap-plants

The exposure periods for trap-plants at Gweek started on 7 February and ended on 2 May 2007. Trap-plants from all exposure periods starting between 7 February and 2 March developed typical white mould lesions within 3 or 4 weeks following the end of exposure, confirming the prolonged availability of white mould spores shown by the spore trap results. Trap-plants that began their exposure on 6 or 14 March took 1 to 2 weeks longer to produce symptoms, and trap-plants from later exposure periods failed to show symptoms. In all cases the symptoms continued to increase progressively. Since carrying out these trap-plant tests, related experiments have been conducted at Wellesbourne and these will be reported in the next report.

Discussion

In 2007 two Cornish farms were monitored for the incidence and severity of white mould. At each site the environmental factors were monitored using a phone-linked data logger with probes measuring temperature, humidity, leaf wetness, rainfall and precipitation impact. The data can be used in conjunction with a mathematical model, which summarises the effect of one or more factors on disease incidence or development, to estimate the disease risk to the crop under different climatic conditions. The use of mathematical models can help in forecasting disease on crops and provide information on the optimal timing of fungicide application for controlling crop disease. Mathematical relationships (models) describing the effect of temperature and leaf wetness duration on *R. vallisumbrosae* infection were developed at Warwick HRI in a project funded under the Horticulture LINK programme, and it is envisaged that these will form the basis of a white mould forecasting or spray-timing system.

During 2007 observational trials were used to compare the output from the model with actual white mould development in the field. The use of the model to predict the risk of white mould development in the field in Cornwall during 2006 indicated that white mould infection conditions were limiting. In 2007, however, white mould infection occurred at both sites where observational trials had been set up. At Gweek persistent weather conditions favouring the development of white mould continued into February, and the beginning of a severe infection developed in February. At Castle Kayle infective weather conditions lessened in February, but crop damage in the February to April period appeared to have resulted in a relatively late infection, in March. The white mould infection scores were much higher in 2007 than in 2006, when only limited white mould epidemics were observed at monitoring sites.

Infection is possible at all infection scores, and the potential infection at low scores may be increased by the occurrence of damage to leaf tissues or the presence of large amounts of white mould inoculum. Environmental data from sites in Cornwall in 2006 gave only low scores when processed through the model. The lack of significant trap plant infection during 2005 and 2006 resulted in little information with which to interpret the model infection scores. However, scores of approximately 20 to 30 appear to be critical and increase the likelihood of white mould infection, provided inoculum is present.

It is possible that there are discrete periods for white mould (scolecospore) inoculum production within the crop. Temperatures of 5–10°C, in conjunction with long periods of high humidity (>95% RH) are required for the production of scolecospores. To facilitate the identification of *R. vallisumbrosae* spores, polyclonal antibodies were developed. The results

obtained in 2007 suggest that white mould development on narcissus crops is closely related to the availability of white mould inoculum. Detecting the presence of white mould in the absence of symptoms on the plant would be a very useful method of reducing the risk of white mould development on narcissus. Detecting white mould inoculum would be particularly useful early in the season as a method of preventing disease transfer between early narcissus crops and those with later emergence dates. However tests that can be conducted in the field are necessary if information on air-borne white mould inoculum concentration is to be of more practical value. The potential exists for linking estimates of white mould inoculum to mathematical models describing infection, and the use of this approach might improve the efficiency of disease forecasts.

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