ANNUAL REPORT

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HDC Project BOF 59
Annual Report (2006)

Narcissus Smoulder Decision Support System

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Grower Summary

BOF 59

Narcissus Smoulder Decision Support System

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Signed on behalf of: Warwick HRI

Signature:…… ***Date***: …….
Name: Professor Simon Bright
Director and Head of Department

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

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

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Headline

The development of a predictive model-based fungicide spray programme against narcissus (daffodil) foliar fungal diseases offers growers the opportunity of achieving better disease management with fewer sprays, reduced costs, and a positive environmental effect. Two years into this four-year project to validate and deliver such a system for controlling smoulder, the results indicate that the smoulder infection model has potential to forecast disease outbreaks accurately.

Background and expected deliverables

The control of pests and diseases is a major factor in meeting the exacting specifications for narcissus required by the export and multiple-retail sectors. There appears to have been an increase in the incidence of the fungal foliar disease smoulder (caused by *Botrytis narcissicola*) in UK crops over the past 10 years or so. It is estimated that the disease regularly exacts losses of yield of the order of 10%. Bulb growers control fungal diseases such as smoulder by applying regular fungicide sprays from emergence until after flowering. In an earlier project, funded through the 'Horticulture LINK' programme, the HDC and ten companies, a smoulder infection model, driven by temperature and leaf wetness duration, was formulated. This indicated the key dates at which fungicides sprays should be targetted for maximal control. Trials indicated that the number of sprays needed could be halved through eliminating those applied on inappropriate dates.

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

Summary of the project and main conclusions

The incidence and severity of smoulder was tracked in non-sprayed second-year narcissus crops growing at Kirton and on commercial farms in Lincolnshire in 2004-2005 and 2005-2006. Site weather was logged and spore trapping was carried out through the use of both mechanical spore traps and by exposing 'trap plants'. The previously developed weather-based predictive infection model was run using the collected weather data, and the predicted results were compared with the observed results from the monitored crops. The information was also related to the findings from spore trapping.

Using the infection model to predict outbreaks of smoulder showed potential for use in forecasting the disease. There was a reasonably close relationship between trap plant infection and the occurrence of higher infection scores. The results confirmed that other factors, probably crop damage, were also important for infection to take place, though the nature of the damage (perhaps related to frost or high windspeeds) needs to be defined. This work is on-going, and in the next two years the practical consequences of applying fungicide sprays according to the prediction model will be evaluated on commercial farms, including a comparison between a conventional (regular) fungicide spray programme. In year 4 it is hoped that a spray timing system will be sufficiently tested to be made available to HDC levy-payers.

Narcissus smoulder decision support system

Introduction

Narcissus (daffodil) is a spring-flowering bulb and flower crop grown in eastern and south-west England, Scotland, the Netherlands and north-western USA. Currently the world area of commercial, field-grown narcissus is over 7000ha, with about 4000, 400, 1800 and 400ha in these three countries, respectively (Hanks, 2002). The UK is the world leader in the production of narcissus bulbs and cut flowers, with annual exports valued at about £20million (O'Neill *et al*., 2004). Narcissus exports from the UK greatly exceed that of any other flower.

In the UK the cultivars mainly grown are those trumpet and large-cup yellow varieties exemplified by 'Golden Harvest' and 'Carlton', while Dutch growers produce a diversity of types but predominantly the dwarf cultivar 'Tête-à-Tête' (Hanks, 2002). Traditionally, growers specialised in either bulb or flower production, but many crops are now regarded as dual-purpose. Large numbers of bulbs are also 'forced' in glasshouses to produce cut-flowers over an extended season.

The control of pests and diseases is of major concern to bulb growers, since the production of healthy bulbs is vital in meeting the exacting specifications of the export trade and multiple-retail sector. Fungal diseases are of particular concern, and it is expected that they should be controlled with only the minimal use of fungicides, with this usage justified on a case-by-case basis. In the past decade, there appears to have been an increase in the incidence of fungal foliar diseases, particularly the widespread 'smoulder' caused by *Botrytis narcissicola*. Smoulder occurs wherever daffodils are grown. It is estimated by major UK growers that the disease regularly exacts an on-going loss of bulb and flower yield of the order of 10%, representing overall losses of some £2.5m *per annum* at farm-gate prices, and flower quality may also be reduced (O'Neill and Mansfield, 1982; O'Neill *et al*., 2004).

The UK narcissus crop is planted and left in the ground for at least two years, with symptoms of smoulder becoming increasingly common after the first year of the crop. The pathogen survives the host's dormant period as mycelium in the bulb neck and sclerotia on bulbs and leaf debris and in the soil (O'Neill *et al*., 2004). Young shoots, termed 'primaries', may emerge obviously infected with smoulder, having their leaf tips withered, distorted, blackened, adhering and bearing a profuse gray mass of sporulating tissue (O'Neill *et al*., 1982). The spores are dispersed by wind or water-splash, infecting other leaves that subsequently develop dark brown lesions bounded by yellowing areas. The lesions often occur on one side of the leaf, the resultant uneven growth resulting in characteristic sickle-shaped leaves. Damaged leaves are susceptible to infection by *B. narcissicola* (O'Neill and Mansfield, 1982). After flower picking, the fungus may cause a stalk-end rot, and the spread of lesions on leaves leads to rapid die-back. When pulled up, such withered leaves often carry sclerotia or a grey mass of spores at the base. Small (1 - 2mm diameter) oval or circular black sclerotia can also be found on leaf debris. Smoulder can also cause flower spotting. *B. narcissicola* is also found in association with neck rot disease of narcissus (Carder, 1999). *Botrytis* spp. produce abundant gray mycelia and long, branched conidiophores bearing clusters of gray conidia.

Materials and methods

Sites for crop monitoring

In each of autumn 2004 and 2005, two second-year Lincolnshire daffodil crops were selected for monitoring (see Table 1). The crops used were considered typical commercial crops for the region. In each crop an area *ca.* 0.2ha in extent was clearly marked with corner posts and other markers, and it was arranged with the owners that no fungicide sprays would be applied during this year of the crops in these designated areas. In all other respects, it was agreed that each crop would be farmed according to its grower's normal commercial practices. The central 0.1ha of each designated area was demarcated for monitoring and observation, leaving the surrounding area as a buffer zone for protection against spray drift from adjacent crops.

Table 1. Smoulder monitoring sites, 2004 to 2006.

Year monitored	*Owner's name and address*	*Site Name**	*Grid reference*	*Cultivar and number of years in ground*
2004-2005	Warwick HRI The Kirton Research Centre (KRC) University of Warwick Kirton Boston Lincolnshire PE20 1NN	Kirton (Jessops 3)	TF300395	'Carlton' 2
2004-2005	O A Taylor & Sons Ltd Weston Spalding Lincolnshire PE12 7PP	Saracen's Head (Whaplode Marsh)	TF337280	'Fortune' 2
2005-2006	Warwick HRI (as above)	Kirton (Asplands 1)	TF300394	'Golden Harvest' 2
2005-2006	Winchester Growers Ltd Herdgate Lane Pinchbeck Spalding Lincolnshire PE11 3UP	Surfleet (Gosberton Marsh)	TF259293	'Fortune' 2

* The Saracen's Head site is *ca*. 14km SE of Kirton, and the Surfleet site *ca*. 11km SW.

Weather data

A meteorological data logger ('Smaartlog'; Intelligent Micro Design Ltd., or equivalent) was set up close to the centre of each monitoring area prior to crop emergence. The loggers, powered by battery and solar panel and downloadable *via* a modem and digital cell telephone, were provided with sensors recording soil and air temperature, relative humidity, surface wetness and rainfall at 30-minute intervals.

Trap plant production

In August 2004 and 2005 daffodil bulbs (grade 12-14cm cv 'Carlton') were allocated for the production of trap plants from a stock grown at KRC. To achieve comparability with the second-year daffodil crops being monitored, these bulbs were not given the usual hot-water treatment (HWT) before planting, nor did they receive any fungicide applications after lifting in June/July.

Each year the bulbs were stored at 17°C until early-October, when they were planted in a standard fashion, five bulbs per 20cm-diameter, 4L-capacity plant-pot, using a blended growing medium of peat, sand and proprietary John Innes compost. After planting the pots were placed outdoors at KRC; the pots were covered with fleece for protection from extreme weather, and kept watered as required.

Disease symptoms

In the early stages of shoot emergence in winter/spring, infected shoots emerged as 'primaries', heavily infested shoots with the leaf tips withered, distorted, blackening, 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 appeared 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. In some cases the lesions spread rapidly late in the growing season. 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 often carried 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 in these trials. In some cases, laboratory investigations showed that the spores were of grey mould, *B. cinerea,* not *B. narcissicola*, but it is not practical to distinguish the two in the field.

Crop and disease monitoring

The allocated areas of crops 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, disease levels were assessed weekly for the incidence and severity of the disease. The central, 0.1ha area of each was walked in a standard fashion in an X-pattern, starting from a marked corner, and on crossing ridges a 0.5m-long sub-sample was delimited with a ruler at the intercept to give 50, 0.5m-long sub-samples for assessment. The incidence and severity of smoulder were scored in each of the sub-samples according to the scale shown in Table 2; overall incidence and severity scores were then calculated by summing the scores for all 50 sub-samples. The crop growth stage and (later in the season) the percentage of foliage that was senescent or dead were also noted.

Table 2. 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 or occasionally >1 lesion per leaf
3	>10 leaves but <50% leaves affected	3	Generally 2 or more lesions per leaf
4	>50% but <100% leaves affected	4	Lesions coalescing to form larger damaged areas
5	All leaves affected	5	Extensive leaf die-back

Spore trapping - use of trap plants

Starting after shoot emergence, pot-grown trap plants were placed adjacent to crop foliage near the centre of each crop for defined exposure periods. In 2005 exposure periods were 24h each, and pots were put out on Monday through Thursday and collected Tuesday through Friday; in 2006 *ca*. 4-day exposure periods were used on a continuous basis. For each exposure period, six plant-pots were used. 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 from the field sites, exposed trap-plants 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 control pots, not exposed in the field, were moved straight to the glasshouse (three pots per week). The three replicate pot-plants in each set were arranged in the glasshouse in three blocks, and all pots were spaced from one another to reduce the liklihood of cross-infection. The pot-plants were kept well watered during this time, using bottom-watering into saucers to avoid spreading infection. Plants were examined for disease lesions at at least weekly intervals, and once symptoms were present the number of leaves with lesions and incidence and severity scores (Table 2) were recorded at 2-weekly intervals over a period of 14 weeks

Spore trapping – use of spore trap

A Burkard seven-day recording volumetric spore trap with air sampling speed of 10L min^{-1} was used for the field experiments (Figure 1). This is a compact unit with built-in pump designed to sample airborne particles continuously over a seven-day period. Particles are impacted on adhesive silicon-coated, transparent Melinex plastic tape supported on a clockwork-driven drum, the Melinex tape being secured around the drum using double-sided adhesive tape.

Figure 1. Burkard seven-day volumetric spore trap.

Spore trapping was carried out from 31 January to 28 March 2005. The recording tape of 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, and mounted on glass slides with double-sided adhesive tape. Each tape was marked at 2mm intervals with a razor blade to indicate one-hour periods for examination.

Use of polyclonal antibody (PAb)

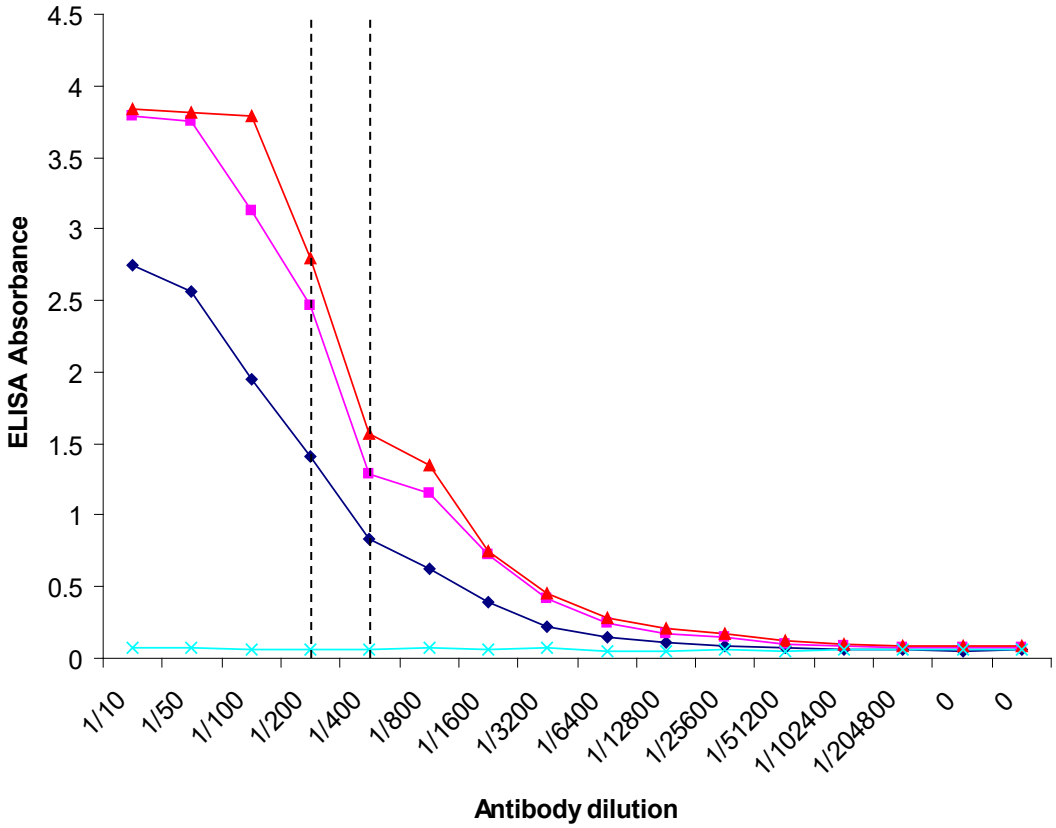
Determination of optimal working dilution of PAb
A PAb which recognised conidia of *B. cinerea* and *B. narcissicicola* (coded Warwick HRI 94/4/3) was titrated against *B. narcissicicola* conidia in an indirect plate-trapped antigen ELISA (PTA-ELISA). Fourteen paired wells of a 96-well Nunc Immunosorbent Polysorp flat-bottomed microtitre plate (catalogue number 475094A; Life Technologies, Paisley, UK) were coated with 100µl per well of a spore suspension of *B. narcissicicola* 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 (pNPP) (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.

Results
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. At a dilution of 1:400 a very sharp response was obtained (as indicated by the broken lines in Figure 2), 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.

Detection of spores of *B. narcissicola* on spore tapes using immunofluorescence (IF)

Tapes from the seven-day volumetric sampler were examined for spores of *B. narcissicola* by bright-field microscopy using a Zeiss binocular microscope (x400). Hourly spore counts were determined by sectioning the exposed portion on the slide and tape into 24, each representing 1h periods. The slides were processed for IF by adding PAb 94/4/3 (1:200 dilution in PBSTC) 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) (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.

Figure 2. Optimisation of working dilution of PAb Warwick HRI 94/4/3.
Absorbance taken after (♦) 30, (■) 40 and (▲) 60min; (x) indicates the PBS control.



Forecasting infection

A smoulder infection model that relates the number of disease lesions to temperature and periods of leaf wetness was developed in a previous 'Horticulture LINK' project. This showed that the critical weather conditions favouring smoulder infection were temperatures between 10 and 15ºC combined with leaf wetness durations of 12 to 24h. The infection model can therefore be run with the weather data obtained from the loggers in the field crops, enabling a comparison to be made between the predicted (modelled) and actual (observed) levels of smoulder symptoms; correspondance of predicted and observed levels would validate the accuracy of the model, while dissimilar results would indicate that the model is inappropriate or needs to be refined. For presentation in Results, the infection score was averaged for 24h periods starting at 00:00 hours.

Results

Year 1 (2004-2005)

Meteorological data

Air temperature and leaf wetness data for the area are shown in Figure 3. Previous work showed that the range of infective temperatures for *B. narcissicola* is 4 - 16°C, with an optimum at 12°C. In 2005 there was a cold period from mid-February to mid-March, during which temperatures were unlikely to result in infection of narcissus by the smoulder pathogen. For most of the rest of the growing season temperatures were between 4 and 12°C, within the infective range, though the optimum infective temperature (12°C) was reached or exceeded only periodically, and only for brief periods, in early -February, late-March and early- and late-May. In contrast, and with the exception of a few scattered days, periods of long leaf wetness duration occurred throughout the growing season. Rainfall (data not shown) occurred throughout the growing season and there were larger rainfall events towards the ends of February and May. In-crop relative humidity (data not shown) was consistently high in the first half of the growing season, but fell below 70% for significant periods later. It is likely that in this situation infection was most likely in late-March, driven by the warmer temperatures at this time.

Crop and disease monitoring

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 4). 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 base. However, the incidence and severity scores at both Kirton and Saracen's Head were low (<50) until early-March, and then increased steadily at both sites from the second half of March.

Figure 3. Weather at the Saracen's Head site in the 2005 growing season.

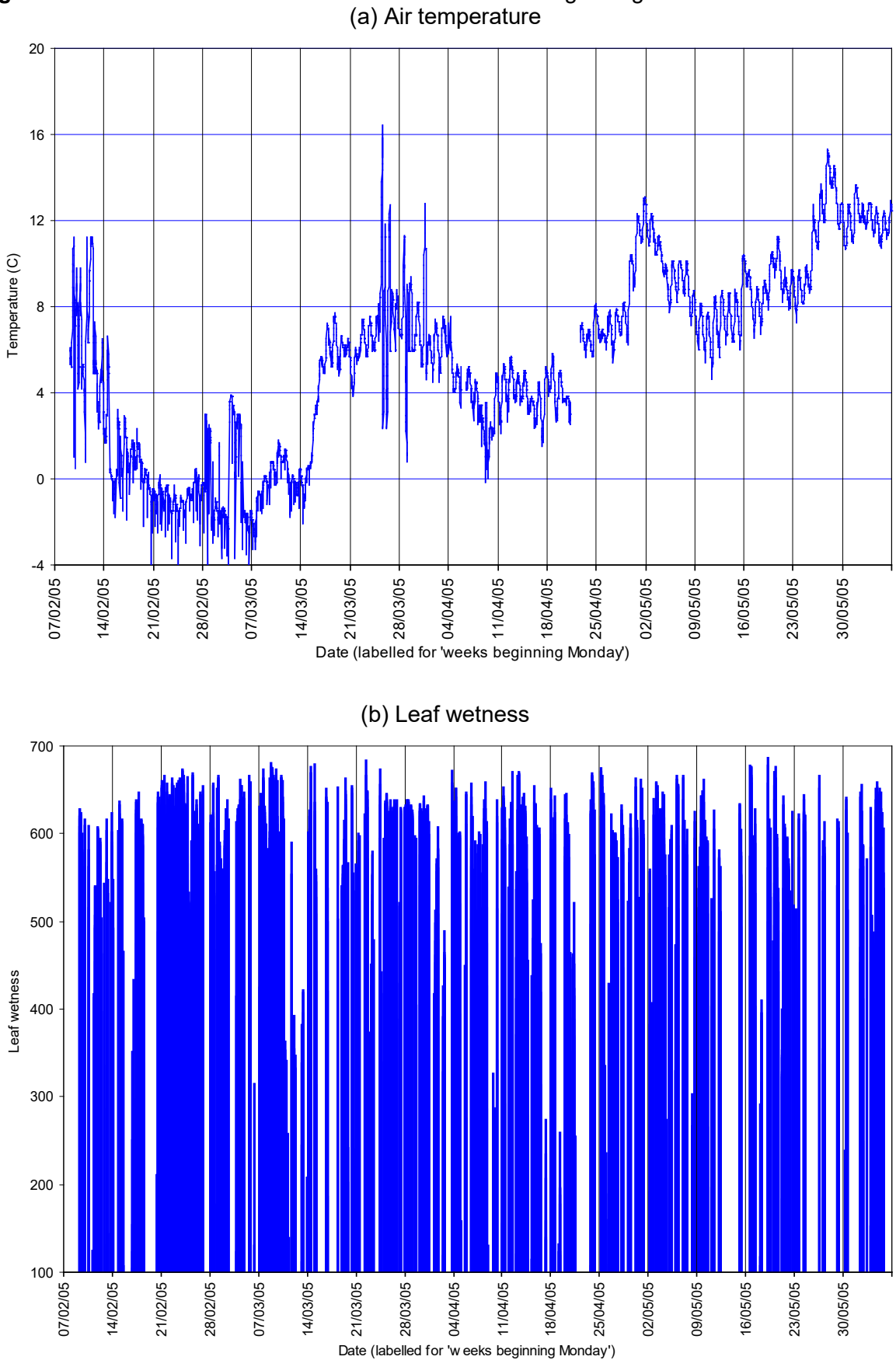
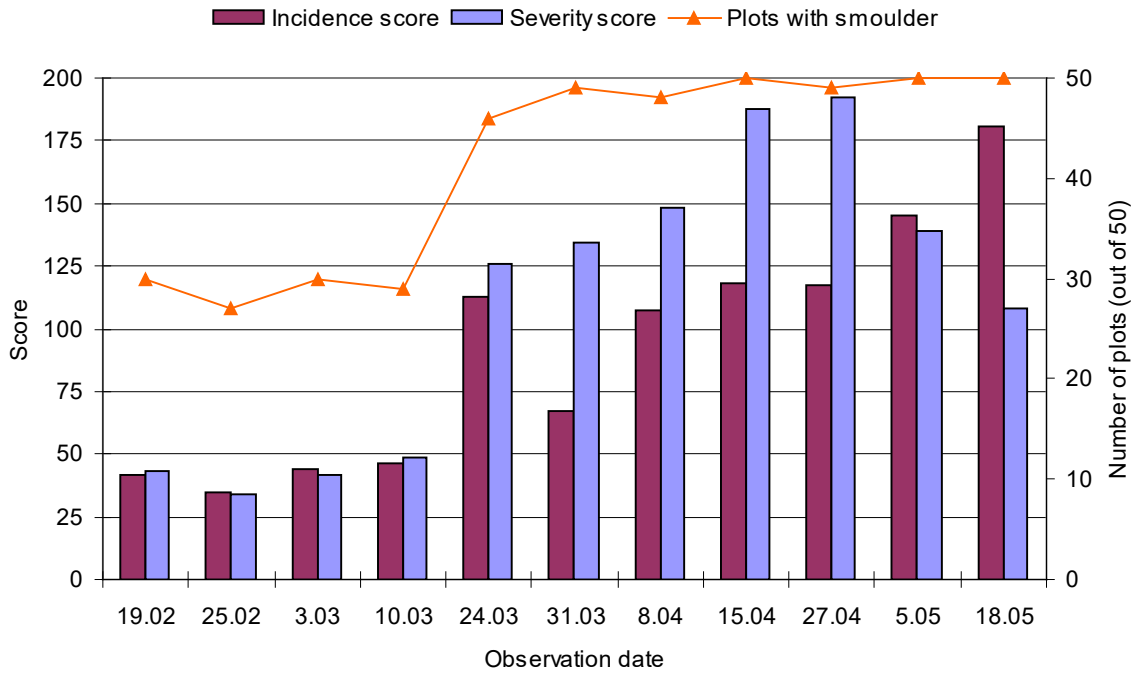
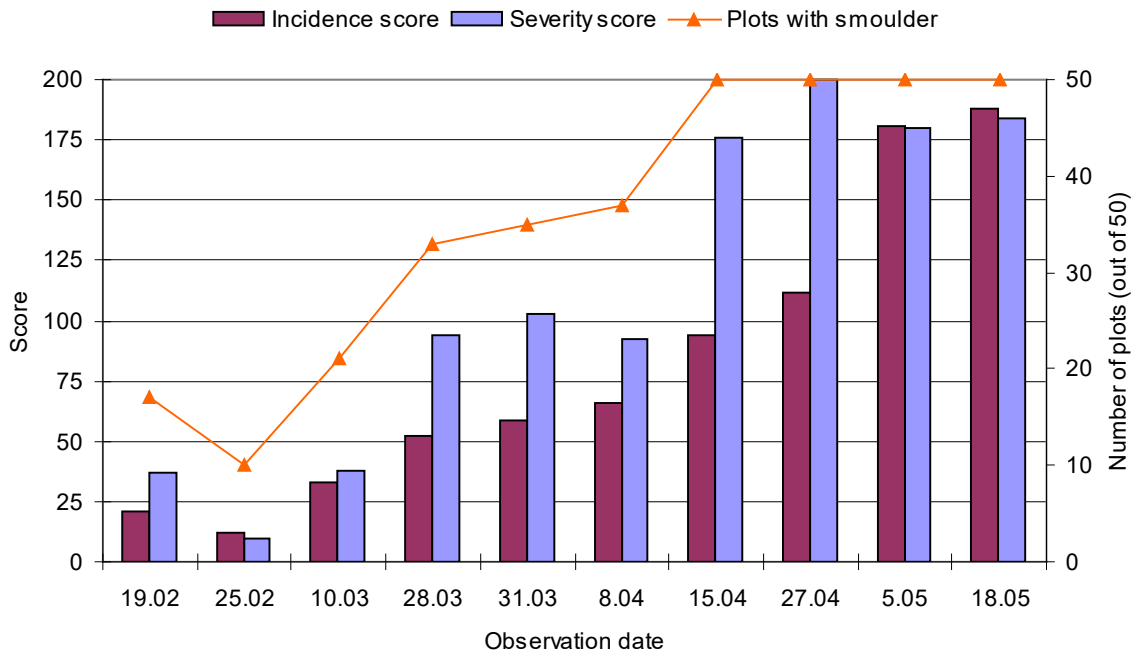


Figure 4. Smoulder monitoring at Kirton and Saracen's Head in 2005: number of plots showing smoulder symptoms and incidence and severity scores. (a) Kirton



(b) Saracen's Head



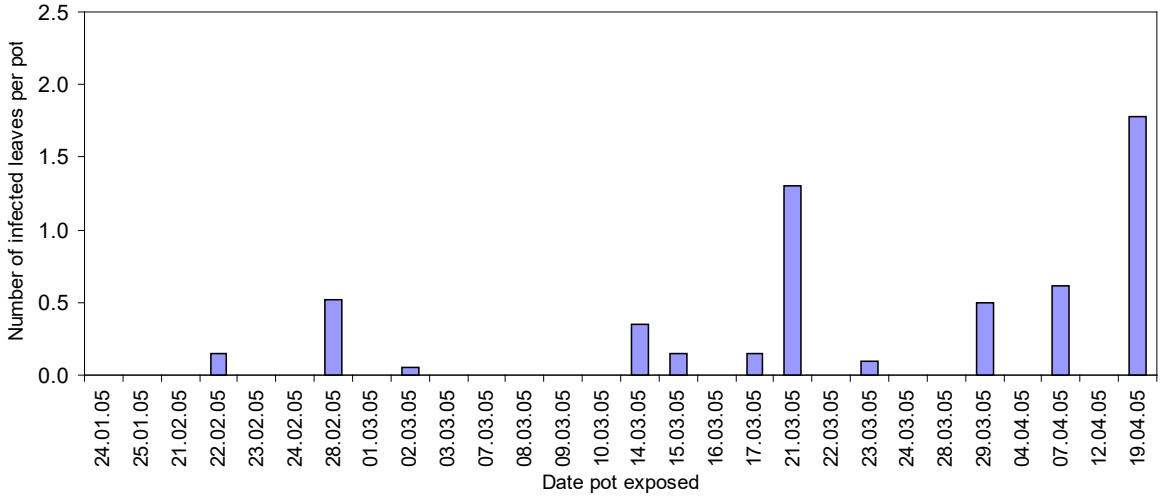
Trap plants

Plants in only eleven out of 168 pots exposed at Kirton developed smoulder lesions, all in pots in which the foliage had been damaged (Figure 5a). 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.

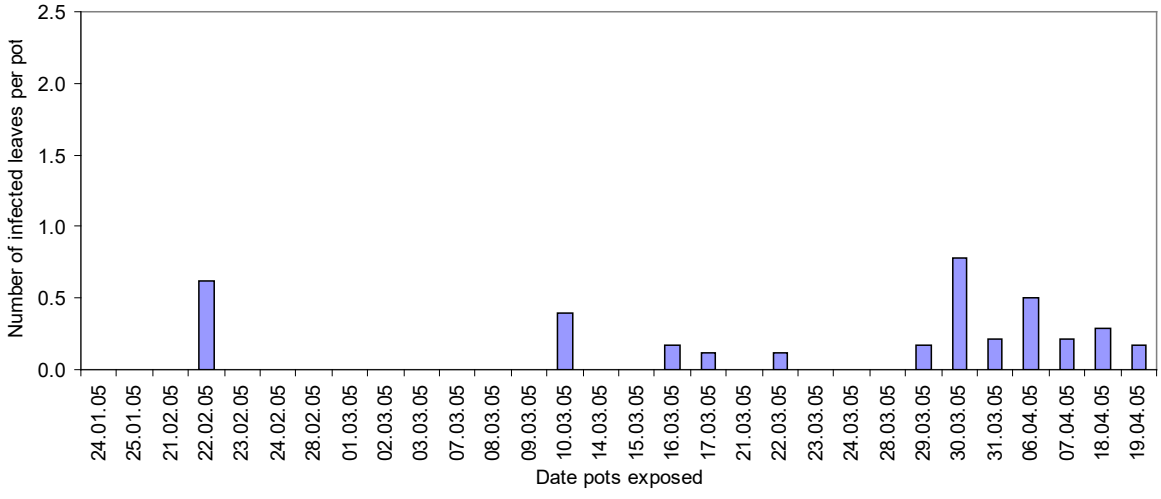
Only twelve out of the 180 pots exposed at Saracen's Head were found to develop smoulder lesions, mostly on plants exposed on 5 May 2005 and all on plants that had had their leaves damaged (Figure 5b). The average number of leaves per pot affected was lower than at Kirton, but as at Kirton there appeared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

Figure 5. The incidence of smoulder lesions on narcissus trap plants with wounded leaves at Kirton and Saracen's Head in 2005. No smoulder lesions were found on the set with non-wounded leaves or on unexposed controls. The values are the average for all five assessment dates, determined from three replicate plant-pots for each exposure period.

(a) Kirton



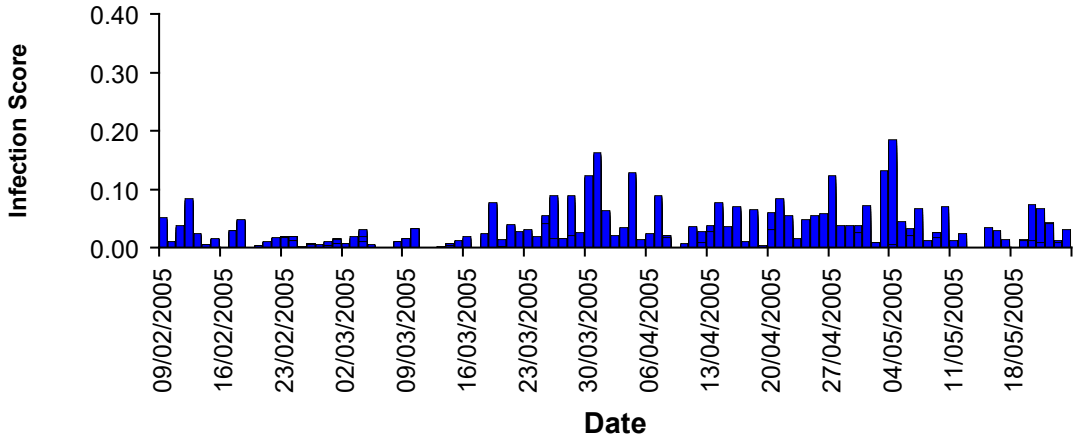
(b) Saracen's Head



Forecasting infection

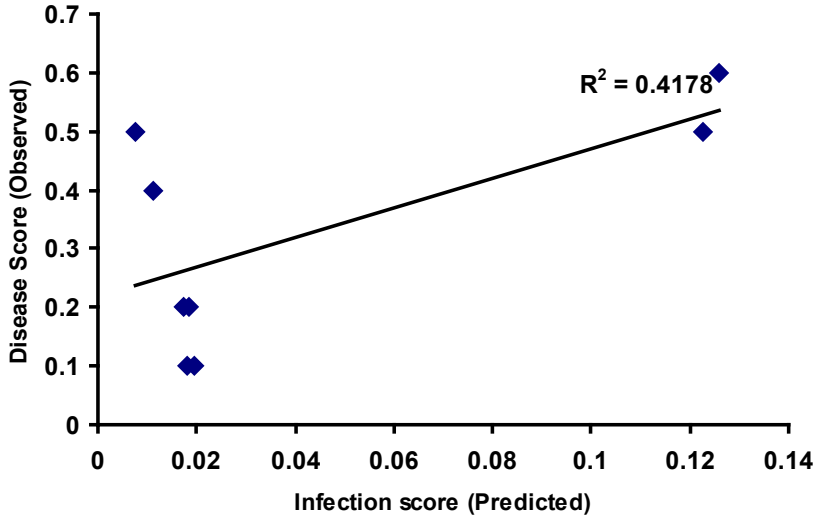
The smoulder infection model was run with the temperature and leaf wetness data for each site, and the predicted infection scores are presented in Figure 6. During 2005 the number of predicted infection periods with scores above 0.2 was low. However, several infection periods with scores above 0.1 were predicted. Predicted infection scores of over 0.1 occurred on 30 and 31 March and 4 and 27 April 2005. Trap-plants were placed within an infected plot on one day and removed to the glasshouse on the following day. For this reason predicted infection on (day +1) was compared to observed infection on each day.

Figure 6. Predicted smoulder infection score in 2005 at Saracen's Head.



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 at Saracen's Head in 2005.



Trap plant and spore trap data

The number of lesions observed on narcissus 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 recording volumetric spore trap was averaged for the same days the plants were exposed (Table 3). On 22 February 2005 the mean number of leaves affected was highest (0.6), however the average spore count was 4.8 spores/m^3/h. The highest average spore count of 125 spores/m^3/h was observed on 22 March 2005, with 0.1 leaves affected.

Table 3. 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

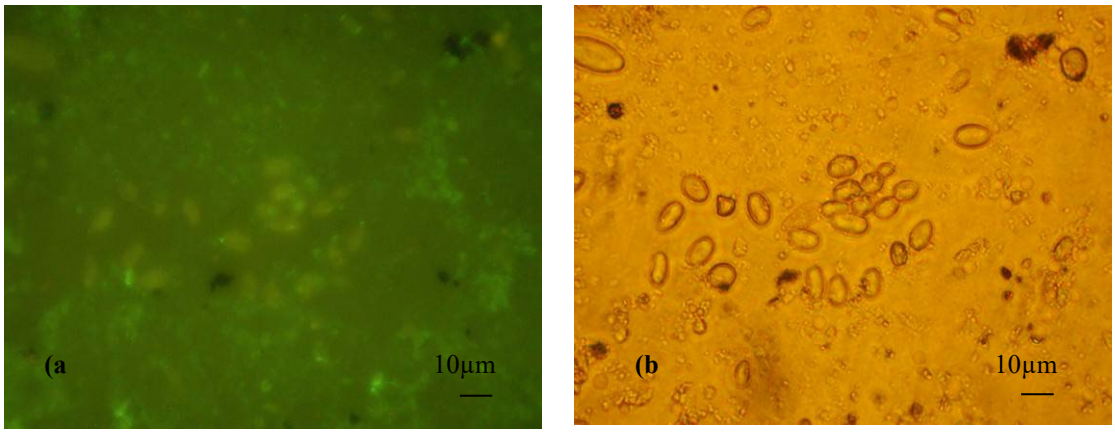
The tapes from 7-day volumetric sampler were processed for IF, and fluorescing conidia of *B. narcissicola* were counted on tapes viewed by a Nikon Optiphot-2 microscope with episcopic-fluorescence (Plate 1). Immuno-detection of conidia of *B. narcissicola* under UV episcopic-fluorescence was low on all the tapes compared

with the number obtained under bright-field microscopy (Table 4). The correlation of the spore count and IF was found to be 0.822.

Table 4. 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. (a) Conidia of *B. narcissicola* under UV light from the 7-day volumetric trap, negative by immunofluorescence. (b) 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.

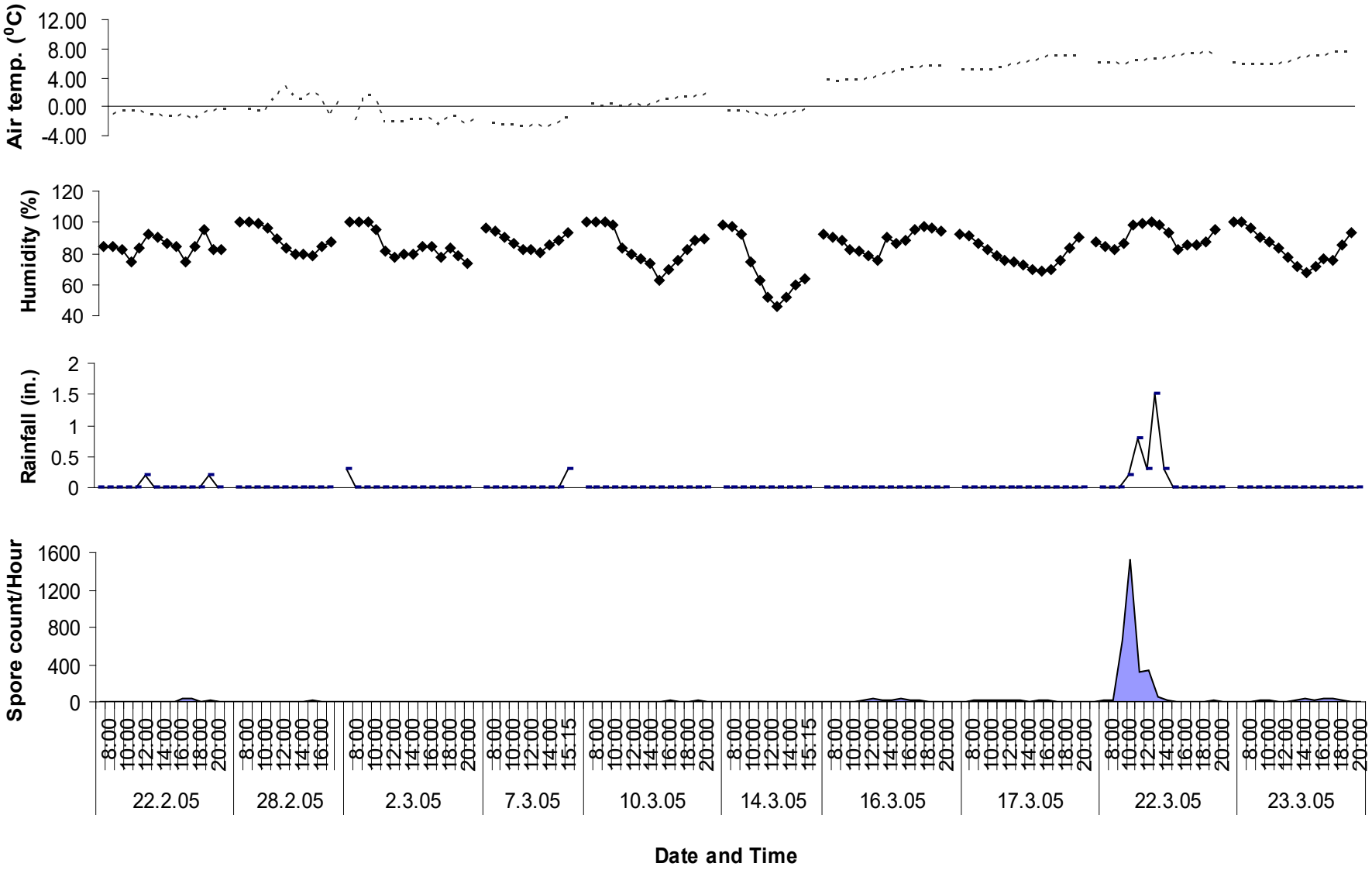


Figure 8 The effect of environment on field trapping of *B. narcissicola*. (Break in line after each trapping date shows data is not continuous).

Results
Year 2 (2005-2006)

Meteorological data

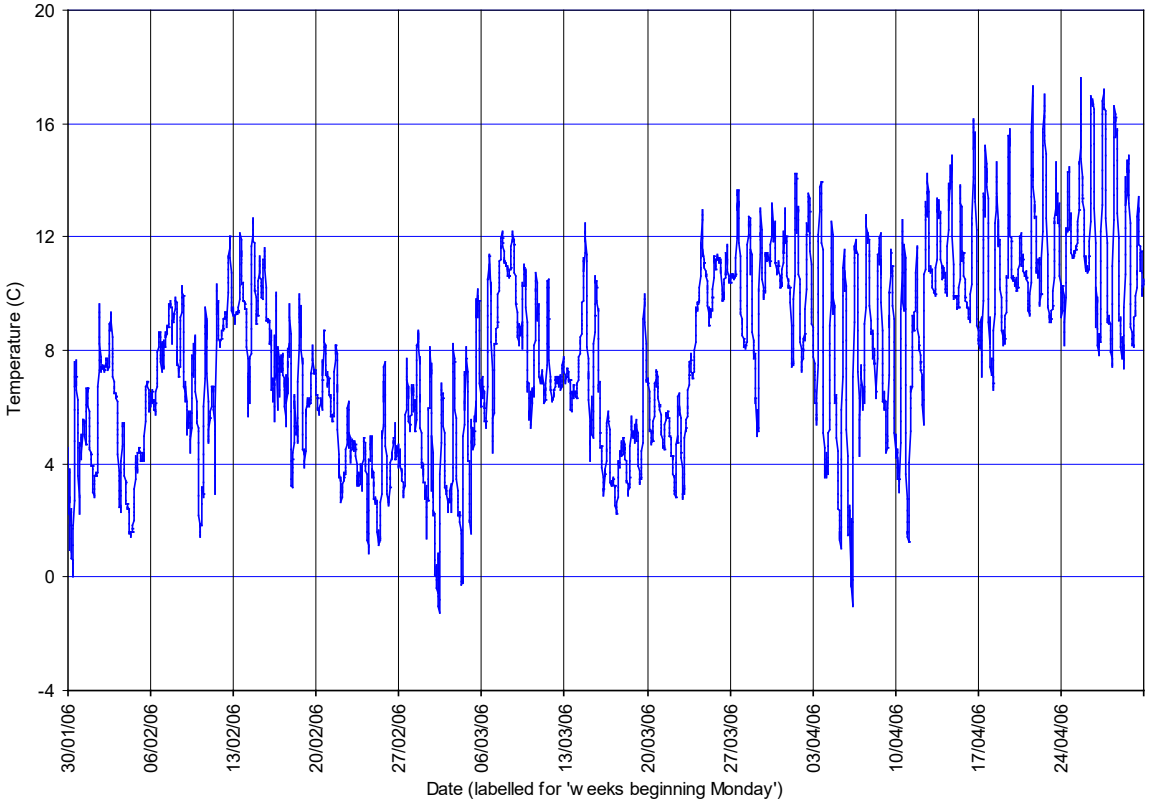
Figure 9 shows the temperature and leaf wetness data for the Surfleet and Kirton sites for the 2006 growing period. As expected for such relatively close locations, the weather was generally similar, although with some marked differences. Comparing temperatures at the two sites, Kirton temperatures were the more extreme, with lower minimum temperatures over most of the growing season and higher temperatures in warm periods (especially in late-March; Figure 9a). At Kirton, temperatures in cooler periods were often <4°C. In contrast, for most of the growing period temperatures at Surfleet remained in the infective range for smoulder (4-16°C), though below the optimum of 12°C, except in the last three weeks where it averaged 12°C. Apart from a dry period in mid-March, leaf wetness durations at Surfleet were consistently long, while there were more dry periods at Kirton (Figure 9b). Rainfall was higher at Surfleet than at Kirton, though relative humidity minima were the lower at Kirton (data not shown).

These data are interpreted as largely due to shelter, with the Surfleet site being relatively sheltered with trees on two sides of the area (warmer, smaller temperature range), and the Kirton being more open with little shelter (larger temperature range, windy site producing lower minimum humidity). These data would suggest that narcissus at the Surfleet site would be more susceptible to smoulder.

Crop and disease monitoring

In 2006 the pattern of smoulder infestation was similar at the two sites (Figure 10) and to the pattern which had occurred in 2005 at Saracen's Head. 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.

Figure 9. Weather at the Surfleet (above) and Kirton (below) sites, 2006 growing season.
(a) Air temperature



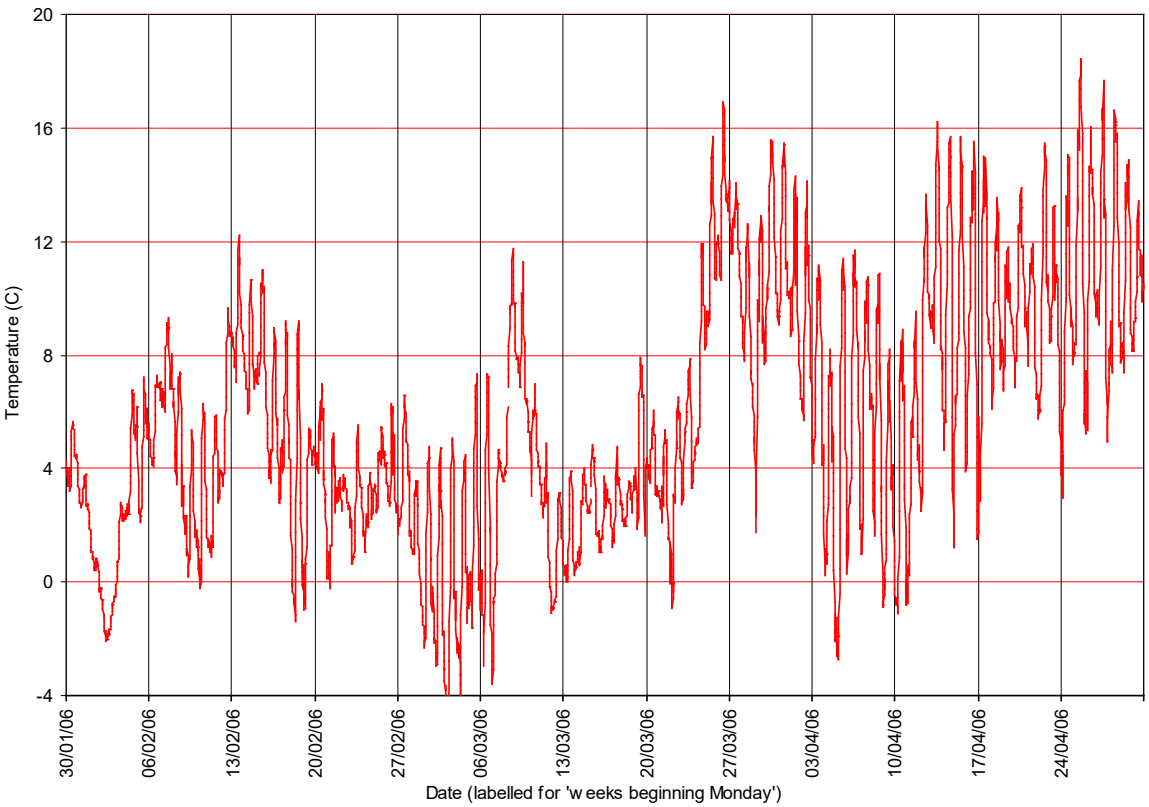
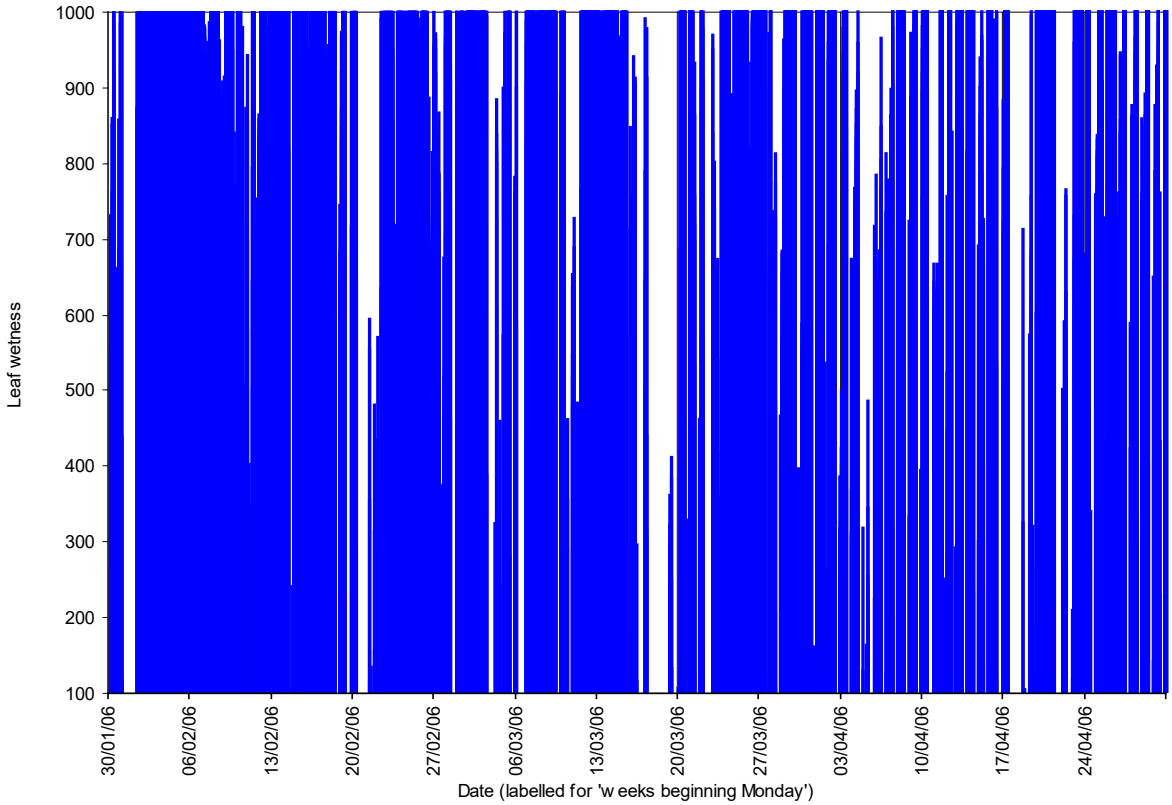


Figure 8(b) Leaf wetness



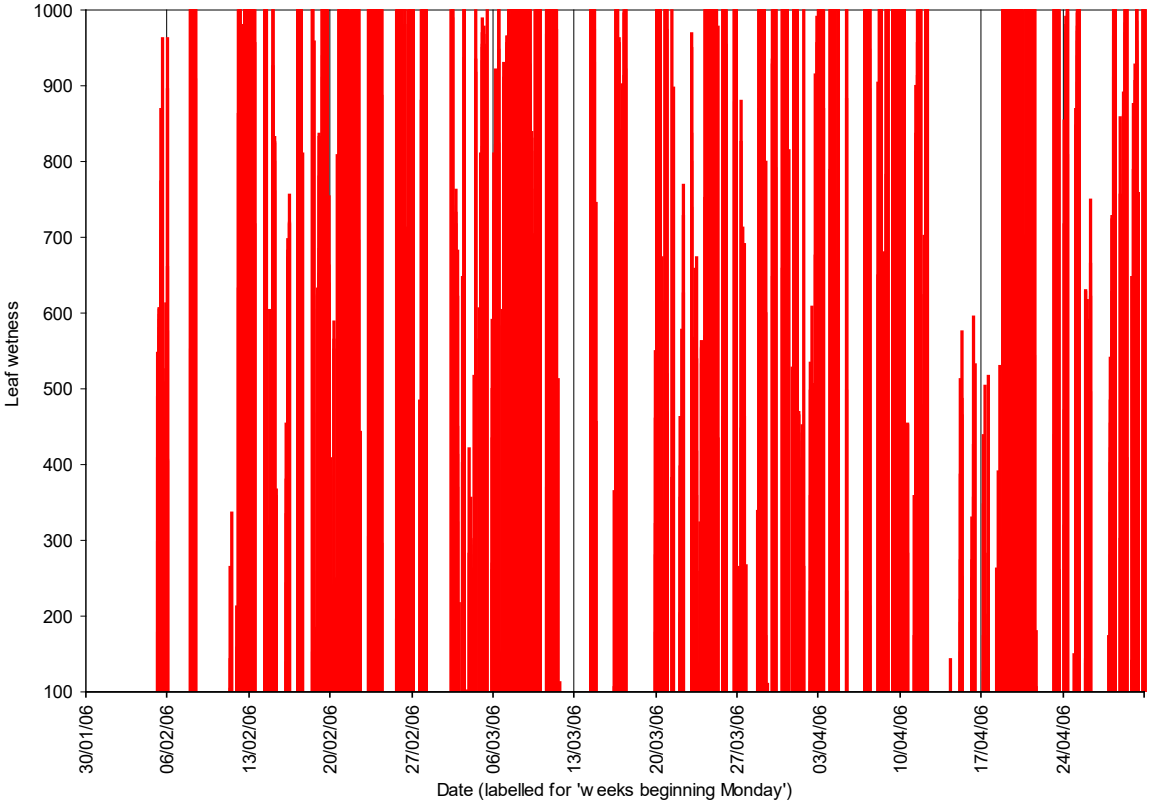
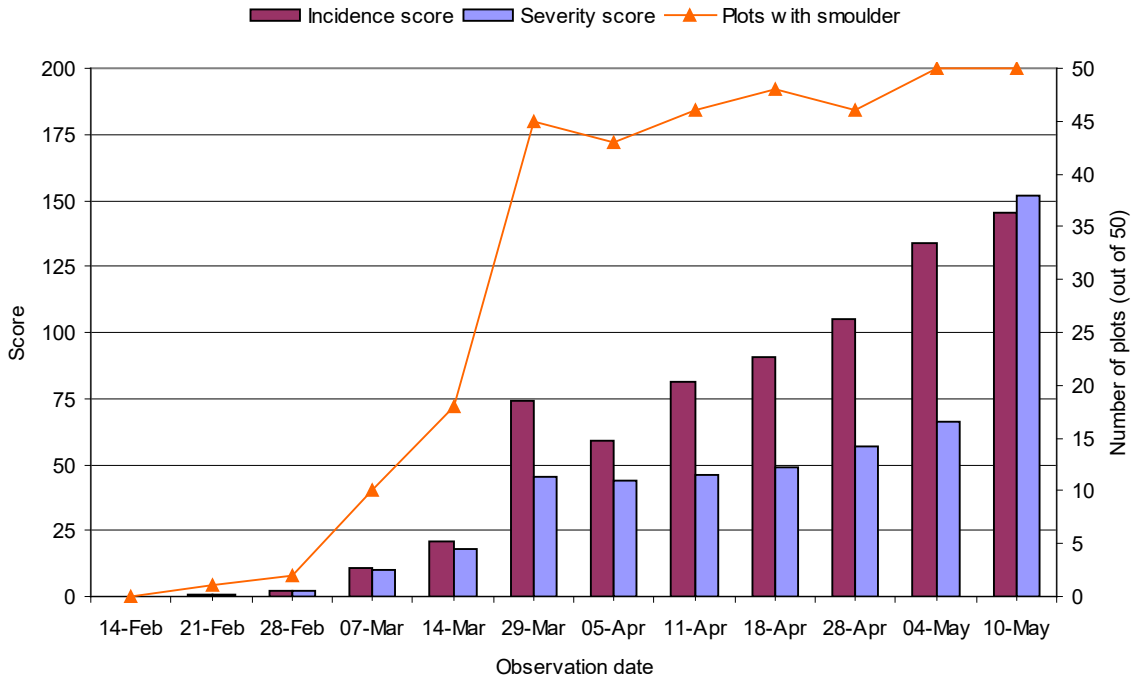
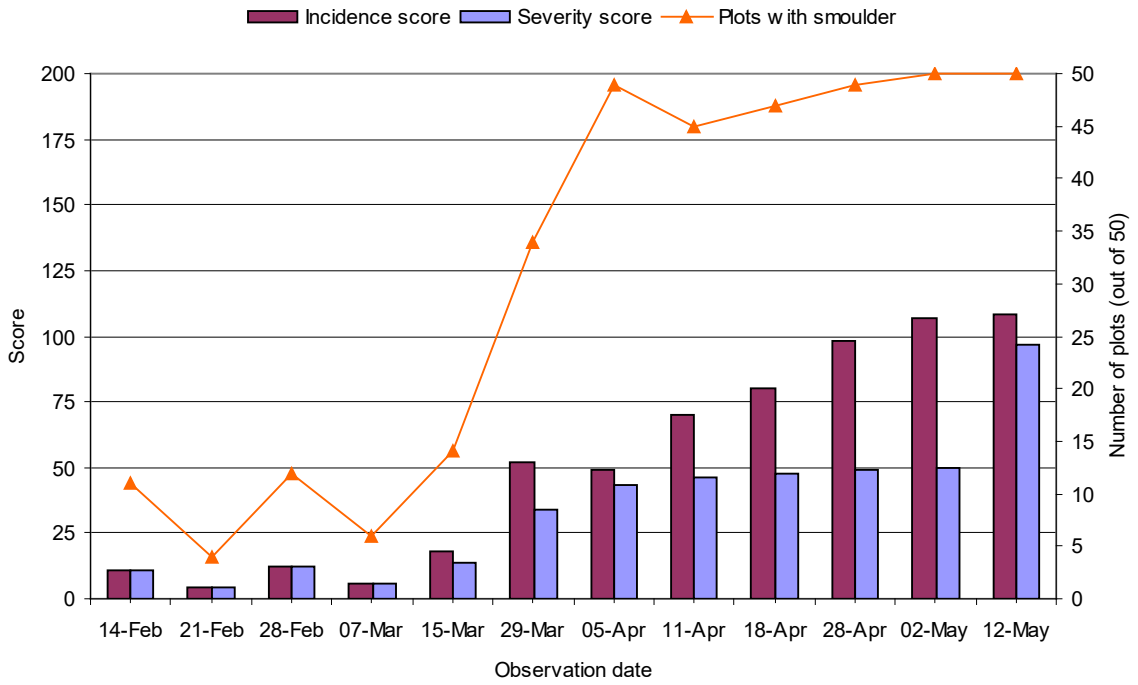


Figure 10. Smoulder monitoring at Kirton and Surfleet in 2006: number of plots showing smoulder symptoms and incidence and severity scores. (a) Kirton



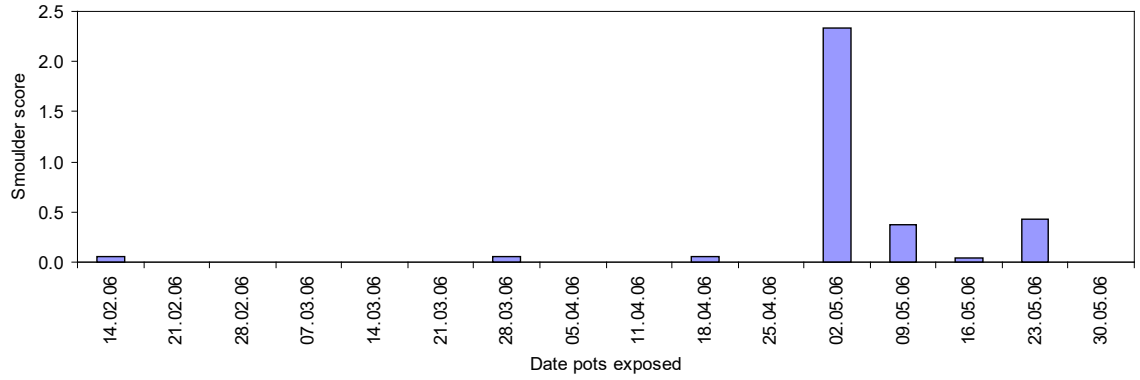
(b) Surfleet



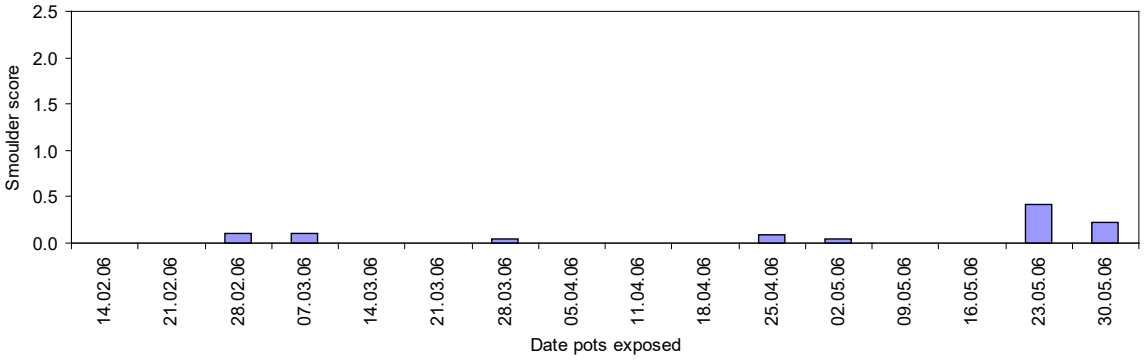
Trap plants

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 11). At Kirton there was a prominent peak of infection in early-May, followed by smaller peaks. At Surfleet, only the late, small peaks occurred.

Figure 11. Smoulder symptoms on narcissus trap plants at Kirton and Surfleet 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. No symptoms were found on non-exposed controls. (a) Kirton



(b) Surfleet

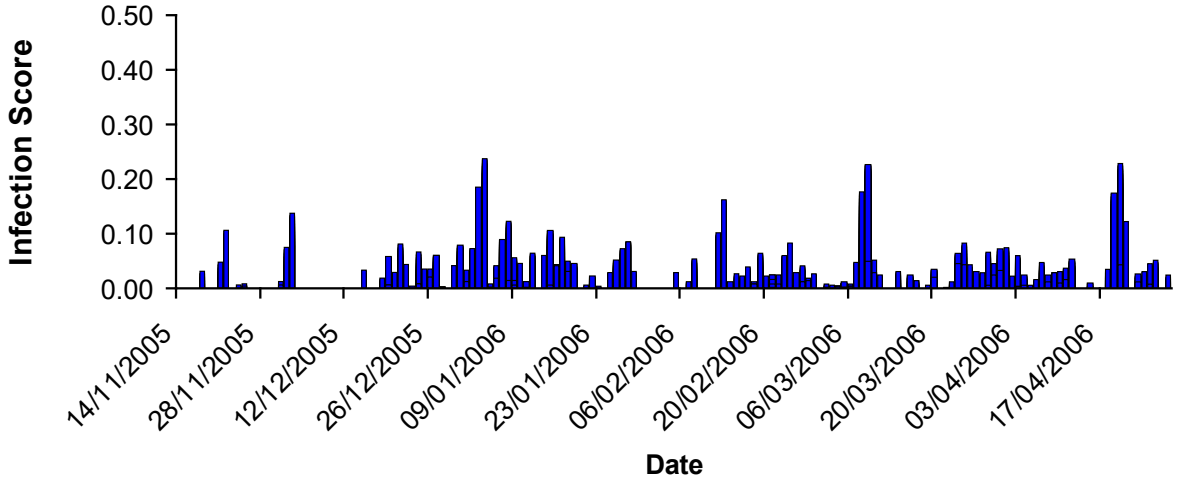


Forecasting infection

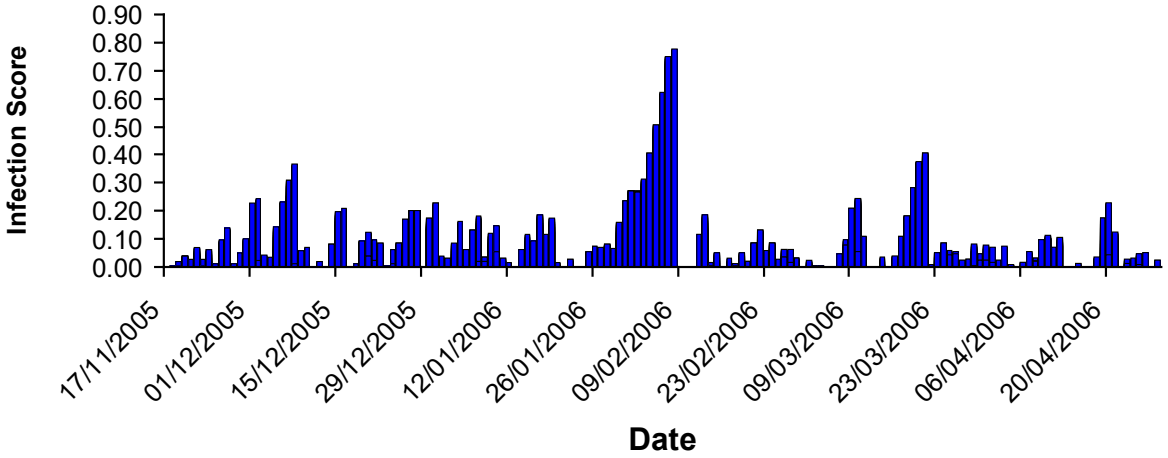
The smoulder infection model was run with the temperature and leaf wetness data for both the Kirton and Surfleet site in 2006. The predicted daily infection scores are presented in Figure 12. Major infection periods (periods where scores were above 0.2) were recorded at Kirton on 3 and 4 January, 8 and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

Figure 12. Predicted smoulder infection scores in 2006.

(a) Kirton



(b) Surfleet

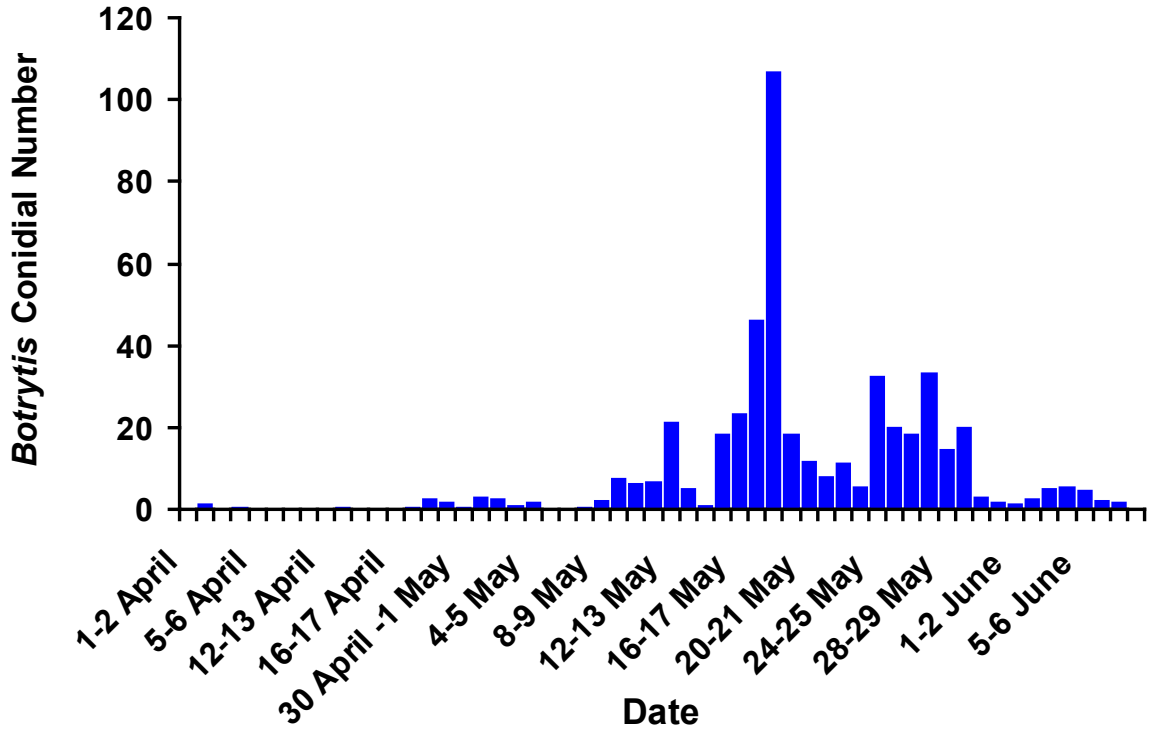


At Surfleet higher predicted infection scores were recorded. Infection scores of above 0.2 were predicted by the model on 1, 2, 6, 7, 8, 27, 28 and 31 December 2005. At Surfleet a continuous predicted infection period with scores above 0.2 was observed from 31 January to 8 February 2006. During this period predicted infection scores of up to 0.7 – 0.8 were recorded, however the stepwise pattern of the predicted infection suggests that the wetness sensors were in contact with either leaf material or the ground which would have resulted in this pattern of predictions. Predicted infection scores above 0.2 were also recorded on 9, 10, 19, 20, and 21 March and 20 April 2006.

Spore traps

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).

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



Discussion

Using the infection model to predict outbreaks of smoulder showed potential for use in forecasting this disease. The results appeared to confirm that other factors were also important on producing *Botrytis* lesions. In most cases, smoulder lesions appeared only on trap plants which were artificially damaged prior to field exposure. The nature of the requisite damage is still unclear, although it could be associated with frost or high windspeeds. Additionally, transmission of spore or inoculum availability could also be a factor which results in variability in disease levels on plants. However, there was a reasonably close relationship between trap plant infection and the higher model scores for the available data, particularly in 2006. Variation between these two measures appeared to be linked to the availability of smoulder inoculum.

The 7-day volumetric air sampler was useful in the field for collecting spores of *B. narcissicola*. It is clear from results of this study that the dispersal of spores of *B. narcissicola* is influenced by environmental factors, particularly rainfall. The high peak in the amount of trapped spores during rainfall may be due to groups of spores 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 *B. 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 splashing water for dispersal. Further, the temperature effect was considerable in field trapping, where there was a high variation in air temperature. Spores were not trapped at sub-zero temperatures, and high peaks in spore number coincided with relatively high temperature. Sporulation is reduced at low temperatures. It has been previously reported that temperature affects the rate of sporulation (Sosa-Alvarez, 1995), therefore it is important in monitoring airborne spore concentrations.

Immunodetection of trapped spores in the field using the IF technique was carried out, and statistical analysis of the results of spore counts using light and UV microscopy gave a correlation coefficient (R^2) of 0.822. This suggests 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 increased age, as the slides were processed a long time after sampling was done, although they were stored at 4ºC.

The results from year one and two of these field trials indicate that the *Botrytis narcissicola* infection model can be used accurately to forecast outbreaks of smoulder on narcissus crops. Further work is required on the impact that plant damage may have on smoulder infection. This work is on-going: in years 3 and 4 the practical consequences of applying fungicide sprays according to the prediction model will be evaluated on three farms, with a comparison of a conventional (regular) fungicide spray programme. In year 4 it is hoped that the disease prediction or spray timing system will be made available to HDC levy-payers for testing.

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