Project title:	**Narcissus White Mould Decision Support System**
HDC project number:	**BOF 56/56a**
Project leader(s):	Malcolm Millar ADAS UK Ltd Boxworth Cambridgeshire CB23 4NN T: 01954 268214 F: 01954 267659 E: malcolm.millar@adas.co.uk
Report:	BOF 56a Final Report (2008)
Previous reports:	BOF 56 Annual Report (2005) BOF 56 Final Report (2006) BOF 56a Annual Report (2007)
Key worker(s):	Malcolm Millar BSc - Project Leader Roy Kennedy BSc, MSc, PhD Gordon R Hanks BSc, MPhil, FIHort, MIBiol, CBiol Pippa Hughes BSc Leanne Cozens BSc, DipBiol, DipChem, MIBiol, CBiol, MRSC
Location:	Commercial narcissus farms in Cornwall (field trials) Warwick HRI, University of Warwick, Wellesbourne, Warwickshire (laboratory work) Warwick HRI, University of Warwick, Kirton Research Centre, Lincolnshire (other work)
Project co-ordinator(s):	Arthur Andrews Winchester Growers Ltd Varfell Farm Long Rock Penzance Cornwall TR20 8AQ
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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.

For accurate reporting, materials may be referred to by the name of the commercial product. No endorsement is intended of products mentioned, nor criticism of those not mentioned.

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AUTHENTICATION FOR BOF 56a

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Mr M Millar

Horticultural Consultant

ADAS

Signature ..... Date .....

Dr R Kennedy

Warwick HRI

Signature ..... Date .....

Report authorised for by:

Dr W E Parker

Horticulture Sector Manager

ADAS

Signature ..... Date .....

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Grower Summary

Headline

Narcissus White Mould spray-warning system for 'at risk' periods offers growers the opportunity to achieve better disease management with fewer sprays, reduced costs and a positive environmental impact.

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 Horticulture LINK funded project (BOF 41 involving the HDC and ten commercial companies), a white mould infection model, driven by temperature and leaf wetness duration, was formulated. The trials indicated the key dates at which fungicide sprays should be targetted for maximal control of infection resulting in the potential to halve the number of sprays required by eliminating those otherwise applied on inappropriate dates.

The aim of this project was to validate (test, confirm or modify) the infection model and deliver it to the industry as a practical 'white mould alert' or '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 monitoring stations 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 for 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.

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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 also corresponded to the observations of white mould within the crop.

These results confirmed the potential value of using the infection model to forecast infective periods, and hence the optimal dates at which to apply fungicides. In 2008 the forecasting system was tested. Each of four narcissus crops areas were designated to receive either (a) the grower's routine spray programme ('commercial spray programme') or (b) a model-based spray programme based on applying fungicides only when a target period of white mould risk is indicated by high infection scores. These field trials demonstrated that the white mould alert system is simple and practical to use in a commercial situation, and it is hoped that white mould alerts will be available to growers in 2010 in a form similar to that of the current HDC Pest Bulletin.

Financial benefits

The 'Horticulture LINK' project (BOF 41) which developed the white mould infection model was subject to independent scrutiny which concluded that very considerable financial savings could be made by using a fungicide spray programme that reduces the total number of fungicide sprays applied. The disease warning system developed in this project will help growers to apply fungicides only when they will be most effective, with the potential to achieve better disease management at lower cost and with a lower environmental impact.

NOTE

Action points for growers

Further information about the decision support system is available in HDC News and through the grower workshops held in 2009. As a result of this project and project BOF 59 (developing a decision support system for smoulder) setting up a white mould and smoulder disease bulletin that will provide weekly alerts for growers when crops should be sprayed is being considered.

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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 predicted using a white mould infection model developed in a 'Horticulture LINK' project, BOF 41. Comparing the predictions with what actually occurs 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.

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 comparisons of observed and predicted white mould levels carried out in 2007 and practical trials of the spray warning system carried out in 2008. The aim is to deliver a disease bulletin or spray warning system for growers.

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Materials and methods

White mould monitoring

In autumn 2006, four Cornish narcissus crops were selected for monitoring (Table 1). These were considered typical commercial crops of the region. In each crop a *ca.* 0.2 ha area was marked with corner posts and appropriate signage, 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 0.1 ha 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. Experience in Projects BOF 41 and 56 had shown that the appearance of white mould was sporadic, and effort was concentrated at the Gweek and Castle Kayle sites, where meteorological monitoring stations were set up, while the Mawnan Smith and Gulval sites were kept as 'reserves' should white mould symptoms fail to appear at the first sites.

Table 1. Crops used for monitoring white mould in 2007

Site name	*Grid reference*	*Cultivars*	*Crop year*
Castle Kayle	SW 585357	'Golden Ducat' and 'Standard Value'	3
Gulval	SW485325	'Cheerfulness'	3
Gweek	SW698285	'Dutch Master'	3
Mawnan Smith	SW775295	'Hollywood'	1

Weather data

Prior to crop emergence a meteorological monitoring station (MMS) ('Smaartlog'; Aardware Design Ltd., Kingston, UK) was set up in the centre of the monitoring areas at Gweek and Castle Kayle (December 2006) and in the centre of the trials areas at Gweek and Rosewarne (December 2007). Where one MMS was 'shared' between two sites, the sites had been considered close and comparable enough for this to be acceptable. The MMS, 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), all at 30-minute intervals. The PI sensor ranked impacts into 14 levels ('bins') from the lowest energy (1) to the highest (14).

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White mould assessments

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

Score	*Incidence*	*Score*	*Severity*
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

Forecasting infection

A white mould infection model that relates the number of disease lesions to temperature and to duration of leaf wetness periods (see Final Report for 'Horticulture LINK' Project 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 MMS in the crops, enabling a comparison to be made between the predicted infection score and the observed, actual extent of white mould symptoms. A correspondance of predicted and observed levels would validate the model, while dissimilar results would indicate that the model was inappropriate or needed to be refined. For interpretation, the infection score was averaged for 24-hour periods starting at 00:00 h. Because white mould infection is facilitated by crop damage, the following were also considered factors in triggering white mould infection: (a)

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the occurence of frost (a screen temperature below -1°C), (b) the occurrence of >2 impact events in 'bin' 7 or higher of the PI sensor or (c) other crop damage (e.g., due to flower cropping). Impacts of bin 7 were the threshold for potential leaf damage by precipitation, derived from drop-impaction energy studies conducted in project BOF 41 (see above).

During 2008 the model was programmed using 'Matlab' modelling software, this being necessary to speed up the rate of processing of meteological data compared with the 'Microsoft Excel' version previously used in BOF 56. This gave improved accuracy and ease of processing. Comparisons were made between the results of using the 'Matlab' and 'Excel' versions, and showed that the two versions of the software produced essentially similar results over the same date range (see example in Figure 1). The Matlab software was therefore used for all further forecasts.

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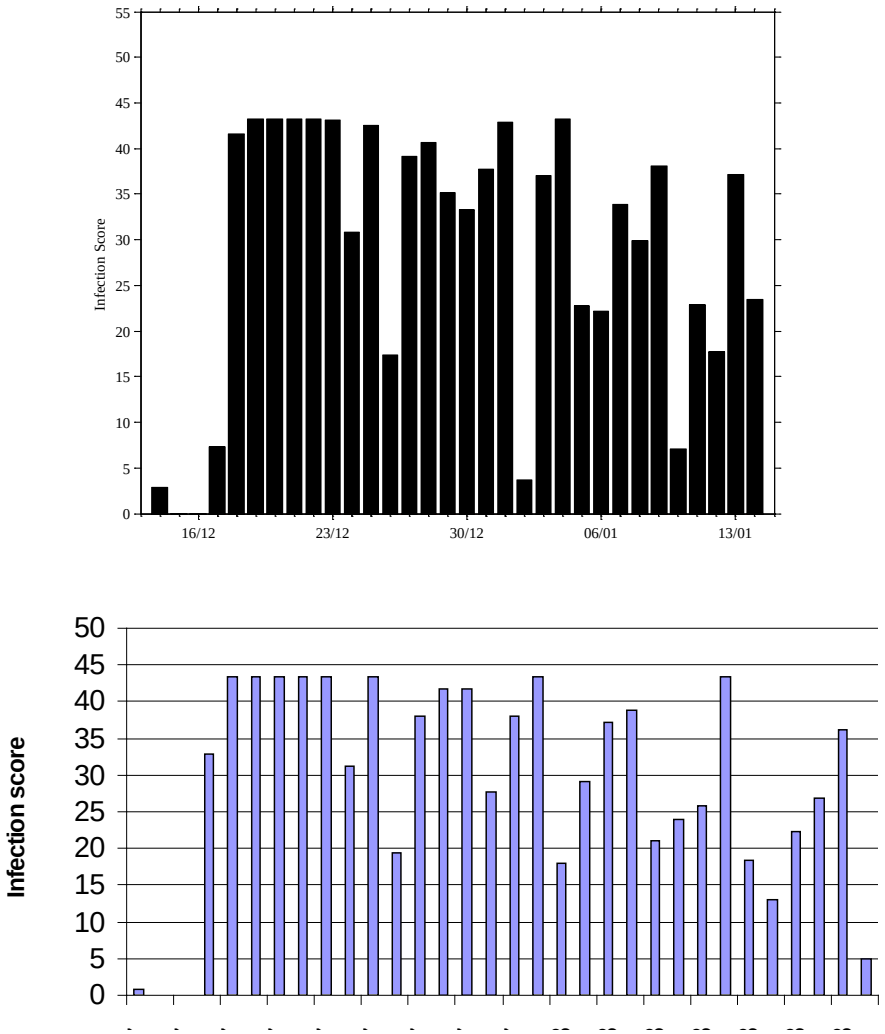
Figure 1. Infection scores produced using ‘Matlab’ (top) and ‘Excel’ software (bottom).



Spore trapping studies

Trap-plant production

In August 2006 bulbs of narcissus ‘Carlton’ (grade 12-14 cm circumference) were allocated from a stock grown at Warwick HRI, The Kirton Research Centre (KRC), Lincolnshire for the production of trap-plants. For 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 20 cm-diameter, 4 L-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 watered as required. In December 2006 the trap-plants were transported to Camborne, Cornwall and grown-on outdoors until used in the field trial at Gweek in 2007.

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In 2007-2008 further trap-plants were produced in the same way as before, but were transported to Wellesbourne in January 2008 for use in a field trial there.

Trap-plant studies

Between 7 February and 2 May 2007, trap-plants were placed adjacent to crop foliage near the centre of the plot being monitored at Gweek for exposure periods of *ca*. 5 days. For each exposure period, three plant-pots were used, and, unlike those used in the earlier study (see Final Report on Project BOF 56), the foliage of these plants was not damaged prior to exposure. 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 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 pot-saucers to avoid spreading infection. The trap-plants were examined for disease lesions at weekly intervals for 12 weeks, recording incidence and severity scores as already described (Table 2).

In spring 2008 white mould developed on a third-year-down field plot of narcissus 'Cheerfulness' growing at Wellesbourne. During April 2008 about five pots of trap-plants per day were distributed around the crop, exposed from 18:00 h until 18:00 h the following day (on some occasions at weekends trap-plants were exposed over 3-day periods) and then moved to a glasshouse (as described above).

Spore trap studies

Following shoot emergence in the monitoring site at Gweek in 2007, a Hirst-type 24-h volumetric spore trap (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) was set up. The sampler consists of a metal body with a rectangular inlet slit (14mm-high x 2mm-wide) through which air is sampled at *ca.* 10 L/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 moving 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 x 400 magnification) and later using a polyclonal antibody (PAb) developed in Project BOF 56.

During the 2007 and 2008 seasons a MTIST air sampler (Burkard Manufacturing) was set up at Gweek in unsprayed parts of the crop to determine the presence of white mould spores

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in the air (Kennedy *et al*., 2000). This sampler impacts air-borne particulates onto microtitre strips which can be processed using enzyme-linked immunosorbant assay (ELISA), having the advantage of being a faster technique for white mould detection and quantification. 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 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. This process was repeated for each coating preparation. After treatment the coated microtitre well strips were secured within ELISA multiframes (Catalogue No. 9503060, Life Technologies Ltd, see 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 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 (see Final Report on Project BOF 56).

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 at 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-

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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.

Field testing the spray-timing system

Four field trials were done in 2008 to test the proposed spray-timing system for white mould. Details of the crops used are shown in Table 3. In each crop a *ca.* 0.4 ha area was designated and divided into two equal treatment areas of *ca.* 0.2 ha each. Each area was marked with corner posts and signage to indicate the spray programme to be used. Apart from applying the specified fungicide spray programmes to these areas, each grower was asked, in all other respects, to grow the crops according to his current commercial practices. The central *ca.* 0.1 ha of each 0.2 ha area was further marked with 50 sampling points, as described for the monitoring sites the previous year.

Table 3. Crops for testing fungicide spray programmes in 2008

Site name	*Grid reference*	*Cultivar*	*Crop year*
Rosewarne	SW637416	'Golden Ducat'	3
Leedstown	SW604337	'Golden Ducat'	2
Gweek	SW698285	'Dutch Master'	4
Mawnan Smith	SW775295	'Hollywood'	2

Two spray programme treatments were tested at each site:

1. *Commercial spray programme* - each grower was asked to apply his routine fungicide spray programme as used on his other daffodil crops, deciding the timing of fungicide applications and also the rates, number and methodology of spraying.
2. *Spray-timing or model-based programme* - each grower was asked to apply the same fungicide programme as just described, but only when triggered by Warwick HRI staff

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after running the white mould model each week. For this programme a maximum of three sprays was to be applied.

In determining when to apply fungicide sprays using the infection model, the following criteria were used:

- When the infection score was 35 or more daily during any week or
- When the infection score was 25 or more daily during any week and there had been frost, high-energy precipitation or other crop damage (such as flower cropping) that week.

Once the infection score had reached the critical level, the grower was asked to apply fungicide to his spray-timing area as soon as practical but also taking account of the following: (a) no sprays were to be applied until sufficient crop foliage was present to make spraying worthwhile (e.g. if a significant proportion of the shoots had reached a height of 5 to 10cm), (b) the minimum interval between applying fungicides as stated by the producer, and (c) sprays were to be delayed if flower cropping was taking place or was shortly to begin, the appropriate harvest interval being observed. In practice, delays in spraying are caused by unsuitable weather and commercial considerations as well as (a), (b) and (c). The dates and other details of the fungicide spray requests and applications are shown in Table 4 (see next page).

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Table 4. Details of fungicide spray applications in commercial and spray-timing fungicide programmes in 2008

Spray programme	*Spray number*		
	1	*2*	*3*
Rosewarne			
Commercial	Applied 29 January Delsene 50 Flo + Folicur + Amistar 1.0L/ha + 0.5L/ha + 0.5L/ha	Applied 02 March Delsene 50 Flo + Folicur + Amistar 1.0L/ha + 0.5L/ha + 0.5L/ha	Applied 15 April Pencozeb + Bravo 500 + Folicur 1.5kg/ha + 3.0L/ha + 0.5L/ha
Model-based	Requested 24 January Applied 29 January Delsene 50 Flo + Folicur + Amistar 1.0L/ha + 0.5L/ha + 0.5L/ha	Requested 15 February Applied 02 March Delsene 50 Flo + Folicur + Amistar 1.0L/ha + 0.5L/ha + 0.5L/ha	Requested 26 February Applied 15 April Pencozeb + Bravo 500 + Folicur 1.5kg/ha + 3.0L/ha + 0.5L/ha
Leedstown			
Commercial	Applied 28 January Delsene 50 Flo + Folicur + Amistar 1.0L/ha + 0.5L/ha + 0.5L/ha	Applied 27 March Dithane NT + Mantra + Bravo 500 1.5kg/ha + 0.6L/ha + 3.0L/ha	Applied 22 April Pencozeb + Bravo 500 + Folicur 1.5kg/ha + 3.0L/ha + 0.5L/ha
Model-based	Requested 24 January Applied 28 January Delsene 50 Flo + Folicur + Amistar 1.0L/ha + 0.5L/ha + 0.5L/ha	Requested 15 February Applied 27 March Dithane NT + Mantra + Bravo 500 1.5kg/ha + 0.6L/ha + 3.0L/ha	Requested 26 February Applied 22 April Pencozeb + Bravo 500 + Folicur 1.5kg/ha + 3.0L/ha + 0.5L/ha

Table 4. Details of fungicide spray applications in commercial and spray-timing fungicide programmes in 2008 (continued).

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Gweek			
Commercial	Applied 30 January	Applied 27 February	-
	Micene DF + Bravo 500	Rovral Flo + CC Celeb	-
	2.5kg/ha + 2.0L/ha	0.5L/ha + 0.5L/ha	-
Model-based	Requested 24 January	Requested 15 February	Requested 26 February
	Applied 30 January	Applied 27 February	Not applied
	Micene DF + Bravo 500	Rovral Flo + CC Celeb	-
	2.5kg/ha + 2.0L/ha	0.5L/ha + 0.5L/ha	-
Mawnan Smith			
Commercial	Applied 25 January	Applied 23 February	Applied 8 April
	Micene DF + Bravo 500	Rovral Flo + CC Celeb	Folicur + Occidor 500 SC
	2.5kg/ha + 2.0L/ha	0.5L/ha + 0.5L/ha	1.2L/ha + 1.0L/ha
Model-based	Requested 24 January	Requested 15 February	Requested 26 February
	Applied 25 January	Applied 23 February	Applied 8 April
	Micene DF + Bravo 500	Rovral Flo + CC Celeb	Folicur + Occidor 500 SC
	2.5kg/ha + 2.0L/ha	0.5L/ha + 0.5L/ha	1.2L/ha + 1.0L/ha

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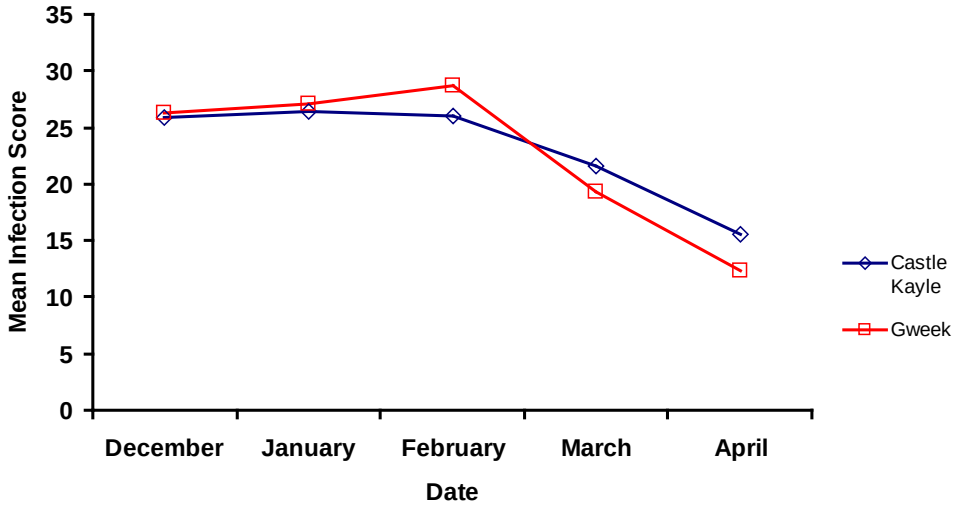
Results

White mould monitoring (2007)

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 MMS in the crops. For easier interpretation, the daily infection scores (see Figure 3) are presented as monthly averages in Figure 2. This shows the somewhat higher infection scores at Gweek in February but at Castle Kayle in March and April.

Figure 2. Mean monthly infection scores predicted using the white mould model for Gweek and Castle Kayle in 2007



In Figure 3 (see below) the daily infection scores are presented in red for days on which there was a high incidence of frost or high-impact precipitation. At Gweek only two leaf damage events were observed, i.e. frosts on 22 December 2006 and 21 March 2007, while in contrast, at Castle Kayle, several damage events (high-impact precipitation) were recorded during February, March and April.

While the white mould model produces an infection score, it cannot determine when fungicide should be applied, and for this a suitable threshold score has to be agreed. In this study average infection scores around 25 appeared to denote a line between higher and lower white mould risk, although higher daily individual scores were observed at both sites.

The observed incidence and severity of white mould infection are shown in Figure 4. 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

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incidence and severity increased rapidly, all 50 sample areas being 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.

At the additional sites - Mawnan Smith and Gulval - white mould infections occurred late in the season and symptoms were relatively slight. White mould is known to occur sporadically, for as yet unknown reasons, though this may be due to local variations in the level of inoculum (see trap-plant results for 2008).

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Figure 3. 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)

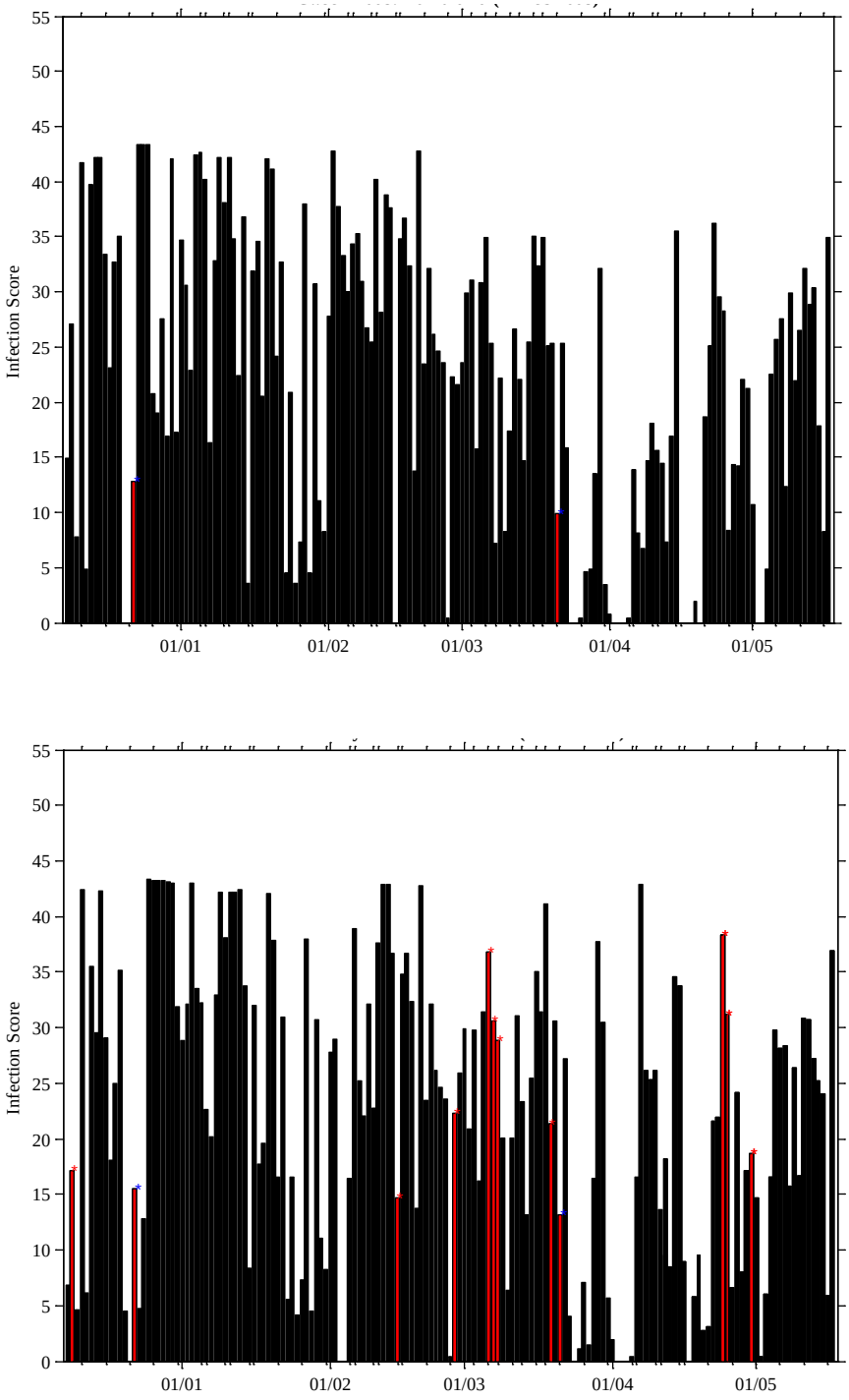

Figure 4. White mould monitoring in 2007 at (from top to bottom) the main sites at Gweek and Castle Kayle and the additional sites at Mawnan Smith and Gulval. Note that the scale of the left-hand axis for the Gweek site is different to the other sites

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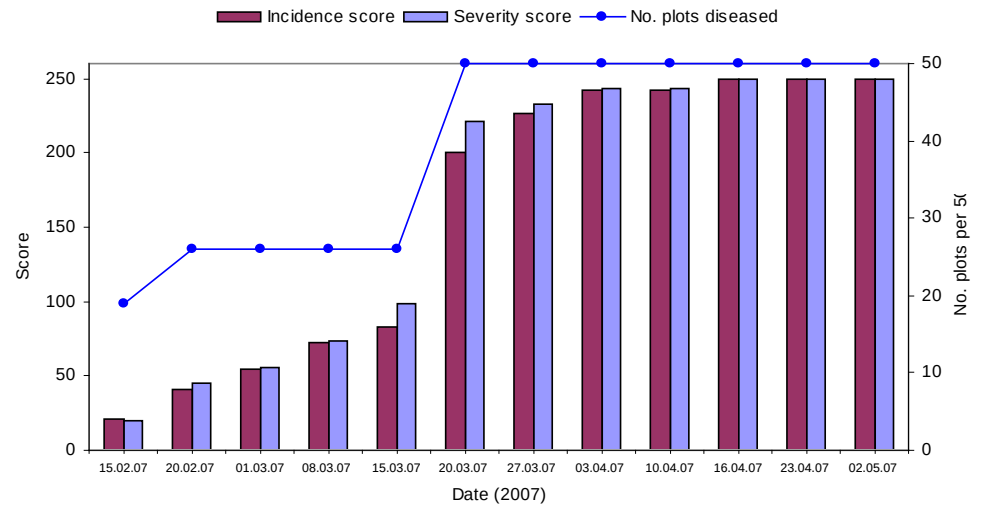

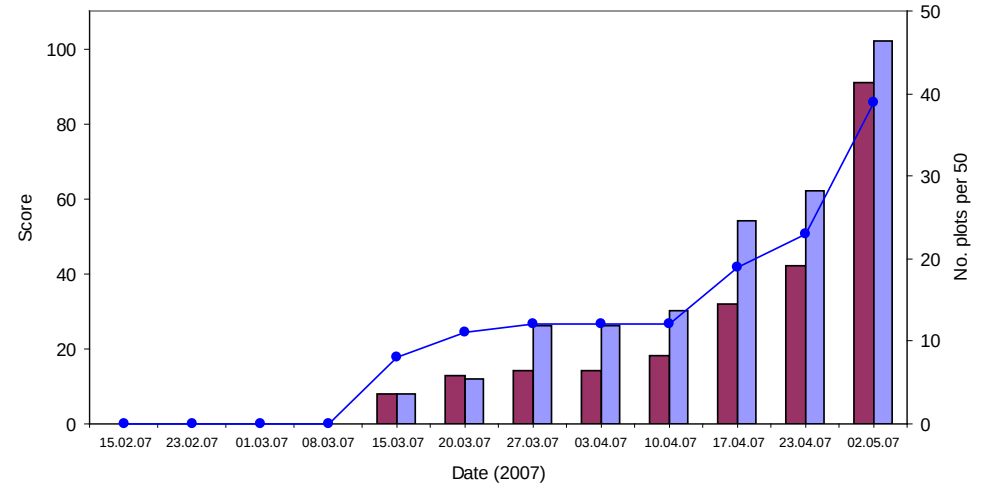

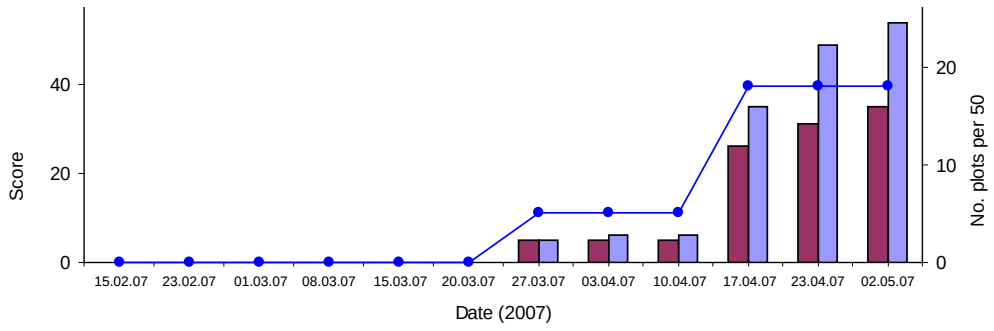

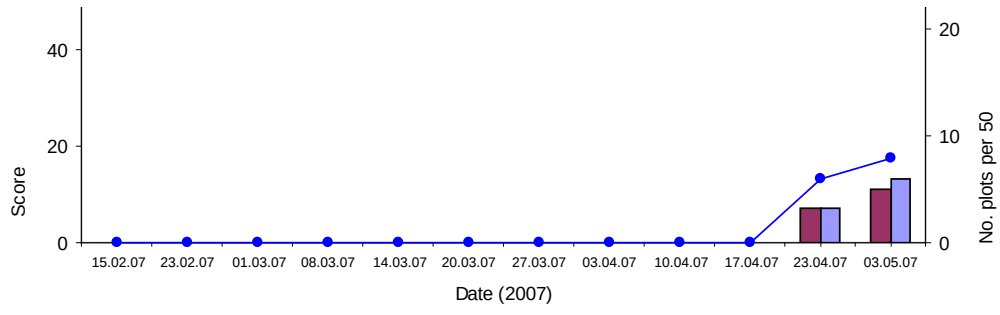

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Spore trapping studies (2007)

The ELISA technique 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 5 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 period 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 two with silicone.

Figure 5 shows that white mould spores were present in the air in large numbers at Gweek over the periods 1–7 and 20–27 February and 1–14 March and after 3 April, 2007. 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. 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 the type of well coating did not significantly affect 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 5. Absorbance values in wells containing particulates from the air after staining with a white mould-sensitive antibody, using wells coated with either silicone (blue line) or sodium azide (red line).

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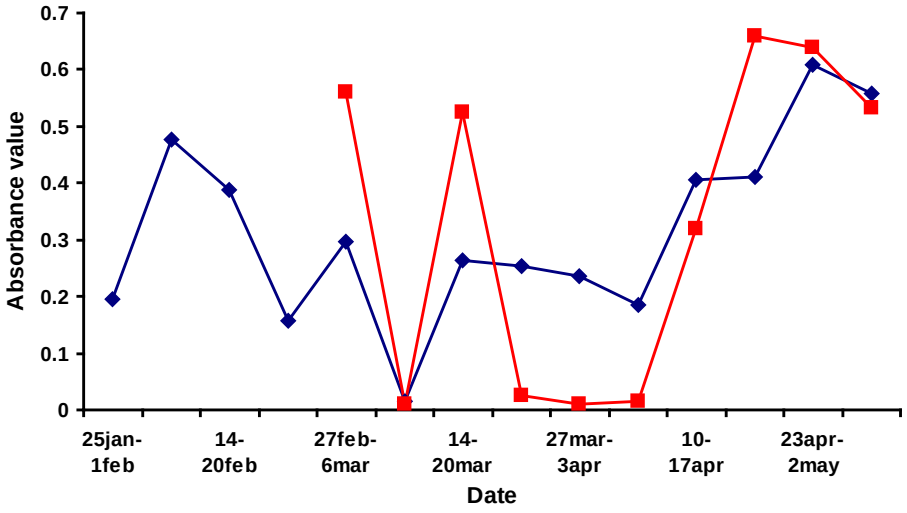

The seven-day Burkard spore trap was operated between 14 March and 14 April 2007 at Gweek. The results were used to confirm the presence of both scolecospores and phragmospores of white mould in air samples. Low daily numbers of both spore types were found in air samples collected during this period. The results from 15 to 16 March are shown in Table 5. Scolecospores were only found during day-time periods, confirming the results obtained at Kirton in 2006 (see Final Report for Project BOF 56). The results also confirmed the pattern of inoculum availability seen using the MTIST trap.

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Table 5. Numbers of white mould scolecospores and phragmospores present on 15–16 March 2007 at Gweek

Time (hh:mm)	*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*

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, while trap-plants from later exposure periods failed to show symptoms. In all cases the symptoms continued to increase progressively.

Spore trapping studies (2008)

The ELISA technique was applied to air samples collected using the MTIST spore trap at Gweek in 2008, where white mould was already present. Figure 6 shows the ELISA absorbance values in microtitre wells from these air samplers. Wells were exposed for approximately 7-day periods in the crop. The sampling wells were coated with sodium azide to improve the collection and retention of air-borne particles within the trap (compared with uncoated wells). The results indicated that white mould spores were present in the air in large numbers at Gweek from 18 February until 7 April 2008. White mould incidence

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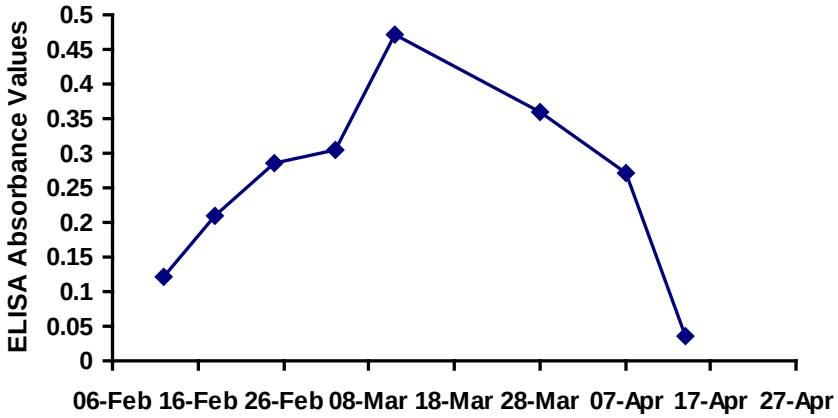
increased markedly after 18 March 2008 in the crop, and these spore trapping studies showed why this increase occurred.

Figure 6. Absorbance values in wells containing particulates from the air after taining with a white mould-sensitive antibody, using wells coated with sodium azide



Trap-plant studies (2008)

In 2008 white mould developed on a third-year-down plot of narcissus 'Cheerfulness' growing at Wellesbourne. During April 2008 trap-plants were exposed in the plot, usually on a 24-hour basis, following which they were placed in a glasshouse to allow any symptoms to develop. The results are shown in Figure 7. Small numbers of white mould lesions resulted following six of the exposure periods, 2–3, 4-7, 9–10, 11-14, 23–24 and 24–25 April 2008. Of these, only three periods (9–10, 23–24 and 24–25 April) had significant amounts of disease.

Figure 7. Trap-plant white mould infection at Wellesbourne during April 2008

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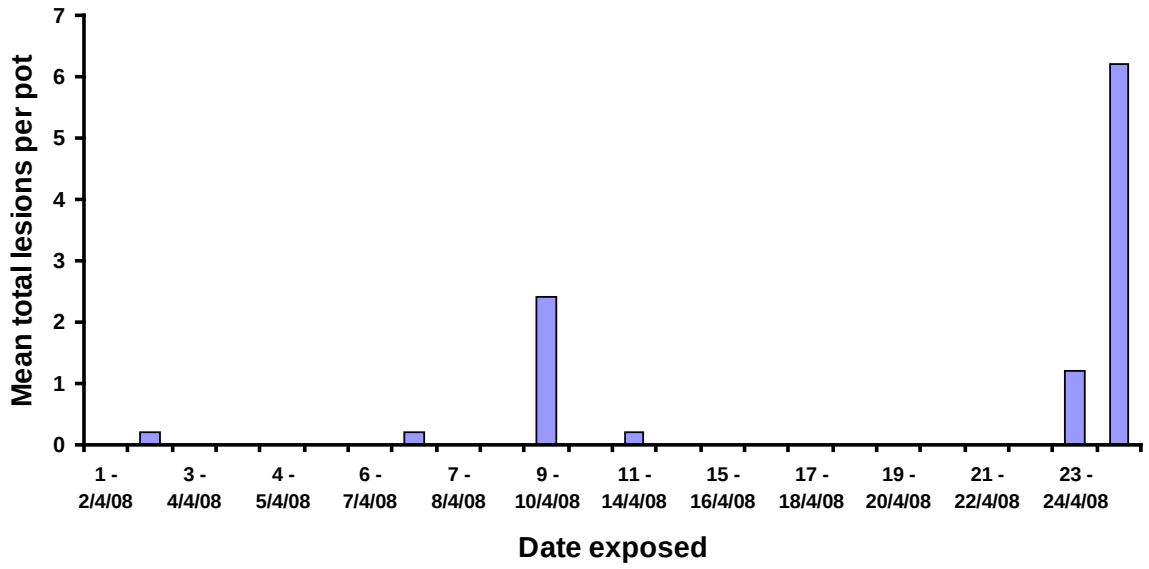

Examination of the weather data associated with the days resulting in the development of reasonable numbers of white mould lesions indicated low night temperatures with frost conditions followed by high day temperatures of over 10°C. It is possible these conditions promoted mist or fog, although this could not be confirmed. On all occasions where trap-plant infection was observed, low night temperatures (with the presence or absence of frost temperatures at the point of recording) were followed by high day temperatures of 12–20°C (Figure 8). Additionally, hail-stones were observed at Wellesbourne on 24 and 25 April. There is little doubt that the elevated levels of white mould infection observed on trap-plants at Wellesbourne during these periods resulted from infection following leaf damage cause by hail. It is clear that damage must occur to the leaf in the field for white mould infection to occur. This can arise from either hail damage or from frost action. Hail damage can be measured using the output from an impact sensor although the threshold value used to assess this might need further investigation. The occurrence of sporadic frosts can be recorded using the output from the weather station. It is likely that the positioning of the weather station in locations prone to frost or hail could help improve the forecasts for white mould in Cornwall.

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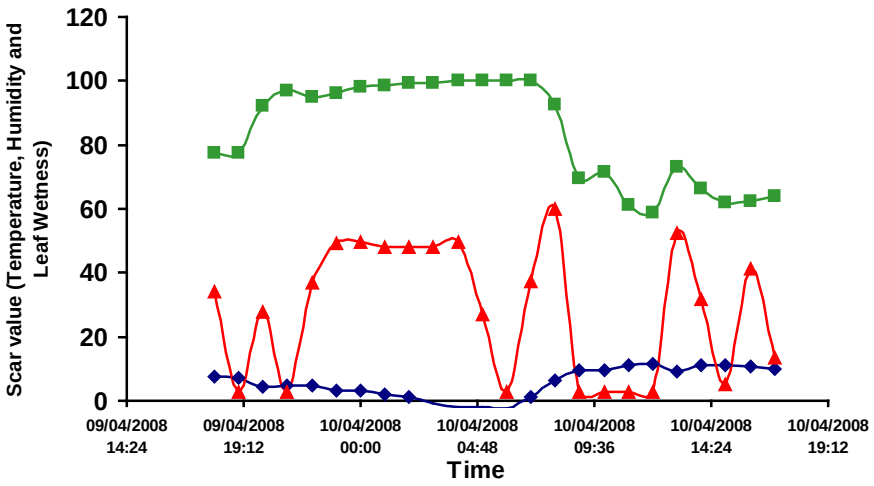
Figure 8. Weather conditions during trap-plant exposure on 9-10 April 2008 at Wellesbourne: humidity (□), leaf wetness (□, threshold for leaf wetness = 40) and temperature (□)



Field testing the spray-timing system (2008)

White mould infection predictions were generated using the model at about weekly intervals, starting once a reasonable foliage area was present and continuing until three spray requests had been made. The predicted white mould infection scores for the Gweek and Rosewarne sites are shown in Figure 9 (see next page; see Table 4 for full details of the fungicide sprays applied). It is clear that at both sites there was an on-going high risk of white mould throughout much of the growing season. The weekly predictions, and actions taken as a result, are summarised in Table 6.

At Gweek, the threshold score of 35 was exceeded in all but one week of the relevant period, i.e. from late-January to the end of February. Because of spray interval considerations, no spray requests were generated two weeks running, so that three sprays were requested over the 5-week period. At Gweek, there were no occasions over this period when frost or high-impact precipitation were recorded. The same spray programme was used at the site at Mawnan Smith.

At Rosewarne, the threshold score of 35 was exceeded in all weeks of the relevant period except the week ending Friday 29 February, when the lower threshhold of 25 was exceeded and frost occurred, triggering a spray request. Because of spray interval considerations, no spray requests were generated two weeks running, so that three sprays were requested over the 5-week period, as at Gweek. The same spray programme was used at the site at Leedstown.

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Table 6. White mould predictions and action taken at two sites in 2008. Predictions were generated at about weekly intervals starting once a reasonable foliage area waspresent, and continuing until three spray requests had been made

Period ending	*White mould prediction and action taken at Gweek and Rosewarne sites*	
	Gweek	*Rosewarne*
24 January 2008	Score ≥35, spray requested	Score ≥35, spray requested
01 February 2008	Score <35, no spray request	Score ≥35, no spray request as within spray interval
15 February 2008	Score ≥35, spray requested	Score ≥35, spray requested
22 February 2008	Score ≥35, no spray request as within spray interval	Score ≥35, no spray request as within spray interval
28 February 2008	Score ≥35, spray requested	Score ≥25 and frost, spray requested

Of the 12 sprays requested, one was not applied (the crop at Gweek was treated with glyphosate to ready the land for the following crop) and the others were applied between 1 and 56 days after the request, with an average delay of 19 days (Table 4). From the experimental point of view this was very regretable, but unfortunately this reflects the lack of 'spray-days' in the region and the growers' economic considerations when organising crop sprays. It was disappointing that there were no differences between the actual application dates for the model-based and commercial spray programmes at any of the sites; again, this reflects commercial considerations: a spray contractor is routinely used, so sprays in any one location tend to be done on the same day, for economy.

Disease development at the four sites is shown in Figure 10. There was a steady increase in white mould incidence over the February to April period, though at Leedstown disease levels were markedly lower than at the other sites. As expected from the similar spray programmes, no clear differences can be determined between disease levels in areas treated with the model-based or commercial spray programmes; those minor differences between treatments that were seen probably lying within the expected parameters of normal biological variation. At all sites, however, successful disease control was indicated by the

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presence of white mould lesions that had ‘dried-up’, and only at Leedstown was there was a later resurgence of white mould.

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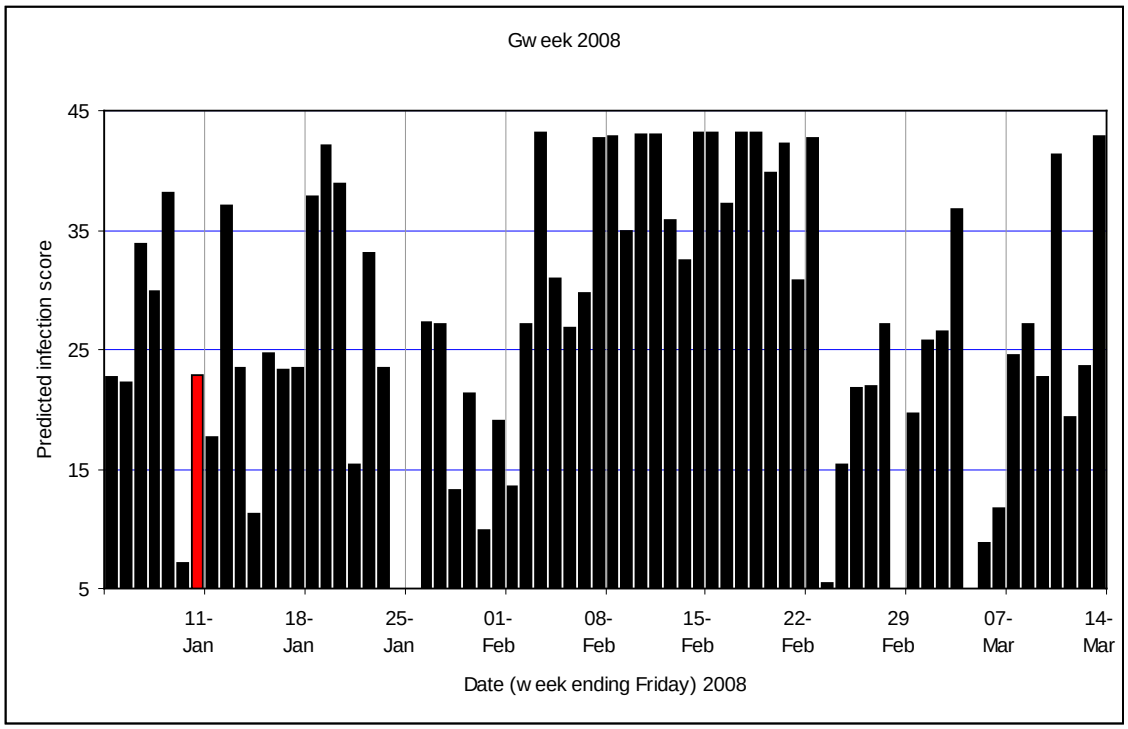
Figure 9. White mould infection scores for the 2008 growing season at Gweek (above) and Rosewarne (below). Blocks in red or blue indicate that high-energy precipitation or frost, respectively, occurred simultaneously



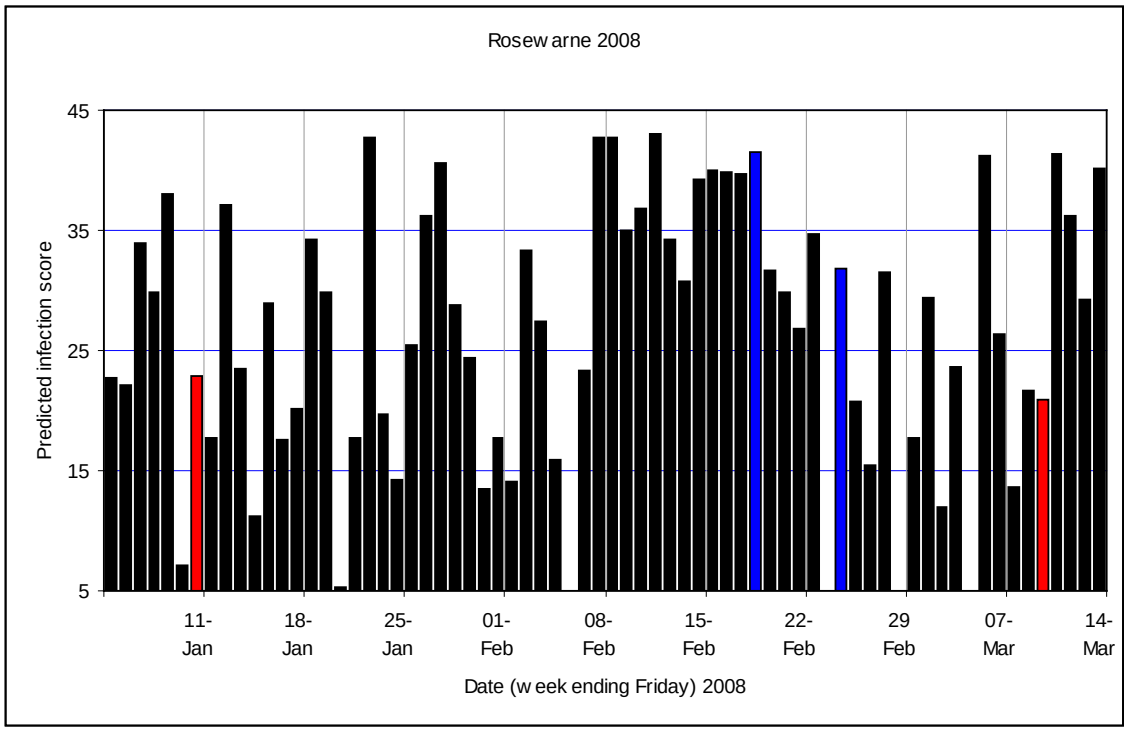


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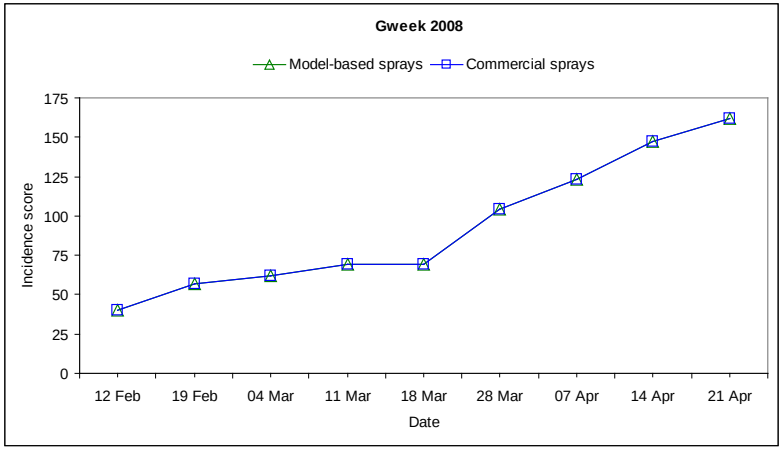
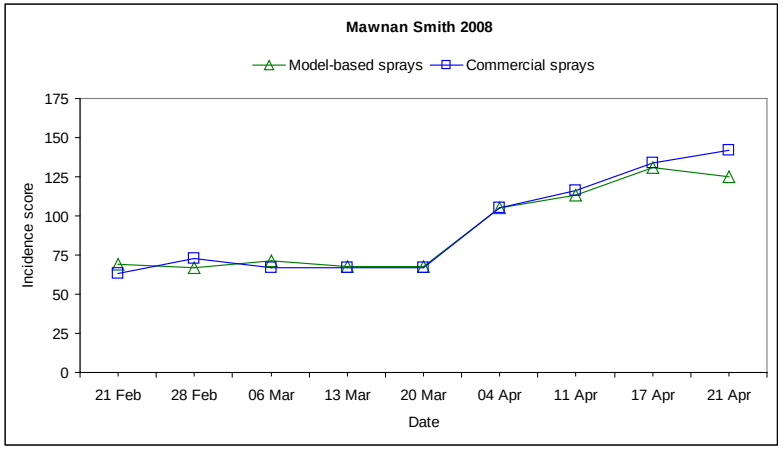
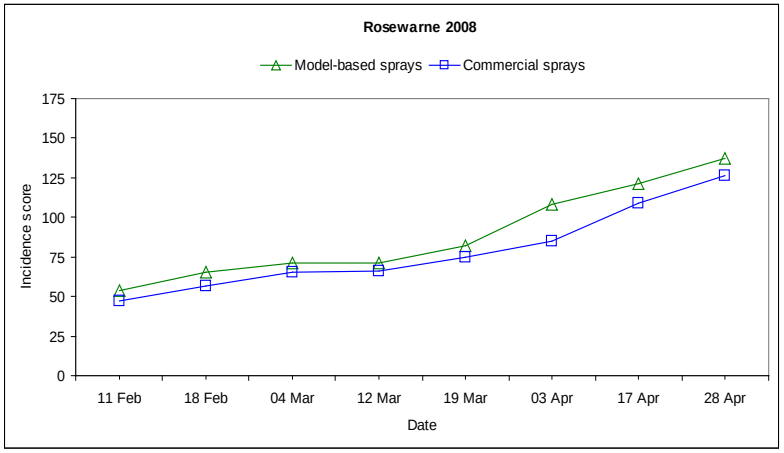
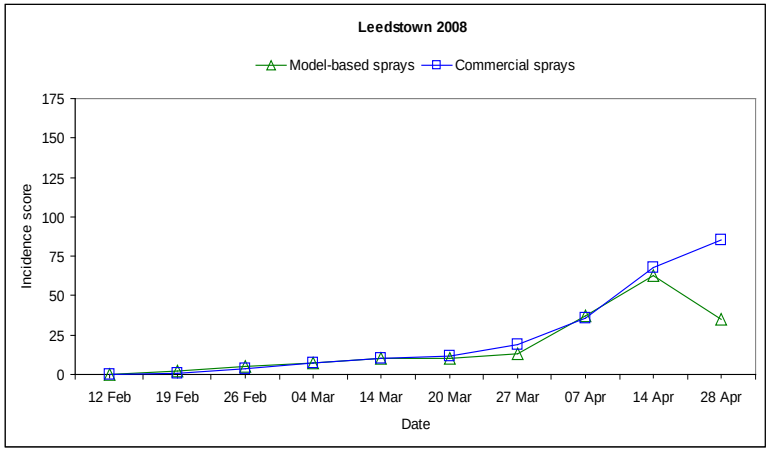
Figure 10. White mould incidence in 2008 in narcissus receiving model-based and commercial spray programmes. Data for sites at Gweek, Mawnan Smith, Rosewarne and Leedstown









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Discussion

In 2005 – 2007, typical commercial narcissus crops were monitored in Cornwall for the incidence, severity and spread of white mould (*Ramularia vallisumbrosae*), a little-studied but periodically epidemic and seriously damaging pathogen of narcissus crops in south-west England. Fungicide applications were excluded from these monitoring sites. In 2005 and 2007 white mould infections were common on Cornish narcissus crops, and occurred on the crops being monitored, though in the intervening year white mould was generally absent and was not seen on the monitored crops.

In 2006, however, in a parallel study by our research group in Lincolnshire of another narcissus foliar disease, smoulder (caused by *Botrytis narcissicola*; HDC- and LEADER+-funded project BOF 59), a white mould epidemic occurred at Kirton, providing useful further data. In a preceding Horticulture LINK study (HDC project BOF 41), the environmental and other requirements for white mould infection and development had been elucidated; these were three-fold: an appropriate temperature, an adequate period of leaf surface wetness, and the presence of a damaged plant surface (for example, resulting from hail, frost or mechanical damage from flower picking). By means of experiments in controlled-environment (CE) cabinets, a predictive model was derived, relating the expected extent of white mould lesions to temperature and leaf wetness duration.

The objective of crop and disease monitoring in Projects BOF 56 and 56a, in 2005 to 2007, was to compare predicted white mould development with actual, observed disease development. Since the development of a disease requires not only suitable conditions, but also presence of infective units, the CE and monitoring studies were supplemented with spore-trapping and trap-plant experiments. Following difficulties encountered in the first year of the study in the quantification of white mould spores, a monoclonal antibody was raised against *R. vallisumbrosae* and was subsequently used in an immuno-fluorescence technique to quantify white mould spores. It is also known that white mould infections can be related to the presence of infective units remaining in the soil or on debris, and, though not studied further in this project, this factor may account for the sporadic nature of white mould epidemics. The white mould pathogen produces at least two types of spores, phragmospores and the very elongate scolecospores, both produced on the same lesions, and it is likely that each spore type requires different conditions for infection; further study is required to ascertain if production of both spore types are required if epidemic development of white mould is to take place.

In 2005 and 2007 the model's predicted white mould infection periods coincided with the appearance of white mould symptoms in field crops and with white mould occurrence using trap plants and inoculum detection. In contrast, running the model indicated that in 2006

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development of a Cornish white mould epidemic was unlikely. Taken together, these results confirmed the validity of the predictive model, suggesting that the model could be used as the basis of a practical 'white mould alert' or 'spray-timing' system to inform bulb growers of the optimal, target dates for fungicide application. Prior to delivering a white mould alert system, it was important to test a prototype system on commercial crops. In 2008, therefore, four commercial crops were used to compare white mould control under (a) typical current 'commercial' (or 'conventional') fungicide spray programmes and (b) a model-based spray programme. Essentially, the only difference between the two programmes were the target dates for fungicide application. The commercial spray programme is essentially, though not entirely, a calendar-based programme, while the model-based programme signals the need for fungicide application only when a 'threshold' infection score is reached or exceeded as a result of running the model with the current weather data on a weekly basis; typically, in the present studies, a spray was signalled once an agreed threshold was reached, or, if crop damage had been likely in the same period (e.g. through high-impact rain or hail, frost or crop damage by flower picking) once a lower threashold had been reached. Based on earlier results (see project BOF 41) it was known that three targeted fungicide applications could be as effective in managing narcissus foliar diseases as up to six fungicide applications applied on a conventional basis. In the evaluation of the model-based spray programme, therefore, it was agreed that no more than three fungicide sprays would be applied in this programme in a growing season.

In 2008 difficulties in applying fungicides at the target dates – due to coincident unsuitable weather for spraying, or to logistical or economic difficulties – meant there was little difference in the two spray programmes delivered to the four crops, and both spray programmes achieved reasonable management of white mould, all crops and treatments developing 'dried-up' white mould lesions that did not progress to the wholesale loss of foliage seen in typical white mould epidemics. Although the difficulties of running field trials in a commercial situation were evident, this in no way detracted from the earlier conclusion that the white mould infection model was valid as a determinant of target spray dates.

The trials in 2008 also provided the opportunity for testing the proposed 'white mould alert' system on a practical level, and confirmed it could be delivered as a useful tool for growers. This could be set up as an HDC White Mould Bulletin, analogous to the present HDC Pest Bulletin (see http://www2.warwick.ac.uk/fac/sci/whri/hdcpestbulletin). This is a web-based service that HDC members can easily access, providing a weekly spray warning for several crop pests throughout the growing season. It was reported recently (*HDC News*, no. 149 (December 2008 – January 2009), p.15) that use of the HDC Pest Bulletin service has jumped by almost 40% in the last year, confirming the value of this approach. As a result of a parallel HDC-funded project (BOF 59), disease forecasting has also been developed and tested for smoulder disease of daffodils (caused by *Botrytis narcissicola*). Since both

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diseases can occur on UK narcissus crops, bulb growers should consider both diseases and spray warnings irrespective of their regional location. A project proposal is therefore being written, involving setting up and running a white mould and smoulder spray-warning alert along the lines of the HDC Pest Bulletin. This would require weekly updates of weather data (air and ground temperature, leaf wetness duration and precipitation impact) from representative bulb-growing regions of the UK (west Cornwall, east Cornwall, the Lincolnshire Fenlands, east Norfolk and the Grampians). Automatic weather stations would be set-up and run in the key regions, because surface wetness duration and precipitation impact are not available in the standard Meteorological Office data-sets that can be purchased. At weekly intervals during the growing season data from the weather stations would be downloaded and used to run the two infection models. Initially this would be done using 'stand-alone' software, though in the longer term it is hoped the models could be incorporated into the existing MORPH decision support software. The resultant 'infection scores' would be interpreted and growers would be advised to 'spray this week' or 'don't spray this week', as appropriate, and the web-page would be updated weekly. The spray-warning page could include information on typical foliar fungal disease symptoms and information on current, suitable fungicides.

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