Project title: **Narcissus Smoulder Decision Support System**

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

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# Narcissus smoulder decision support system

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# Grower Summary

## Headline

A spray-warning system for narcissus smoulder disease offers growers the opportunity to achieve better smoulder management with fewer sprays, reducing costs and having a positive environmental impact.

## Background and expected deliverables

Smoulder, caused by *Botrytis narcissicola*, is the most widely experienced fungal foliar disease of narcissus exacting a steady reduction in yield to the order of up to ten per cent. The control of smoulder has been largely through using a fungicide spray programme, and a programme of pre- and post-flowering sprays has long been used, though with little definite knowledge of the effectiveness of individual active ingredients and times of application. Cultural methods of control may be ineffective or impractical, and few other disease control strategies have been reported. To give a better understanding of smoulder epidemiology and management, a research programme was initiated with a 'Horticulture LINK' project (BOF 41). The results showed that factors leading to smoulder infection and spread were largely temperature, leaf wetness periods, and crop damage. From the findings a predictive model was proposed. The model indicates the dates when fungicide applications should be targetted to obtain the most effective control, and in trials it was shown that the number of fungicide sprays applied in one growing year could be reduced from six to three by the expedient of applying these sprays only at the dates predicted by the model. The present project, BOF 59, was set up in 2006 to test and validate the smoulder infection model from the 'LINK' project, and to develop a 'spray-warning system' for 'at risk' periods that could be used to inform growers of the best dates for fungicide applications.

The main expected deliverable was a 'spray-warning system' for 'at risk' periods, a decision-support system that growers could use to:

- Improve the management of smoulder (and incidentally of other fungal foliar diseases)
- Produce enhanced yields of better quality bulbs and flowers
- Reduce production costs through using fewer fungicide applications
- Justify fungicide applications
- Support more sustainable growing through lowering environmental inputs.

## Summary of the project and main conclusions

Previous work showed that the smoulder pathogen can infect daffodil leaves at temperatures between 4 and 16°C, with an optimum of 12°C. At 12°C a minimum period of leaf wetness of 6 hours is needed. Longer periods of leaf wetness, up to 24h, are required for infection if temperatures are sub-optimal. Infection is much more likely if the leaf surface is damaged.

This information was developed into a predictive 'smoulder infection model' that related the likely severity of smoulder in a crop to temperature and leaf wetness data. In 2005 and 2006, predicted and observed smoulder data from commercial daffodil crops were compared, to determine the validity of the model. There was a reasonable correspondance between the predicted and observed infections, indicating that the model was a valid way to predict disease levels, and hence to guide growers and consultants when to apply fungicides to their crops.

In 2007 and 2008 the smoulder infection model was tested on six crops, using it to determine when fungicides should be applied, and comparing the smoulder control achieved using this model-based spray programme with that obtained using a conventional spray programme (consisting of regular fungicide applications). Better control of smoulder was obtained using the model-based system.

## Financial benefits

The ‘LINK’ project was subject to independent scrutiny and assessors concluded that considerable financial savings could be made by using a fungicide spray programme that reduces the total number of fungicide sprays applied. The present project will help growers to apply these fewer fungicide sprays to crops at the best, most effective time to control smoulder, thereby improving crop quality and reducing wastage due to foliar disease.

## Action points for growers

As a result of this project and project BOF 56/56a, a proposal is being prepared for submission to the HDC to set up a smoulder and white mould disease bulletin that will provide weekly alerts for growers when crops should be sprayed
Further information about both decision support systems is available in *HDC News* (no. 150, February 2009) and through grower workshops.

# Science Section

## Introduction

Smoulder is the most widely experienced fungal foliar disease of narcissus (daffodil), and probably occurs everywhere narcissus are grown, exacting a steady reduction in yield, perhaps of the order of ten per cent. The disease, caused by *Botrytis narcissicola* (sometimes called *Sclerotinia* or *Botryotinia narcissicola*) spreads via infected bulbs and sclerotia in the soil (for general accounts of the disease, see Bergman *et al*., 1978; Gould and Byther, 1979; Moore *et al*., 1979). Infected plants become evident at (or shortly after) shoot emergence, with brown or black leaf tips above a yellowing zone, the leaves stuck together, crooked and torn; these are known as 'primaries'. In severe cases the plants have pale, broken leaves, dwarfed stems and misshapen flower buds. As a result of contact infection as the leaf grows up through the neck of the bulb, characteristic lesions appear on one edge of a leaf, with a brown or black area surrounded by a yellowing zone, and the death of the leaf tissue unilaterally results in uneven growth producing a curved, sickle-shaped leaf. Under damp conditions a mass of grey spores is formed on the primaries, spreading by wind and rain-splash to cause secondary infections showing as leaf and flower spotting throughout the crop. The fungus also colonises the cut end of stems when flowers are cropped. Sclerotia develop on and in the bulb. The bulb skins may become 'greasy' and the base plate corky, and the bulb may rot. The ubiquitous grey mould (*Botrytis cinerea*) also occurs on narcissus, and results in grey, sporulating areas on leaf and stem bases. The symptoms of *B. cinerea* are not easy to distinguish from those of *B. narcissicola*.

Studies of the epidemiology of smoulder were carried out in Scotland in the 1970s and 1980s. The development of the characteristic lesions was enhanced by wet soil conditions, cold wet weather near the time of emergence, and harvest damage, and by the presence of multiple-nosed bulbs (Gray and Shiel, 1975, 1987). In one study, using a stock with a low level of smoulder, disease incidence did not increase over 4 years, unless the flower-heads were removed or flowers were cropped, when smoulder levels increased by the third and fourth year, respectively (Dixon, 1986). The pathogen does not easily penetrate undamaged tissue, and infection was linked to tissues already colonised by the bulb-scale mite (Gray and Shiel, 1975; Gray *et al*., 1975). *B. narcissicola* is therefore considered a wound pathogen, and crops should be sprayed with fungicide following damage, such as that caused by flower picking, wind, frost or chemical damage. Investigations showed that infection was enhanced by the light mechanical wounding of leaf and bulb tissues and by the addition of nutrients, particularly at higher temperatures (O'Neill and Mansfield, 1982; O'Neill *et al*., 1982).

The control of smoulder has been largely through using a fungicide spray programme on the growing crop, though it is supposed that fungicide dips and hot-water treatment also aid the control of fungi generally. A programme of pre- and post-flowering sprays has long been used, though with little definite knowledge of the effectiveness of individual active ingredients and times of application, until more recently (O'Neill *et al*., 2004). Few other disease control strategies appear to have been reported. There are some cultural methods of managing smoulder (and other fungal foliar diseases of narcissus), but these may be considered ineffective or impractical (see Discussion and Table 13). Few other disease control strategies have been reported. A literature review on *B. narcissicola*, updated in November 2008, confirmed the authors' earlier views that little smoulder research is being carried out worldwide, other than studies of the antifungal properties of novel compounds, such as saponins, other plant extracts and chitosan, by the Skierniewice group in Poland (e.g., Saniewska, 2001; Saniewska *et al*., 2004, 2006).

With the aim of better understanding smoulder epidemiology and developing more rational and sustainable ways of managing the disease, a research programme was initiated with a 'Horticulture LINK' project (CSA 4716; BOF 41) funded by Defra, the HDC and ten companies (Hanks *et al.,* 2003). The factors leading to smoulder infection and spread were studied with the aim of developing a decision-support system. Smoulder development was shown to be driven largely by temperature, leaf wetness periods, and crop damage, and a predictive infection model was proposed. Following the ‘Horticulture LINK’ project, the present project, BOF 59, was set up in 2006 to test and validate the smoulder infection model, and to develop from it a ‘spray-warning system’ that could be used to inform growers and consultants of the most effective dates for fungicide applications.

Project BOF 59 was funded by the HDC and the Lincolnshire Fenlands ‘LEADER+’ programme, with the in-kind support of three bulb-growing companies. The first Annual Report (2006) further outlined the rationale for the work, and described the monitoring of smoulder in commercial Lincolnshire daffodil crops that had not been treated with fungicides. This enabled the ‘natural’ development of the disease to be recorded, and these (observed) data were compared with disease development predicted using the infection model. Comparing the observed and predicted data on smoulder infection and development enabled the accuracy (or otherwise) of the model to be ascertained, and showed that there was potential to use the model as the basis of a disease-forecasting or spray-timing system. In 2007 and 2008 the ‘smoulder (or at-risk) periods’ predicted using the model were used as the basis of a ‘model-based’ fungicide spray programme, and the effectiveness of using this spray programme was compared with the effectiveness of ‘conventional’, ‘commercial’ or ‘grower’s’ spray programmes, i.e. the current practice of using spray programmes based on regular (date-based) fungicide applications. This work validated the use of the infection model in a variety of crop situations, providing the opportunity to develop practical methods for using the model and considering how best it could be delivered to growers.

## Materials and methods

### Weather data

Because weather can vary over relatively small distances, meteorological monitoring stations (MMS) (‘Smaartlog’; Aardware Design, Walton-on-Thames, KT12 3PL, UK) were set up close to the centre of monitoring and trial areas each year prior to crop emergence. The MMS provided the local and real-time weather data needed for running the smoulder infection model. They were powered by batteries and solar panels, were downloadable via a modem and digital cell telephone, and had sensors recording soil and air (screen) temperature, relative humidity, surface wetness (SW), rainfall and precipitation impact (PI) at 30-minute intervals. The SW sensors were designed to simulate leaf wetness. The PI sensor ranked impacts into 14 levels (referred to as ‘bins’), from the lowest impact energy (1) to the highest (14).

### Using the infection model to forecast ‘at risk’ periods

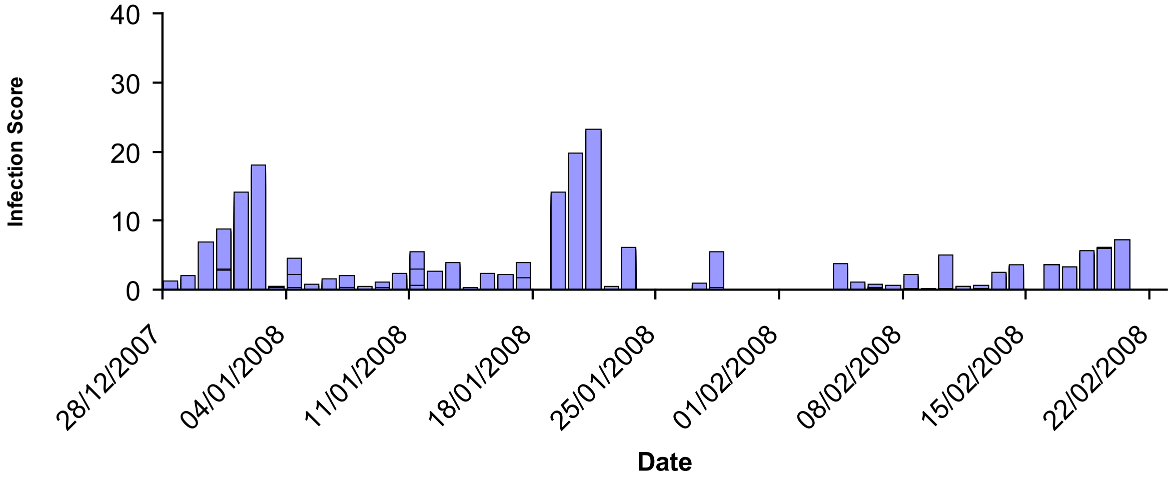
The smoulder infection model predicts the number of disease lesions likely to occur, based on the temperature and the duration of periods of surface (leaf) wetness (for details, see the Final Report on HDC Project BOF 41). Previous experiments had shown that the optimum temperature favouring smoulder infection was 12ºC, which required a 6h period of leaf wetness to be effective, and that infection would take place at temperatures between 4 and 16ºC with longer leaf wetness durations, up to 24h. The infection model was run with the current, local weather data obtained from the MMS, enabling a comparison to be made between the predicted (modelled) and actual (observed) levels of smoulder symptoms in crops: the correspondance of predicted and observed levels would validate the accuracy of the model, and dissimilar results would indicate that the model is inappropriate or needs to be modified. In these tests the results are not *directly* comparable, since symptoms often take time to appear; however, there should be some correspondence between the observed and predicted occurrence of smoulder in the field, for example an increase in disease observed after high predicted infection scores.

Using weather data downloaded from each MMS, the model was run at weekly intervals to produce an infection score, this being averaged across 24-h periods starting at 00:00 hours. The model gives an infection score, but does not determine what score should be used as the threshold to trigger fungicide application, so the threshold was investigated by applying different thresholds at different sites (see below). As crop damage has also been shown to favour the spread of smoulder, both the infection score and the extent of any crop damage were taken into account when determining target spray dates. Relevant crop damage would include that due to frost, high-energy rainfall or flower picking (involving breaking of leaves and stems and general trampling). Crop damage could be used as a spray criterion in its own right, or it could be used to confirm a recommendation to spray when the infection score itself was borderline.

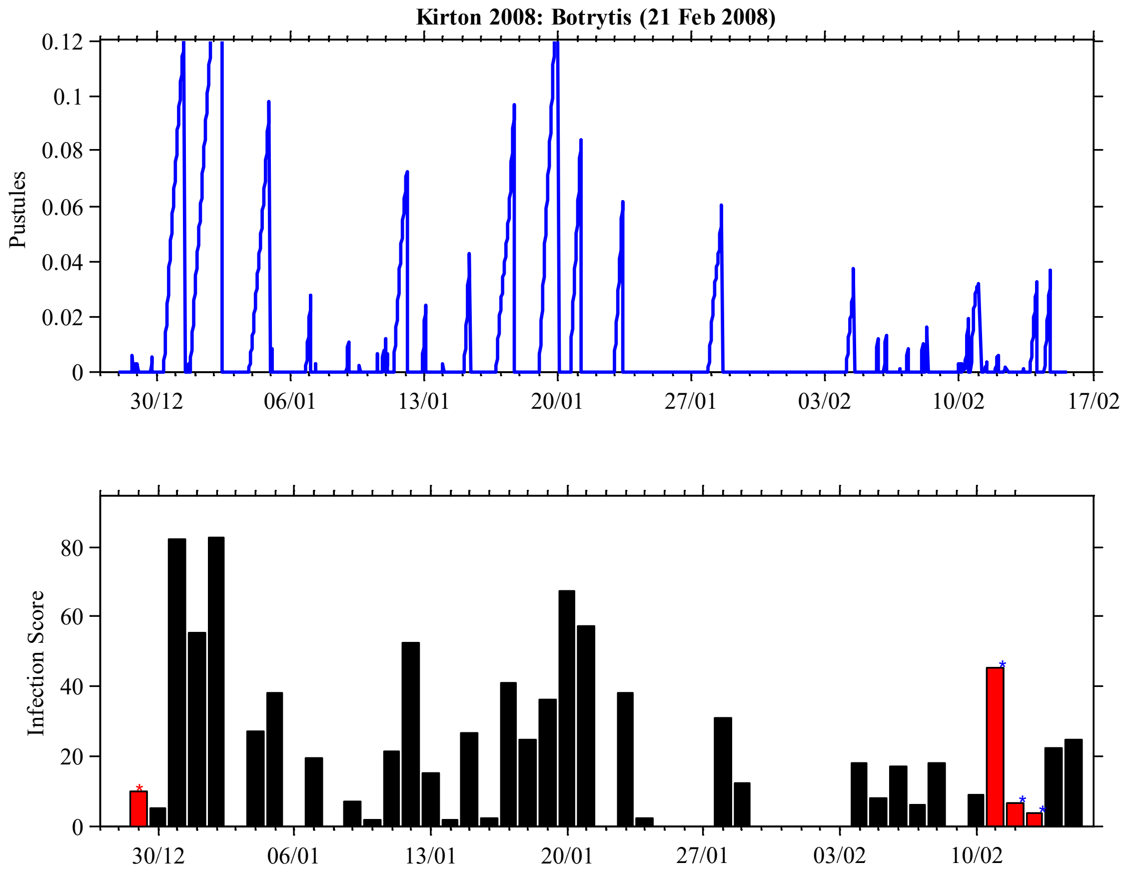
Two versions of the model were used over the course of this project, and in 2008 comparisons of the two versions, using Excel and MATLAB software respectively, showed that each produced similar results (Figure 1). Because of its speed of processing the data and the enhanced presentation of outputs, the MATLAB version was adopted for further use. In the presentation of results it should be noted that the infection scores produced by the MATLAB software are, for ease of presentation, 100-fold greater than those from the Excel software, so that, for example, scores of 20 (using MATLAB) and 0.2 (using Excel) are equivalent.

**Figure 1. Comparison of (a) Excel and (b) MATLAB smoulder model outputs from 27 December2007 to 15 February 2008. Note that the scores from the Excel model have been multipled by 100 (see text).**

**(a) Excel output**



**(b) MATLAB output**



## Crop and disease assessments

The selected daffodil areas were checked at weekly intervals from December onwards, and the date of first appearance of smoulder symptoms was recorded. Following the appearance of first symptoms, the level of smoulder symptoms were assessed weekly. Each area was walked along an X-pattern (see below), and the incidence and severity of smoulder were scored (see Table 1) in each of 50 sampling zones. The number of smoulder primaries (shoots emerging from the ground already infected) in each sampling zone was also recorded. Overall incidence and severity scores for each area were calculated by summing the scores for all 50 sampling zones. The stage of crop growth was recorded weekly and, later in the season, the percentage of foliage that was senescent or dead was estimated and foliage was also scored as being erect (1), beginning to lodge (2) or lodged (3).

The symptoms of smoulder are as follows. In the early stages of shoot emergence in winter/spring, infected shoots emerged as primaries, heavily infested shoots with the leaf tips withered, distorted, blackened, adhering and bearing a profuse grey mass of sporulating tissue. Later, lesions appeared on the leaves, classically on one side of the leaf or at the tip, with a darkening area of leaf perhaps 2cm or more in length bearing grey sporulating material that appears fluffy under a hand-lens. The one-sided lesions resulted in the leaf bending at this point due to restricted growth. The lesions were bounded by yellowing areas. Late in the growing season the lesions sometimes spread rapidly. Leaves often died-back from the lesions, resulting in a yellowish senescent or blackened area across the whole leaf or in a longitudinal tract of it, which could extend to the withering and death of the whole leaf lamina with an appearance of premature leaf senescence. When pulled up, such withered leaves were often seen to carry sclerotia or a grey mass of spores at the base. Small (1 - 2mm diameter) oval or circular black sclerotia were found on leaf debris. Smoulder can also cause flower spotting, though this was not observed during this project. In some samples, laboratory investigations showed that the spores were of grey mould, *B. cinerea,* not *B. narcissicola*, but it was impractical to distinguish the two in the field.

**Table 1. Smoulder incidence and severity scales.**

| *Score* | *Incidence* | *Score* | *Severity* |
|---|---|---|---|
| 0 | None | 0 | None |
| 1 | 1 or 2 leaves affected | 1 | Single lesions |
| 2 | >2 but <10 leaves affected | 2 | Single lesions, occasionally >1 lesion/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 into larger areas |
| 5 | All leaves affected | 5 | Extensive leaf die-back |

Smoulder monitoring (2005 and 2006)

During November and December 2004 and 2005, two crops, considered typical of commercial crops of the South Lincolnshire region, were selected for crop and disease monitoring in 2005 and 2006. Details of these crops are shown in Table 2. In each crop an area *ca.* 0.2ha in extent was designated and marked with corner posts and signage, and it was arranged with each grower 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 entirely according to its grower's normal commercial practices.

The central *ca.* 0.1ha of each 0.2ha area was further marked out for monitoring and observation, leaving the surrounding 'picture frame' area as a buffer zone guarding against spray drift from adjacent crops. Within each central area 50 sampling zones were marked in an X-pattern: starting from a marked corner and moving diagonally across the area and then back along the other diagonal, 50 evenly spaced 0.5m-long sections of ridge were marked with numbered canes as sampling zones where the diagonals crossed the planted ridges.

**Table 2. Smoulder monitoring sites in 2005 and 2006.**

| *Year* | *Site name* | *Grid reference* | *Cultivar* | *Crop year* |
|---|---|---|---|---|
| 2005 | Kirton | TF300395 | 'Carlton' | 2 |
| | Saracen's Head | TF337280 | 'Fortune' | 2 |
| 2006 | Kirton | TF300394 | 'Golden Harvest' | 2 |
| | Surfleet | TF259293 | 'Fortune' | 2 |

## Associated field and laboratory studies (2005 and 2006)

### *Spore trapping using trap-plants*

To provide suitable trap-plants daffodil bulbs (grade 12-14cm cv 'Carlton' from a stock grown at the Kirton Research Centre, KRC) were allocated in August 2004, 2005 and 2006. To produce trap-plants similar to the second-year field-crops being studied, the bulbs did not receive the usual hot-water treatment before planting, nor did they receive any fungicide applications following lifting in June/July. The bulbs were stored at 17°C until early-October when they were planted in a standard fashion, five bulbs per 20cm-diameter, 4L-capacity plant-pot, using a peat growing medium. The pots were placed on a standing ground outdoors at KRC, covered with fleece for protection from extreme weather, and kept well watered in dry weather.

In the monitoring crops in 2005 and 2006, starting after shoot emergence, pot-grown trap-plants were placed adjacent to crop foliage near the centre of each area for an exposure period. In 2005 the 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 periods were used on a continuous basis. For each exposure period, six plant-pots were used. To investigate the effects of leaf damage, before exposure 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, the other three pots remaining undamaged as controls.

Following collection of the exposed trap-plants from the field sites, they were placed in a frost-protected glasshouse at KRC (minimum maintained temperature 3ºC, ventilated at 10ºC, and free of other potentially infective plant material). Further pots, not exposed in the field, were moved straight to the glasshouse (three pots per week) as controls. The three replicate pot-plants in each set were arranged in the glasshouse in three blocks, and all pots were spaced from one another to limit the liklihood of cross-infection. The pot-plants were well watered into saucers, to avoid spreading infection in water splash. Plants were examined for disease lesions at weekly intervals, and once symptoms were present the number of leaves with lesions and incidence and severity scores (Table 1) were recorded at two-weekly intervals over a period of 14 weeks

### *Spore trapping using spore traps*

A 7-day recording volumetric Hirst-type spore trap (Burkard Manufacturing Co. Ltd., Rickmansworth, WD3 1PJ, UK), with an air sampling speed of 10L/min, was used in the field experiments. This unit is designed to sample airborne particles continuously over a seven-day period, particles being impacted on adhesive, silicon-coated, transparent 'Melinex' plastic tape supported on a clockwork-driven drum, the tape being secured around the drum using double-sided adhesive tape.

Spore trapping was carried out from 31 January to 28 March 2005. The tape from the spore trap was replaced at weekly intervals and the exposed tape was refrigerated and sent to Warwick HRI, Wellesbourne, for examination. Tapes were cut into 48mm-lengths, representing 24-h periods; they were mounted on glass slides with double-sided adhesive tape and marked at 2mm intervals (using a razor blade) to indicate one-hour periods for examination.

### *Detection of spores of* Botrytis narcissicola *on spore trap tapes using immuno-fluorescence*

Tapes from the spore trap were examined for spores of *B. narcissicola* by bright-field microscopy using a binocular microscope at a magnification of 400x. Spore counts were made for each marked, 1-h period.

Slides were processed for immuno-fluorescence (IF) by adding a polyclonal antibody (PAb) 94/4/3 (1:200 dilution in PBSTC; see Appendix 1) over the entire surface. After incubation in a moist chamber at 37°C for 1h, slides were washed carefully with PBSTC and air-dried. A solution of anti-rabbit IgG FITC conjugate (Sigma F-0382; Sigma-Aldrich Company Ltd., Gillingham, SP8 4XT, UK) (diluted 1:80 in PBSTC) and two drops of Evan's blue (Sigma E-0133) (0.2% in PBS) and eriochrome black (Sigma E-2377) (0.5% in PBS) was added to cover slides. Slides were incubated in a dark moist chamber at 37°C for 30min after which they were carefully washed, air-dried and mounted in Dakocytomation fluorescent mounting medium and viewed by episcopic-fluorescence microscopy.

### Field testing the spray-timing system (2007 and 2008)

#### *Sites for testing the spray-timing system*

Three field trials were carried out in each of 2007 and 2008 to test the proposed spray-timing system for smoulder. Details of the crops used are shown in Table 3, those selected being considered typical of crops in the region. In each crop an area *ca.* 0.6 or *ca.* 0.4ha in extent was designated and divided into two or three equal treatment areas of *ca.* 0.2ha each. Each area was marked with corner posts and signage and the growers instructed their sprayer operatives on how these areas should be managed. 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.* 0.1ha of each 0.2ha area was further marked for monitoring and observation, as described previously for the monitoring sites, except that one MMS was shared between the Kirton and Kirton End sites because of their proximity.

**Table 3. Field testing sites for fungicide spray programmes in 2007 and 2008.**

| *Year* | *Site name* | *Grid reference* | *Cultivar* | *Crop year* | *Treatments tested* | | |
|---|---|---|---|---|---|---|---|
| | | | | | *Non-sprayed control* | *Commercial sprays* | *Spray-timing system* |
| 2007 | Kirton | TF302395 | 'Golden Harvest' | 2 | ✓ | ✓ | ✓ |
| | Holbeach Marsh | TF388306 | 'Carlton' | 2 | ✓ | ✓ | ✓ |
| | Surfleet | TF259293 | 'Fortune' | 3 | ✓ | ✓ | ✓ |
| 2008 | Kirton | TF301396 | 'Golden Harvest' | 2 | - | ✓ | ✓✓ |
| | Kirton End | TF296404 | 'Carlton'` | 2 | - | ✓ | ✓ |
| | Surfleet | TF256292 | 'Golden Ducat' | 2 | - | ✓ | ✓ |

#### *Fungicide spray programmes tested in 2007*

Three treatments were tested at each site.

1. *Control* - no fungicide sprays applied.
2. *Commercial spray programme* - each grower was asked to apply his routine fungicide spray programme as used on his other daffodil crops, deciding the fungicides, rates and number, timing and methodology of sprays. It was anticipated that growers would apply up to six sprays to these areas over the growing season.
3. *Spray-timing system* - each grower was asked to apply an agreed fungicide spray programme to these areas as triggerred by Warwick HRI staff running the smoulder infection model at weekly intervals. The fungicide used on each occasion was tank-mix Amistar (0.5L product/ha) plus Folicur (0.5L product/ha). It was anticipated that probably no more than three sprays would be applied to these areas.

In determining when to apply fungicide sprays using the spray-timing system, different criteria were used at the three sites.

1. At Kirton, fungal foliar infections were expected to be high, partly because of the presence of diseased crops on-farm as part of the work at the research site. Therefore a

‘cautious’ criterion was used, triggering fungicide application for the spray-timing plot when the infection score was 0.15 or more daily during any week.
2. At Surfleet an ‘economical’ criterion was used, spraying the spray-timing plot only when the infection score was 0.25 or more daily during any week.
3. At Holbeach Marsh, a more exposed site, treating the spray-timing plot was triggered *either* when the infection score reached or exceeded 0.25 in any one day, irrespective of precipitation impact (PI) levels, *or* when the score reached 0.15 *and* there was a ‘heavy rain event’ in that week. A heavy rain event was defined as producing PI in bins 7 to 14 in any rolling 24-h period.

The infection scores above refer to values obtained using the Excel software. Dates and other details of the fungicide sprays applied are shown in Table 4.

Once the model indicated a smoulder risk period, the grower was asked to apply fungicide to his spray-timing area as soon as practical, but taking account of the following factors: (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, these sprays were applied up to about a week after each request was made, delays being caused mainly by unsuitable weather and commercial considerations.

**Table 4. Details of fungicide spray applications in commercial and spray-timing fungicide programmes in 2007.**

| *Spray programme* | *Spray number* | | | |
|---|---|---|---|---|
| | *1* | *2* | *3* | *4* |
| **(a) Surfleet** | | | | |
| Commercial | 14 February<br>Ronilan Fl<br>0.72 L/ha | * | * | * |
| Model | 09 March<br>Folicur + Amistar<br>0.5L + 0.5L/ha | 26 March<br>Folicur + Amistar<br>0.5L + 0.5L/ha | 09 April<br>Folicur + Amistar<br>0.5L + 0.5L/ha | - |
| **(b) Holbeach Marsh** | | | | |
| Commercial | 16 February<br>Bravo + Dithane 945<br>2.0L + 2.5kg/ha | 28 March<br>Folicur + Bravo + Dithane 945<br>0.5L + 1.5kg + 1.5kg/ha | 16 April<br>Amistar + Folicur<br>0.5L + 0.25L/ha | - |
| Model | 02 March<br>Folicur + Amistar<br>0.44L + 0.44L/ha | 28 March<br>Folicur + Amistar<br>0.58L + 0.58L/ha | - | - |
| **(c) Kirton** | | | | |
| Commercial | 1 February<br>Folicur + Delsene Flo<br>0.5L + 0.5L/ha | 17 February<br>Scala + Folicur<br>2.0L + 0.5L/ha | 8 March<br>Dithane + Delsene Flo<br>1.5kg + 0.5L/ha | 28 March<br>Folicur + Amistar<br>0.5L + 0.5L/ha |
| Model | 02 March<br>Folicur + Amistar<br>0.5L + 0.5L/ha | 28 March<br>Folicur + Amistar<br>0.5L + 0.5L/ha | 11 April<br>Folicur + Amistar<br>0.5L + 0.5L/ha | 5 May<br>Folicur + Amistar<br>0.5L + 0.5L/ha |

* A commercial decision was taken by the grower to make no further sprays.

*Fungicide spray programmes tested in 2008*

In 2008 the testing programme was further developed. Two or three spray programmes were tested at each site, and the criteria for evoking the spray-timing system were also refined.

1. *Commercial spray programme* – as in 2007.
2. *Spray-timing system with a maximum of three sprays* - the first three sprays triggered by the spray-timing system were applied. The details of these spray applications were as for

2007, except that each grower was asked to apply the same fungicides as used for his commercial spray programme.

3. *Spray-timing system with a maximum of two sprays* - the first two sprays triggered by the spray-timing system were applied, otherwise as programme 2.

At these sites spraying the spray-timing plot(s) was triggered *either* when the infection score exceeded 50 in any one day, *or* when the infection score exceeded 30 in any one day *and* any of the following applied on the same day or on any day of the previous week: (a) a period with a screen temperature of 1°C or lower, (b) PI sensors recording two or more 'hits' in 'bin 7' or higher, or (c) flower cropping had taken place. Once the model indicated a spray was needed, the grower was asked to apply fungicide to his spray-timing plot as soon as practical, again bearing in mind the additional safeguards mentioned above under the 2007 trial (spray and harvest intervals, etc.).

The infection scores above refer to values obtained using the MATLAB software. The dates and other details of the fungicide sprays applied are shown in Table 5.

**Table 5. Details of fungicide spray applications in commercial and spray-timing programmes in 2008.**

| *Spray programme* | *Spray number* | | | |
|---|---|---|---|---|
| | *1* | *2* | *3* | *4* |
| **(a) Kirton** | | | | |
| Commercial | 28 January<br>Amistar<br>0.75L/ha | 13 February<br>Folicur<br>1.0L/ha | 27 February<br>Delsene 50 Flo + Bravo<br>1.0 + 2.0L/ha | 27 March<br>Amistar<br>1.0L/ha |
| Spray timing (3 sprays) | 04 February<br>Amistar<br>0.75L/ha | 14 March<br>Folicur<br>1.0L/ha | 27 March<br>Delsene 50 Flo + Bravo<br>1.0 + 2.0L/ha | - |
| Spray-timing (2 sprays) | 04 February<br>Amistar<br>0.75L/ha | 14 March<br>Folicur<br>1.0L/ha | - | - |
| **(b) Kirton End** | | | | |
| Commercial | 28 January<br>Amistar<br>0.75L/ha | 13 February<br>Folicur<br>1.0L/ha | 27 February<br>Delsene 50 Flo + Bravo<br>1.0 + 2.0L/ha | 27 March<br>Amistar<br>1.0L/ha |
| Spray-timing (3 sprays) | 04 February<br>Amistar<br>0.75L/ha | 14 March<br>Folicur<br>1.0L/ha | 27 March<br>Delsene 50 Flo + Bravo<br>1.0 + 2.0L/ha | - |
| **(c) Surfleet** | | | | |
| Commercial | 04 March<br>Folicur + Delsene Flo<br>0.5L + 0.5L/ha | * | * | * |
| Spray-timing (3 sprays) | 12 February<br>Folicur + Amistar<br>0.5L + 0.5L/ha | 04 March<br>Folicur + Amistar<br>0.5L + 0.5L/ha | 03 April<br>Folicur + Amistar<br>0.5L + 0.5L/ha | - |

* A commercial decision was taken by the grower to make no further sprays.

## Associated field studies (2007)

### *Spore trapping using trap-plants*

To complement the 2007 testing of the spray-timing system, trap-plants were used to obtain information on the incidence of smoulder spores aerially. The trap-plants were raised and used as described earlier. Between 6 March and 7 May 2007, trap-plants (damaged and undamaged) were placed adjacent to crop foliage near the centre of each trial crop for exposure periods of 7 days. After exposure and collection from the field sites the trap-plants were grown-on in a glasshouse as described before, except that, to provide better conditions for infection, during the initial 48-hour period the pots were placed under high humidity provided by a humidifier running under a polythene-film cover within the glasshouse, after which they were moved to the body of the glasshouse. The trap-plants were examined for disease lesions at weekly intervals, and incidence and severity scores were recorded as previously described.

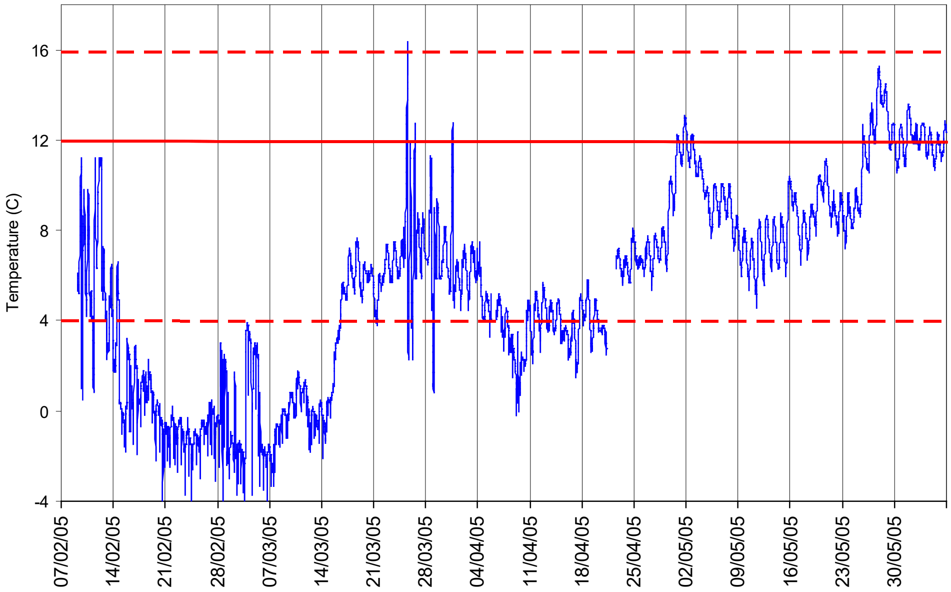
## Results

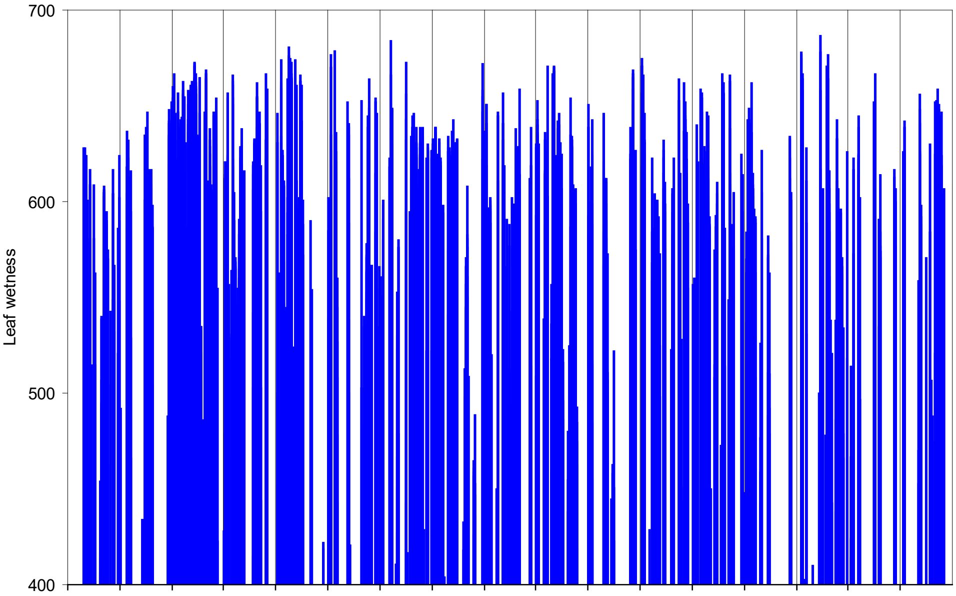
### Smoulder monitoring and spore trapping, 2005

*Weather records*

Air temperatures and leaf wetness duration data for the Saracen's Head site are shown in Figure 2. Previous work showed that the range of infective temperatures for *B. narcissicola* is 4 to 16°C, with an optimum of 12°C which requires a 6-h period of leaf wetness. In 2005 there was a cold period from mid-February to mid-March, during which temperatures were unlikely to have resulted in a serious smoulder problem. For most of the rest of the growing season temperatures were within the infective range, though the optimum temperature was reached only periodically and only for brief periods. The infection model predicts that longer periods of leaf wetness, up to 24h, are required for infection if temperatures are sub-optimal. Significant periods of leaf wetness occurred throughout the season. It is likely that in this situation infection would be most likely from late-March onwards.

**Figure 2. Screen temperature (above) and leaf wetness (below, in mvolt) at the Saracen's Head site in 2005. The broken and solid red lines indicate, respectively, the limits of infective temperatures and the optimum infective temperature.**

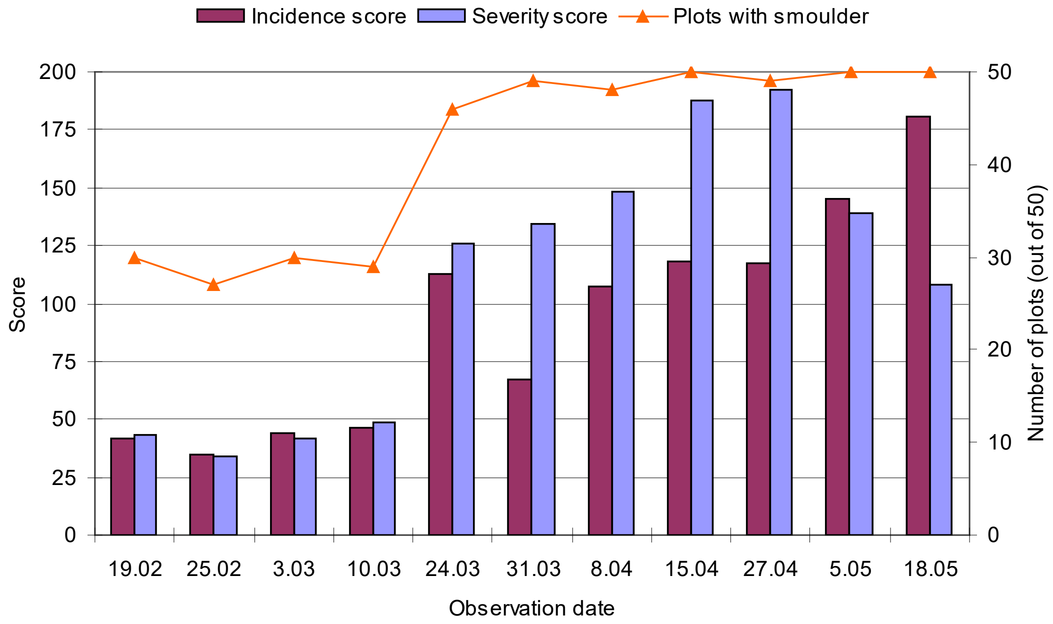


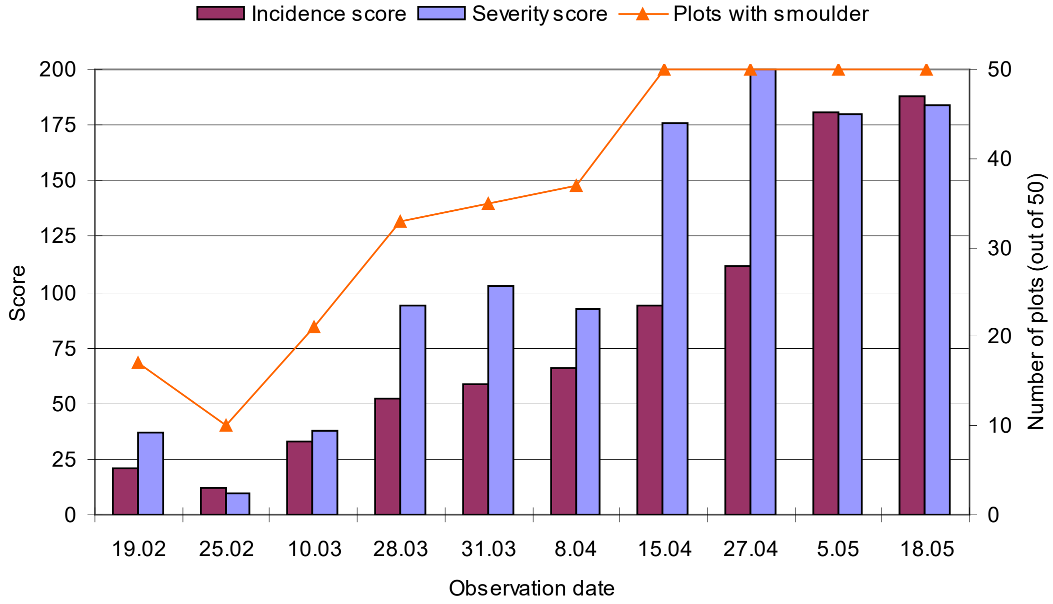


*Disease levels*

The number of plots showing smoulder symptoms was high at both the Kirton and Saracen's Head sites in 2005, though the pattern of development differed (Figure 3). At Kirton, more plots were affected from an early date than at Saracen's Head, where the number of plots with symptoms increased more gradually and from a lower baseline. However, the incidence and severity scores at both Kirton and Saracen's Head were relatively low until early-March, and then increased steadily at both sites from the second half of March.

**Figure 3. Smoulder monitoring at Kirton (above) and Saracen's Head (below) sites in 2005. Disease levels expressed as the number of plots with symptoms and as incidence and severity scores.**

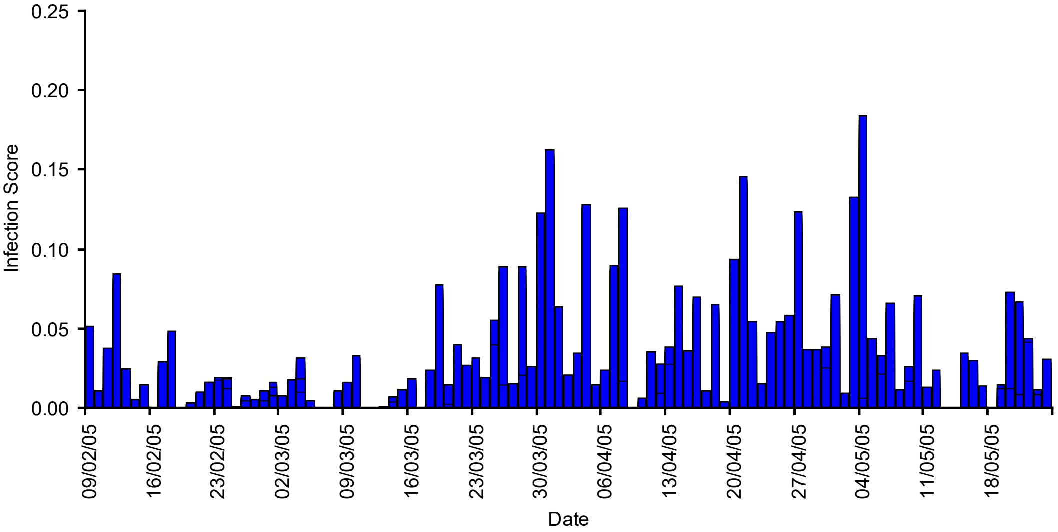

Incidence score
Severity score
Plots with smoulder
Score
Number of plots (out of 50)
Observation date
0
25
50
75
100
125
150
175
200
0
10
20
30
40
50
19.02
25.02
3.03
10.03
24.03
31.03
8.04
15.04
27.04
5.05
18.05


Incidence score
Severity score
Plots with smoulder
Score
Number of plots (out of 50)
Observation date
0
25
50
75
100
125
150
175
200
0
10
20
30
40
50
19.02
25.02
10.03
28.03
31.03
8.04
15.04
27.04
5.05
18.05

### *Infection predictions*

The infection model was run with temperature and leaf wetness duration data for each site, and the predicted infection scores are presented in Figure 4. There were several possible infection periods when scores >0.1 were predicted, at the end of March, on 4 and 27 April and on 3-4 May 2005. The generally higher infection scores around the end of March correspond with the onset of the higher temperatures seen in the weather data, and with the increase in smoulder incidence and severity that begins at this time.

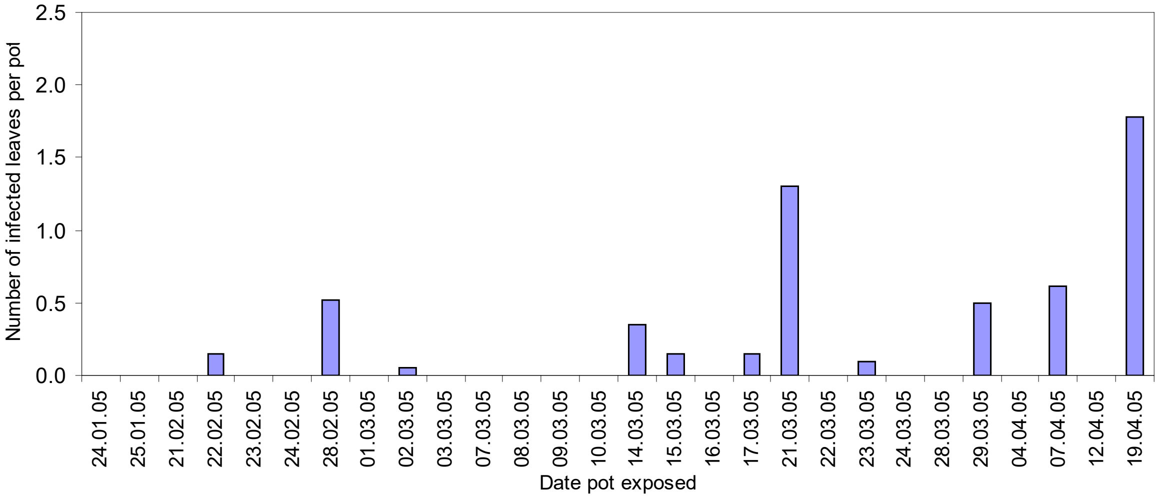
**Figure 4. Predicted smoulder infection score in 2005 at Saracen's Head site.**



### *Trap-plant and spore trap data*

Plants in only eleven out of 168 pots exposed at the Kirton site developed smoulder lesions, all in pots in which the foliage had been damaged (Figure 5). The average number of leaves per pot affected was low, varying from 0.3 to 3.0, but nevertheless there appeared to be three or four peaks of activity over the growing season. These peaks centred on 28 February, 21 March and late-March onwards. Trap-plants were placed in an infected plot on one day and removed to the glasshouse on the following day; for this reason the predicted infection on (day +1) was compared with the observed infection on each day. No smoulder lesions were found on the plants with non-wounded leaves or on unexposed control plants.

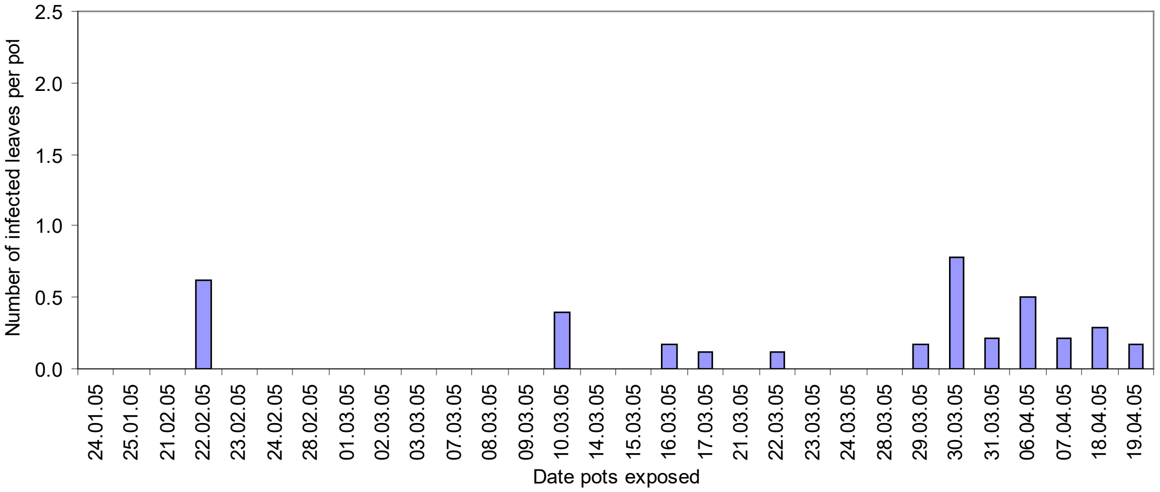
**Figure 5. The incidence of smoulder lesions on trap-plants with wounded leaves at Kirton in 2005. The values are the average for all five assessment dates, determined from three replicate plant-pots for each exposure period.**



Only twelve out of the 180 pots exposed at the Saracen's Head site were found to develop smoulder lesions, mostly on plants exposed on 5 May 2005 and all on plants that had had their leaves damaged (Figure 6). The average number of leaves per pot affected was lower than at Kirton, but as at Kirton there appeared to be three or four peaks of activity. These peaks centred on 22 February, 10 March and late-March onwards.

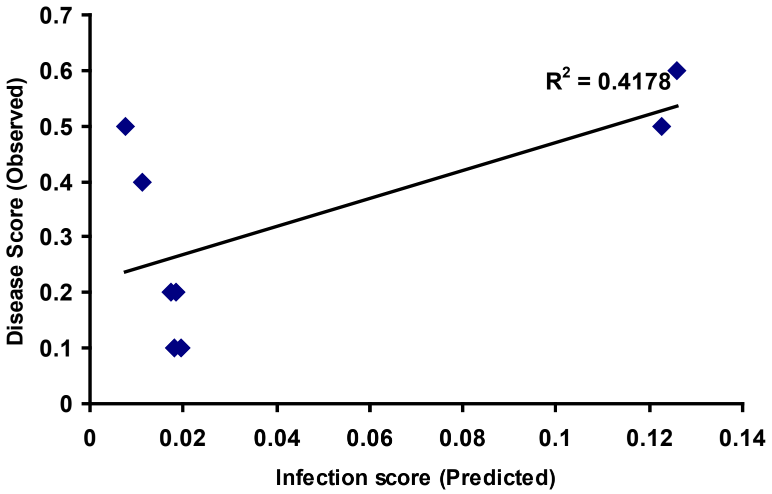
Infection of trap-plants was very low on non-damaged plants and sporadic on trap-plants which had received damage. As infection on trap-plants depends on both inoculum availability and infection conditions, it is possible that the trap-plants infection levels corresponded more closely to periods where smoulder inoculum and infection conditions were not limiting.

**Figure 6. The incidence of smoulder lesions on trap-plants with wounded leaves at Saracen's Head in 2005. The values are the average for all five assessment dates, determined from three replicate plant-pots for each exposure period.**



There was a poor relationship between predicted infection score and observed disease on trap-plants (Figure 7). An $R^2$ value of 0.4178 was obtained when comparing observed and predicted infection. This was due to the low levels of infection observed. An improved $R^2$ value of 0.5116 was obtained if the data for the 7 March 2005 were omitted from the analysis. Spore trapping studies indicated that there were too few conidia of *B. narcissicola* present on that date for symptom expression to be caused by smoulder. However, on two occasions high predicted infection from the model appeared to correspond to higher observed infection scores on trap-plants.

**Figure 7. Predicted and observed smoulder infection on trap-plants at the Saracen's Head site in 2005.**



The number of lesions observed on trap-plants was averaged for the six-week period following exposure in the field. Only five out of all the pots exposed during the two-month period of air sampling at Saracen's Head were found to develop smoulder lesions, mostly on plants exposed on 22 February 2005 and all on plants that had had their leaves damaged. Also, the number of conidia of *B. narcissicola* on each slide representing 24h from the 7-day spore trap was averaged for the same days the plants were exposed (Table 6). On 22 February 2005 the mean number of leaves affected was at its highest (0.6), however the average spore count was only 4.8 spores/m$^3$/h. The highest average spore count, 125 spores/m$^3$/h, was observed on 22 March 2005, but with only 0.1 leaves affected.

**Table 6. Mean number of leaves affected by smoulder after exposing trap-plants to infective conditions, and the mean number of spores of *B. narcissicola* trapped per hour for the same day.**

| *Date of exposure* | *Mean number of leaves affected* | *Mean number of spores trapped /m$^3$/h* |
|---|---|---|
| 22/02/05 | 0.6 | 4.800 |
| 28/02/05 | 0 | 1.528 |
| 02/03/05 | 0 | 0.344 |
| 07/03/05 | 0 | 0.600 |
| 10/03/05 | 0.4 | 2.144 |
| 14/03/05 | 0 | 0.120 |
| 16/03/05 | 0.2 | 7.628 |
| 17/03/05 | 0.1 | 6.344 |
| 22/03/05 | 0.1 | 125.452 |
| 23/03/05 | 0 | 9.428 |

*Immunodetection of* B. narcissicola *on tapes from spore traps*

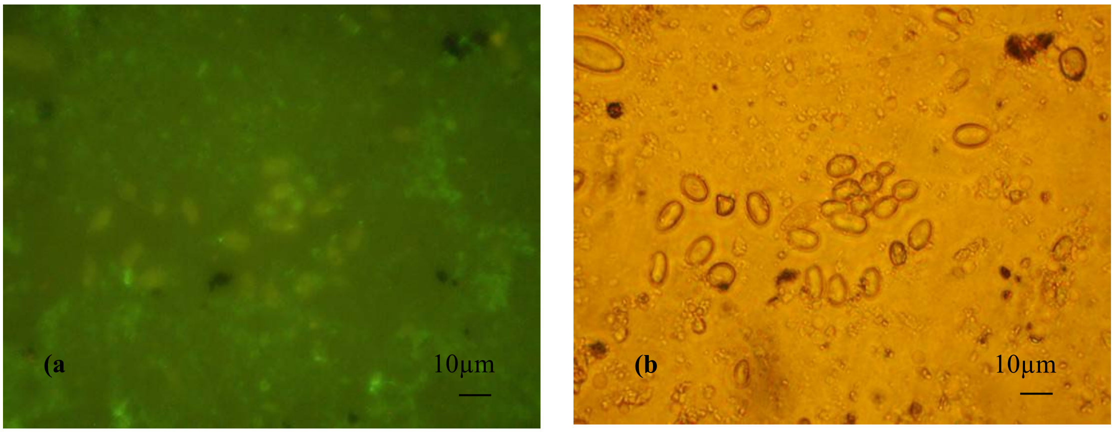
The tapes from 7-day spore trap were processed for IF, and fluorescing conidia of *B. narcissicola* were counted on tapes viewed by a Nikon Optiphot-2 microscope with episcopic-fluorescence (Plate 1). Immuno-detection of conidia of *B. narcissicola* under UV episcopic-fluorescence was low on all the tapes compared with the number obtained under bright-field microscopy (Table 7). The correlation of the spore count and IF was found to be high, 0.822.

**Table 7. The total number of spores counted for each date of sampling using light**

**microscopy and UV microscopy.**

| *Sampling date* | *Light microscopy* | *UV microscopy* |
|---|---|---|
| 22/02/05 | 112 | 30 |
| 28/02/05 | 28 | 24 |
| 02/03/05 | 8 | 8 |
| 07/03/05 | 10 | 4 |
| 10/03/05 | 50 | 30 |
| 14/03/05 | 2 | 0 |
| 16/03/05 | 178 | 70 |
| 17/03/05 | 148 | 32 |
| 22/03/05 | 2974 | 156 |
| 23/03/05 | 220 | 112 |

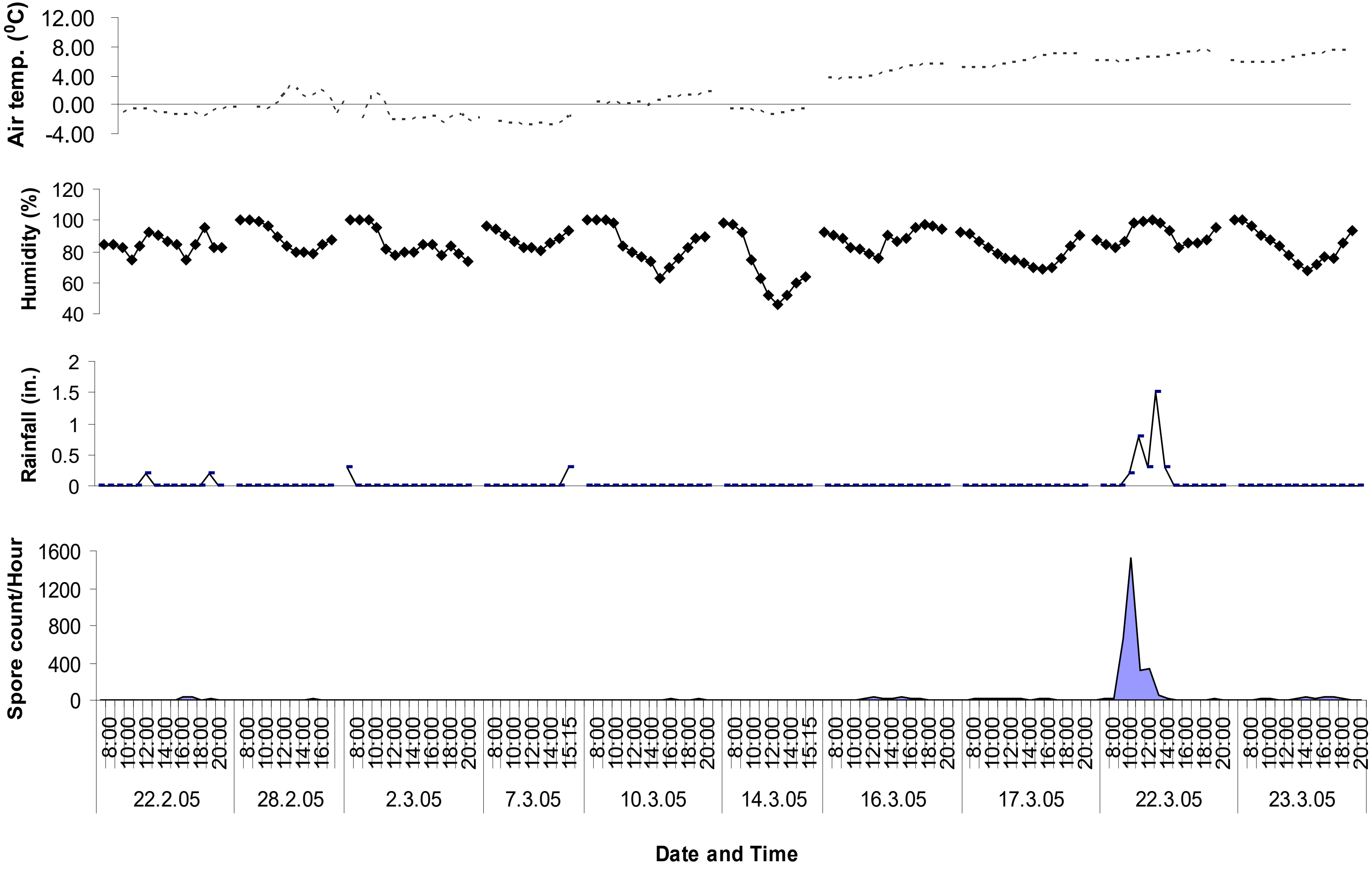
**Plate 1. Left: conidia of *B. narcissicola* under UV light from the 7-day volumetric trap, negative by immuno-fluorescence. Right: bright-field view of the same portion of tape.**



### *The effect of the environment on field trapping of* B. narcissicola

Air temperature, humidity and rainfall measurements were averaged over 1h periods and the effect of the meteorological data on trapping was observed. There was a correlation in the rainfall and spore count. The highest hourly spore count coincided with the highest rainfall recorded. There was a high peak on 22 March 2005, when rainfall was 0.2 to 1.5 inches and spore count within this time ranged from 56 to 1522 spores per hour (Figure 8). Temperature also affected the amount of trapped spores. High spore numbers were recorded at high temperatures and spores were not trapped at temperatures below 0°C.

**Figure 8. The effect of environment on field trapping of *B. narcissicola* spores. The break in lines after each trapping date shows the data are not continuous.**



## Smoulder monitoring and spore trapping, 2006

### *Weather records*

Figures 9 and 10 show the temperature and leaf wetness duration data for the Surfleet and Kirton sites in the 2006 growing season. As expected for such relatively close locations, the weather was generally, but not entirely, similar. Comparing temperatures at the two sites, Kirton temperatures were the more extreme, with lower minimum temperatures over most of the growing season but some higher temperatures especially in late-March/early-April. At Kirton, temperatures were more often <4°C than at Surfleet. However, at both sites temperatures more conducive to smoulder infection occurred in early-February, early-March and late-March onwards. There were relatively dry periods in mid-February, mid-March and mid-April at both sites, not corresponding to the warmer periods (Figure 10). These differences between sites are interpreted as largely due to shelter, with the site at Surfleet being relatively sheltered with trees on two sides of the field, and the Kirton more open. The data suggest that narcissus at the Surfleet site would be more susceptible to smoulder, with overall warmer and wetter weather.

**Figure 9. Temperatures at the Kirton (above) and Surfleet (below) sites in the 2006 growing season.**

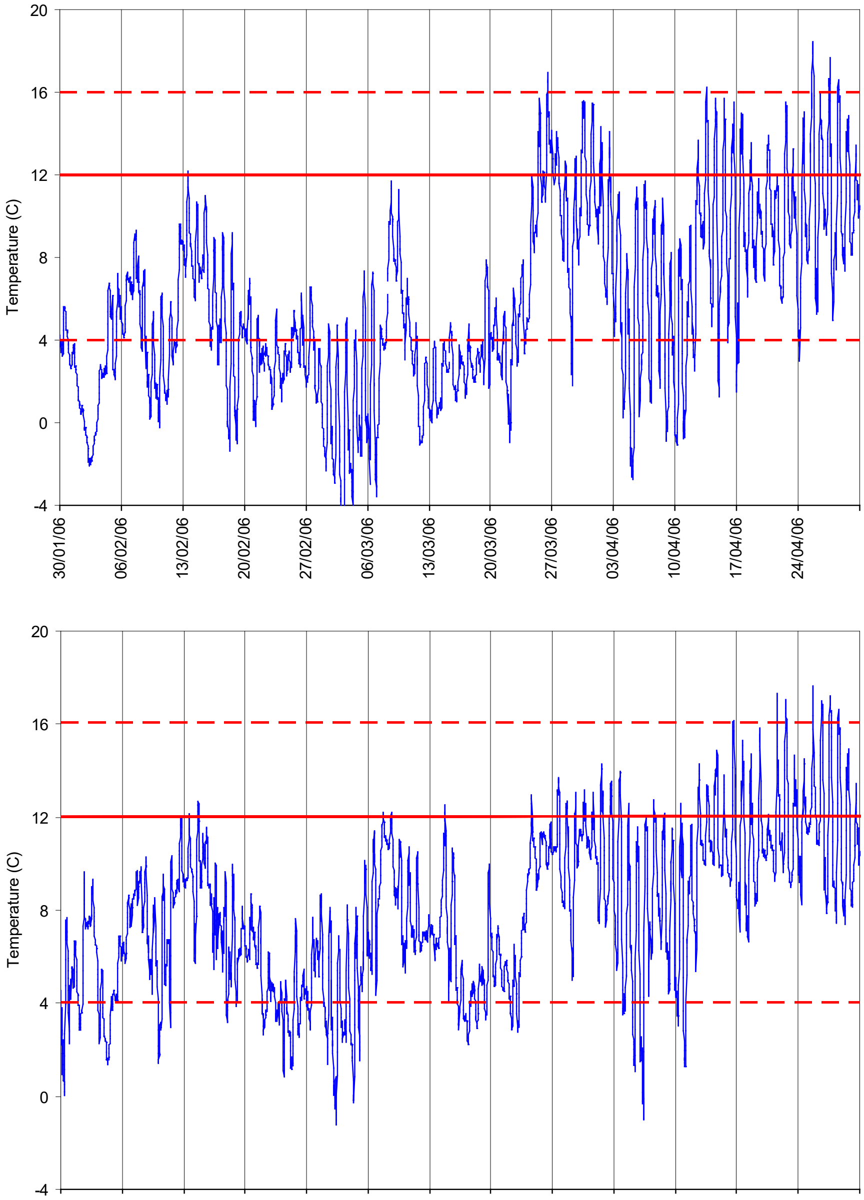
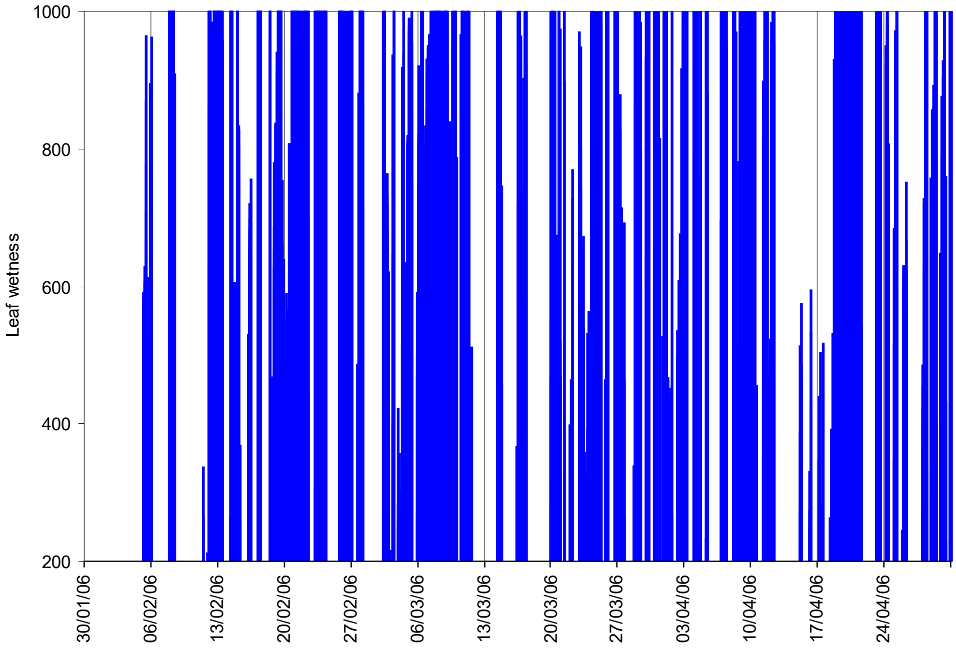
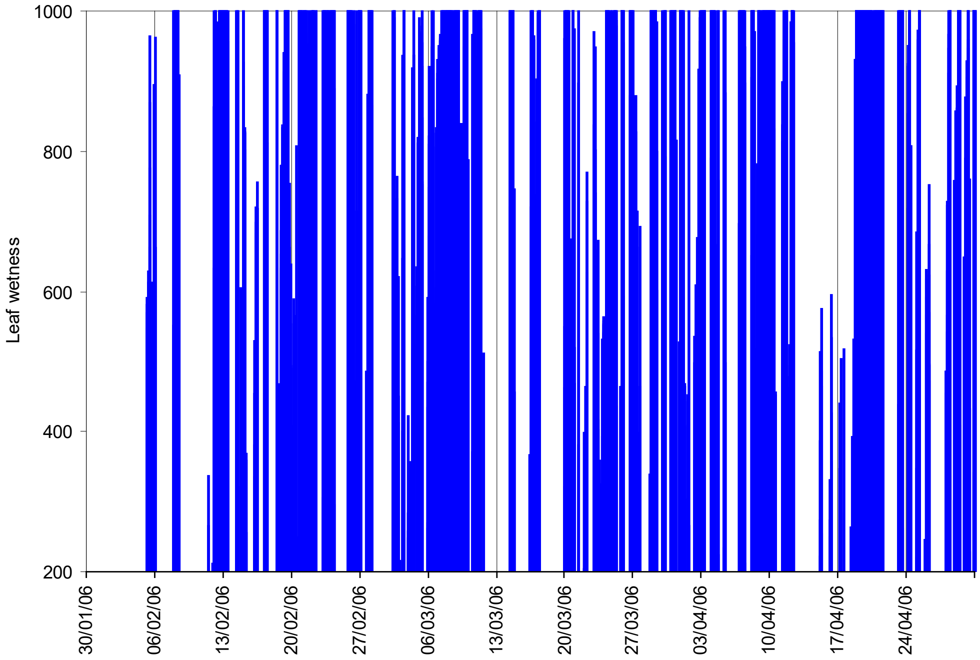


**Figure 10. Leaf wetness (in mvolt) at the Kirton (above) and Surfleet (below) sites in the 2006 growing season.**

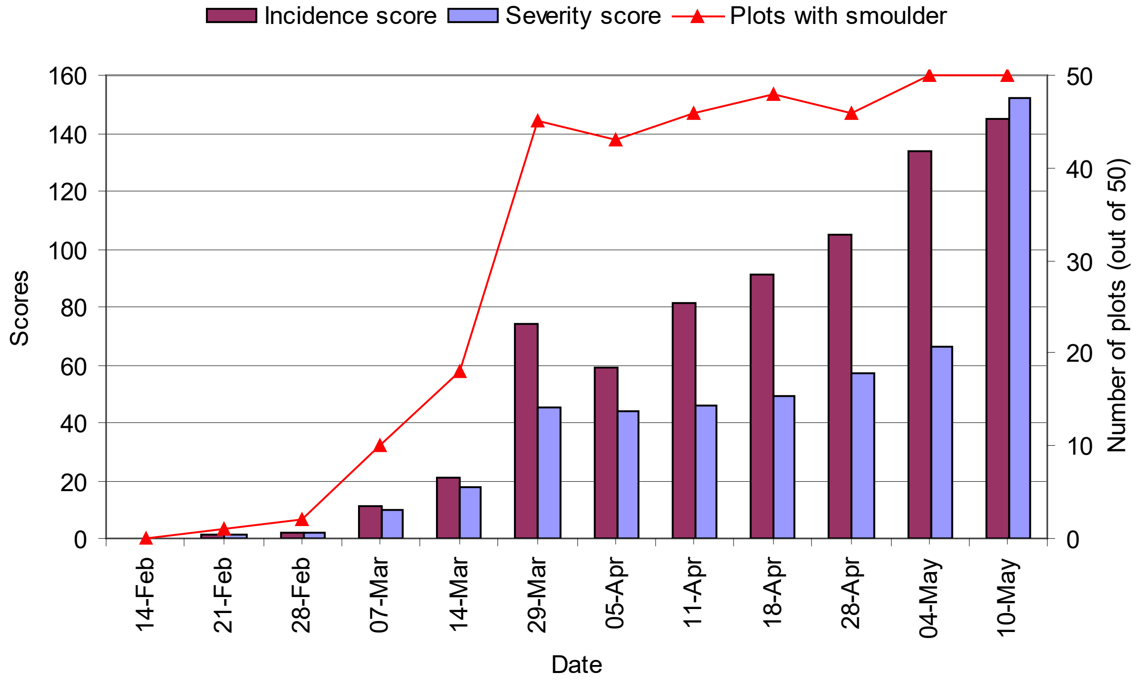


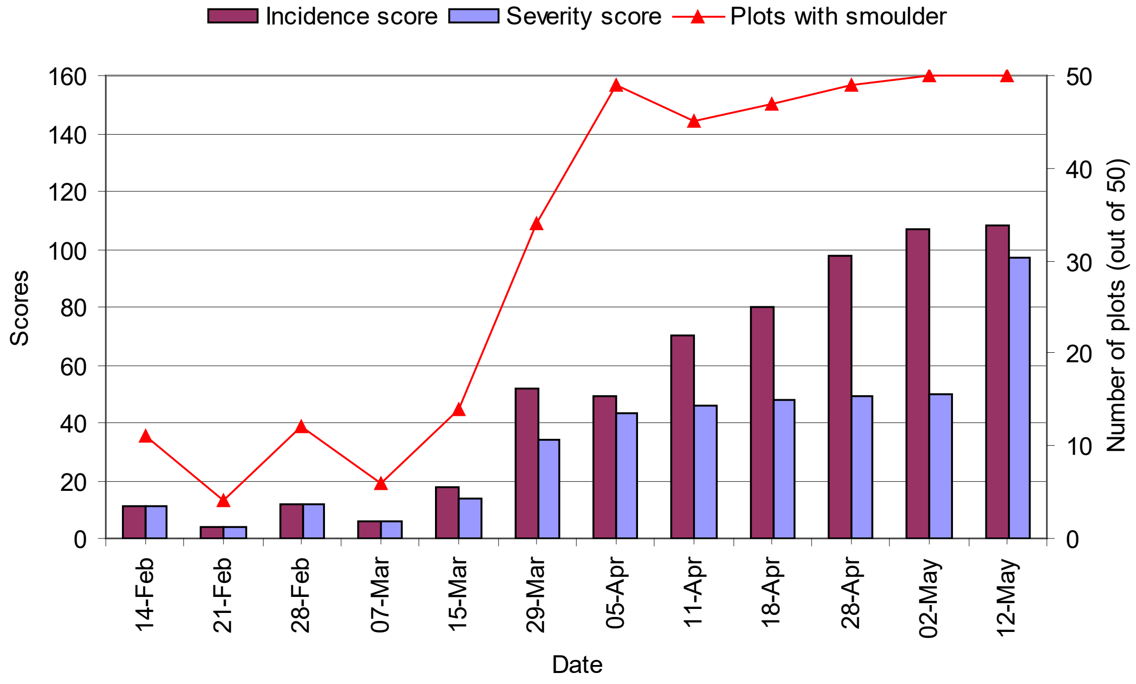


*Disease levels*

Despite the differences between the two sites, the pattern of smoulder incidence and severity was similar (Figure 11) and to the pattern seen in the 2005 data. Smoulder levels initially increased slowly, with a large increase in incidence and severity starting in late-March. The final incidence and severity of the disease was lower at Surfleet in 2006 than in the other cases, which may heve been related to cultural or varietal diifferences between the sites.

**Figure 11. Smoulder monitoring at Kirton (above) and Surfleet (below) sites in 2006. Disease levels expressed as the number of plots with symptoms and as incidence and severity scores.**





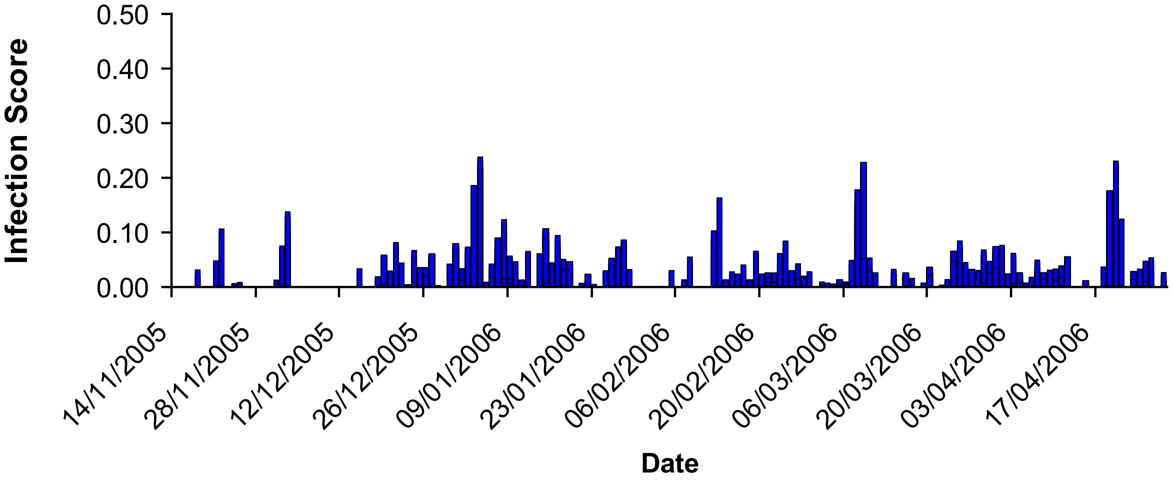
*Infection prediction*

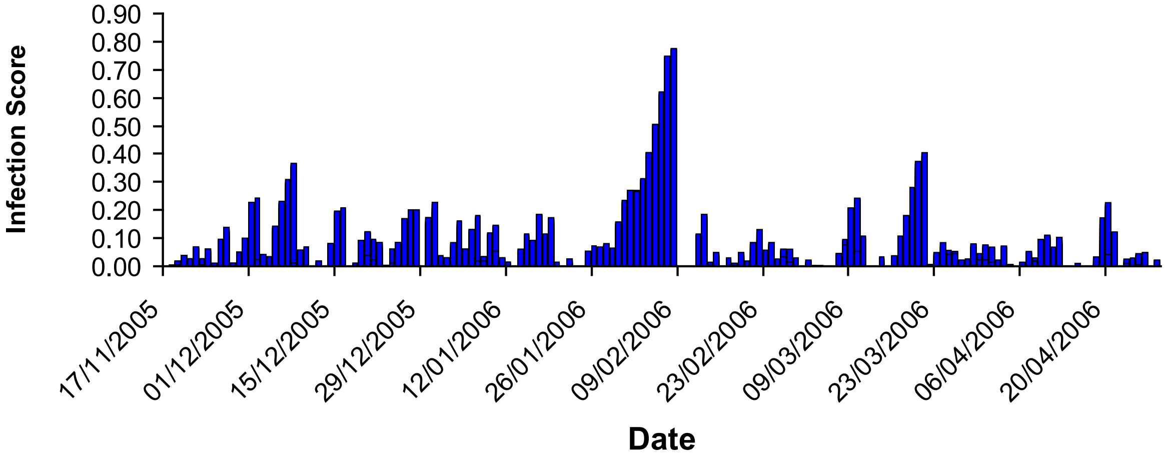
The smoulder infection model was run with the temperature and leaf wetness duration data for both sites, and the predicted daily infection scores are presented in Figure 12. Major infection periods (with scores of about 0.2) were recorded at Kirton on 3 and 4 January, 8 and 9 March and 19 and 20 April 2006. Additionally, scores of >0.1 were predicted on 22 November, 3 December, 8 and 15 January and 12 and 13 February 2006.

At Surfleet higher predicted infection scores were given. Infection scores >0.2 were predicted for 1, 2, 6, 7, 8, 27, 28 and 31 December 2005, and a continuous infective period with scores reaching well above 0.2 (up to 0.7 or 0.8) predicted from 31 January to 8 February 2006. Predicted infection scores >0.2 were also recorded on 9, 10, 19, 20, and 21 March and 20 April 2006.

In the period leading up to 8 February there was a step-wise pattern in the predicted infection score which may have been an artefact. It suggests the wetness sensors had shifted and had come into contact with leaves or the ground. This error in data collection is a general problem and has been seen in the collection of wetness data from other crops.

**Figure 12. Predicted smoulder infection scores at Kirton (above) and Surfleet (below) in 2006.**





*Trap-plant and spore trap data*

Maximum numbers of *B. narcissicola* conidia were observed on spore tapes during 18–19 May 2006. Smaller but significant peaks in conidial numbers were observed on 17–18, 24–25 and 27–28 May 2006 (Figure 13).

As in the previous year, few trap-plants developed smoulder symptoms, but in contrast to 2005 symptoms developed on both wounded and non-wounded leaves, so the figures presented have been averaged across both treatments (Figure 14). At Kirton there was a prominent peak of infection in early-May, followed by smaller peaks. At Surfleet, only the later, small peaks occurred. No symptoms were found on non-exposed control plants.

**Figure 13. Numbers of B. *narcissicola* spores trapped in air samples at Kirton in 2006.**

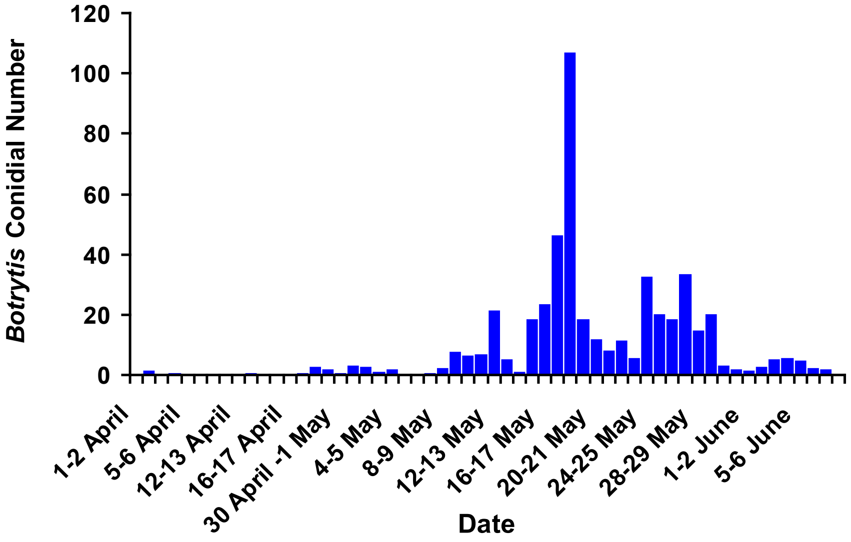
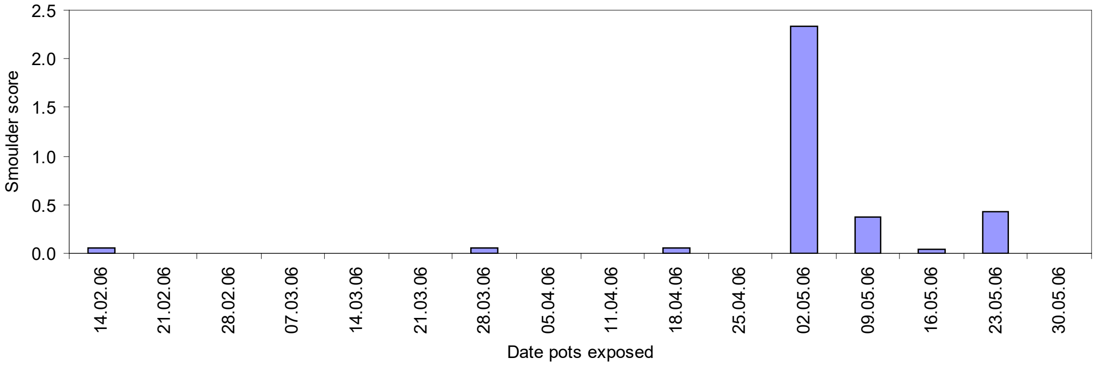
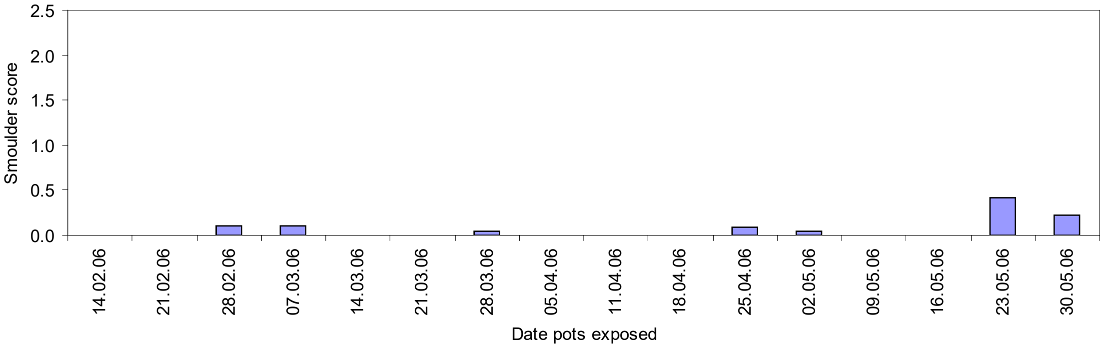


**Figure 14. Smoulder symptoms on trap-plants at Kirton (above) and Surfleet (below) in 2006. The smoulder score used is the product of incidence and severity scores (see text). Figures are averages of wounded and non-wounded plants across the last five assessment dates for three replicate plant-pots for each treatment.**

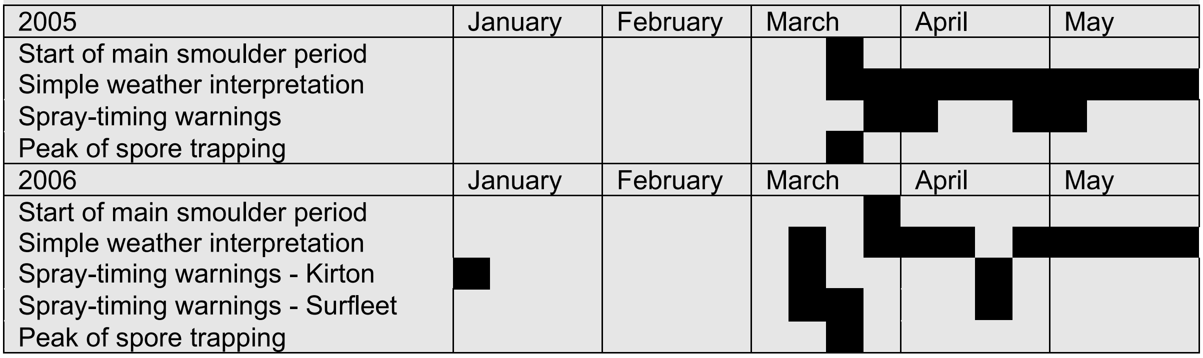




*Comparison of observed and predicted smoulder levels, 2005 and 2006.*

Observed and predicted measures of smoulder levels are summarised in Table 8. In general these results demonstrate that three spray indicators – spray-timing predictions, general weather observations and spore-trap results – all effectively warn of the onset of the main smoulder period. Of course, only the spray-timing prediction is convenient and automated.

**Table 8. Summary of observed and predicted smoulder indicators, 2005 and 2006.**



| 2005 | January | February | March | April | May |
|---|---|---|---|---|---|
| Start of main smoulder period | | | ■ | | |
| Simple weather interpretation | | | ■ | ■ | ■ |
| Spray-timing warnings | | | ■ | ■ ■ | ■ |
| Peak of spore trapping | | | ■ | | |
| **2006** | **January** | **February** | **March** | **April** | **May** |
| Start of main smoulder period | | | ■ | | |
| Simple weather interpretation | | | ■ ■ | ■ ■ | ■ |
| Spray-timing warnings - Kirton | ■ | | ■ | ■ | |
| Spray-timing warnings - Surfleet | | | ■ | ■ | |
| Peak of spore trapping | | | ■ | | |

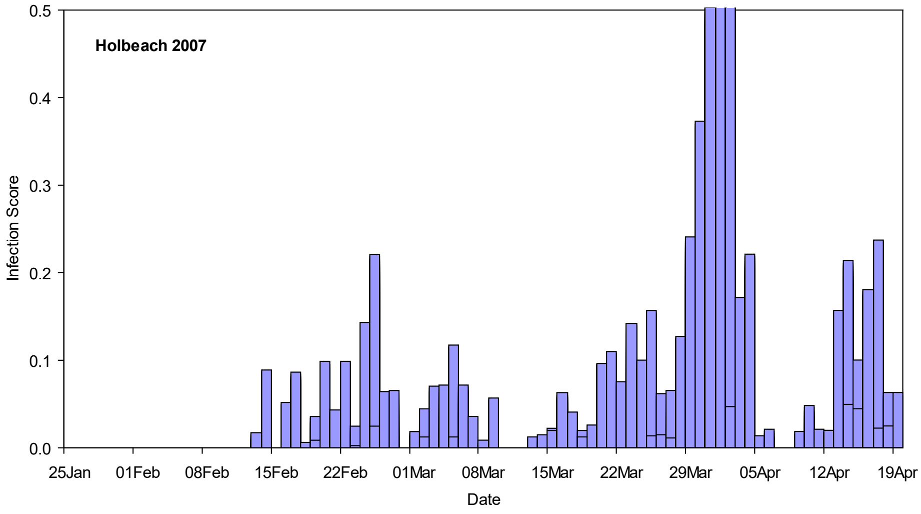
Field testing the smoulder spray-timing system, 2007

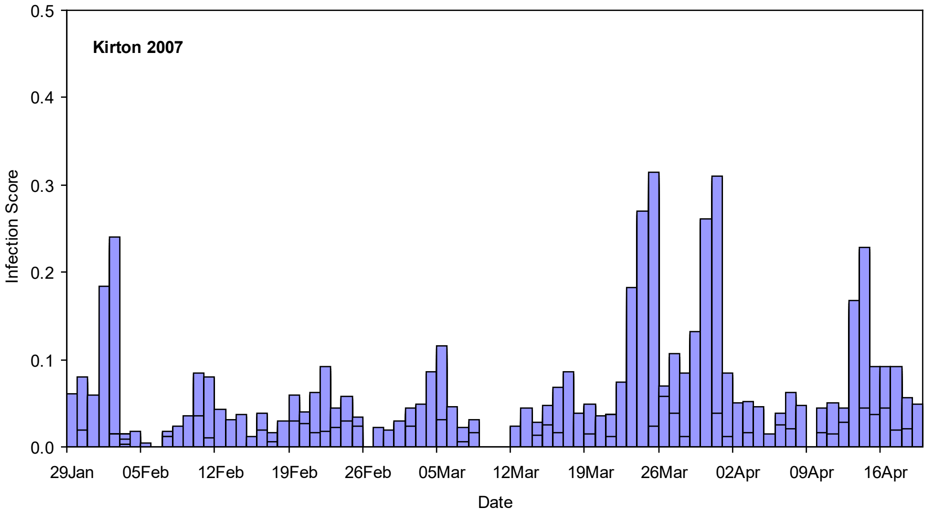
The aim of field testing in 2007 was to compare crop and disease development in (a) non-sprayed ‘control’ areas, (b) areas treated according to a conventional, ‘commercial’ fungicide spray programmes (i.e. regular spray applications ‘by the calandar’), and (c) areas treated according to the ‘grower spray-timing system’ (with spray dates determined from damage criteria (e.g. flower cropping) and (or) that week’s disease development prediction given by the smoulder infection model). Field trials were carried out at three sites, at the Warwick HRI site at Kirton and at commercial grower sites in Holbeach Marsh and Surfleet.

As described above the smoulder infection model was run weekly for each of the three trial sites, using the air temperature and leaf wetness duration data recorded at each site and taking account of crop damage factors. In the trials at Kirton and Surfleet a spray was requested for the spray-timing plots when the infection score exceeded a level of 0.15 or 0.25, respectively, in any one week. Since heavy rainfall produces damage that enhances smoulder infection, at the Holbeach Marsh site a combination of critical infection score and heavy rainfall was used to trigger a request for fungicide application (when *either* the infection score reached 0.25 in a week, irrespective of PI levels, *or* when the score reached 0.15 *and* there was a ‘heavy rain event’ in that week).

The predicted infection scores are shown for the three sites in Figure 15, and the PI reading for Holbeach Marsh in Figure 16. At all three sites the first spray-timing fungicide application in 2007 was triggerred, in the absence of high infection scores, by flower cropping. Despite the proximity of the three trial sites, there were considerable variations in weather patterns across the sites, requiring the use of locally produced weather data. It was found that spray alerts could be triggerred throughout the growing season, including early in the year when frost damage might also occur.

**Figure 15. Smoulder infection scores derived from the predictive model using air temperature and leaf wetness duration data from each site in 2007, Holbeach Marsh (top), Kirton (middle) and Surfleet (bottom).**





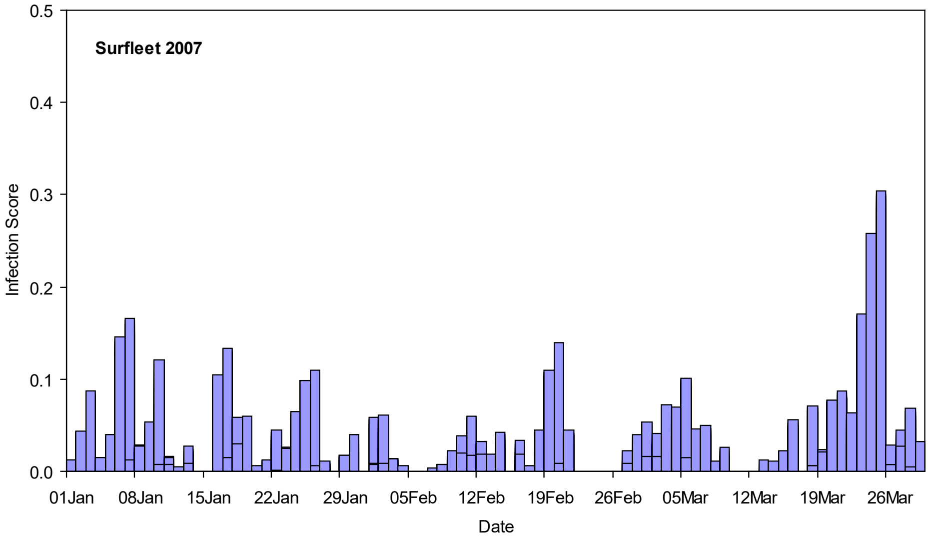
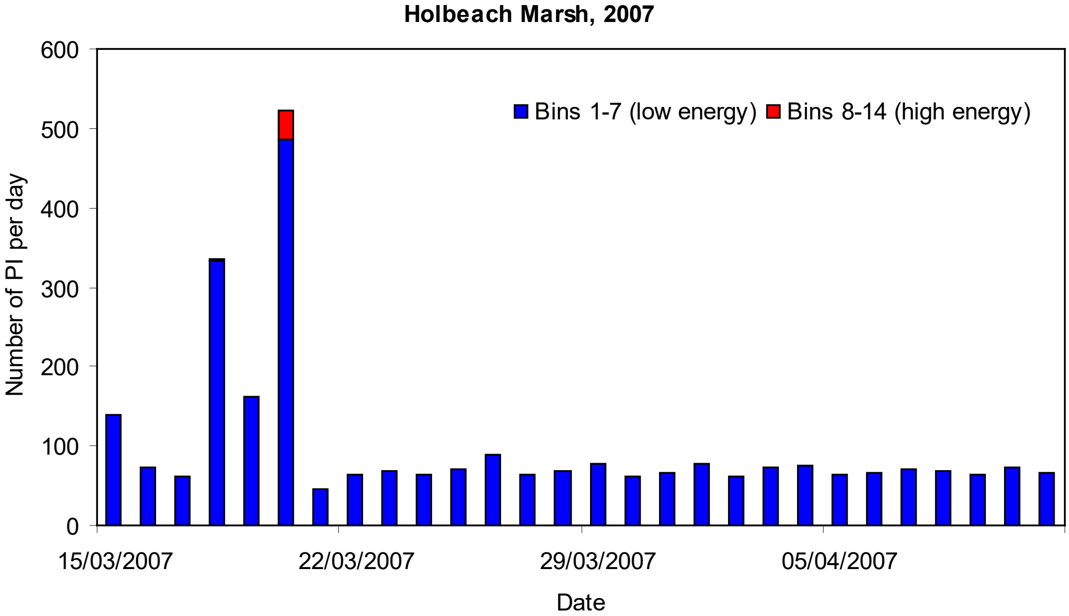


**Figure 16. Daily 'precipitation impacts' (PI) at Holbeach Marsh site in mid-March to mid-April 2007. PIs have been split between low and high energy levels ('bins').**



For the commercial spray programme, four sprays were applied at Kirton, three at Holbeach Marsh and one at Surfleet. The starting dates varied from 1 February at Kirton to 16 February at Holbeach Marsh, probably a reflection of how different disease levels were perceived by individual growers. The number of commercial sprays was less than the six or so that would have formerly been expected, probably due to a combination of changing commercial practice, the greater effectiveness of the fungicides now being used, and the lack of suitable weather for crop spraying.

For the spray-timing system spraying at all sites started between 2 and 9 March, triggered (in the absence of high infection scores) by the damage caused by flower cropping (dates shown in Figure 17). The later sprays were triggered by high disease development scores alone, and at Kirton, Holbeach Marsh, Surfleet there were totals of four, two and three sprays, respectively.

*Case 1 – Kirton site*

At Kirton, the critical infection score of 0.15 was exceeded on four occasions, 2-3 February, 23-25 March, 30-31 March and 13-14 April. Fungicide sprays were applied to the model spray programme plot following the last three warning scores, but technical problems with accessing weather data earlier in the year prevented the first warning score being used. The spray-timing plot received one spray due to flower cropping damage and three more due to high infection scores. The commercial spray programme plot also received four applications, though at different timings - starting and finishing earlier than the model programme.

At Kirton the incidence of smoulder increased slowly from early-February, becoming relatively more severe than at the Holbeach Marsh or Surfleet sites (Figure 17). This high level of smoulder at this experimental site in 2007 may have been partly due to the greater concentration there of diseased crops used in trials, and indeed more smoulder primaries were recorded at Kirton in 2007 than at the other sites (Table 10).

In the area treated using the grower spray-timing system, the omission of an early spray was clearly detrimental. Nevertheless, the non-sprayed control area had a higher smoulder incidence than the plots receiving either of the fungicide spray programmes, while in the spray-timing system area the crop remained greener than in the commercial spray programme area towards the end of the growing season. At Kirton, the spray-timing system was considered a success, despite the high initial levels of smoulder at the site.

*Case 2 – Surfleet site*

At Surfleet, the infection score also exceeded 0.15 early in the season, in January, but, as described above, it had been decided to operate on a warning level of 0.25 at this site, a level exceeded only on 24-25 March, following which two fungicide sprays were applied to the spray-timing plot. The model plot therefore received one spray due to flower cropping damage and two more due to high infection scores. At this site only one fungicide spray was applied to the commercial spray programme plot, due to commercial considerations, this spray being applied in mid-February.

As at Kirton and Holbeach Marsh the incidence of smoulder increased slowly from early-February (Figure 17). There was no evidence for treatment effects on smoulder incidence over most of the growing season, but towards the end of this period the beneficial effects of fungicide sprays were evident, the spray-timing system giving the better disease control. Using the spray-timing system, as at Kirton, the crop remained greener than in the commercial spray programme area at the end of the growing season. Here, the early curtailment of the conventional spray programme inevitably meant that the model spray programme was the more effective.

*Case 3 – Holbeach Marsh site*

At Holbeach Marsh the critical level of 0.15 was first exceeded on 25 February, and also later in the season, but as these scores were not accompanied by heavy rain events no sprays were applied as a result. However, the higher warning level, 0.25, was greatly exceeded over the period 29 March to 4 April, resulting in fungicide application. The spray-timing plot therefore received only two fungicide sprays in all, one due to flower cropping damage and one more due to a high infection score. The commercial spray programme plot received three applications, starting earlier and finishing later than the model programme.

As at Kirton the incidence of smoulder increased slowly from early-February (Figure 17). There was no evidence for treatment effects on smoulder incidence over most of the growing season, but towards the end of this period the beneficial effects of fungicide sprays were evident, the commercial spray programme being the better of the two. At this site, waiting for a high infection score before spraying proved, in retrospect, unwise: it would have been better to have used a lower critical infection score and not to have waited for high-impact precipitation to occur.

For the more reliable control of smoulder, these results suggest that a relatively low predictor score (0.15 in this case) should be used to trigger fungicide applications. Fungicide applications early in the growing season are also important. Until the effects of weather-induced damage are better understood, perhaps it would be better generally to rely on predicted disease development alone, taking frost and heavy rain into account as damage factors only when the infection score itself was boarderline.

Incidence is only one way of expressing the amount of disease in a crop, so the severity (the degree to which affected leaves are affected) and distribution (the number of sample areas with symptoms) of smoulder were also recorded. For the Kirton site severity scores and the number of sample areas affected by smoulder, confirmed the conclusions obtained using incidence scores – most smoulder was found in the control blocks, and least where sprays were applied according to the model programme (Figure 18). Attempts were made to combine incidence and severity scores in order to give a more comprehensive means of expressing the level of smoulder in crops, but were no improvement over using the unamended incidence scores alone. For the less diseased sites at Holbeach Marsh and Surfleet, differences between the three treatments, expressed as severity or number of plots affected, were small (data not shown).

The most obvious effect of different fungicide treatments on daffodil crops is a delay in leaf senescence, partly a result of control of foliar diseases and partly due to a direct effect on the leaves themselves. By the end of the period of regular assessments, the mean percentage of leaf die-back was still very low, averaging 3.0 over all the sample areas, a figure too low to provide meaningful comparisons. However, later observations showed clear differences in die-back between the three treatments at the Kirton and Surfleet sites, with advanced senescence in the control plots and greenest foliage in the model spray area (Plates 2 and 3). At Holbeach Marsh the foliage in all three plots died-back relatively early and at a similar rate.

**Figure 17. Smoulder incidence scores for non-sprayed daffodils and daffodils receiving a fungicide programme as part of the growers' standard treatment or applied according to the predictions of the smoulder infection model. Crops at Holbeach Marsh (top), Kirton (middle) and Surfleet (bottom), 2007. Fungicide application dates and the flower cropping period are also shown.**

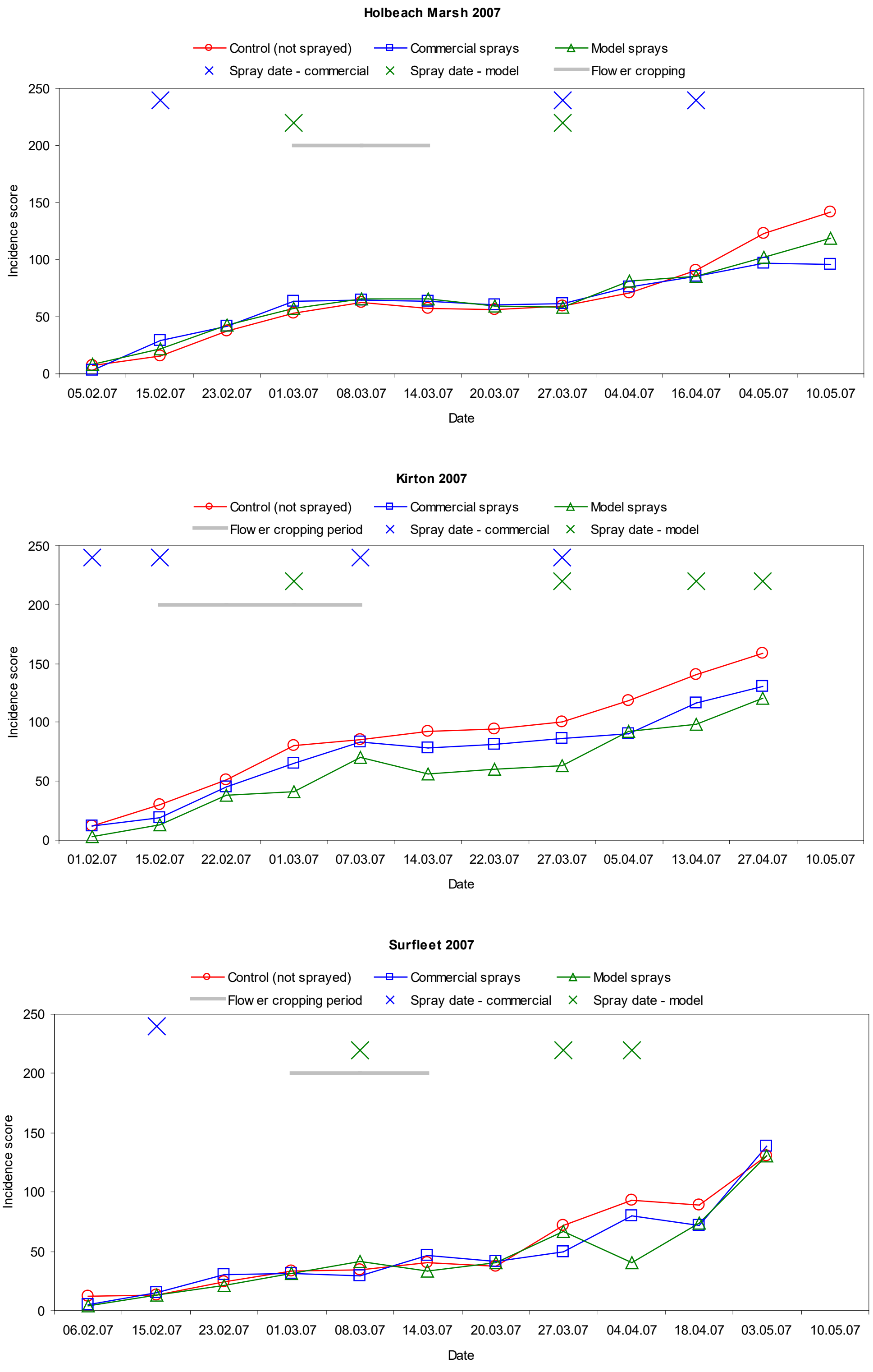


Figure 18. Smoulder in three treatments at Kirton in 2007: (top) severity score, (bottom) number of sample areas affected by smoulder.

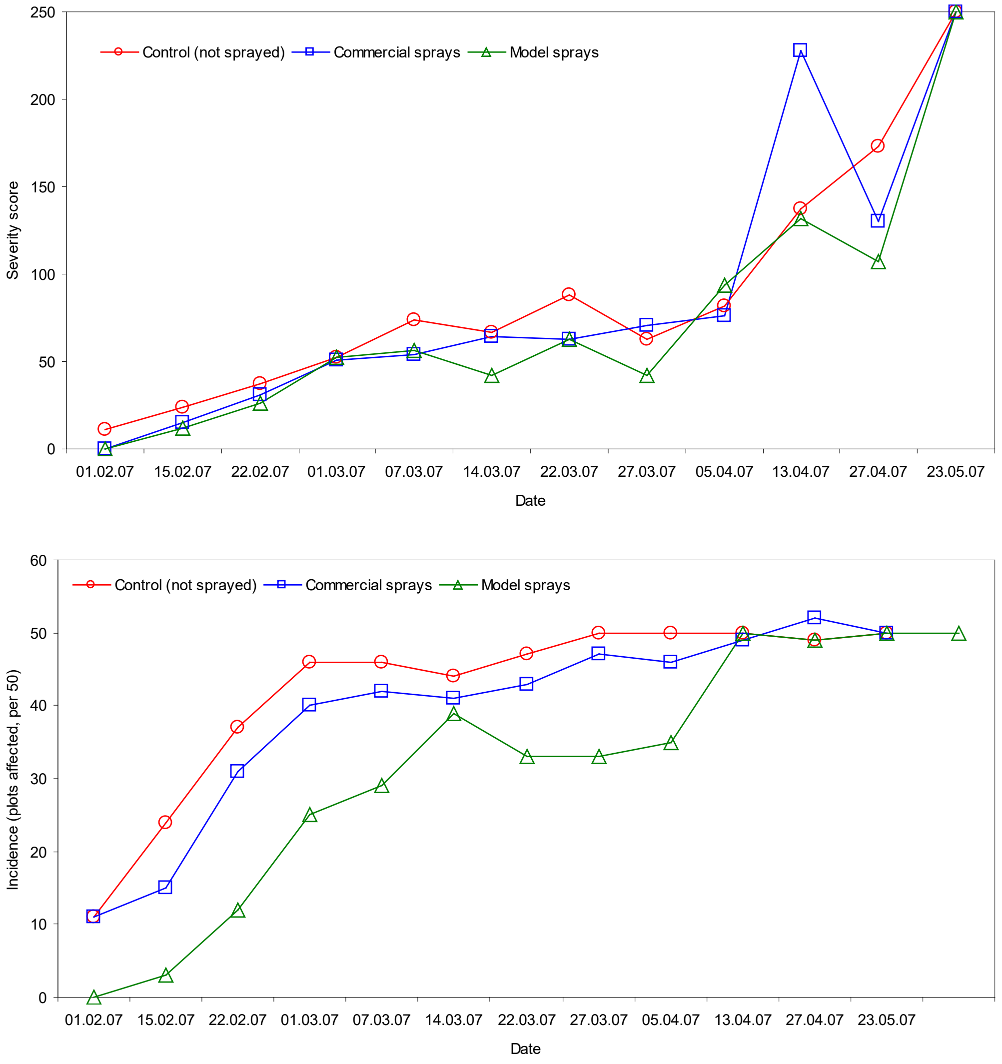


**Plate 2. Effects of fungicide programmes on leaf senescence at Kirton, photographed on 24 May 2007. Top, non-sprayed control plot (brown foliage, 0 sprays); middle, conventional spray programme plot (green foliage, 4 sprays); bottom, model spray programme plot (green foliage, 4 sprays). Details of foliage shown on the right-hand side.**

**Plate 3. Effects of fungicide programmes on leaf senescence at Surfleet, photographed on 24 May 2007. Foreground, conventional spray programme plot (brown foliage, 1 spray); middle, model spray programme plot (green foliage, 3 sprays); distance, non-sprayed controls (brown foliage, 0 sprays).**

Field testing the smoulder spray-timing system, 2008

In 2008 the aim of field testing was again to compare crop and disease development under 'commercial' fungicide spray programmes (regular spray applications 'by the calendar') and the 'grower spray-timing system' (with spray dates derived from the smoulder infection model). No control (non-sprayed) blocks were included in the trials in 2008, since the unrestrained development of fungal foliar diseases was self-evident from the earlier fieldwork, and the omission of 'controls' also circumvented the possibility of disease spreading from control blocks into treated blocks.

There were three trials, with two at Warwick HRI sites (Kirton and Kirton End) and a third at a grower site in Surfleet (for details of crops see Table 3). At all sites one block of crop received the commercial spray programme and the other was sprayed using the grower spray-timing system, the latter being limited to three sprays only. At Kirton there was a third block which was also sprayed using the grower spray-timing system, but with the number of sprays restricted to two instead of three. For details of sprays, see Table 5.

The smoulder infection model was run at weekly intervals for the Kirton/Kirton End sites (which were relatively close together) and for the Surfleet site, and the infection scores produced are shown in Figures 19 and 20.

**Figure 19. Smoulder infection scores for the Kirton/Kirton End sites in 2008. Data expressed as the predicted number of lesions per leaf (above) and as an infection score (below).**

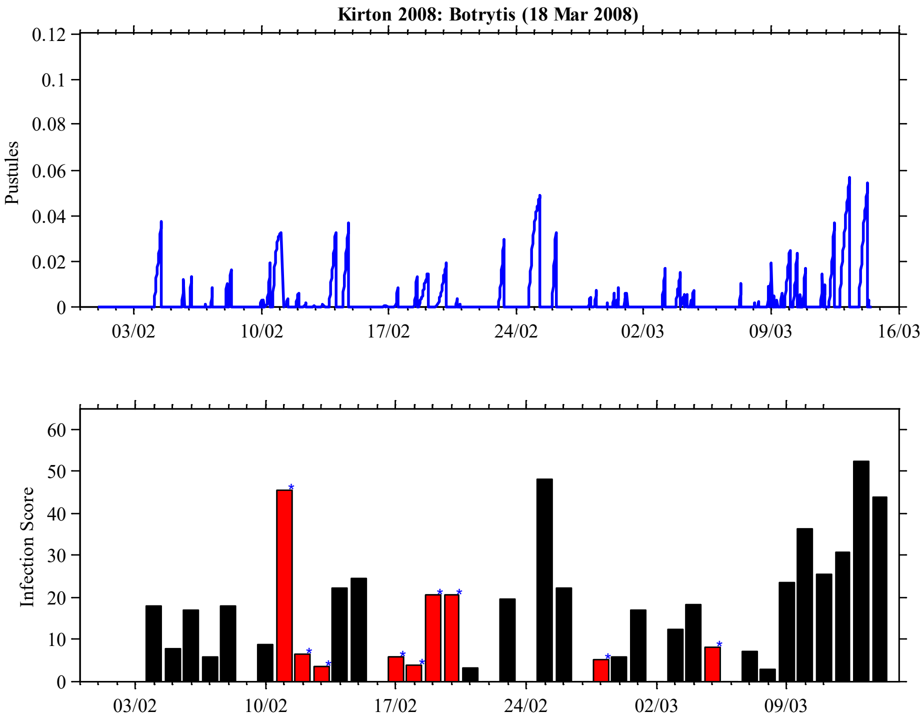
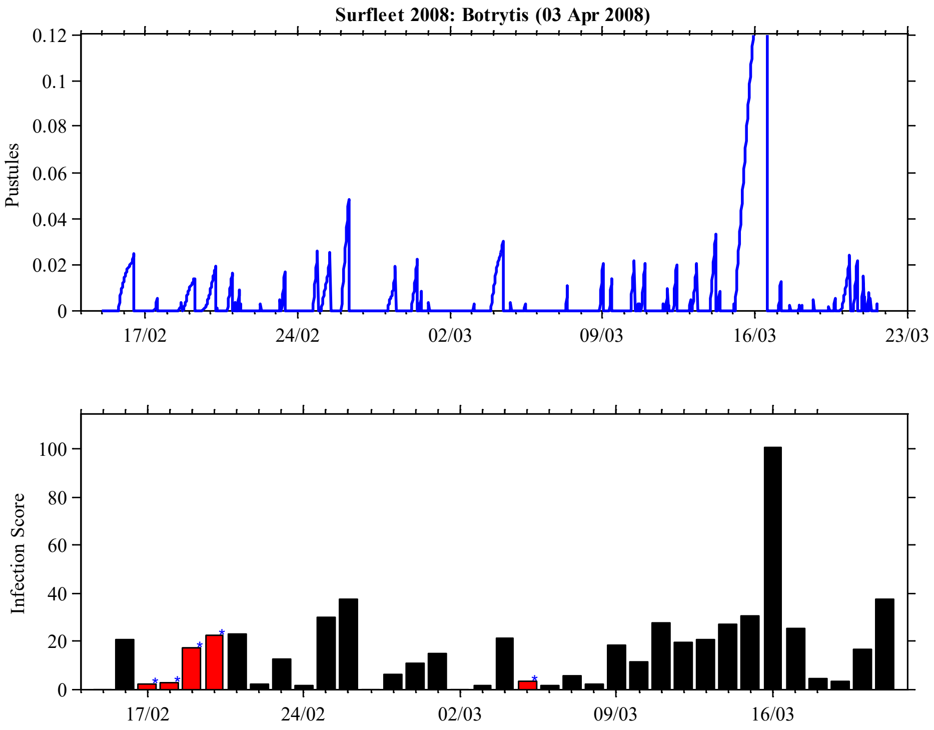


**Figure 20. Smoulder infection scores for the Surfleet site in 2008. Data expressed as the predicted number of lesions per leaf (above) and as an infection score (below).**



*Case 4 – Kirton site*
The areas treated using the grower spray-timing system were sprayed on 4 February (the trigger being a threshold score >50), 14 March (the trigger being a threashold score >30 with accompanying crop damage due to flower cropping) and, for the area receiving three sprays, 27 March 2008 (the trigger being a threshold score >50). The area receiving the commercial spray programme were sprayed four times, on 28 January, 13 February, 27 February and 27 March 2008.

Until early-March 2008 the incidence and severity of smoulder increased at similar rates in all three treatment blocks at Kirton. From March onwards the incidence and severity of smoulder increased faster where the commercial spray programme was being used, with smoulder development slowed where the grower spray-timing system was used (Figure 21). By May, at the end of the growing season, smoulder levels were lowest where the three-spray grower timing system had been used, with the two-spray-timing system giving levels of smoulder intermediate between the three-spray system and the commercial spray programme.

In the May to June period the block that had received the three-spray system showed a slower rate of foliar senescence and lodging than those that had either the commercial or two-spray programmes (Table 9).

*Case 5 – Kirton End site*
The spray dates and criteria for the areas receiving the commercial spray programme and the three-spray grower spray-timing system were the same as for the Kirton site.

Here, the incidence and severity of smoulder increased steadily throughout the growing season. There was a slower increase in disease symptoms where the spray-timing system was used, compared with the commercial spray programme, though by June, against the trend, disease levels increased even under the grower spray-timing system, perhaps indicating a general loss of disease control by the end of the growing season (Figure 21). Using the spray-timing system slowed foliar senescence at the end of the growing season, but did not clearly reduce the rate of lodging (Table 9).

*Case 6 – Surfleet site*
The area treated using the grower spray-timing system was sprayed on 12 February (threshold score >50), 4 March (score >30 with accompanying crop damage due to flower cropping) and 3 April 2008 (score >30 accompanied by late frost). Plots receiving the commercial spray programme were sprayed only once, on 4 March 2008.

From mid-March onwards the incidence of smoulder was less when the spray-timing system was used, with the benefit evident right to the end of the growing season (Figure 21). However, there were no clear differences in smoulder *severity* levels between the two treatments. In May to June the area receiving the spray-timing system showed a slower rate of foliar senescence and lodging than those receiving the commercial spray programme (Table 9).

At all three sites there was clear evidence that using the spray-timing system, with three sprays, gave slower and less disease development, usually accompanied by slower foliar senescence and slower lodging, compared with using the commercial spray programme. For the trial at Kirton, the relatively green foliage of plants in the under the spray-timing system with three sprays, can be seen in Plate 4. Using the spray-timing system with only two sprays appeared to provide inadequate control of smoulder. While fungicide applications all controlled smoulder to some extent, all were far from fully effective in controlling the development of the disease. However, using the spray-timing system (with an adequate number of sprays) achieved better disease management, and at lower cost, than using a conventional spray programme.

**Figure 21. Smoulder incidence for the Kirton (top), Kirton End (middle) and Surfleet (bottom) sites in 2008. Fungicide application dates and the flower cropping period are also shown.**

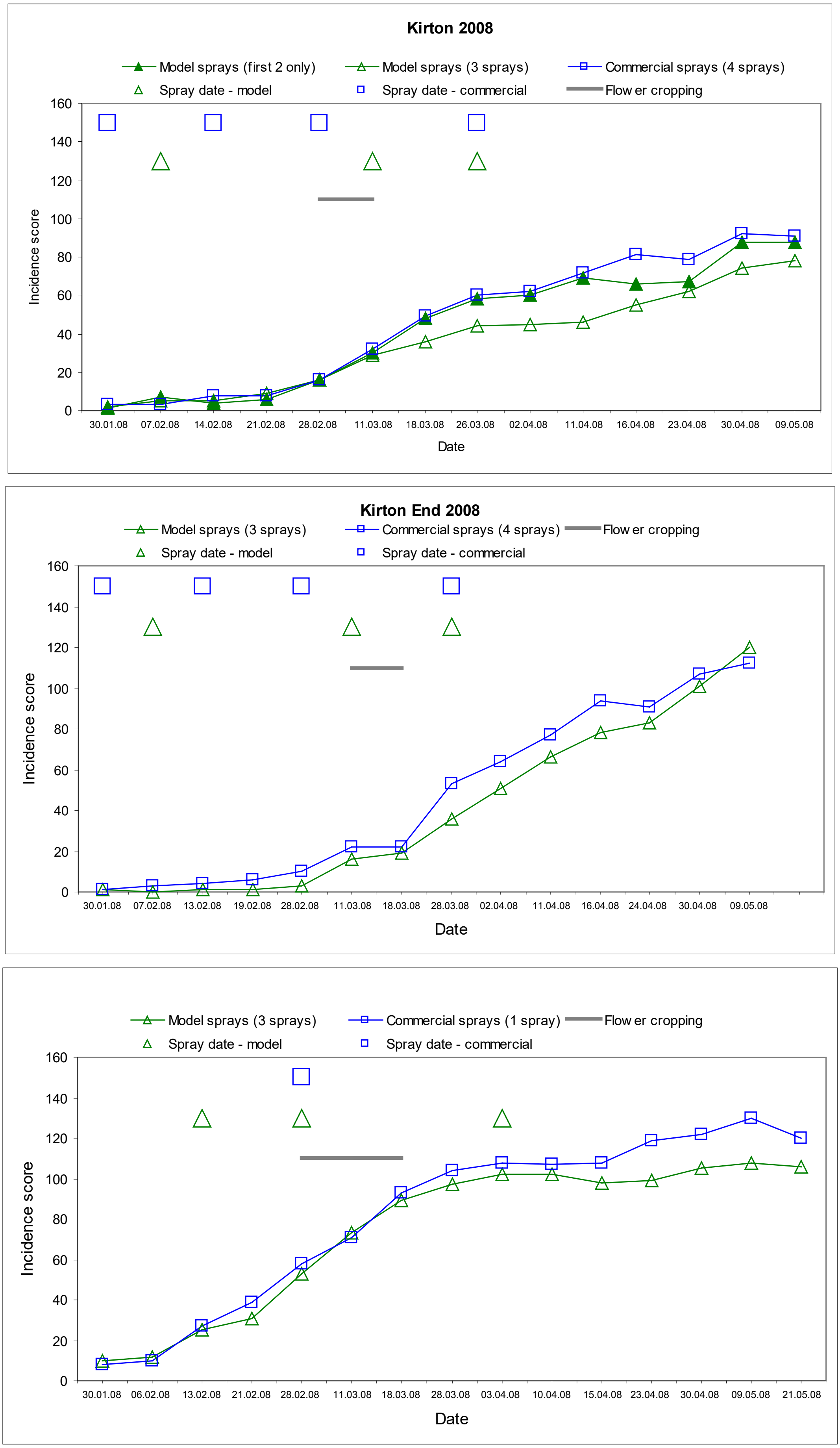


**Plate 4. Foliage in the spray programme trial at Kirton on 10 June 2008 (top photographs) and 19 June (bottom photographs)**

| Model spray programme (2 sprays) | Model spray programme (3 sprays) | Commercial spray programme (4 sprays) |
|---|---|---|
|  |  |  |
|  |  |  |

**Table 9. Percentage foliar senescence and lodging scores for daffodil crops under different fungicide spray programmes[1].**

| Fungicide spray programme | Kirton | | Kirton End | | Surfleet | |
|---|---|---|---|---|---|---|
| | % Senescence | Lodging score[2] | % Senescence | Lodging score | % Senescence | Lodging score |
| Commercial programme (4 sprays) | 4.2 | 2.5 | 6.6 | 2.5 | 21.1 | 2.4 |
| Spray-timing system (3 sprays) | 3.3 | 2.1 | 5.1 | 2.6 | 11.9 | 2.1 |
| Spray-timing system (2 sprays) | 4.2 | 2.5 | n.a.[3] | n.a. | n.a. | n.a. |

[1] figures are the means for 50 sampling points assessed on 4 June 2008

[2] scored from 1 (not lodging) through 2 (partially lodged) to 3 (lodged)

[3] n.a., not applicable

### Associated field studies (2007)

#### *Smoulder primaries*

The extent of smoulder infestation in second-year and older daffodil crops is probably also related to the amount of inoculum already present in bulbs and debris and on or in the ground. This inoculum is unlikely to be controlled by fungicide applications, except any applied early in the growing season. The number of primaries present was recorded in each area used in field trials, so that any large differences in inocula between sites could be accounted for (Table 10). This showed that the number of smoulder primaries were generally low in both years (<1 primary per 0.5m-long sampling area), but was higher than usual at Kirton in 2007, perhaps reflecting the higher disease levels of an experimental site where diseased crops are used in trials.

**Table 10. Numbers of smoulder primaries in daffodil crops used to test fungicide spray programmes in 2007 and 2008[1].**

| Year | Site | Maximum smoulder primaries per sampling area for different treatments | | | |
|---|---|---|---|---|---|
| | | Non-sprayed | Commercial spray programme | Spray-timing system (3 sprays) | Spray-timing system (2 sprays) |
| 2007 | Kirton | 1.1 | 0.6 | 0.4 | n.a.[2] |
| | Holbeach Marsh | 0.3 | 0.3 | 0.4 | n.a. |
| | Surfleet | 0.1 | 0.2 | 0.2 | n.a. |
| 2008 | Kirton | n.a. | 0.2 | 0.1 | 0.2 |
| | Kirton End | n.a. | 0.1 | <0.1 | n.a. |
| | Surfleet | n.a. | 0.4 | 0.3 | n.a. |

[1] figures are the means for 50 sampling points assessed when numbers of primaries were maximal (in the first few weeks following shoot emergence)

[2] n.a., not applicable

*Spore trapping using trap-plants*

The infection of 'trap-plants' was used as a further means of assessing likely infective periods. In the previous two years' experiments, only a small number of exposed trap-plants had developed typical smoulder lesions. This could have been due to the relatively short exposure durations or to low humidity under glass where they were grown after exposure. Therefore in 2007 trap-plants were exposed with the crop for 7 days and were then placed in a high-humidity atmosphere for the first 48 hours in the glasshouse. This resulted in a much greater number of exposed plants developing smoulder lesions (Table 11).

In 2007 the smoulder scores recorded for trap-plants with damaged leaves were markedly higher than for those from non-damaged plants, as had been found in trials in 2005 (though not in 2006), a difference possibly associated with the longer exposure period in the field and the possible occurrence of *Botrytis cinerea* on damaged tissues. Figure 21 shows the smoulder scores for damaged trap-plants in 2007. Smoulder symptoms developed on trap-plants at all three sites, and, as noted before, the peaks of infection occurred at different dates at the different sites.

The timing of these peaks was examined in relation to the occurrence of known infective weather conditions, predicted infective conditions, and the findings from spore traps (see below). At Kirton a high, sharp peak of infection occurred in mid- to late-March, with a broader peak over most of April, corresponding with infective periods determined using the smoulder infection model (Figure 6). At Surfleet too, there was a broad peak over most of April, but with no large, single peak until late-March. At Holbeach Marsh there were infection peaks in mid-March, corresponding to very high infection scores (Figure 1) and in the second half of April.

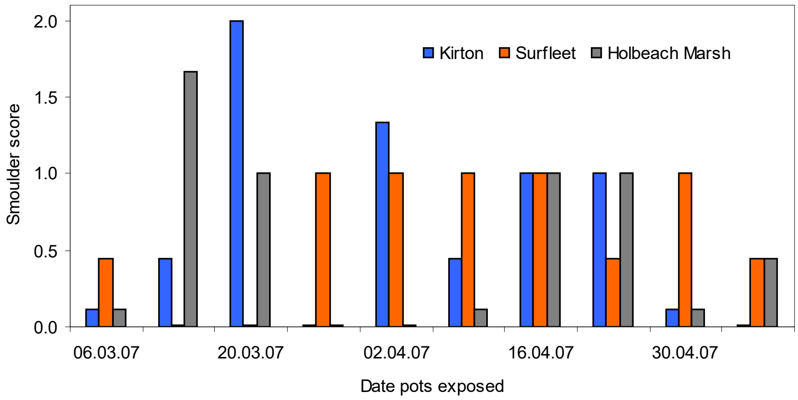
As a result of three years' trials, it appears that using trap-plants may be an inconsistent way of assessing smoulder risk. Additionally, the contamination of the trap-plant by *Botrytis* may not be visible in a way that is easy to distinguish. Contaminated plants may show the result of *Botrytis* infection after relatively long periods. There would certainly appear to be a poor relationship between spore numbers that trap-plants were exposed to and the number of lesions that developed subsequently.

**Table 11. Overall mean smoulder scores for trap-plants exposed at three trial sites in 2007.***

| *Site* | *Smoulder score* | |
|---|---|---|
| | *Damaged leaves* | *Non-damaged leaves* |
| Kirton | 0.64 | 0.09 |
| Surfleet | 0.63 | 0.27 |
| Holbeach Marsh | 0.54 | 0.02 |

* For meaning of smoulder score, see legend of Figure 21.

**Figure 22. Smoulder scores for trap-plants with damaged leaf surfaces at three trial sites in 2007. Exposure periods started at the dates shown. The score is the mean of three replicates of the product of incidence and severity scores, recorded 3 weeks after the end of each exposure period. Only low smoulder scores were recorded on trap-plants with non-wounded leaves (data not shown), and no smoulder symptoms were recorded on non-exposed controls.**



## Discussion

A better approach to smoulder control

Loss of yield and quality due to smoulder seems to occur almost universally in daffodil crops, and the cost of managing the disease chemically – presently the only practical option available to growers - is significant. As a means of reducing the inputs of fungicides to crops, pest and disease prediction is a widely used technique that enables growers to apply pesticides to affected crops when, and only when, those applications are most likely to be effective. Disease prediction was applied to daffodil smoulder in an earlier 'Horticulture LINK' project, and a smoulder infection model, driven by temperature, leaf wetness duration and crop damage, was proposed (BOF 41). An independent financial assessment of project BOF 41 concluded that the benefits to daffodil growers of reduced fungicide usage and targetted fungicide application were very high, and the industry appears already to have responded to the findings of that study by adopting more effective, modern fungicides for use on its daffodil crops, leading to fewer sprays overall and a genuine environmental benefit.

Testing the smoulder infection model

The present project, BOF 59, was designed to test and validate the smoulder infection model, and to develop it to a state in which it could be delivered to growers as a practical, spray-timing system or 'at risk' alert. The main task in the first two years of the project was to compare the observed levels of smoulder in non-fungicide treated, typical daffodil crops, with the levels of smoulder predicted by the model using real-time, local weather data. These studies showed that the predicted onset of widespread smoulder infection in the crops corresponded with the observed increase in incidence and severity of smoulder. Equally important to the development of the spray-timing system, this field-work enable experience to be gained in the practical usage of the system, including the running of meteorological monitoring stations (MMS), data interpretation and interpretation of the 'infection score' produced by the model.

Studies of *Botyrytis* spores

Since outbreaks of smoulder depend not only on having appropriate conditions for the spread and infection of the smoulder pathogen, but also on the presence of infective units, spore-trapping was carried out so that this risk too could be quantified. The use of air samplers, based on sticky tape exposed for fixed durations, showed clearly that the dispersal of spores of *Botrytis narcissicola* is influenced by environmental factors, particularly rainfall. A peak in the number of spores trapped during rainfall may be due to aggregations of spores being carried in rain droplets onto the tapes. The effect of rain on spore dispersal has been reported in previous studies: for example, dry spores of *Botrytis cinerea* were shown to be dispersed on air shock and turbulent currents, and large groups (of about a hundred spores) have been observed on spore-trap slides which are dispersed on droplets of water, small enough to be carried on air currents (Jarvis, 1962). *Botrytis* conidia are released by a hygroscopic mechanism in association with a rapid change in relative humidity, and require air currents or splashed water for dispersal. It has been reported that temperature affects the rate of sporulation, with sporulation reduced at low temperatures (Sosa-Alvarez, 1995). Hence, temperature is also important in monitoring airborne spore concentrations, and in the field there can be considerable variations in air temperature. Spores were not trapped at sub-zero temperatures, and peaks in spore number coincided with relatively high temperatures.

As part of the project a polyclonal antibody was raised against *Botrytis*, enabling spores to be quantified using an immuno-fluorescence (IF) technique. A statistical analysis of the results of spore counts using light- and UV-microscopy gave a correlation coefficient ($R^2$) of 0.822. This suggested that the use of IF for detection of airborne spores has great potential. Many of the spores recognised under the light microscope as *B. narcissicola* fluoresced weakly when observed under the UV microscope. This may be due to loss of spore viability with increasing age, as, although spores were stored at 4ºC after collection, the slides were processed some time later.

As an alternative to using an air sampler, pot-grown daffodils were used as ‘trap-plants’, exposed in the field for specific periods and then monitored for the appearance of smoulder lesions. The results appeared to confirm that factors other than temperature and leaf wetness duration were important in producing *Botrytis* lesions: in most cases smoulder lesions appeared only on leaves that had been mechanically damaged prior to exposure. The extent of the damage required is unclear, though it could be associated with frost or high windspeeds. There was a reasonably close relationship between trap-plant infection and the higher predicted infection scores, particularly in 2006.

The smoulder model in practice

In the second two years of the project the smoulder infection model was applied to further commercial daffodil crops, and comparisons were made between smoulder levels on crops treated conventionally (with fungicides applied at regular intervals) and those on crops treated according to the infection scores produced by the model and the developing spray-timing system (where fungicides are applied only at target dates). The infection model indicated clear peaks of smoulder activity. It was still necessary, however, to understand how the predicted infection scores should be used in practice – what was the score or threashold that indicated a need to apply a fungicide? In 2007, two critical threshold levels were applied, and it was shown that it would have been more appropriate to have used the lower, safer threshold. At the Saracen’s Head site, where (in the absence of heavy rain) a high threshold was being applied, smoulder became rampant later in the season, and, by the time this threshold was exceeded, it was hugely exceeded. Smoulder levels were also relatively high at the Kirton site, perhaps because the site and bulb stocks carried a higher disease inoculum than the commercial sites because of their previous usage in disease-control experiments. At the third site, Surfleet, despite using the higher threshold, disease was reasonably well contained. The results showed that it was necessary to use a low threshold early in the growing season, though later a higher threshold might be used.

In the trials in 2008 a more comprehensive spray-timing system was evaluated. Fungicide applications were triggered *either* when the infection score (threshold) exceeded 50 in any one day, *or* when the infection score exceeded 30 in any one day *and* any of the following applied on the same day or on any day of the previous week: (a) a period with a screen temperature of 1°C or lower (potential frost damage), (b) PI sensors recording two or more ‘hits’ in ‘bin 7’ or higher (potential damage from hail or heavy rain), or (c) flower cropping had taken place (causing general damage and crop trampling). It was further decided that a maximum of three fungicide applications would be given to the plots treated according to the spray-timing system (and in one area the number of applications was reduced to two). At all three trial sites there was clear evidence that using the infection model and spray-timing system, with three sprays, gave slower and less disease development, usually accompanied by slower foliar senescence and slower lodging, compared with using the commercial spray programme. Using the spray-timing system with only two sprays, however, gave inadequate control of smoulder in this instance. It is not clear whether the crucial factor is the number of sprays or when they were applied, and possibly sprays should have been applied beyond March.

It is important to consider some additional factors in assessing the rational need to apply fungicides. The spread of smoulder has been shown to be dependent on crop damage, whereby only damaged leaf surfaces will allow penetration by the fungus. While daffodil leaves suffer marked natural damage over the course of the growing season, for example through chafing caused by the wind, or by the breakdown of the protective cuticle through normal fungal activity, frost, flower picking and heavy rain or hail all cause damage. Several other factors need to be taken into account before deciding to spray a crop, and these considerations may result in slippage beyond the target spray dates. Thus, (a) no sprays should be applied until sufficient crop foliage is present to make spraying worthwhile (e.g. if a significant proportion of the shoots has not reached a height of 5 to 10cm), (b) the minimum interval between applying fungicides is governed by the producer's stated spray intervals, and (c) sprays must be delayed if flower cropping is taking place or is shortly to begin, the appropriate harvest interval being observed. Unfortunately unsuitable weather conditions for spraying may add further delays.

## A smoulder alert system

Despite the practicalities associated with the foregoing riders, the project demonstrated that the smoulder infection model, developed into a spray-timing system or warning, could be a useful tool for growers. Bulb growers with whom the project has been discussed would prefer a regular disease forecast or spray warning, in preference to running models themselves using their own PCs and MMS. One solution would be to set up an HDC Smoulder Bulletin, analogous to the present HDC Pest Bulletin run by Dr Rosemary Collier of Warwick HRI (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 usefulness of this approach.

As a result of a parallel HDC-funded project (BOF 56 and 56a), disease forecasting has also been developed and tested for another disease of daffodils, white mould (caused by *Ramularia vallisumbrosae*). Though white mould is sporadic in its occurrence, once infested crop foliage can die-back within a few days. Formerly considered as confined to the South-West, white mould does occur on daffodils in eastern England and elsewhere (O'Neill *et al*., 2002), and bulb growers should consider both diseases and spray warnings irrespective of their regional location.

A project proposal is being written involving setting up and running a smoulder and white mould spray-warning alert, tentatively called 'DAFFspray', along the lines of the successful HDC Pest Bulletin. DAFFspray 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). MMS would be set-up and run in the key regions, because surface wetness duration and precipitation impact are not available in standard Meteorological Office data-sets that could 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. The DAFFspray web-page would be updated weekly. The spray-warning page could include information on typical smoulder and white mould symptoms and information on current, suitable fungicides.

## Fungicide choice and integrated control for smoulder

In the absence of effective non-chemical means of managing smoulder, fungicide crop sprays are likely to remain the key element in the control of smoulder (and other foliar fungal diseases) for some years. Until recently, it had been relatively easy to list a number of active ingredients and products known to be suitable for this purpose, and Millar (2008) was able to list products containing azoxystrobin, carbendazim, chlorothalonil, kresoxim-methyl, mancozeb, pyrimethanil, tebuconazole and vinclozolin under a number of specific off-lable approvals (SOLAs), provisional approvals and the Long Term Arrangements for Extension of Use (LTAEU). Since then, some SOLAs have expired and some products have been withdrawn (e.g. Ronilan FL containing vinclozolin), and carbendazim products are no longer included under the current LTAEU.

At the time of writing (December 2008) the Pesticides Safety Directorate (PSD) on-line database includes two fungicides from the list above as having on-lable approvals for use in outdoor ornamental plant production generally, kresoxim-methyl (as Stroby WG and other products) and tebuconazole (as Bezel). Relevant current SOLAs listed for outdoor ornamental plant production include Amistar (a.i., azoxystrobin). Both Stroby WG and Amistar have been tested on daffodil foliar disease control, and although the tebuconazole fungicide Folicur has also been tested (Hanks *et al*., 2003), Bezel has not (as far as the authors know). PSD has announced that the LTAEU in respect of non-edible crops and plants will cease once key uses have been converted to SOLAs, a process currently underway. For the present, bulb growers will need to use the LTAEU to extrapolate other on-label approvals to ornamental plant production. Before making decisions about pesticides to use, the current regulatory situation should always be checked, for example by going to http://www.pesticides.gov.uk/home.asp and following the links to PSD Databases.

This emphasis on chemical control does not deny the economic and environmental desirability of developing non-chemical alternatives, and the targetted use of fungicides should ideally be integrated with other elements of Good Practice including the cultural measures listed in Table 12. Such techniques are also important because it is inescapable that even the most effective use of fungicides against smoulder does not eliminate the disease but merely reduces it, so there is still a need to enhance fungicidal and other controls beyond what is possible at the present time. Indeed, the shortage of ‘spray-days’ in the bulb-growing regions of the UK – days without significant windspeeds and rainfall – argues for the development of alternative fungicide delivery systems and (or) non-chemical methods. Using current climate change scenarios, it is likely that the numbers of ‘spray-days’ will become less in the next decades (O’Neill *et al*., 2004).

**Table 12. Non-chemical means of managing smoulder and similar diseases.**

☑ Avoid planting too many multiple-nosed bulbs – which seem to be susceptible to smoulder.

☑ Site first-year crops away from previous plantings – spread from older crops is likely.

☑ Ensure use of adequate rotations.

☑ Rogue (remove and destroy) bulbs with smoulder primaries.

☑ Apply commonsense hygeine – removal and destruction of debris such as old foliage and diseased bulbs. Both smoulder and white mould are carried on leaf debris, but only smoulder is carried in the bulb.

☑ Ensure careful bulb handling to reduce bulb injury and bruising which provide infection sites.

☑ Use prompt bulb drying and correct storage conditions to reduce disease spread.

☑ When giving hot-water treatment use the recommended temperature, duration, timing and additives. Although geared to controlling stem nematode and basal rot, it is presumed it gives some incidental control of other pests and pathogens.

Notes

1. The most effective non-chemical way of managing foliar fungal diseases of daffodils is to grow the crop ‘one-year-down’, thereby avoiding a build-up of infective material and giving an annual opportunity for bulb cleaning, inspection, dipping and hot-water treatment. This is why smoulder is a relatively minor concern of Dutch growers. However, one-year-down growing is uneconomic in the UK.
2. Flower cropping (and de-heading) also increase the incidence of smoulder by providing entry points.
3. There may be some varietal differences in susceptibility to smoulder (e.g. ‘Golden Harvest’ is more susceptible than ‘Dutch Master’), but all varieties are probably susceptible to some extent. Late-flowering varieties (such as ‘Cheerfulness’, Double White’ and ‘Actaea’) are susceptible to white mould, along with some earlier cultivars (‘Dutch Master’, Magnificence’ and ‘Fortune’).

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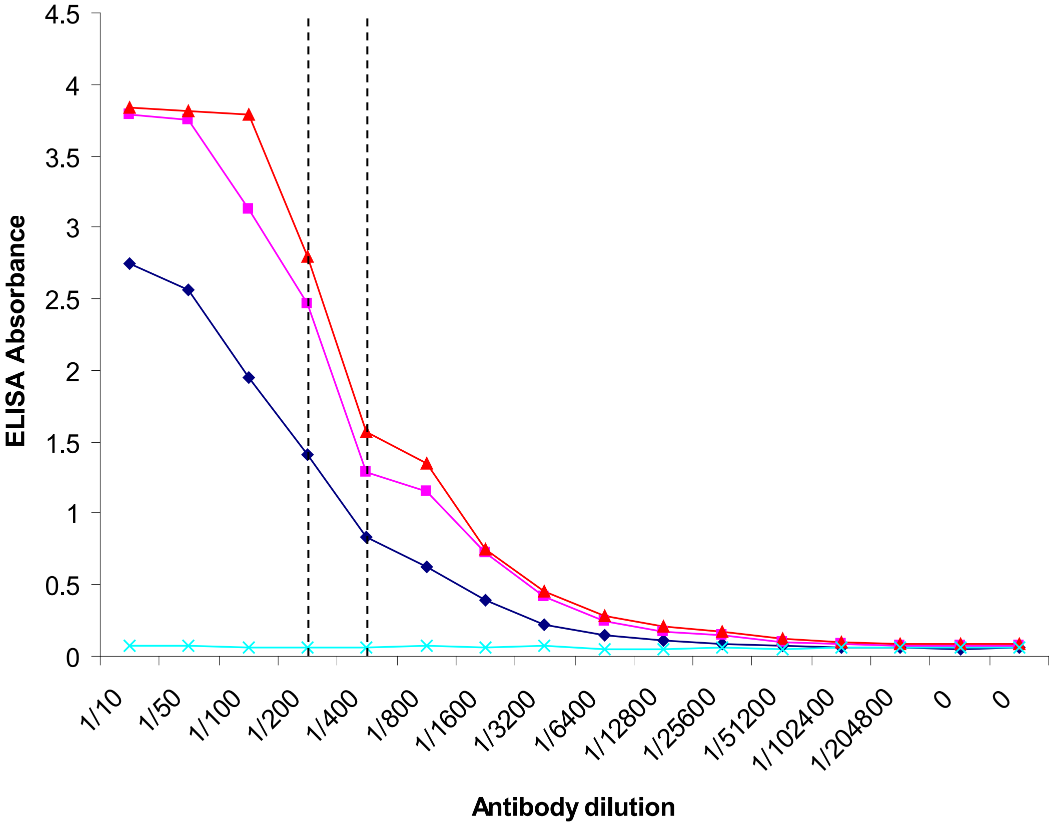
## Appendix 1

Method of determination of the optimal working dilution of polyclonal antibody

**Appendix Figure 1. Optimisation of the working dilution of polyclonal antibody. Absorbance was recorded after (♦) 30, (■) 40 and (▲) 60 minutes; (x) indicates the PBS control (see text).**



A polyclonal antibody (PAb) (coded Warwick HRI 94/4/3) that recognised conidia of *B. cinerea* and *B. narcissicola*, it was titrated against *B. narcissicola* conidia in an indirect plate-trapped antigen ELISA (PTA-ELISA). Fourteen 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 a spore suspension of *B. narcissicola* in phosphate-buffered saline (PBS). As a control, 14 paired wells received 100µl per well of PBS alone. Following overnight incubation at room temperature (RT), unbound antigen was removed by inverting the individual microtitre plates and tapping them dry onto absorbent towelling. The wells were washed with PBS (100µl per well) for 1min. Wells were blocked with 200µl 1% casein (1% casein in PBS, w/v) and incubated in a Wellwarm shaker incubator (Denley Instruments Ltd, Sussex, UK) at 30°C for 30min. Residual blocking buffer was removed and wells were washed once for 1min with 200µl per well of PBS, 0.05% Tween 20 and 0.1% casein (PBSTC). The polyclonal antibody was diluted 1:10 in PBSTC and 1:50 and subsequent doubling dilutions made to 1:102400. The respective serum dilutions were applied to paired wells at 100µl per well and incubated in the shaker incubator at 30°C for 45min. Unbound material was removed and wells washed three times for 1min each with PBSTC. Aliquots of 100µl goat anti-rabbit IgG (whole molecule) alkaline phosphatase (Sigma A-3687) diluted in PBSTC (5µl in 30ml PBSTC) were added to each well and incubated as above. After three washes, 100µl per well of 1mg $ml^{-1}$ *p*-nitrophenyl phosphate (*p*NPP) (Sigma N-2770), freshly dissolved in deionised water, was added. The plates were incubated at RT in darkness for 40min and absorbance values were read at filter wavelengths of 405 and 630nm in a Biohit BP 800 ELISA plate reader (Alpha Laboratories, Eastleigh, Hampshire, UK). Mean values were calculated for each of the paired wells.

Determination of the optimal working dilution of the PAb by PTA-ELISA was carried out and the absorbance values were taken at three time intervals, 30, 40 and 60min after colour development with the substrate (Appendix Figure 1). At a dilution of 1:400 a very sharp response was obtained (indicated by the broken lines in Appendix Figure 1), with a significant difference in absorbance values for all three curves. Therefore, 1:400 was used as the antibody dilution for all further PTA-ELISA carried out using this antibody throughout this study.