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Project leader(s):	Malcolm Millar ADAS UK Ltd Boxworth Cambridgshire CB23 4NN T: 01954 268214 F: 01954 267659 E: malcolm.millar@adas.co.uk
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Key worker(s):	Malcolm Millar BSc – Project Leader (ADAS) Dr Roy Kennedy BSc PhD (Warwick HRI) Gordon R Hanks BSc, MPhil, FIHort, MIBiol, CBiol (Warwick HRI) Pippa Hughes BSc (Warwick HRI)
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Project co-ordinator(s):	Arthur Andrews Winchester Growers Ltd Varfell Farm Penzance Cornwall TR20 8AQ
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Narcissus white mould decision support system

Summary

Headline

The project has contributed towards the evaluation and improvement of the white mould infection model, which in turn will lead to a decision support system that will give the timing of fungicide sprays needed to control white mould in a cost-effective and sustainable way.

Background and expected deliverables

The management of narcissus leaf diseases is of major concern to bulb growers in Cornwall and the Isles of Scilly, since the production of disease-free bulbs and foliage is important in meeting the high quality specifications demanded by the export trade and the multiple-retail sector. In today's marketplace it is expected that pests and diseases will be managed with a minimal, and always justified, use of pesticides.

In the past decade UK bulb growers have become concerned about an increase in the incidence of fungal foliar diseases, particularly white mould (caused by *Ramularia vallisumbrosae*).

For many years the control of narcissus foliar diseases has been by a more or less non-specific programme of fungicide sprays. Up to seven sprays, often tank-mixes, are used on narcissus crops each year. In a Defra-, HDC- and industry-funded 'Horticulture LINK' project completed in 2002, the factors leading to the infection of narcissus leaves with white mould were examined. The results showed that infection was dependent on temperature and leaf wetness duration, and a mathematical model was formulated to describe this relationship. The model allows likely infective periods to be identified through interrogating data from temperature and surface wetness sensors sited in the crop, enabling growers to target fungicide applications to key periods when they will be most effective. Field trials, part of the same project, showed that the number of fungicide sprays used on a narcissus crop could be

reduced by half, without loss of control, through such targeting, also ensuring that fungicide spray programme is made in an environmentally friendly way.

The current study aimed to translate the findings of the Horticulture LINK project into a practical system that growers and advisors can use. This involved validating the model and delivering it as a user-friendly 'spray-timing system'.

The expected deliverables from this project are:

- Clear guidelines on the timing and number of fungicide sprays needed to control white mould.
- An evaluation of spray-timing to establish a decision support ('spray-timing) system.

Summary of the project and main conclusions

In 2005 and 2006, commercial narcissus crops in Cornwall were monitored for the incidence and severity of white mould (*Ramularia vallisumbrosae*) in the absence of fungicide spray applications. In 2005 white mould infections were common in Cornwall and occurred on the monitored sites, while in 2006 white mould did not occur generally in Cornwall and was also not observed on the monitored crops. In a parallel study of smoulder disease (*Botrytis narcissicola*) in Lincolnshire narcissus crops, a white mould epidemic occurred at Kirton, so data from this site were also utilised. To further study the epidemiology of white mould, spore traps and trap plants were placed in the monitored crops and meteorological data were logged in the crops. Following difficulties encountered in the first year of the study in the quantification of white mould spores, a laboratory technique was developed in the second year of the work. A predictive model of white mould infection was run for each site using the site's temperature and leaf wetness data, and the predicted and observed infection levels were compared in order to validate, or be able to improve, the infection model.

The observations in 2005 indicated that the model's predicted white mould infection periods coincided with observed infection. In 2006 the laboratory technique was applied to spore samples collected from Kirton, the only site where white mould

developed fully and successful spore trapping was carried out. The results indicated that white mould spores were present in large numbers at Kirton from mid-May onwards, with the greatest counts observed on 17–18 May and smaller peaks occurring later. *R. vallisumbrosae* produces two types of spores and both types were observed on the traps, with larger numbers of type 1. The numbers of type 1 spores trapped also increased during the hours of daylight. Analysis of weather conditions during this peak in spore numbers indicated that the following conditions were necessary for type 1 production:

- Mean daily temperature (06.00-18.00h) above 12°C (optimal above 16°C)
- Daily rainfall of at least 0.3 mm
- No night frosts
- High daily white mould infection scores (>2)

The data indicated that several days of optimal conditions were required for production of both spore types, and optimal infection conditions had to coincide with these criteria if white mould epidemics were to develop. The production of type 2 spores was similar to that of type 1, though the lower numbers of type 2 trapped indicated that wind was not the primary agent of dispersal for them. Both spore types are produced on the same lesion and it is likely that each spore type requires different conditions for infection. It is possible that type 1 require only short periods of leaf wetness for infection to occur. Further analysis is required to ascertain if production of both inoculum types are required on white mould lesions if successful epidemic development is to take place.

The results from the white mould infection model appeared to match closely observations of white mould occurrence using trap plants and inoculum detection. Running the model indicated that, in 2006, development of a Cornish white mould epidemic was unlikely. This result contributed to confirming the validity of the proposed infection model. The cumulative infection score at each site, calculated from crop emergence onwards, may be a useful indicator of the onset of white mould epidemics.

Financial benefits

The early leaf senescence that white mould causes can be very dramatic: it is estimated by growers in Cornwall that the disease exacts a yield-loss of bulbs and flowers of about 10% annually, and losses can be much greater than this in some situations. The development of a decision support system that will give the timing of fungicide sprays needed to control white mould in a cost-effective and sustainable way will reduce crop losses, pesticides costs and the associated labour costs of pesticide application. It will also enable growers to better meet the high quality specifications demanded by the export trade and the multiple-retail sector and therefore help secure continued trade.

Action points for growers

Growers should continue to monitor their crops for symptoms of disease and consider applying fungicides preventatively after cool (4-16C) wet conditions (at least 6 hours) or crop damage. See HDC factsheet 14/03 for further guidance.

Narcissus white mould decision support system

Introduction

Narcissus (daffodil) growing is an important component of the agriculture and horticulture industry in Cornwall and the Isles of Scilly. Bulb crops have been grown there commercially since the late-19th century, becoming a part of the landscape as well as a source of employment, income and enjoyment. Since the 1970s, entrepreneurial growers have developed a healthy export trade in both the bulbs and the cut-flowers. UK narcissus are held in high esteem and are exported all over Europe and to North America.

The control of fungal diseases is of major concern to bulb growers, since the production of disease- and pest-free bulbs is vital in meeting the exacting specifications of the export trade and the multiple-retail sector. Also, it is expected that pest and disease management will be achieved with only minimal use of pesticides, with each use justified on a case-by-case basis. As an added concern over the past decade, bulb growers in Cornwall have become concerned by epidemics of the fungal foliar disease white mould caused by *Ramularia vallisumbrosae*.

For many years the control of narcissus foliar diseases has been by a more or less non-specific programme of fungicide sprays. Up to about seven sprays, often mixtures of active ingredients, may be used on these crops each year. As little specific information is known about the control of white mould, fungicides have been applied more in the hope of a general fungistatic efficacy than on any assurance of their fungicidal effectiveness in this situation. This changed following a research project carried out between 1998 and 2002 under the 'Horticulture LINK' programme, and funded by Defra, the Horticultural Development Council (HDC), nine bulb growing companies (considered to represent 70% of the UK narcissus acreage) and Aardware Design, a producer of environmental sensing devices (Hanks, Kennedy & O'Neill, 2003).

The 'LINK' project included experiments in controlled environment cabinets. In these, the factors leading to infection of narcissus leaves with the white mould fungus were studied. It was demonstrated that infection was dependent on two environmental factors - temperature and leaf wetness duration - and mathematical

relationships (models) were formulated to describe this relationship. These models allow infective periods to be predicted through interrogating data from temperature and surface wetness sensors sited in the crop, enabling growers to target fungicide sprays to these key periods when they will be most effective. Field trials, part of the same project, showed that the number of fungicide applications used on a narcissus crop might be reduced by half, without loss of control, through such targeting, ensuring that fungicide applications are made in an environmentally friendly and sustainable, and also highly effective and specific, way. The current project aimed to translate these research findings into a practical, decision support system that growers and advisors could use. This was achieved by:

- Validating (testing) the model, and refining it if necessary, to ensure it works in practice;
- Delivering the validated model as a user-friendly 'spray-timing system' for bulb growers, informing them when it is best to apply fungicides and, just as importantly, when it is unnecessary or inappropriate to do so.

The Horticulture 'LINK' project was subjected to economic scrutiny by independent assessors as part of a quinquennial review of the HDC. This assessment showed the project would have a very high economic benefit if carried through to the development phase that enabled its findings to be applied by the bulbs industry. The project described here has been jointly funded by the HDC (as BOF 56) and Cornwall Horticultural Enterprises (administrators of EU 'Objective 1' funds) over the period 2004-2006, and the results of the two years' work are described in this report. Subsequently, the HDC has agreed to provide funding to carry out further work (as BOF 56a) over the period 2006-2008.

Materials and methods

Sites for crop monitoring

In autumn 2004 and 2005, five second-year Cornish narcissus crops were selected for monitoring (see 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 owners that no fungicide sprays would be applied during this year of the crops 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.

Table 1. Cornish white mould monitoring sites.

Year monitored	*Owner's name and address*	*Site name*	*Grid reference*	*Cultivar and year*
2004-2005	Duchy College Rosewarne Camborne Cornwall TR14 0AB	Rosewarne	SW 643413	'Carlton', 2
2004-2005	Winchester Growers Ltd Varfell Farm Long Rock Penzance Cornwall TR20 8AQ	Nancedd an	SW 504339	'Planet' and 'Golden Ducat', 2*
2005-2006	Winchester Growers Ltd	Ludgvan	SW 500332	'Cheerfulness', 2
2005-2006	Winchester Growers Ltd	Catchall	SW 423283	'Veryan', 2
2005-2006	Winchester Growers	Varfell	SW 505323	Variety collection, 2

	Ltd

* The area was part of a field with 'Planet' on one side and 'Golden Ducat' on the other.

Weather data

A meteorological data logger ('Smaartlog'; Aardware Design Ltd., Kingston, UK) was set up close to the centre of each monitoring area in November or December 2004 and 2005, 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 and rainfall at 30-minute intervals. Since leaf damage (e.g. through hail impact) has been shown to be a predisposing factor for white mould infection, precipitation impact (PI) sensors were added for the 2006 season; PI sensors were mounted under a guard giving a 40mm-diameter impact area (Hanks *et al*., 2003). In January 2006 malfunctions of the logger at Varfell necessitated interpolating data from the Ludgvan logger over the missing days.

Trap plant production

In August 2004 and 2005 narcissus bulbs 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, second-year crops being monitored, the bulbs were not given the usual hot-water treatment nor any fungicide applications from lifting in June/July. The bulbs used were grade 12-14cm (circumference) 'Carlton' (in 2004) and grade 10-12cm 'Cheerfulness' (in 2005).

Each year 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 blended growing medium of peat, sand and proprietary John Innes compost. 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.

Disease symptoms

White mould leaf lesions appear in spring, often singly, in the upper one-third of the leaf, and near the mid-line. The lesions are elongated areas often 5 – 10mm in length, with the degraded leaf surface presenting as sunken grey-green to yellowish areas. When sporulating, the lesion surface appears powdery and characteristically creamy-white in colour. Later, rows of minute black sclerotia-like bodies (visible with a hand-lens) are present in the lesions. Sometimes the affected area becomes degraded, leaving a ragged hole in the leaf. Further lesions appear elsewhere on the leaves, becoming elongated and coalescing. White mould is often first seen in prominent patches in the crop 1 – 2m across. As the disease progresses, the leaves die-back from the tip and become dry and brown, sometimes in a matter of a few days. Instances have been seen where the flower stalks are similarly affected, although in other cases they did not appear to be attacked and remained erect among the dead foliage. No symptoms are seen on the flowers.

Crop and disease monitoring

The allocated 0.2ha areas of crops were checked at weekly intervals from December onwards, and the date of first appearance of white mould symptoms was recorded. Following the appearance of first symptoms, disease levels were assessed weekly for the incidence and severity of disease. The central, 0.1ha area of each was walked in an X-pattern starting from a marked corner, and on crossing ridges a 0.5m-long section (sub-sample) was delimited with a ruler at the intercept, producing 50, 0.5m-long sub-samples for assessment. 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 calculated by summing the scores for all 50 sub-samples. The crop growth stage and percentage of foliage senescent or dead were also noted.

Table 2. White mould incidence and severity scales

Score	*Incidence per 0.5m sub-sample*	*Score*	*Severity within each 0.5m sub-sample*
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		

Spore trapping - mechanical

After shoot emergence, and continuing until the end of the growing season, a sticky-tape spore trap was set up at the test sites. The Hirst-type Burkard 24-h volumetric trap (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) has been described elsewhere (British Aerobiology Federation, 1995; Lacey & Venette, 1995). 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^{-1} 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 (applied using a glass edge coated with silicone and drawn over the surface of the slide). The glass slide is attached to a 24-h clock which moves the slide progressively. The overall efficiency of this type of sampler is known to be high (Stedman, 1978).

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 below).

Production of PAb

Following an initial pre-immune bleed, a female New Zealand White rabbit was given an intra-muscular injection of 500□ Freund's complete adjuvant mixed with

500□ of a *Ramularia* spore suspension ($5x10^4$ spores ml^{-1}) produced on oatmeal agar. Thereafter, four further injections of 500□ Freund's incomplete adjuvant mixed with 500□ of a spore suspension (as above) were administered at weekly intervals. Immune bleeds, collected from the marginal ear-vein of the rabbit, were taken 4 and 6 weeks after the initial immunisation. Following collection the pre-immune and immune bleeds were stored at 36°C for 2h, after which they were centrifuged at 100xg for 20min and the pellets discarded. Serum samples were centrifuged as before and the supernatants collected and stored at -20°C in 250µl aliquots until required.

Determination of PAb working dilution

The immune sera (coded 96/10/2 and 96/10/3) were titrated against spores of *R. vallisumbrosae* ($5x10^4$ spores ml^{-1} phosphate buffered saline (PBS)) in a plate-trapped antigen indirect ELISA (PTA-ELISA). Ten paired wells of a 96-well Nunc Immunosorbent Polysorp flat-bottomed microtitre plate (catalogue number 475094A; Life Technologies, Paisley, UK) were coated with 100µl per well of spores of *R. vallisumbrosae* ($5x10^4$ spores ml^{-1} PBS). As a control for each serum type, 10 paired wells received 100µl per well of PBS alone. Following overnight incubation at 4°C, unbound antigen was removed by inverting the individual microtitre plates and slapping them onto absorbent toweling. The plates received four, one-minute washes of 200µl per well 0.3% tris-casein buffer (TCB) (154mM NaCl, 0.3% w/v casein, 10mM tris/HCL). Wells were blocked with 200µl 0.3% TCB for 40min at room temperature. Residual blocking buffer was removed and wells washed four times for 1min each with 200µl per well 0.3% TCB. Pre-immune and immune sera were diluted 1:10 in 0.5ml tincture of merthiolate (1mg ml^{-1} thimerosal, 1mg ml^{-1} pararosaniline in ethanol) L^{-1} PBS and 0.3% TCB (10:1 v/v) (PBST) at 1:50 PBST v/v, and subsequent doubling dilutions made to 1:51200. One hundred µl per well of the respective serum dilution was applied to paired wells and incubated in a Wellwarm 1 shaker incubator (Denley Instruments Ltd., Sussex, U.K.) at 30°C for 30 min. Unbound material was removed and wells washed four times for 1min each with 0.3% TCB. Aliquots of 100µl of goat anti-rabbit IgG (whole molecule) alkaline phosphatase (Sigma A-8025) diluted in PBST and 0.3% TCB (10:1 v/v) to an activity of 0.94 units ml^{-1} was added to each well and incubated as above. After washing, 100µl per well of 1mg ml^{-1} p-nitrophenyl phosphate (Sigma N-9389) freshly dissolved in 9.7% diethanolamine

buffer (pH 9.8) was added. The plate was incubated at room temperature in darkness for 60min. Absorbence values were read at 405nm in an ELISA plate reader (HT11; Anthos Labtec Instruments, Salzburg, Austria). Mean values and percentage coefficient were calculated for each of the paired wells.

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.

Spore trapping – trap plants

Starting after shoot emergence, pot-grown trap plants were placed adjacent to crop foliage near the centre of each crop for defined exposure periods. In 2005, exposure periods were 24h each and pots were put out on Monday through Thursday and collected Tuesday through Friday; in 2006 *ca*. 4-day exposure period were used on a continuous basis. For each exposure period, six plant-pots were used. In 2005, the plant leaves in three pots of each batch were damaged by drawing a stiff bristle nail-brush across the leaves in a standard fashion, while the other three pots remained undamaged as controls. In 2005, wounding leaves in this manner did not appear to increase the liklihood of leaf infection, therefore this treatment was no longer used in 2006.

Following collection from the field sites, the exposed trap-plants were placed in a frost-protected (minimum maintained temperature, 3ºC), well ventilated (at 10ºC) glasshouse, free of other potentially infective plant material, at Duchy College, Rosewarne, Camborne, Cornwall. Further control pots, not exposed in the field, were moved straight to the glasshouse (three pots per week). The three replicate pot-plants in each set were arranged in the glasshouse in three blocks, keeping all pots well spaced from one another. The pot-plants were kept well watered during this time, using bottom-watering into saucers to avoid spreading infection. Plants were examined for disease lesions at at least weekly intervals, and once symptoms were

present the number and extent of lesions in each pot was recorded at 2-weekly intervals over a period of 14 weeks using the scale given in Table 3.

Table 3. Scoring for white mould lesions in trap-plants.

Score	*Description*
0	No lesions
1	1 or 2 leaves per pot with single lesions
2	2 to 10 leaves per pot with 1 or 2 lesions each
3	>10 leaves per pot with 2 or more lesions each
4	>10 leaves per pots with coalescing lesions
5	*ca*. 50% of leaf surface affected
6	Most of leaf surface affected

Forecasting infection

A white mould infection model that relates the number of disease lesions to temperature and durations of leaf wetness periods was developed in the previous 'Horticulture LINK' project (Hanks, Kennedy & O'Neill, 2003). This showed that the critical weather conditions favouring white mould infection were temperatures between 5 and 10ºC combined with leaf wetness durations of 12 to 24h. The infection model can therefore be run using the weather data obtained from loggers in field crops, enabling a comparison to be made between the predicted (modelled) and observed (actual) levels of white mould symptoms. 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 presentation under Results, the infection score was averaged for 24-h periods each starting at 00:00h.

Results

Year 1 (2004-2005)

Meteorological data

Meteorological data for the Rosewarne and Nanceddan sites for spring 2005 were presented in the previous Annual Report. At Rosewarne, during the period studied, average air temperatures fell generally into the critical 5-10ºC band over the periods 17-19 January and 28 January to 5 February 2005, while there were periods of leaf wetness of >12h on 17 and 19-23 January and 1-3 and 11 February. These periods corresponded to periods of RH >95% and days with heavy rainfall. Looking for coincident conditions of suitable temperatures *and* suitable leaf wetness durations suggested that 17 and 19 January and 1-3 February would be critical periods for white mould infection at Rosewarne.

Carrying out a similar analysis of the data for Nanceddan, the likely infective periods indicated were 11-13 March, 16-19 March, 28-29 March, 3-7 April and 12-17 April 2005.

Crop and disease monitoring

The numbers of plots showing white mould symptoms are shown in Figure 1. At Rosewarne white mould symptoms were first seen in early-March 2005 (when 14 out of 50 sub-samples were affected), some 6 weeks after the highest predictive score had been recorded (see below). The number of sub-samples with symptoms increased to >40 out of 50 over the next three weeks. Incidence and severity scores increased rapidly from mid-March and peaked at the end of March.

At Nanceddan, white mould symptoms were first seen at the beginning of March 2005 (on 19 out of 50 sub-samples), steadily increasing over the growing season such that all plots showed symptoms by late-April. The incidence and severity scores at Nanceddan increased steadily from the beginning of March. Although predictive scores for the early part of the year were not available, the frequently high

predictive scores seen over late-March to mid-April may be related to the very high disease incidence and severity seen at this site.

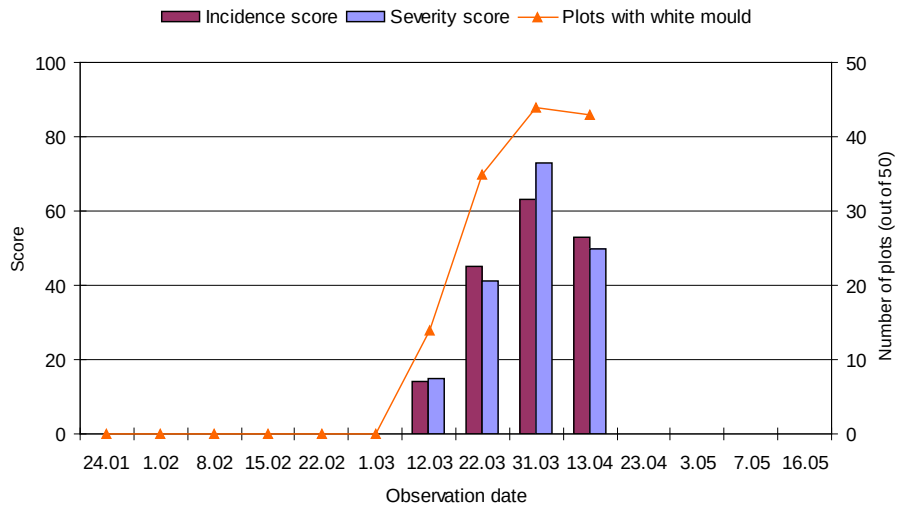
Trap plants

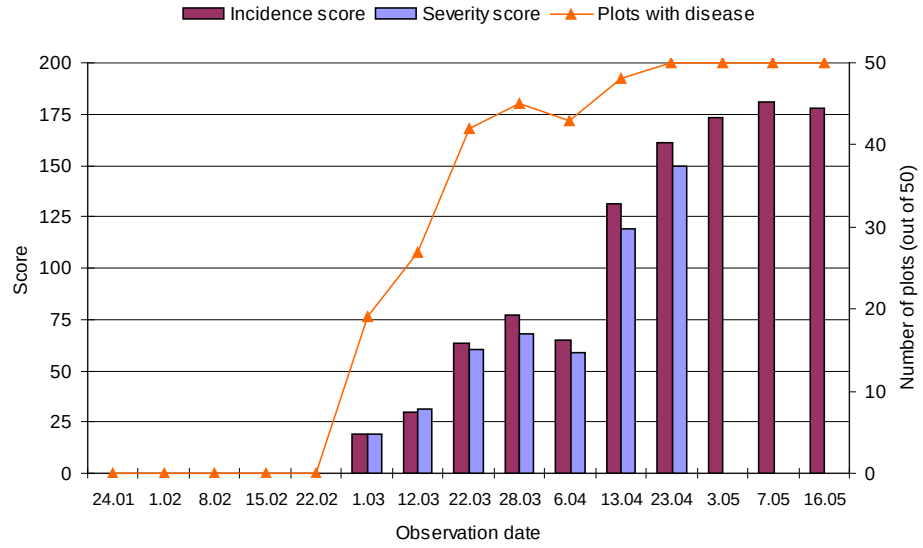
Although large numbers of trap-plants were exposed in the crops over a long period, white mould lesions were seen on only three of the trap plants from Nanceddan, on plants exposed starting 12 March 2005 (on one undamaged and one damaged plant) and 13 April (on one damaged plant). No lesions were seen on the trap-plants from Rosewarne. No lesions were seen on any non-exposed control pots. This is surprising, given the high levels of infection seen on the field crops, and the reason for this apparent discrepancy needs to be investigated.

Spore traps

Only two dates having resulted in the infection of trap plants, and only at Nanceddan, sticky spore tapes from this site for 12 March and 13 April 2005 were examined for the presence of conidia of *Ramularia*. Long, thin *Cercosporella*-type spores, tentatively identified as scolecospores of *R. vallisumbrosae*, were observed on spore tapes from both these dates, suggesting that *R. vallisumbrosae* spores were present in the air during the two dates when trap plant infection occurred. At this time there was no additional method of confirming the identity of these structures, or of determining if phragmospores of *R. vallisumbrosae* were also present.

Figure 1. White mould monitoring in 2005 at Rosewarne (above) and Nanceddan (below). *Note difference in scales of left-hand axes.* Disease data shown as (1) the number of sub-samples showing white mould symptoms, (2) incidence scores and (3) severity scores.



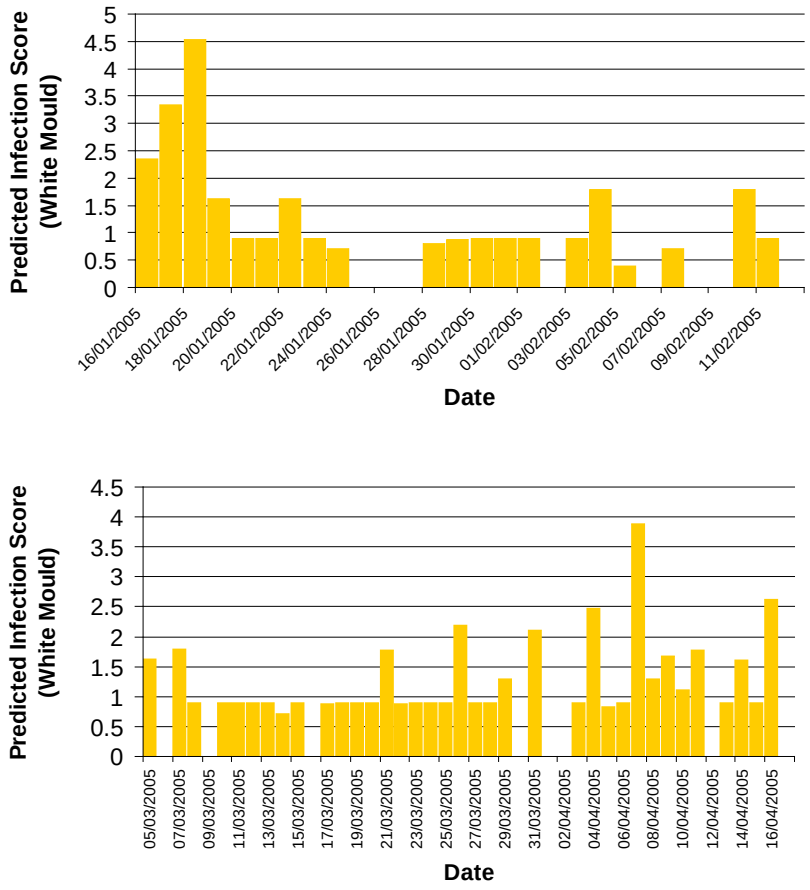


Forecasting infection

The white mould infection model was run using the temperature and leaf wetness data for each site, and the predicted infection scores are presented in Figure 2. At Rosewarne the highest predicted infection score occurred on 18 January 2005, corresponding with one of the two likely infective periods derived in an empirical way from meteorological data (see above).

At Nanceddan, the highest score occurred on 7 April 2005, again coinciding with a postulated infective period. At this site, however, whether working empiracally or from the predictive model, there appeared to be a high liklihood of infection over the whole late-March and April period. As reported above, at Nanceddan the level of white mould infection did in fact continue to increase over this two-month period, with very high levels of infection by late-April.

Figure 2. Predicted white mould infection score in 2005 at Rosewarne (above) and Nanceddan (below).



This initial validation exercise in spring 2005 appeared to indicate that the model's predicted white mould infection periods did indeed coincide with observed infection, though it is clearly necessary to obtain further confirmatory data-sets. It was also clear that further developments in the practical use of the model would require (1) a better understanding of the role of the *R. vallisumbrosae* spore types,

and (2) improved reliability of remote weather data loggers. These requirements were further investigated in the next year of the project.

Year 2 (2005-2006)

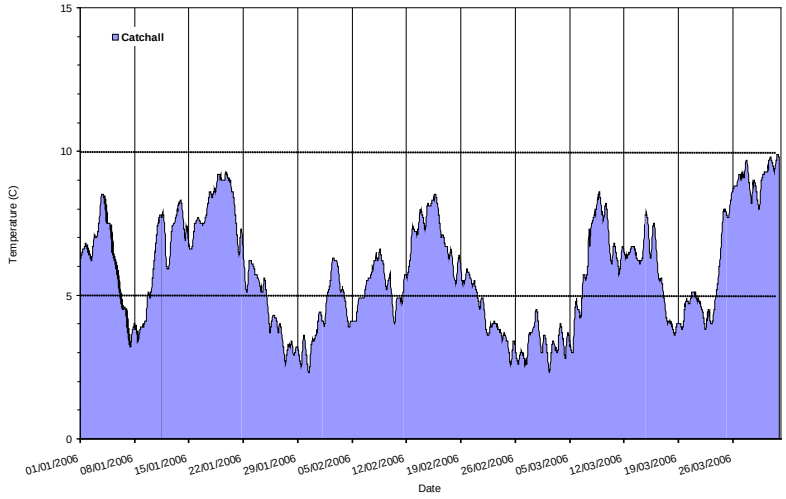
Meteorological data

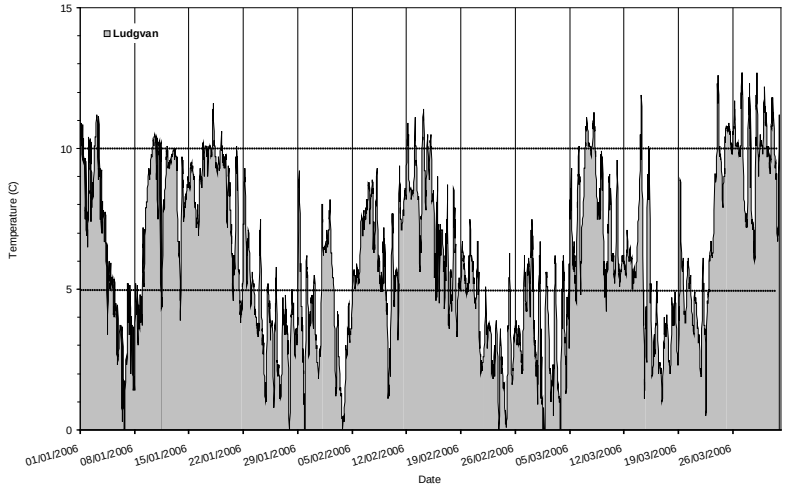
As shown for the critical January through March period, temperatures at the Catchall, Ludgvan and Varfell sites first cycled between periods at 5-10°C (i.e. predicted infective conditions according to the white mould infection model) and cooler periods; by late-March, temperatures generally were rising above 10ºC (Figure 3). The infection model also requires leaf wetness periods of 12 to 24h (Figure 4). By comparing temperature and leaf wetness duration data it can be seen that the predicted conditions for white mould infection occurred on a number of periods around:

- 1 - 4 January
- 10 - 23 January
- 1 - 2 February
- 5 - 21 February (only at Varfell for the first part of this period)
- 11 - 15 March

It can be seen from Figures 5 and 6 that rainfall and humidity data would not be helpful in assessing infective periods. Thus, RH in the crop remained generally high throughout (>70%, apart from a brief drier period in the latter part of January) and fluctuated diurnally, while rainfall was sporadic and was not related in an obvious way to humidity levels or leaf wetness.

Figure 3. Air temperatures at Catchall (top), Ludgvan (middle) and Varfell (bottom) sites in January to March 2006 (first month's data for Varfell interpolated). Infective temperatures lie between the dotted lines.





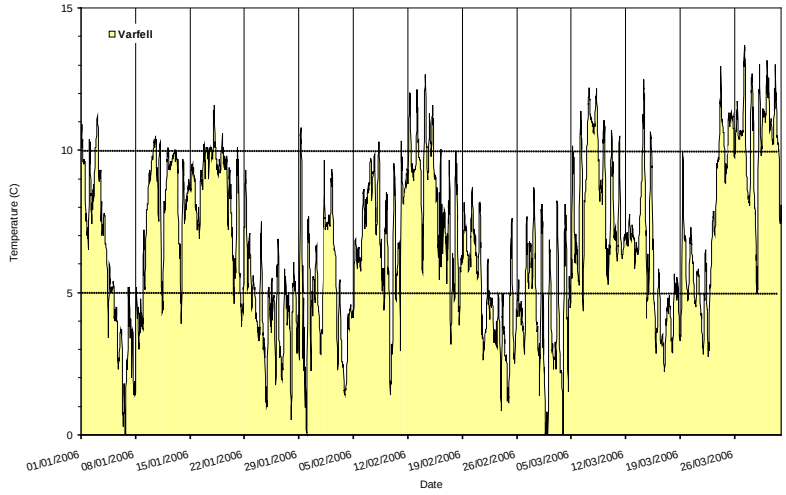


Figure 4. Periods of leaf wetness (indicated by coloured segments) at Catchall (top), Ludgvan (middle) and Varfell (bottom) sites, January to March 2006.

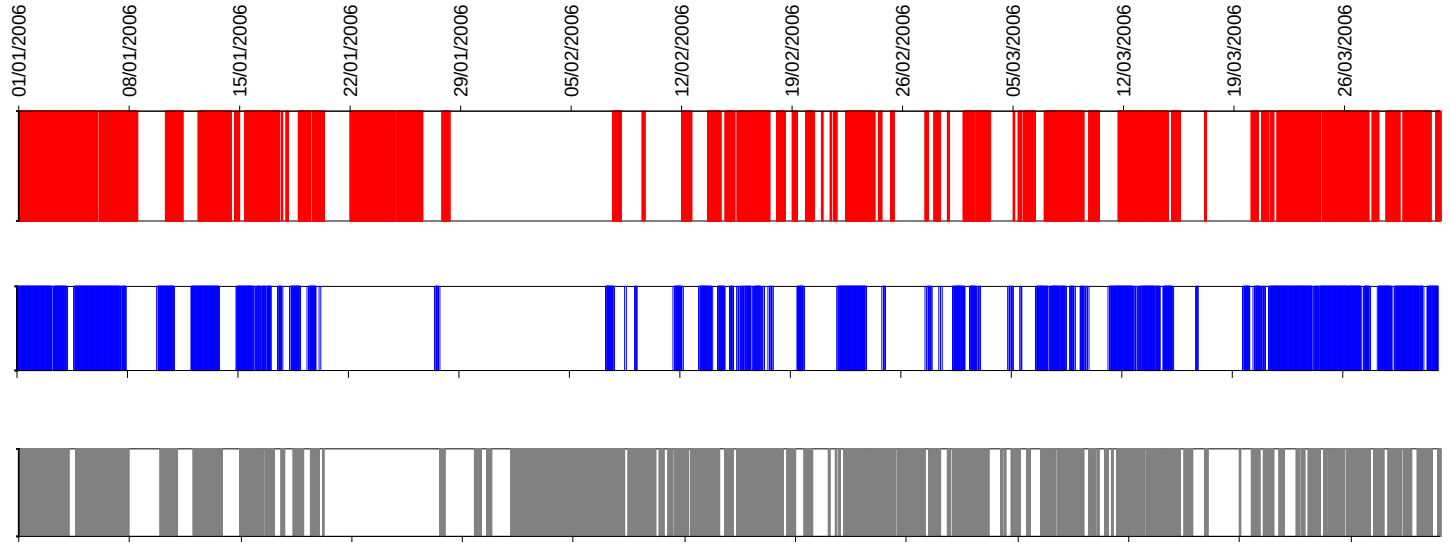


Figure 5. Precipitation at Catchall (top), Ludgvan (middle) and Varfell (bottom) sites in January to March 2006.

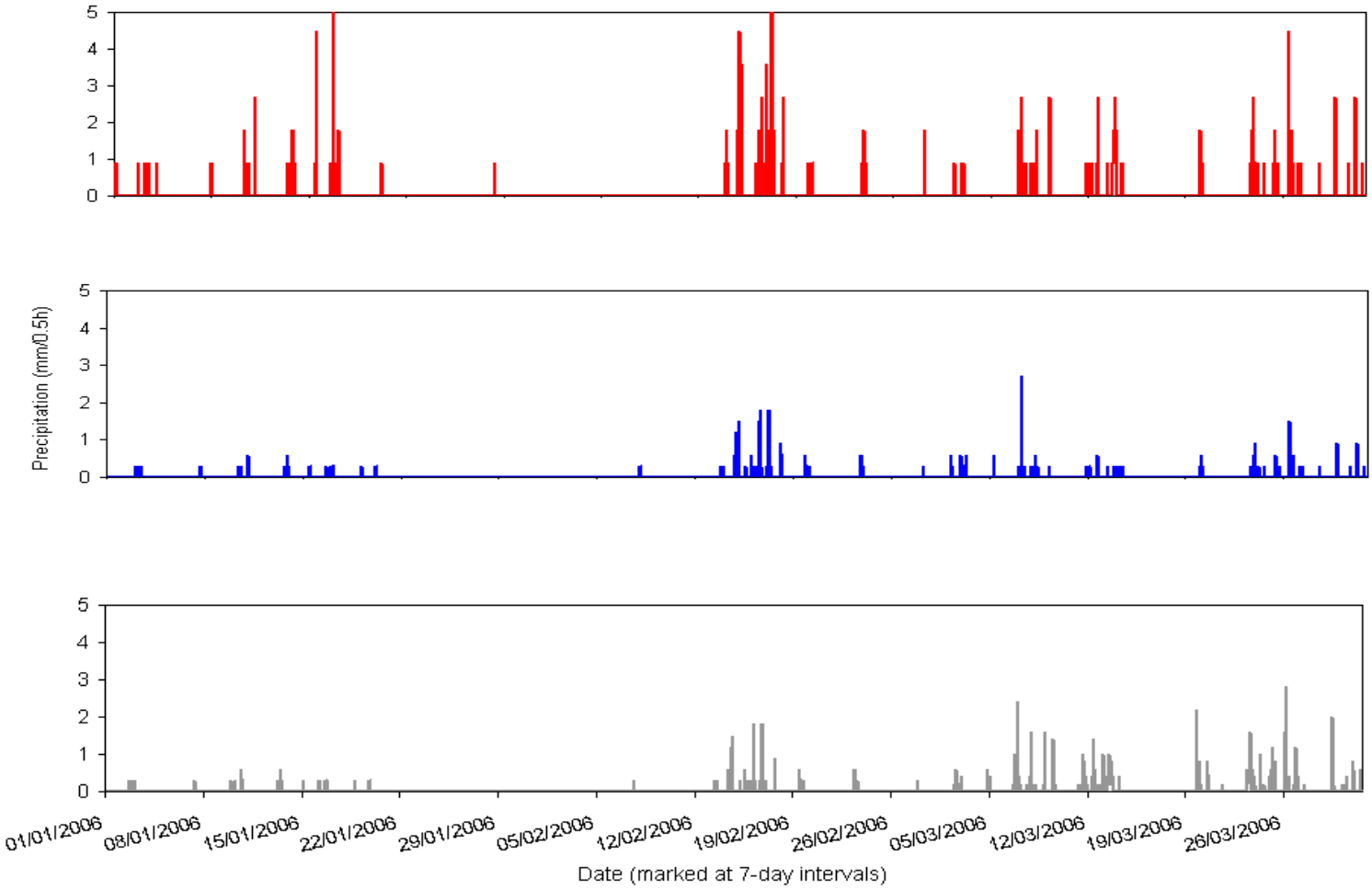
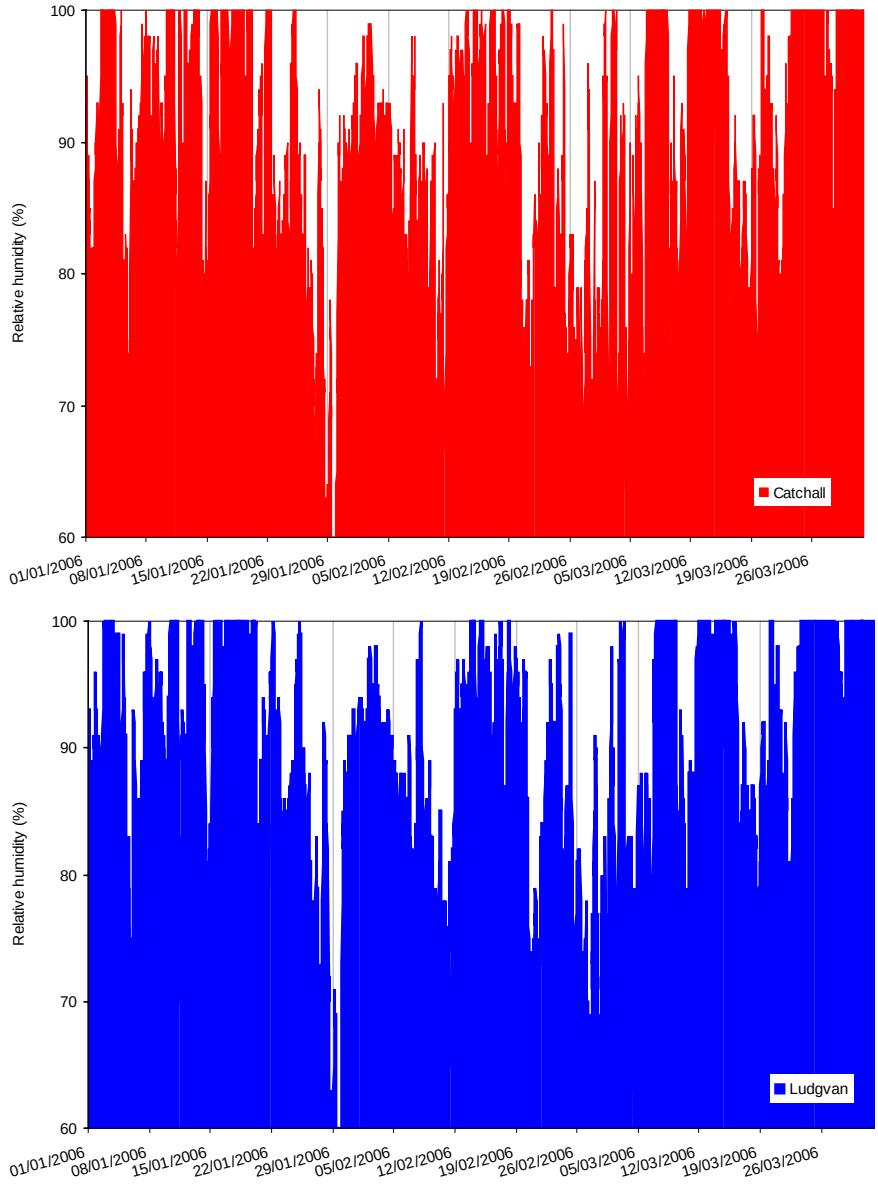
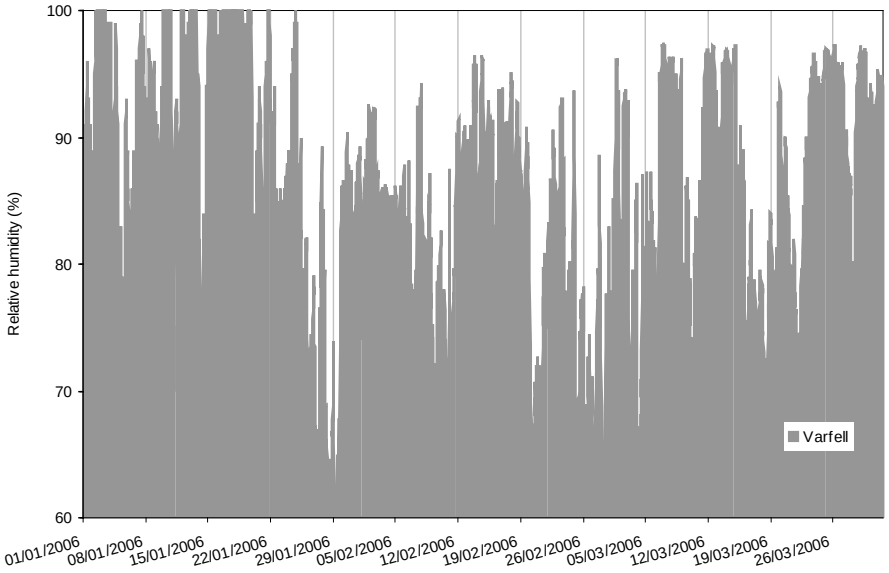


Figure 6. RH at Catchall (top), Ludgvan (middle) and Varfell (bottom) sites in January to March 2006.





The Catchall site lies about 9km south-west of Ludgvan and Varfell (which are about 1km apart). In general temperature trends showed a consistency across the three sites, though Catchall was relatively cool (no periods at >10°C and longer periods at <5°C) and Varfell was relatively warm (least time at <5°C). In terms of leaf wetness, Varfell was the wettest site, and particularly so in the early part of February. Throughout the period studied, Catchall was the site with the most frequent rain events. Relative humidities were similar at the three sites, except that at Varfell the average RH was lower and it was never >95%.

Catchall's more westerly location largely explains this site's rainier and cooler climate and suggests that narcissus crops might be the more susceptible to white mould infections of the three sites. Compared with other sites, Varfell was warmer and, despite relatively low rainfall and humidity, had more periods of leaf wetness, perhaps related to this field being sited close to the company's extensive facilities and probably, therefore, having a slightly more protected microclimate.

Crop and disease monitoring

In contrast to the previous year, no confirmed white mould symptoms were seen at any of the trial sites in 2006. Suspect leaf lesions from the sites were collected and sent for examination to Dr TM O'Neill, ADAS Arthur Rickwood, Mepal, Cambridgeshire, who confirmed that no growths or spores characteristic of *R. vallisumbrosae* were present in the samples, though spores of *Botrytis narcissicola* (the smoulder pathogen) were present. Discussions and visits with several growers across the west Cornwall area confirmed that white mould was not occurring this year.

Trap plants

White mould lesions were seen on trap plants only from the exposure periods 5 – 15 May 2006 (at Ludgvan) and 3–13 May (at Catchall). In these cases the lesions appeared promptly after exposure, as shown in the Table 4. No lesions were seen on plants from exposure periods prior to 3 May or after 15 May. Neither were lesions seen on any non-exposed control pots.

Table 4. Trap plant exposure periods at Catchall and Ludgvan that subsequently resulted in leaf lesions.

Exposure period	*Date lesions first seen in trap plants from:*	
	Ludgvan	*Catchall*
3-5 May	---	5 May
5-8 May	8 May	15 May
8-13 May	13 May	18 May
13-15 May	15 May	---

Observations on narcissus crops at Kirton, Lincolnshire

In another project by the same research team, investigating smoulder disease of narcissus in the east of England, a serious white mould infection occurred in a non-fungicide-sprayed second-year crop of narcissus 'Carlton' growing at KRC, Lincolnshire. Classic white mould lesions (Figure 7a, b) were first noted on 28 April 2006, and subsequently the presence of *R. vallisumbrosae* conidia was confirmed by Dr TM O'Neill. By 17 May the disease was spreading rapidly, covering much of the about 1ha field over the next three weeks (Figure 7c, d). White mould was not, however, reported from other commercial narcissus crops in the area, while in another monitored, non-sprayed crop ('Fortune', second-year-down) at Gosberton, some 11km south of Kirton, suspect white mould lesions were seen but the pathogen was not confirmed in laboratory tests: although a white mycelium was found, no spores of either *R. vallisumbrosae* or *B. narcissicola* were seen.

Figure 7. Narcissus 'Carlton' crop at Kirton in 2006.

(a) Typical white mould lesions.

(b) Lesions spreading and coalescing.

(c) Left-hand area of crop in middle distance sprayed with fungicide programme as normal. Area on right had received no fungicide sprays.

(d) Extensive premature die-down of narcissus foliage resulting from white mould infestation.

Weather data were available from the Kirton site, and are shown in comparison with data from the most westerly Cornish site, Catchall, in Figures 8 (temperature) and 9 (leaf wetness periods and RH). As expected, the temperature records illustrated the milder temperatures in the south-west. The amount of time that temperatures at Kirton were in the critical 5–10°C range for white mould infection was less than in Cornwall, although in cooler (<5°C) periods the daytime temperatures at Kirton often spiked to reach 5 or even 10°C, perhaps partly explaining the unexpected infection

at Kirton. Apart from early-April, both sites experienced many periods of leaf wetness, but wet periods were less consistent at Kirton.

Figure 8. Air temperatures at Catchall (Cornwall) and Kirton (Lincs.) sites, January to March 2006. Infective temperatures lie between the dotted lines.

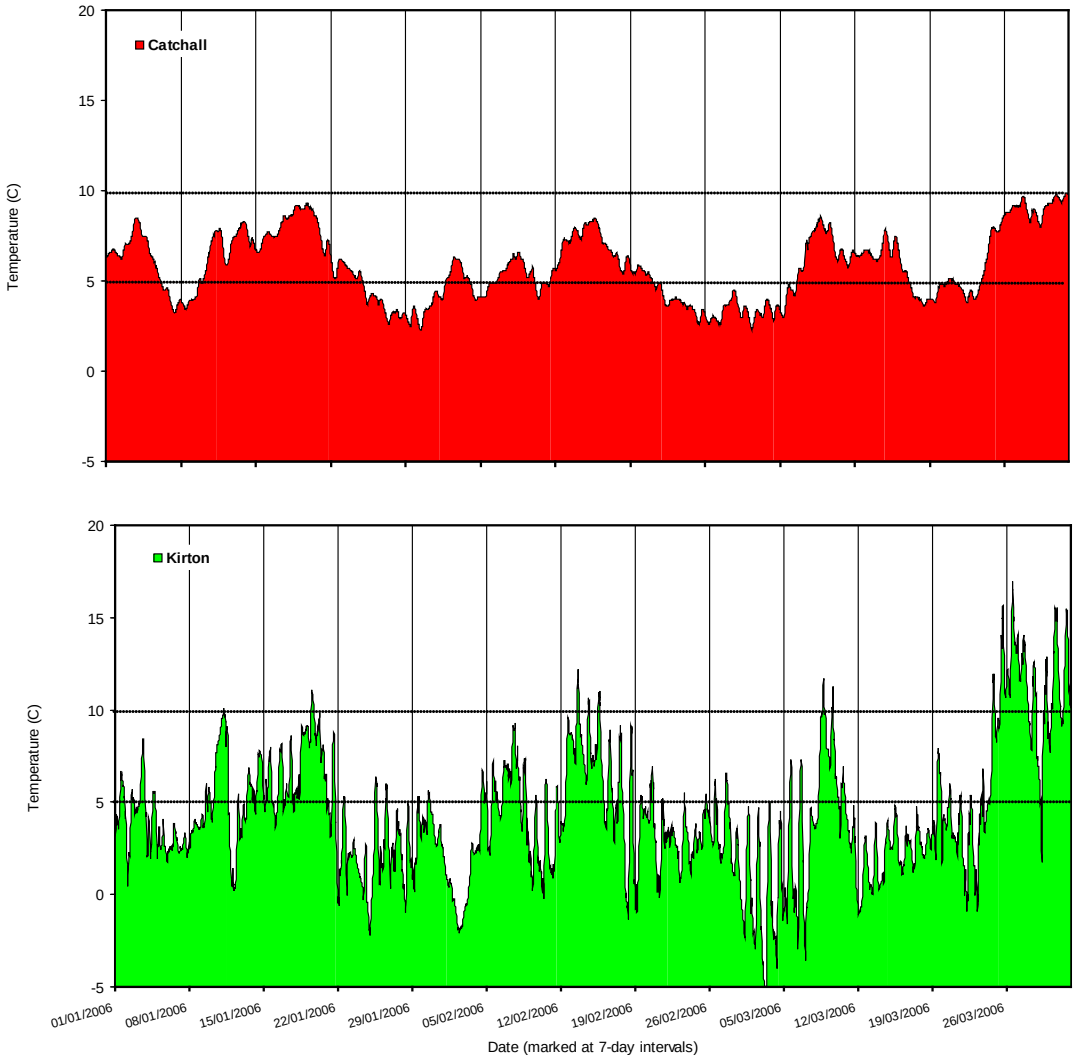
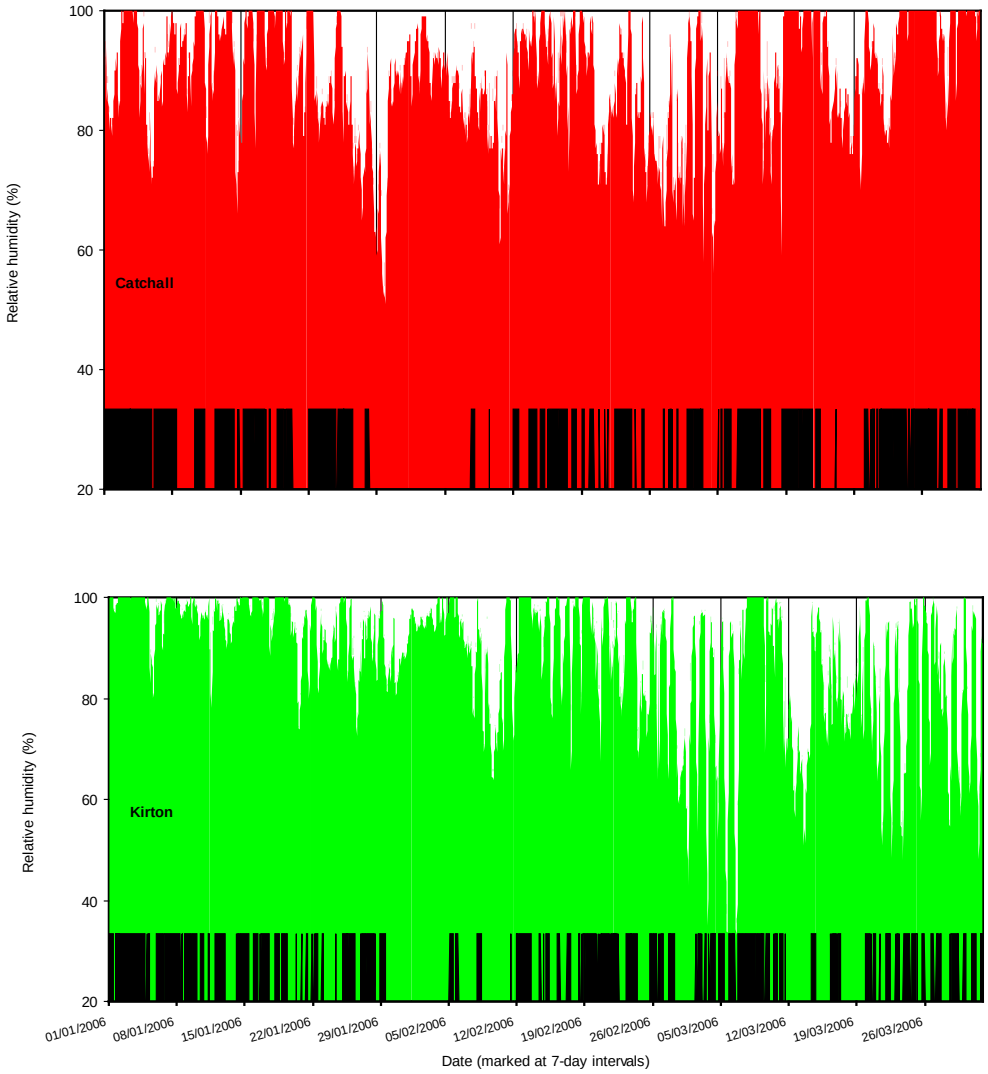


Figure 9. RH and leaf wetness periods at Catchall (Cornwall) and Kirton (Lincs.) sites, January to March 2006. Periods of leaf wetness indicated by black bars.



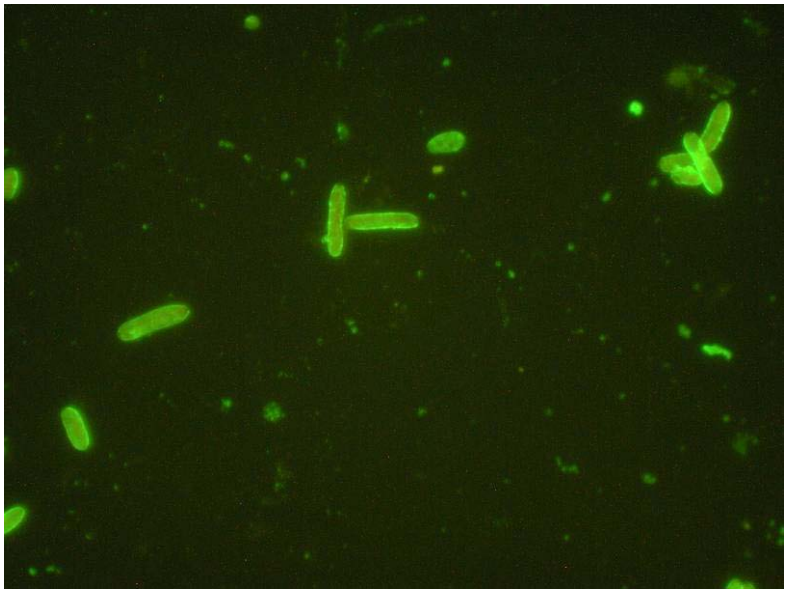
Detecting white mould inoculum

The immuno-fluorescence technique described above was applied to spore samples collected from Kirton, the only site in 2006 where white mould fully developed and successful spore trapping was carried out. Figure 10 shows the staining of both scolecospores and phragmospores using the antibody technique. The scolecospores were longer and hyaline, although their dimensions varied

considerably and they were generally shorter that previously reported. The phragmospores were round to oval in shape.

The results indicated that white mould spores were present in large numbers at Kirton from 13 May 2006 until the end of the monitoring period (June 2006). The greatest counts were observed on 17–18 May, though further peaks in numbers of both types of spores were observed on 26–27 May and again on 4–5 June (Figure 11). Both scolecospores and phragmospores were observed on the traps, however there were larger numbers of scolecospores, indicating that this type of spore is wind dispered.

Figure 10. White mould scolecospores and phragmospores (sampled in June 2006 at Kirton) observed using immuno-fluorescence.



The numbers of scolecospores trapped increased during the onset of daylight (Figure 12). Analysis of the weather conditions during the period 06:00 – 18:00h during this peak in spore numbers indicated that the following conditions were required for scolecospore production:

- Mean daily temperature (06.00-18.00h) above 12°C (optimal above 16°C)
- Daily rainfall of at least 0.3 mm
- No night frosts
- High daily white mould infection scores, probably >2

Preliminary data indicated that several days of optimal conditions were required for scolecospore and phragmospore production, and optimal infection conditions need to coincide with these criteria if white mould epidemics were to develop. There was some evidence that smaller peaks in spore production also occurred during the period 15:00–19:30h, but the low numbers of spores produced during these periods made further analysis difficult. Production of phragmospores was similar to that of scolecospores, however the lower numbers of phragmospores trapped indicated that wind dispersal is not the primary agent involved in their dissemination. Both spore types are produced on the same lesion and it is likely that each spore type requires different conditions for infection. It is likely that infection studies conducted under the earlier Horticulture LINK project, which used inoculum from naturally produced lesions, contained varying amounts of both spore types. This could account for the observed infection after only very short periods of leaf wetness. It is possible that scolecospores require only short periods of leaf wetness for infection to occur. Further analysis is required to ascertain if production of both inoculum types are required on white mould lesion if successful epidemic development is to take place.

Figure 11. Numbers of white mould scolecospores and phragmospores observed using immuno-fluorescence technique at Kirton in May (above) and June (below) 2006. Note difference in scales of y-axes.

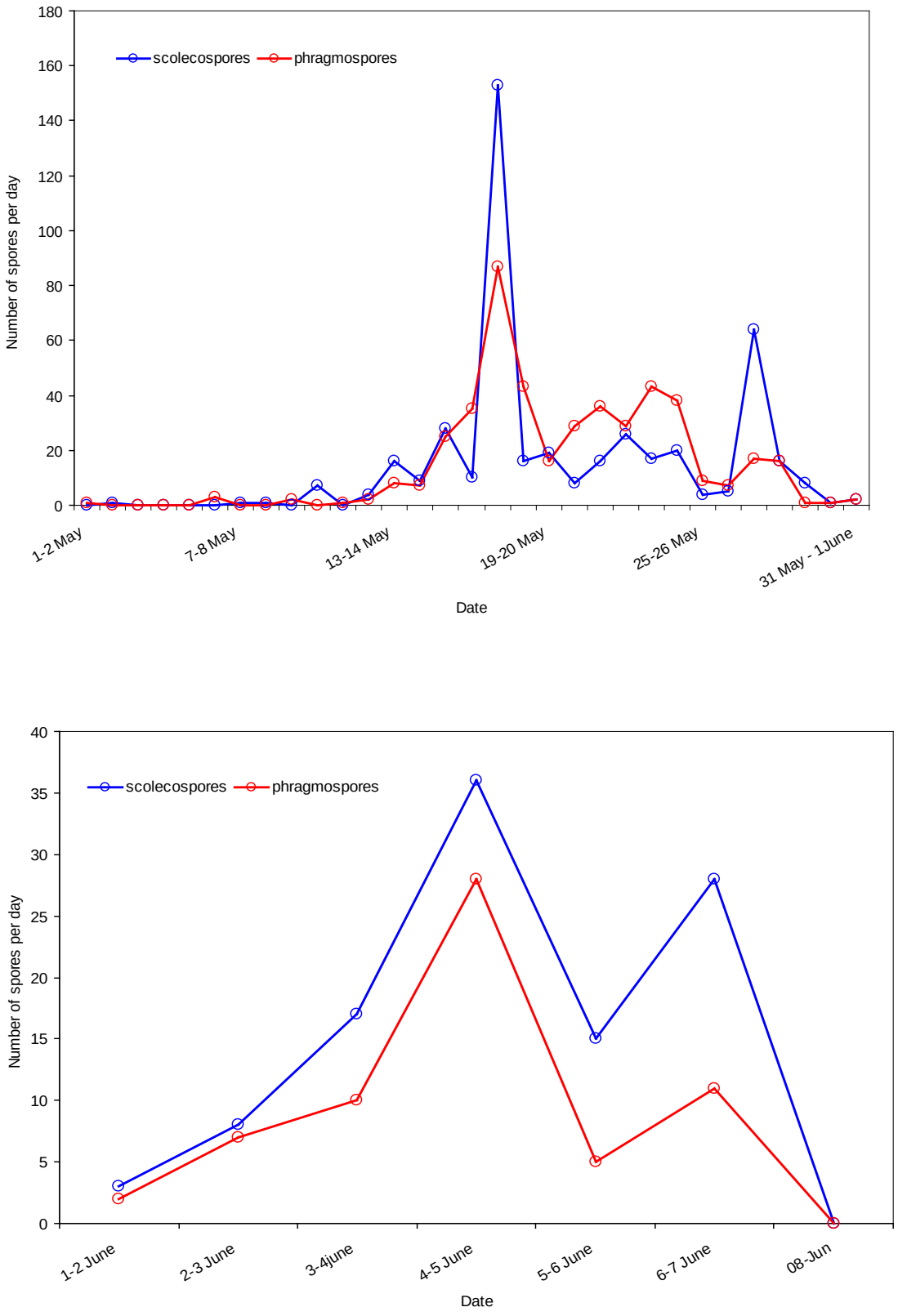
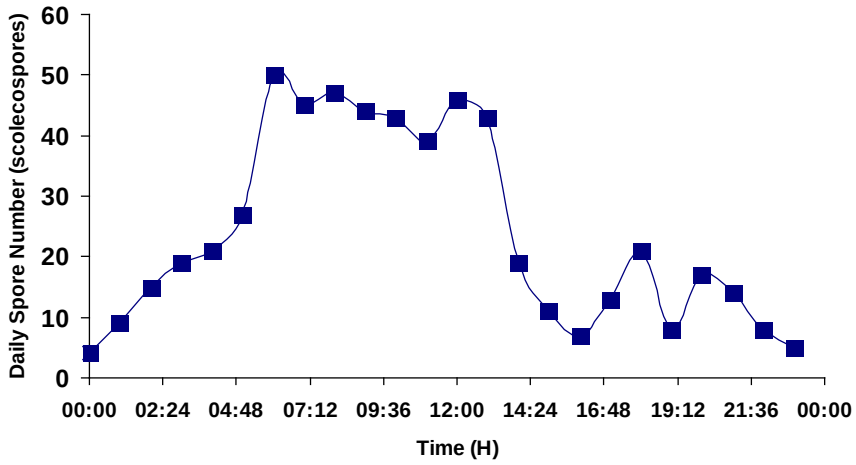


Figure 12. White mould scolecospores observed using immuno-fluorescence during daytime at Kirton in 2006, showing peaks in trapped spore numbers.



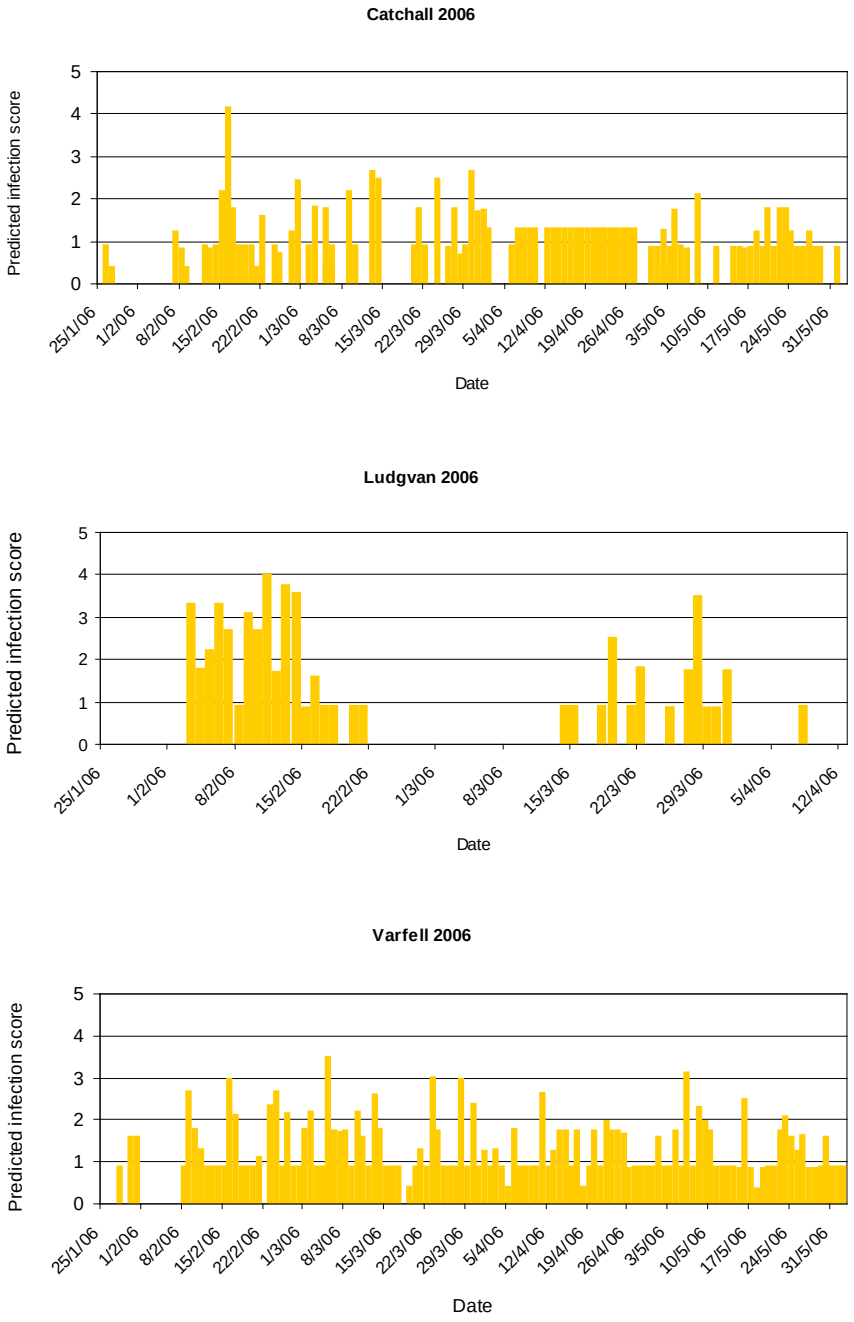
Predicting white mould disease development

White mould infection scores were calculated using the model and temperature and leaf wetness data for each site, and the predicted infection scores are presented in Figure 13. Consecutive infection scores >2 are required, in association with conditions promoting inoculum production, to initiate epidemic development. During 2006 infection conditions were limiting at the sites monitored in Cornwall In 2006 white mould was not observed at the Cornish sites, including Catchall and Ludgvan where a little trap plant infection was seen during early- and mid-May, and Varfell, where suspect lesions were seen. At both Varfell and Kirton there was a more or less continuous background of infective conditions, but only at Kirton did infection scores reaching 3 on several occasions. At Ludgvan there were several instances of infection scores >2, but these occurred over two quite restricted periods, while at Catchall infection scores exceeded 2 on only one occasion.

Comparison of sites proved difficult due to the varying amount of environmental data available at each site. At the Varfell site the data logger was sited within a narcissus variety trial and only relatively small numbers of plants were present within the adjacent plot. Nevertheless, the results from the white mould infection model appeared to match closely observations of white mould occurrence using trap plants and inoculum detection. Running the model indicated that in 2006 development of a Cornish white mould epidemic was unlikely. This result contributed to confirming the validity of the proposed infection model.

The cumulative infection score at each site, calculated from crop emergence onwards, may be a useful indicator of the onset of white mould epidemics. Cumulative infection scores for 2006 are shown in Figure 14.

Figure 13. Predicted white mould infection scores in 2006 at Catchall, Ludgvan and Varfell (Cornwall) and Kirton (Lincs.).



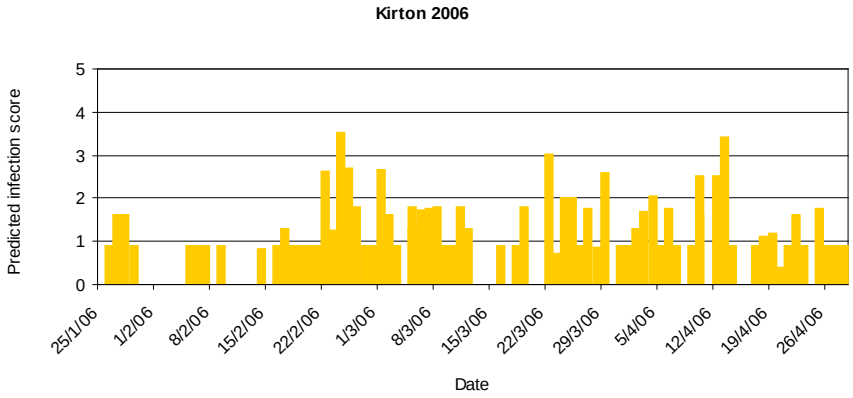
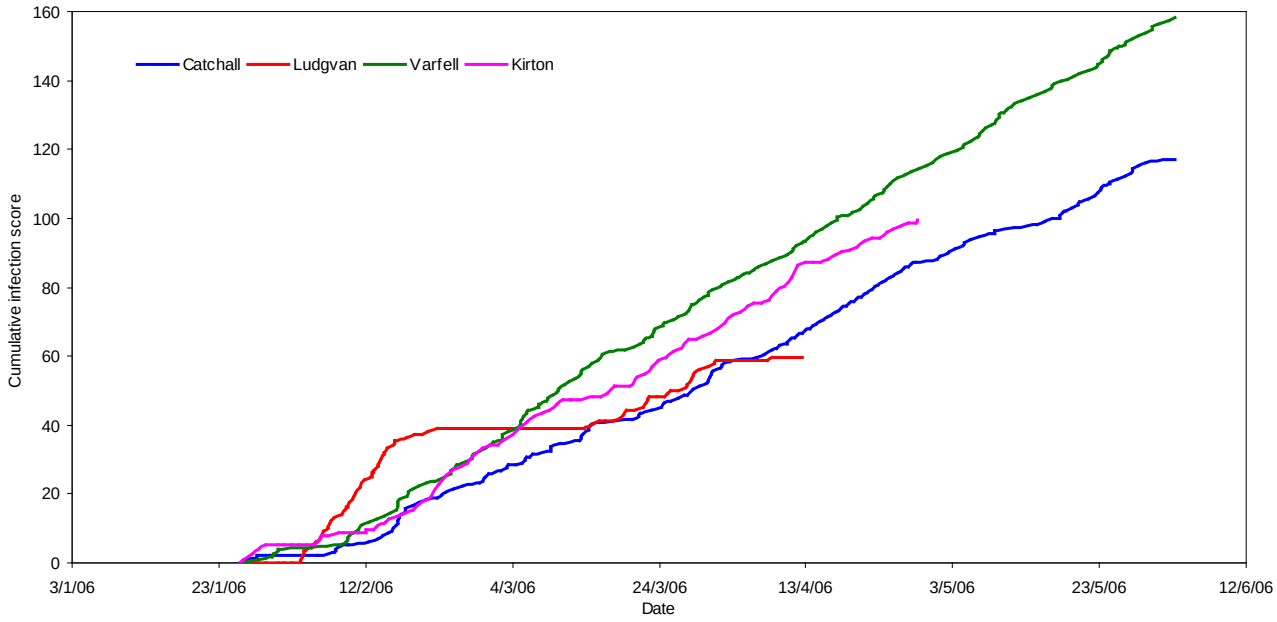


Figure 14. Cumulative white mould infection scores at Catchall, Ludgvan, Varfell and Kirton in 2006.



Discussion

Disease forecasting models can provide information on the optimal timing of fungicide application for controlling crop disease. By developing specific models which summarise important life-cycle stages, and using in-field weather data, information can be obtained to show when conditions are critical for pathogen development in crops. By monitoring the environmental conditions necessary for infection, the infection risk can be determined. Mathematical relationships (models) describing the effect of temperature and wetness 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.

Use of the white mould infection model to predict infection in Cornwall during 2006 indicated that white mould infection conditions were limiting. While infection is possible at all infection scores, the potential infection at low scores (approximately 1) may be possible only in the presence of large amounts of inoculum or at specifically susceptible narcissus growth stage. Conversely, infection is more likely at higher infection scores in the presence of small 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 insufficient information with which to interpret the model infection scores. However scores of approximately 2 and over appear to be important and increase the likelihood of white mould infection, provided inoculum is present.

Data collected in 2006 at Kirton has shown the potential for the involvement of more than one spore type in white mould epidemic development. Although the larger spore detected was similar to that approximating a white mould scolecospore, it could not be confirmed. Previous studies have indicated that scolecospore formation requires lower temperatures that those indicated within this study. However, cycles of wetting and drying and the presence of light also appear to be important in the production and release of these spores.

The results from 2005 tended to confirm the 2006 results. Despite the white mould levels that occurred on the monitored field crops in 2005, white mould lesions were only infrequently seen to develop on trap plants and only limited numbers of *R. vallisumbrosae*-like scolecospores were found on spore traps, though these corresponded with the infection of trap plants. If scolecospores are required for infection, these large spores would require specific conditions for their transmission from the crop onto trap and crop plants. It is likely that there are discrete periods for scolecospores production within the crop. Temperatures of 5–10ºC, in conjunction with long periods of high humidity (>95% RH) are required for the production of scolecospores. Scolecospores were found on spore tapes from both days when trap plant infection was observed, and they appeared not to be present at other times. Conditions conducive to scolecospore production were observed prior to each trap plant period when white mould infection was observed. Additionally, on one occasion (13 April 2005), conditions favourable for scolecospores transmission were also observed. These results suggest that the presence of the scolecospore spore type maybe necessary for the spread of white mould infection. To facilitate the identification of *R. vallisumbrosae* spores, polyclonal antibodies were developed.

Results obtained in 2006 suggest that both scolecospores and phragmospores are involved in white mould development on narcissus crops. However, it is possible that each spore type has differing requirements for infection. Scolecospores appear ascosporic in nature, and maybe the sexual stage of the white mould fungus. Many ascospores of other ascomycetes usually require only short periods of leaf wetness to infect tissues. The infection model developed under the previous Horticulture LINK programme used inoculum collected from naturally occurring lesions in Cornwall. It is likely that it contained both scolecospores and phragmospores, and this could explain why even short periods of leaf wetness resulted consistently in white mould infection.

Trap plants in both years showed only limited amounts of infection. However, the method employed could be improved to make it more responsive to the presence of small amouts of white mould inoculum in the field. One possible way of achieving this would be to mist trap plants for 48h after exposure in the field, which would have the effect of ensuring that any inoculum present in the field infected the trap plant leaf. In this way the narcissus trap plants could be used to check for the presence of

even small amounts of white mould inoculum. It would appear, from the results obtained in 2005 and 2006, that white mould infection conditions are generally not limiting in crops of narcissus. The presence of white mould inoculum has proven to be more sporadic than expected, and represents an important element in white mould epidemic development.

Acknowledgements

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