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Contents

GROWER SUMMARY

Headline

- Novel fungicide spray programmes improve foliar disease control and boost yields in narcissus
- Understanding the relationship between temperature, leaf wetness and infection opens the way for a cost-effective Spray Timing System for narcissus foliar diseases

Background and expected deliverables

UK growers are world leaders in the production of narcissus (daffodil) bulbs and cut-flowers. A high proportion of the crop is exported, while UK sales are increasingly targeted to the high-quality multiple retailer sector. The control of pests and diseases remains a very high priority for bulb growers, a problem intensified by the (otherwise highly efficient) growing and handling systems developed for growing bulbs in the UK, such as ‘two-year-down’ growing which allows a build-up of pests and diseases. While considerable R&D spend had been devoted to understanding and controlling bulb rots and pests, foliar fungal diseases, which also have a significant impact on narcissus quality and yield, have been little studied. These diseases – mainly smoulder (caused by *Botrytis narcissicola*) and white mould (caused by *Ramularia vallisumbrosae*) – are usually ‘controlled’ by a routine programme of fungicide sprays. In practice, little is known of how effective these programmes are – smoulder always seems to be around to some extent, and there have been serious epidemics of white mould on Cornish crops in recent years. In the case of white mould, the last research on the biology of the disease was carried out in the 1930s! These diseases are not a problem in other narcissus-producing countries, where the growing system and climate are different.

The aim of this Horticulture LINK project – funded by DEFRA, the HDC, individual bulb-producing companies and Intelligent Micro Design Ltd (Aardware Design) – was to understand the meteorological and other factors that lead to smoulder and white mould infections. By identifying key events in the development of these diseases, it should be possible to target fungicide treatments more rationally and cost-effectively, reducing the number of sprays applied. It is hoped that this will lead to the development of an easy-to-use ‘alerts’ system for bulb growers, warning them when fungicides need to be used.

The expected deliverables from this project included the following:

- Understanding how weather factors – probably mainly temperature and leaf wetness – affect the infection of narcissus plants by *B. narcissicola* and *R. vallisumbrosae*
- Formulation of these relationships into predictive mathematical models
- Understanding other factors that may influence the infection by and development of smoulder and white mould, such as:
  - ◊ Other weather events
  - ◊ Pathogen carry-over from the first-year of crops
  - ◊ Resting body (sclerotium) germination
  - ◊ Crop husbandry factors
- Data on the effectiveness of newer fungicides on smoulder and white mould

- Understanding how precipitation impact (PI) and surface wetness (SW) sensors can be used to identify key weather events leading to infection
- Assembling the information – infection models and knowledge of other predisposing factors – that will enable Spray Timing Systems to be developed

Summary of the project and main conclusions

The project included:

- Carrying out 'enabling' tasks such as selecting pathogenic isolates of *B. narcissicola* and *R. vallisumbrosae*, developing reliable culture methods, and establishing disease assessment protocols
- Studying the carry-over of pathogen inoculum from commercial first-year narcissus crops to subsequent years
- Investigating the germination characteristics of *R. vallisumbrosae* resting bodies ('sclerotia') in field and laboratory experiments, in order to identify risk periods
- Monitoring smoulder and white mould development in commercial narcissus crops in Cornwall, Lincolnshire and Cambridgeshire, and relating this to weather
- Testing PI sensors in narcissus crops for their use in identifying conditions favouring disease via splash dispersal and crop damage
- Testing SW sensors in relation to the wetness characteristics of narcissus leaves
- In controlled environment experiments, investigating the effects of leaf wetness and temperature on the infection of narcissus by *B. narcissicola* and *R. vallisumbrosae* condia, and developing and validating mathematical models relating temperature, wetness and infection
- Laboratory and field experiments testing the effectiveness of novel and standard fungicides in controlling smoulder and white mould
- Formulating all the above into a Spray Timing System that could be developed into a practical system for growers

The main findings of the project are set out below.

<u>Causal fungi, symptoms and crop monitoring</u>

- *R. vallisumbrosae* was confirmed in samples from Cornwall only (however, it was subsequently identified in Lincolnshire, see below), while *B. narcissicola* was found in samples from both areas. Isolates of each pathogen were selected for further work on the basis of high pathogenicity and abundant sporulation.
- Isolates of *B. narcissicola* and *R. vallisumbrosae* were grown on a range of nutrient media and under different conditions to optimise the production of conidia. A supply of resting bodies of *R. vallisumbrosae* was obtained from naturally infested leaves, which proved more reliable than producing resting bodies by the inoculation of leaves or in culture.
- A range of narcissus crops was examined, disease symptoms described, and a protocol for the efficient monitoring of white mould and smoulder was developed for use in the project.

Disease carry-over

- Commercial narcissus crops in Cornwall and eastern England were examined at the end of their first growing season and again during the next growing season, to determine if disease levels at the end of the first growing season were related to subsequent disease incidence. There were no clear relationships between the incidence of smoulder or white mould at the end of the first crop year, and levels at the beginning of the next crop year.
- In experiments on the germination of resting bodies of *R. vallisumbrosae* in 1999-2000 at Penzance (Cornwall) and Mepal (Cambridgeshire), scolecospores were first seen on 7 January 2000, with little difference between the two sites. In 2000-2001, germination was first observed on 13 December 2000 at Mepal and one week later at Penzance. Low temperatures and moist conditions favoured early germination.

Disease progress and weather

- Over three years of monitoring smoulder in commercial crops, there were no consistent differences in smoulder development between the three sites in eastern England. Differences between sites did not appear to be related to differences in temperature and humidity, although smoulder levels appeared to increase with the general seasonal rise in temperature. A greater incidence of rain and surface wetness appeared associated with more smoulder at one site in 1998-1999, but in other cases the relationship was either more complex or similar meteorological data were obtained at different sites.
- Over three years of monitoring white mould in commercial crops, there were no consistent differences in white mould development between the three Cornish sites. Differences between sites did not appear to be related to differences in temperature, although white mould levels seemed to increase with the general seasonal rise in temperature.

Damage that predisposes crops to disease

- PI sensors set up in a narcissus crop at Penzance showed that few of the many rainfall events that occurred would be likely to cause either significant damage or spread of disease. Increasing sensor shield diameter had a significant effect on the number of rain impacts assigned to higher impact levels. Rainfall events in experiments at Kirton (Lincolnshire) were insufficient to damage leaves of the exposed plants, and attempted infection by inoculating *B. narcissicola* conidia to rain-exposed plants were unsuccessful in all cases.
- In narcissus plants damaged by hail, larger brown and smaller white leaf lesions developed. *Botrytis* could be isolated from the brown lesions whether surface-sterilised or not, but only from white lesions that had not been surface-sterilised. This suggests that hail damage can lead to both direct and indirect colonisation of tissues.
- Narcissus plants were exposed to high energy water droplets in rainfall simulator and rain tower facilities, after which they were inoculated with *B. narcissicola* conidia and incubated. The rainfall simulator was used at its maximum output for two, 15-minute periods. This treatment did not increase the number of lesions produced, probably because too few drop of high energy impacted the leaves at any one point. Further tests were carried out in a rain tower, in which metered drops fell >8 m to impact a fixed point on the leaf surface at terminal velocity, with *ca.* 150 drops in a 5-minute treatment period. Cultured leaf segments from treated leaves developed *Botrytis* infections, whereas inoculated control segments did not.

- Frost as well as hail damage is thought to cause leaf damage leading to infection with foliar pathogens. Narcissus plants were either damaged (by brushing), left undamaged as controls, or were exposed to -2°C for 2, 8 or 22 hours, before inoculating with *R. vallisumbrosae* conidia and incubating in cool, humid conditions. Control plants had very low levels of white mould lesions developing, and the number of lesions increased significantly in 'frosted' plants but much more so in mechanically damaged plants.
- Experiments established that leaf damage (produced by using pins or a bristle brush on the leaf surface) was required for the successful infection of narcissus leaves with *B. narcissicola* and enhanced infection by *R. vallisumbrosae* conidia. Optimal inoculation and incubation conditions for both pathogens included high humidity.
- Although flower cropping causes crop damage, there was no consistent relationship between cropping date and an increase in the frequency of white mould or smoulder symptoms.

Leaf wetness, temperature and infection

- Standard flat surface wetness sensors were compared with a novel design incorporating a well-shaped response surface of various sizes. The time taken for water droplets of different sizes to evaporate from the sensor surface and from narcissus leaves were measured under standard conditions. The evaporation times for the 5 or 6 mm-diameter well-type sensors corresponded most closely with those of the undamaged leaf.
- In controlled environment (CE) experiments, narcissus plants were inoculated with *B. narcissicola* conidia following leaf damage, placed in CE cabinets at 4 - 24˚C, and either sprayed to maintain leaf wetness or maintained at 96% relative humidity. At intervals, samples of plants were removed and the number of lesions recorded 2 weeks later. At short wetness durations (6 hours), temperatures of 12˚C were optimal for infection. At longer wetness durations (about 24 hours) a wider range of temperatures (4 - 16˚C) was effective. Analysis of data showed there was a quadratic relationship between temperature and infection and a linear relationship between wetness duration and infection, within the ranges 4 - 24°C and 1 – 72 hours of wetness. A mathematical model was developed relating temperature, leaf wetness duration and the number of smoulder lesions developed per leaf.
- CE experiments showed the presence of tissue damage was advantageous for infection by *R. vallisumbrosae* on narcissus leaves, and the presence of free water increased the severity of this infection. Temperatures of 5 – 10°C and wetness durations of 12 – 24 hours were optimal for infection. Phragmospores were the most common spore form produced on white mould lesions. The optimal temperature for phragmospore production was 5 – 15°C, while scolecospores were produced at 5 – 10°C. A mathematical model was developed relating temperature, leaf wetness duration and the number of white mould lesions per leaf.

Predicting disease development

- Using the model developed, there was a strong relationship between the predicted cumulative number of smoulder lesions and the observed % die-back of crops in eastern England. Crop die-back was observed when the predicted cumulative lesion number increased above 200. However, there was a poor relationship between the predicted cumulative number of lesions and the observed number of smoulder lesions, perhaps because it is difficult to count individual disease lesions numbers accurately later in the season as the lesions merge and the leaf dies. Hence the infection model would be useful for predicting the severity of smoulder infections, when this is expressed as the premature loss of green leaf area due to disease.

- The assumption that frost periods are required for successful infection of narcissus tissues by *B. narcissicola* was tested. If frost periods were assumed to be necessary, there was *no* relationship between the predicted cumulative number of lesions and the observed % die-back in crops. Further, there was strong *negative* relationship between the predicted cumulative number of lesions and the observed number of smoulder lesions after frost periods. These findings indicate that frost is not likely to be a major factor in smoulder epidemiology.
- Using the model developed, there was a positive relationship between the predicted cumulative number of white mould lesions and the observed % die-back in crops in Cornwall. There was no relationship between the predicted cumulative number of white mould lesions and the observed number of white mould lesions, probably for the same reason as for the smoulder model.

<u>Fungicide activity</u>

- In leaf assays with *B. narcissicola*, all fungicides tested reduced lesion size when applied as protectant sprays. Ronilan, Scala, Unix, Folicur, Opus and Punch C were particularly effective, resulting in >90% reduction in lesion area. The fungicides were generally less effective when applied as curative sprays, and several treatments gave little or no control. However, Amistar, Folicur, Ronilan, Scala and Unix resulted in a reduction of lesion area of >60%. Benlate and Dithane 945 applied alone each gave only slight control, but the mixture showed synergistic protectant activity. These experiments demonstrated, for the first time, good activity against *B. narcissicola* by anilinopyrimidine fungicides (Frupica, Scala and Unix) and triazole fungicides (Folicur, Opus and Plover), and some activity by a strobilurin fungicide (Amistar).
- In leaf assays with *R. vallisumbrosae*, Amistar, Benlate + Dithane 945 and Scala were all very effective, completely preventing the establishment of *R. vallisumbrosae* when applied as protectant sprays. Ronilan and Dithane 945 were ineffective. The same three fungicides or mixtures were the most effective treatments against *R. vallisumbrosae* when applied after inoculation, while Bravo, Stroby WG, Unix, Frupica and Shirlan appeared only slightly less effective. These results demonstrated, for the first time, activity of anilinopyrimidine (Scala) and strobilurin fungicides (Amistar) against *R. vallisumbrosae*, and confirmed the good activity of Benlate + Dithane 945.

<u>Field trials - smoulder</u>

- In replicated smoulder trials at Mepal and Kirton in 2000, four new fungicides (Amistar, Folicur, Scala and Unix) were compared with three standard products or mixtures (Benlate + Dithane 945, Bravo 500 and Ronilan) in six-spray fungicide programmes. No reduction in smoulder primary lesions was observed, but most treatments reduced both secondary lesions on leaves and flower stalk rot. Leaf die-back was noticeably delayed by Amistar, Benlate + Dithane 945, Folicur, Ronilan, Scala and Unix. All fungicide treatments resulted in increased bulb yields compared with untreated controls, increases being greatest with Folicur (48%) and Scala (50%) at Mepal, and Unix (12%) at Kirton. Yield increases were strongly negatively associated with leaf area die-back.

- In further trials in 2002, eight spray programmes, ranging from two to six sprays each, were devised and tested. Sprays were directed at three growth stages: 1, shoot emergence; 2, around flowering, and 3, after flowering, with two sprays at each stage. Up to three products (Folicur, Ronilan and Scala) were used in each programme. Disease control, as judged by foliage die-back, was greatest when either a full, six-spray programme was used, or using two sprays around flowering and two sprays after flowering. However, bulb yield increase was greatest using a four-spray programme comprising two sprays of Ronilan during emergence followed by two sprays of Folicur around flowering.
- A separate investigation to determine whether fungicide sprays used for foliar disease control influenced the incidence of neck rot was described in the Final Report of the associated HDC-funded project BOF 41a.
- In replicated white mould trials at Mepal and Kirton, no white mould developed despite inoculation with *R. vallisumbrosae*, but useful results were obtained on smoulder control following natural attacks of this disease. In 2000, four novel fungicides (Amistar, Folicur, Scala and Stroby WG) were compared with three standard products or mixtures (Bravo 500, Bavistin DF + Dithane 945 and Benlate + Dithane 945). Treatments at both sites reduced secondary smoulder, with Amistar being outstanding at Mepal and Stroby WG at Kirton. At both sites Folicur and Stroby WG resulted in prolonged green leaf retention. All fungicide treatments increased bulb yield, with Folicur providing the greatest benefit at Mepal (58% increase over untreated controls) and Stroby WG at Kirton (35% increase). Bavistin DF + Dithane 945 resulted in similar yield increases to Benlate + Dithane 945 (26-31% increases at Mepal, 19-23% increases at Kirton).
- In further work at Mepal and Kirton in 2001, again no white mould developed despite inoculation with *R. vallisumbrosae*, but useful results were obtained following a natural attack of smoulder. Programmes of five or six sprays of some novel fungicide mixtures were compared with Bavistin DF + Dithane 945. Fungicide treatments significantly reduced *Botrytis* stem rotting from 47% (untreated controls) to 22 to 34%. They also reduced leaf die-back and increased bulb yield. Bavistin DF + Folicur and Amistar +Folicur were particularly effective at both sites. The greatest bulb yield increase over untreated controls was 59% at Mepal (with Bavistin DF + Folicur) and 24% at Kirton (with Amistar + Folicur).

<u>Field trials – white mould</u>

- In trials at Penzance on white mould in commercial crops of cv. Yellow Cheerfulness (cv. Cheerfulness in 2002), severe white mould occurred in the untreated control plots each year. In 1999, programmes of one to five sprays of Benlate + Dithane 945 were compared in an attempt to determine the importance of different spray timings. Programmes of one or two sprays in February/March (leaves 10-15 cm long) gave little control of white mould or leaf die-back. Programmes of three to five sprays all gave very good control of white mould and leaf die-back. A three-spray programme, commencing in late-March when white mould was at a low level, was as effective as a five-spray programme starting one month earlier.
- In further work in 2001, spray programmes of Bavistin DF + Dithane 945 commencing at different growth stages (0-5 and 10-15 cm leaf length), or at first symptoms of white mould, were compared with some novel fungicide mixtures (Bavistin DF + Folicur, Bavistin DF + Ronilan, Bavistin DF + Scala and Amistar

+ Folicur) all starting at the 10-15 cm stage. White mould did not occur until mid-April, and there was no benefit of spraying early at the 0-5 cm stage. Commencing with Bavistin DF + Dithane 945 at first symptoms resulted in a saving of four sprays, but gave inferior disease control when compared with a preventative programme starting at the standard first spray time (10-15 cm stage). Two of the novel fungicide mixtures (Amistar + Folicur and Bavistin DF + Folicur) gave very good disease control, and appeared to be slightly better than Bavistin DF + Dithane 945 applied at the same timings.

- In further work in 2002, the Amistar + Folicur mix was compared with Bavistin DF + Dithane 945 in each of four spray programmes: every 10-14 days from the 10-15 cm stage; every 21-28 days from the same stage; every 14 days from first symptoms; and one spray at 10-15 cm then every 14 days from first symptoms. Amistar + Folicur applied at 21-28 day intervals (three sprays in total) gave control equal to that of Bavistin DF + Dithane 945 every 10-14 days (six sprays in total), resulting in a saving of three sprays over the current standard fungicide programme. Amistar + Folicur applied at 14 day intervals from first symptoms (three sprays) resulted in significantly better control of white mould and reduction in leaf die-back than Bavistin DF + Dithane 945 applied at the same timings, indicating that the former treatment is probably the better choice to treat established white mould.

<u>Bulb yield increases</u>

- In the circumstances of these trials, conducted around 2000 with high levels of secondary smoulder development and associated early foliage senescence, and assuming a return of £300 per tonne and £450 per tonne for bulbs of cultivars Carlton and Cheerfulness, respectively, all treatments resulted in a positive margin over the cost of fungicide. The cost of spray application was excluded. Nevertheless, the increase in bulb yields over chemical costs were large, ranging from £394 to £1359 per ha at Kirton and from £1994 to £4849 per ha at Mepal for cv. Carlton. The margins on cv. Cheerfulness ranged from £602 to £2850 per ha at Kirton and from £1246 to £6234 per ha at Mepal. The greatest margins in each experiment were given by Folicur on cv. Carlton at Mepal, by Unix on cv. Carlton at Kirton, and by Folicur on cv. Cheerfulness at both Mepal and Kirton.

<u>Spray Timing System</u>

- A Spray Timing System (STS) was formulated, using many of the elements described above. The STS would operate by detecting events in the field that would cause damage to the crop. Environmental conditions subsequent to damage events would then be used to predict the amount of smoulder and white mould likely to arise, via models of the effects of leaf wetness duration and temperature on the numbers of lesions of smoulder or white mould developing. Identifying these critical periods of crop damage, wetness and temperature through local environmental logging would provide a system alerting the grower or advisor of the need to apply fungicide sprays as soon as practical after the warning. Other factors favourable to disease infection and spread would augment this warning, for example perhaps reinforcing or deferring the urgency of crop spraying under borderline conditions. Factors that would reinforce the need to spray would include (a) adverse weather conditions likely to cause damage to leaves, primarily hail or heavy prolonged rain (and possibly frost for white mould), (b) the crop being grown for longer than the usual two-year cycle, and (c), for white mould only, the occurrence of suitable conditions for resting body germination (temperatures of <10˚C and rainfall of >20 mm during the previous week in the mid-December to early-March period).

Unexpected findings

- During the course of this project, white mould caused by *R. vallisumbrosae* was found and confirmed in several crops in Lincolnshire. It had previously been thought to be absent from this area, so the finding has implications for future narcissus growing in the region.
- Also in studies in eastern England, late-season epidemics of smoulder, confirmed as caused by *B. narcissicola*, were described. This symptom has not previously been noted in standard texts, and may have been confused in the past with physiological premature leaf senescence. Again, this has implications for future disease control strategies.

Financial benefits

The full benefits of the project will not be available until the 'Spray Timing System' has been validated and is deliverable as a simple 'alerts' system. However, the results so far indicate that savings can be made by cutting the numbers of fungicide sprays applied, perhaps from six or seven sprays to about three annually. Using the newly tested fungicides, the number of sprays can be reduced, still with better disease control. The increase in bulb yields obtained compensates, in any case, for the additional fungicide costs, as shown by the cost-benefit analysis above. This takes account only of the benefit of increased bulb yields and the costs of the pesticides (not application costs). Of course this financial benefit will be dependent on prices and costs, of which the most volatile is likely to be bulb price. However, even with average bulb prices having fallen to considerably lower than at the time of the start of this project – say £250 per tonne in 2002 compared with £400 to £500 per tonne – a financial benefit is evident. Bulb prices are likely to continue to be cyclical. In addition to the relatively easily assessed financial benefits, there will be environmental and marketing benefits of reducing pesticide usage in line with current expectations.

Action points for growers

Full implementation of the findings of the project must also largely await the full 'Spray Timing System' being available. However, in the meantime growers can take the following actions on their fungicide spray programmes:

- For the improved control of smoulder, the following fungicides offered good results: Amistar, Benlate + Dithane 945, Bavistin DF + Dithane 945, Folicur, Ronilan, Scala, Unix and Stroby WG. Overall, the new mixtures Bavistin DF + Folicur and Amistar +Folicur gave excellent results on disease control, delayed leaf senescence and bulb yield. A programme of two sprays of Ronilan around shoot emergence plus two sprays of Folicur around flower cropping is also effective.
- For the improved control of white mould, Amistar + Folicur and Bavistin DF + Folicur, applied at a shoot height of 10 – 15 cm, was useful. Three sprays of

Amistar + Folicur applied at 21 – 28 day intervals was a good treatment for established white mould, and was as good as six sprays of Bavistin DF + Dithane 945 applied at 10 – 14 day intervals. Spray programmes consisting of three sprays starting at a low level of white mould were as effective as a five-spray programme starting earlier. Very early sprays were ineffective.

- Where practical, apply fungicides promptly after weather events that cause crop damage.
- Growers in the eastern counties should be aware of (a) the possibility of white mould occurring in the region, and (b) late-season smoulder attacks, and should be prepared to take remedial action.

Frontispiece

1. Meteorological station and logger in crop at Holbeach Marsh
2. Irrigating trial areas at Kirton to encourage foliar disease development
3. Foliage die-back as a result of smoulder in a monitored commercial crop
4. Fungicide trial, with control plots died-down (left foreground) and treated plots green
5. Rain simulation experiment at IACR
6. White mould lesions on inoculated leaves from controlled environment experiment

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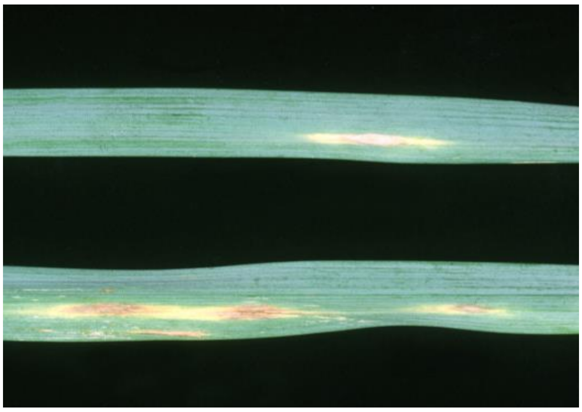
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SCIENCE SUMMARY

White mould (caused by *Ramularia vallisumbrosae*) and smoulder (caused by *Botrytis narcissicola*) are important foliar fungal pathogens of narcissus crops in the UK. The project was set up with the aim of improving understanding of the spread of white mould and smoulder, and developing better control strategies through disease forecasting techniques. At present 'control' consists of using repeated fungicide sprays. There has been little or no recent research on these diseases. This project is the first known in which a disease forecasting system has been developed for the control of foliar diseases of flower crops in the UK. The project is innovative in that the target, narcissus, is a perennial crop grown over at least two years, introducing complications compared with annual, seed-raised crops.

Objective 1: To produce methods for the reliable production of resting bodies and conidia of *R. vallisumbrosae* and conidia of *B. narcissicola*, using typical pathogenic isolates.

<u>1.1. Pathogenicity and selection of isolates</u>
Fifty samples of diseased narcissus leaves were collected and examined from narcissus crops in Cornwall and eastern England in 1998. *R. vallisumbrosae* was confirmed in samples from Cornwall only, while *B. narcissicola* was found in samples from both areas. Isolates of each pathogen were selected for further work on the basis of high pathogenicity and abundant sporulation.

<u>1.2. Culture of pathogens to supply conidia and resting bodies</u>
Isolates of *B. narcissicola* and *R. vallisumbrosae* were grown on a range of media and under different conditions to optimise the production of conidia. Protocols were established for the reliable production of conidia by both fungi, and of resting bodies by *R. vallisumbrosae*, for use in further experiments. A supply of resting bodies of *R. vallisumbrosae* was obtained from naturally infested leaves, which proved more reliable than producing resting bodies by the inoculation of leaves or in culture.

Objective 2: To determine the effect of initial inoculum and environmental factors (including rainsplash for *R. vallisumbrosae*) on the epidemiology of white mould and smoulder within second-year daffodil crops.

<u>2.1. Disease recognition and assessment</u>
A range of narcissus crops was examined and descriptions of disease symptoms were prepared. A protocol for the efficient monitoring of white mould and smoulder in crops was developed and tested for later use in the project.

<u>2.2. Pathogen carry-over from first-year crops</u>
Commercial narcissus crops in Cornwall, Lincolnshire and Cambridgeshire were examined at the end of their first growing season in 1999 and 2000. Crops were examined again during the next growing season, to determine if disease levels at the end of the first growing season were related to subsequent disease incidence. There were no clear relationships between the incidence of smoulder or white mould at the end of the first crop year and levels at the beginning of the second crop year.

2.3. Germination of *R. vallisumbrosae* resting bodies

Experiments on the germination of resting bodies of *R. vallisumbrosae* were set up in 1999 and 2000 at Penzance (Cornwall) and Mepal (Cambridgeshire). In 1999-2000, scolecospores were first seen on 7 January 2000, germination peaking on 1-7 February with little difference between the two sites. In 2000-2001, germination was first observed on 13 December 2000 at Mepal and one week later at Penzance, and continued until late-January 2001. When resting bodies were incubated in damp conditions immediately after recovery, germination occurred earlier. Low temperatures and moist conditions favoured early germination.

2.4. Disease monitoring

Over the period 1998 to 2001 the incidence of smoulder and white mould were monitored regularly in second-year commercial narcissus crops in Cornwall (three crops per year) and Lincolnshire and Cambridgeshire (three crops per year), and trial plots were monitored at Kirton (Lincolnshire) and Mepal (Cambridgeshire).

2.4.1. Smoulder in eastern counties

Over the three years, there were no consistent differences in smoulder development between the three commercial sites in the east. Further, over the two-year growth cycle the rate of disease development was not consistent at any one site. Any differences between sites did not appear to be related to differences in temperature and humidity, although smoulder levels appeared to increase with the general seasonal rise in temperature. This could account for the faster disease development (by 2 – 3 weeks) that occurred at Mepal compared with Kirton. A greater incidence of rain and surface wetness appeared associated with more smoulder at one site in 1998-1999 only; in other cases the relationship was either more complex or similar meteorological data were obtained at different sites. There was no clear relationship between the date of flower cropping and any increasing incidence of smoulder symptoms.

2.4.2. White mould in Cornwall

Over the three years, there were no consistent differences in white mould development between the three commercial sites in Cornwall. Further, over the two-year growth cycle the rate of disease development was not consistent at any one site. Any differences between sites did not appear to be related to differences in temperature, although white mould levels seemed to increase with the general seasonal rise in temperature. The crop at one site in 1998-2000 was unusual in that the site was sheltered on two sides by trees. Compared with the other sites, white mould developed initially more quickly here in the second crop year, but disease development towards the end of the growing season was slower. Kept down for a third year, white mould developed on this crop very quickly. Later in the growing season, white mould developed at Truro in 1998-1999 and 2000-2001 more quickly than at other sites. This may have related to higher levels of surface wetness compared with the other sites. There was no clear relationship between the date of flower cropping and any increasing incidence of white mould symptoms.

During the course of this project, white mould due to *R. vallisumbrosae* was found and confirmed in several crops in Lincolnshire. It had previously been thought to be absent from this area, so the finding has implications for future narcissus growing in the region. Also in studies in eastern England, late-season epidemics of smoulder, confirmed as caused by *B. narcissicola*, were described; this symptom has not previously been noted in standard texts and could have been confused with physiological premature leaf senescence, again with implications for disease control.

2.5. Evaluation of precipitation impact (PI) sensors and crop damage

2.5.1. Natural rainfall and infection by *B. narcissicola*

PI sensors are relevant both to the rain-splash dispersal of disease and to potential damage-related disease occurrence. In 1998, PI sensors were set up in a narcissus crop at Penzance. The outputs showed that few of the many rainfall events that occurred – the highest outputs corresponding to hail - would be likely to cause significant damage or spread of disease. Different sensor shield size was investigated for optimising sensor output, and showed that increasing shield diameter had a significant effect on the number of rain impacts assigned to higher ‘bins’ (higher impact energy). Successive batches of plants were exposed to natural rainfall at Kirton, but the rainfall events in these experiments proved insufficient to damage leaves of the exposed plants, and attempted infections by inoculating *B. narcissicola* conidia were unsuccessful in all cases. In other narcissus plants damaged by hail, and producing white and brown lesions, *Botrytis* could be isolated from larger brown lesions whether surface-sterilised or not, but only from smaller white lesions that had not been surface-sterilised, suggesting that hail damage can lead to both direct and indirect colonisation of tissues.

2.5.2. Simulated rainfall and infection by *B. narcissicola*

Narcissus plants were exposed to higher energy water droplets in rainfall simulator and rain tower facilities at IACR, Long Ashton, after which they were inoculated with *B. narcissicola* conidia and incubated to observe the development of smoulder lesions. The rainfall simulator was used at its maximum output (2.5 bar delivering >90 mm/hour), plants being exposed for two, 15-minute periods. This treatment did not increase the number of lesions produced, probably because too few drops of high energy impacted the leaves at any one point. Further tests were carried out in a rain tower, in which metered drops fell >8 m to impact a fixed point on the leaf surface at terminal velocity (5.94 m/s), using *ca.* 150 drops in a 5-minute treatment period. Cultured leaf segments from treated leaves developed *Botrytis* infections, whereas inoculated control segments did not.

2.5.3. Frost, leaf damage and infection by *R. vallisumbrosae*

Frost as well as hail damage is thought to cause leaf damage leading to infection with foliar pathogens. Narcissus plants were either damaged (by brushing), left undamaged, or were exposed to -2°C for 2, 8 or 22 hours, before inoculating with *R. vallisumbrosae* conidia and incubating in cool, humid conditions. Control plants had very low levels of white mould lesions developing, and the number of lesions increased significantly in ‘frosted’ plants but much more so in damaged plants.

Objective 3: To construct and test models relating tissue wetness duration and temperature to infection of narcissus tissues by *R. vallisumbrosae* and *B. narcissicola.*

3.1. Leaf wetness characteristics

Standard, flat surface wetness sensors were compared with a novel design incorporating a well-shaped response surface of different sizes. The time taken for water droplets of different sizes (0.1 – 1.1 ml) to evaporate from the sensor surface and from narcissus leaves were measured under standard conditions. The evaporation times for the 5 or 6 mm-diameter well-type sensors corresponded most closely with those of the undamaged leaf.

3.2. Temperature, leaf wetness duration and infection

Experiments established that leaf damage (produced by using pins or a bristle brush on the leaf surface) was required for the successful infection of narcissus leaves with *B. narcissicola* conidia, and was very advantageous for infection by *R. vallisumbrosae* conidia. Optimal inoculation and incubation conditions for both pathogens included high humidity.

3.2.1. Temperature, wetness and infection models for *B. narcissicola*

In controlled environment (CE) experiments narcissus plants were inoculated with *B. narcissicola* conidia following leaf damage and placed in CE cabinets at 4 - 24˚C, and either sprayed to maintain leaf wetness or maintained at 96% relative humidity. At intervals (6 – 72 hours) samples of plants were removed and the number of lesions recorded 2 weeks later. At short wetness durations (6 hours), temperatures of 12˚C were optimal for infection. At longer wetness durations (about 24 hours) a wider range of temperatures (4 - 16˚C) was effective. Analysis of data showed there was a quadratic relationship between temperature and infection and a linear relationship between wetness duration and infection, within the ranges 4 - 24°C and 1 – 72 hours of wetness. The following relationship was highly significant in describing the effect of temperature (°C) and leaf wetness duration *w* (hours) on the number of smoulder lesions per leaf, *S*:

$$S = 0.351 + 0.0065w + 0.277t - 0.011\, t^2$$

3.2.2. Temperature, wetness and infection models for *R. vallisumbrosae*

CE experiments showed the presence of tissue damage was advantageous for infection on immature narcissus leaves, and the presence of free water increased the severity of this infection. Temperatures of 5 - 10°C and wetness durations of 12 - 24 hours were optimal for infection. Phragmospores were the most common spore form produced on white mould lesions. The optimal temperature for phragmospore production was 5 - 15°C, while scolecospores were produced at 5 - 10°C. Phragmospores and scolecospores may have different environmental criteria for infection of narcissus leaves. The following relationship was highly significant in describing the effect of temperature *t* (°C) and leaf wetness duration *w* (hours) on the number of white mould lesions per leaf, *R*:

$$\sqrt{R} = ((0.9509 + F) - (F \text{ x } 0.3283^{w})$$

$$\text{where } F = \left(\frac{0.3428}{(1 + \exp(-0.379 \text{ x t } -19.91))}\right)$$

and *w* is between 0 and 30 hours and *t* is between 5 and 30ºC.

3.3. Validation of temperature, wetness duration and infection models

3.3.1. Smoulder model

Using the infection model, there was a strong relationship between the predicted cumulative number of lesions and the observed % die-back, using the data obtained from crops in eastern England in 1998/1999 and 2000/2001. Crop die-back was observed when the predicted cumulative lesion number increased above 200. However, there was a poor relationship between the predicted cumulative number of lesions and the observed number of smoulder lesions, perhaps because it is difficult to count individual disease lesions numbers accurately later in the season as the lesions merge and leaves die. The assumption that frost periods are required for successful infection of narcissus tissues by *B. narcissicola* was tested. If frost periods were assumed to be necessary there was *no* relationship between the predicted cumulative number of lesions and the observed % die-back in crops. Further, there was strong *negative* relationship between the predicted cumulative number of lesions and the observed number of smoulder lesions after frost periods. These findings imply that the infection model would be useful for predicting the severity of smoulder infections, when expressed as the premature loss of green leaf area due to disease, and that frost is unlikely to be a major factor in smoulder epidemiology.

3.3.2. White mould model

Using the infection model, there was a positive relationship between the predicted cumulative number of white mould lesions and the observed % die-back in crops in Cornwall in 1998/1999 and 2000/2001. However, as in the case of the smoulder model, there was no relationship between the predicted cumulative number of lesions and the observed number of white mould lesions, probably for similar reasons.

Objective 4: To use tissue wetness models and other disease development criteria to provide a system for alerting growers to the timing of effective spray applications of appropriate fungicides.

4.1. Fungicide trials

4.1.1. Leaf assays – *B. narcissicola*

All of the fungicides tested reduced lesion size when applied as protectant sprays. Ronilan, Scala, Unix, Folicur, Opus and Punch C were particularly effective, resulting in >90% reduction in lesion area. The fungicides were generally less effective when applied as curative sprays, and several treatments gave little or no control. However, Amistar, Folicur, Ronilan, Scala and Unix resulted in a reduction of lesion area of >60%. Benlate and Dithane 945 applied alone each gave only slight control, but the mixture showed synergistic protectant activity. These experiments demonstrated, for the first time, good activity against *B. narcissicola* by anilinopyrimidine fungicides (Frupica, Scala and Unix) and triazole fungicides (Folicur, Opus and Plover), and some activity by a strobilurin fungicide (Amistar).

4.1.2. Leaf assays – *R. vallisumbrosae*

Amistar, Benlate + Dithane 945 and Scala were all very effective, completely preventing the establishment of *R. vallisumbrosae* when applied as protectant sprays. Ronilan and Dithane 945 were ineffective. The same three fungicides were the most effective treatments against *R. vallisumbrosae* when applied after inoculation, while Bravo, Stroby WG, Unix, Frupica and Shirlan appeared only slightly less effective. These results demonstrated, for the first time, activity of anilinopyrimidine (Scala) and strobilurin fungicides (Amistar) against *R. vallisumbrosae*, and confirmed the good activity of Benlate + Dithane 945.

4.1.3. Smoulder trials at Mepal and Kirton

Two replicated field trials on second-year-down cv. Carlton were each carried out twice in experiments at Kirton and Mepal. Severe attacks of smoulder occurred in all cases.

In 2000, four new fungicides (Amistar, Folicur, Scala and Unix) were compared with three standard products or mixtures (Benlate + Dithane 945, Bravo 500 and Ronilan) in six-spray fungicide programmes. No reduction in smoulder primary lesions was observed, but most treatments reduced both secondary lesions on leaves and flower stalk rot. Leaf die-back was noticeably delayed by Amistar, Benlate + Dithane 945, Folicur, Ronilan, Scala and Unix. All fungicide treatments resulted in increased bulb yields compared with untreated controls, increases being greatest with Folicur (48%) and Scala (50%) at Mepal, and Unix (12%) at Kirton. Yield increases were negatively associated with the area of leaf die-back.

In 2001, eight spray programmes, ranging from two to six sprays each, were devised and tested. Sprays were directed at three growth stages: 1, shoot emergence; 2, around flowering, and 3, after flowering, with two sprays at each stage. Up to three products (Folicur, Ronilan and Scala) were used in each programme. Disease control, as judged by foliage die-back, was greatest when either a full, six-spray programme was used, or using two sprays around flowering and two sprays after flowering. However, bulb yield increase was greatest using a four-spray programme comprising two sprays of Ronilan during emergence followed by two sprays of Folicur around flowering.

4.1.4. White mould trials at Mepal and Kirton

Two replicated field trials on second-year-down cv. Cheerfulness were each carried out twice in experiments at Kirton and Mepal. No white mould developed despite inoculation with *R. vallisumbrosae*, but useful results were obtained on smoulder control following natural attacks of this disease.

In 2000, four novel fungicides (Amistar, Folicur, Scala and Stroby WG) were compared with three standard products or mixtures (Bravo 500, Bavistin DF + Dithane 945 and Benlate + Dithane 945). Treatments at both sites reduced secondary smoulder, with Amistat being outstanding at Mepal and Stroby WG at Kirton. At both sites Folicur and Stroby WG resulted in prolonged green leaf retention. All fungicide treatments increased bulb yield, with Folicur providing the greatest benefit at Mepal (58% increase over untreated controls) and Stroby WG at Kirton (35% increase). Bavistin DF + Dithane 945 resulted in similar yield increases to Benlate + Dithane 945 (26-31% increases at Mepal, 19-23% increases at Kirton).

In further work at Mepal and Kirton in 2001, again no white mould developed despite inoculation with *R. vallisumbrosae*, but useful results were obtained following a natural attack of smoulder. Programmes of five or six sprays of some novel fungicide mixtures were compared with Bavistin DF + Dithane 945. Fungicide treatments significantly reduced *Botrytis* stem rotting from 47% (untreated controls) to 22 to 34%. They also reduced leaf die-back and increased bulb yield. Bavistin DF + Folicur and Amistar +Folicur were particularly effective at both sites. The greatest bulb yield increase over untreated controls was 59% at Mepal (with Bavistin DF + Folicur) and 24% at Kirton (with Amistar + Folicur).

4.1.5. White mould trials at Penzance

Three experiments were carried out in commercial crops of second-year-down cv. Yellow Cheerfulness (cv. Cheerfulness in 2002) at Penzance. Severe white mould occurred in the untreated control plots each year. In 1999, programmes of one to five sprays of Benlate + Dithane 945 were compared in an attempt to determine the importance of different spray timings. Programmes of one or two sprays in February/March (leaves 10-15 cm long) gave little control of white mould or leaf die-back. Programmes of three to five sprays all gave very good control of white mould and leaf die-back. A three-spray programme, commencing in late-March when white mould was at a low level, was as effective as a five-spray programme starting one month earlier.

In 2001, spray programmes of Bavistin DF + Dithane 945 commencing at different growth stages (0-5 and 10-15 cm leaf length), or at first symptoms of white mould, were compared with some novel fungicide mixtures (Bavistin DF + Folicur, Bavistin DF + Ronilan, Bavistin DF + Scala and Amistar + Folicur), all starting at the 10-15 cm stage. White mould did not occur until mid-April, and there was no benefit of spraying early at the 0-5 cm stage. Commencing with Bavistin DF + Dithane 945 at first symptoms resulted in a saving of four sprays, but gave inferior disease control to a preventative programme starting at the standard first spray time (10-15 cm stage). Two of the novel fungicide mixtures

(Amistar + Folicur and Bavistin DF + Folicur) gave very good disease control, and appeared to be slightly better than Bavistin DF + Dithane 945 applied at the same timings.

In 2002, Amistar + Folicur was compared with Bavistin DF + Dithane 945 in each of four spray programmes: every 10-14 days from the 10-15 cm stage; every 21-28 days from the same stage; every 14 days from first symptoms; and one spray at 10-15 cm then every 14 days from first symptoms. Amistar + Folicur applied at 21-28 day intervals (three sprays in total) gave control equal to that of Bavistin DF + Dithane 945 every 10-14 days (six sprays in total), resulting in a saving of three sprays over the standard fungicide programme. Amistar + Folicur applied at 14 day intervals from first symptoms (three sprays) resulted in significantly better control of white mould and reduction in leaf die-back than Bavistin DF + Dithane 945 applied at the same timings, indicating that the former treatment is probably the better choice to treat established white mould.

4.1.6. Cost-benefit analysis of treatment for smoulder in eastern England

In the circumstances of these trials, conducted around 2000 with high levels of secondary smoulder development and associated early foliage senescence, and assuming a return of £300 per tonne and £450 per tonne for cultivars Carlton and Cheerfulness, respectively, all treatments resulted in a positive margin over the cost of fungicide. The cost of spray application was excluded. Nevertheless, the margins over chemical costs were large, ranging from £394 to £1359 per ha at Kirton and from £1994 to £4849 per ha at Mepal for cv. Carlton. The margins on cv. Cheerfulness ranged from £602 to £2850 per ha at Kirton and from £1246 to £6234 per ha at Mepal. The greatest margins in each experiment were given by Folicur on cv. Carlton at Mepal, by Unix on cv. Carlton at Kirton, and by Folicur on cv. Cheerfulness at both Mepal and Kirton. In these experiments, treatment with Folicur was notable for maintaining green leaf area. Even with narcissus bulb prices having fallen considerably since the start of the project – say to an average of £250 per tonne in 2002, compared with £400 to £500 per tonne initially – there is a clear financial benefit of treatment. Bulb prices are likely to continue to be cyclical.

4.2. Formulation of Spray Timing System (STS)

The STS would operate by detecting events in the field that would cause damage to the crop. Environmental conditions subsequent to damage events would then be used to predict the amount of smoulder and white mould likely to arise, via models of the effects of leaf wetness duration and temperature on the numbers of lesions of smoulder or white mould developing. Identifying these critical periods of crop damage, wetness and temperature through local environmental logging would provide a system alerting the grower or advisor of the need to apply fungicide sprays as soon as practical after the warning. Other factors favourable to disease infection and spread would augment this warning, for example perhaps reinforcing or deferring the urgency of crop spraying under borderline conditions. Factors that would reinforce the need to spray would include (a) adverse weather conditions likely to cause damage to leaves, primarily hail, heavy prolonged rain or frost, (b) the crop being grown for longer than the usual two-year cycle (in normal two-year-down growing the incidence of smoulder or white mould in the second crop year does not appear to be closely correlated with the incidence of disease in the first crop year), and (c), for white mould only, the occurrence of suitable conditions for resting body germination (temperatures of <10˚C and rainfall of >20 mm during the previous week in the mid-December to early-March period).

Technology transfer and exploitation

Technology transfer

The project showed that significant increases in bulb yield and disease control could be achieved through using novel fungicides even with fewer applications than previously used. The individual industry partners (representing 70 - 80% of the UK narcissus area) were able to make use of this information to modify their fungicide spray programme for the control of foliar diseases. General information about the project was made available through articles in HDC News and the Agriculture Link newsletter and presentations at meetings for bulb growers, subject to safeguarding the interests of the industrial members of the Consortium. Further technology transfer is being undertaken, including articles for HDC News, the Agriculture Link newsletter and the commercial press, presentations at meetings for bulb growers, papers for refereed scientific journals, and the preparation of HDC fact-sheets on the control of foliar diseases of narcissus.

Exploitation

The industry partners made it clear that they would prefer the development of a simple 'spray alert' system, operating rather like the HDC narcissus fly forecast, rather than a more complex web-, PC- or MORPH-based system. To deliver this, a two-year, industry funded project would be necessary. This would comprise:

- Validating the smoulder and white mould infection models in the field at selected sites where the diseases occur in most years, the sites being identified by growers. Monitoring precipitation impact events, frost and other criteria at these sites would be important in determining the primary causes of leaf damage. The models should also be tested at these sites to determine their validity at different stages of crop growth.
- Validating the STS, including confirming the levels of precipitation impact and demonstrating the practical use of PI and SW sensors
- Designing a delivery system for the STS, a simple system of alerting growers and consultants delivered by automated fax or email systems and including advice on the appropriate fungicides to use at each spray occasion
- Testing of the delivery system by the industrial partners
- Training in the use of the system

Proposals for a 'product development and technology transfer' project are being prepared for submission to Cornwall Horticulture Enterprises Ltd under the EU 'Objective 1' scheme for Cornwall and the Isles of Scilly. Discussions are under way with the HDC, growers and others to secure industry funding. At an appropriate stage there would be scope for marketing the STS to other UK bulb growers and growers in other countries where narcissus are grown under broadly similar conditions to the UK.

SCIENCE SECTION

Introduction

UK Bulbs

UK bulb growers are the world leaders in the production of daffodil (narcissus) bulbs and cut-flowers. Bulb production in the UK is mechanised and highly efficient, producing a saleable output of some 30,000 tonnes annually (Hanks, 2002), and different climatic areas (from the Isles of Scilly to Scotland) are used to produce flowers over a long season. Over the last few years, the area of field-grown narcissus in England and Wales has consistently been about 4,000 ha (DEFRA, 2002a). Additionally, some 390 ha of narcissus are grown in Scotland (Hanks, 2002), and a further significant area (perhaps 200 - 300 ha) of narcissus bulbs has been grown in recent years for the extraction of galanthamine, a promising drug now approved for use in Alzheimer's disease. Currently, nearly 3000 t of narcissus bulbs (about 50 million bulbs) are forced annually under glass for out-of-season flowers and several million bulbs are forced as pot-plants (DEFRA, 2002b).

By sales value, narcissus rank fifth for flower sales in the UK (figures from Flowers & Plants Association), despite being a relatively low-value flower available for only part of the year. As well as serving a traditional home market and, increasingly the high-quality multiple retailer sector, bulbs and flowers are sold throughout Europe, to the USA, and elsewhere. Exports of narcissus bulbs (including those both to EU countries and to third countries) are currently valued at £5m, and the addition of narcissus flowers brings the total of narcissus exports to around £20m annually (estimated from DEFRA, 2002a). This is a remarkable achievement for the bulbs and outdoor flowers sector, an industry with a total primary gate value of about £44m (of which narcissus bulbs, flowers, forced flowers and pot-grown bulbs contribute about £25m) (figures for 1999, estimated from DEFRA, 2002a). Using DEFRA conversion rates, these gate values equate to estimated *added retail values* in excess of £275m for bulbs and outdoor flowers and £156m for narcissus alone. Narcissus production is an essential part of a much larger UK business involving bulb and flower imports, exports and added value.

The problem

The yield and quality of narcissus bulbs are often seriously reduced by bulb rots (basal rot and neck rot, associated primarily with *Fusarium oxysporum* f. sp. *narcissi*), and, since the persistent insecticide aldrin was withdrawn in 1989, by damage caused by larvae of the large narcissus fly (*Merodon equestris*). The understanding and control of bulb rots and narcissus fly have been of high priority and the focus of research on narcissus over the last 20 years, and continue to be so, with three DEFRA-funded strategic research projects in progress (basal rot, HH1008SBU; neck rot, HH1727SBU; and narcissus fly, HH1747SBU).

More recently, there has been increasing concern among UK bulb growers about the foliar diseases of narcissus, primarily smoulder and white mould. Smoulder is a widespread disease occurring wherever daffodils are grown. In the standard text, it was stated that "Opinions differ on the economic importance of the disease" (Moore *et al*., 1979), but the loss of yield was estimated by Consortium members as perhaps 10%, a consistent, if non-dramatic, loss. The smoulder pathogen, *Botrytis narcissicola*, can also cause bulb rot during prolonged storage (Moore *et al*., 1979) and is implicated, with other fungi, in bulb neck rot (Linfield & Hanks, 1994) and skin diseases (Bergman *et al*., 1978). In contrast, white mould (*R. vallisumbrosae*) has caused epidemics in crops in Cornwall in recent years, with considerable loss of bulb and flower yields, but although it occurs on commercial crops in Cornwall, Devon and the Isles of Scilly, it is not a problem in other bulb-growing areas.

It is likely that smoulder and white mould have become more significant in the last 20 years because of changes in the methods used by the UK bulb industry. For example, higher planting densities are used, flower are cropped even by growers who once grew narcissus only for the bulbs, and less roguing (removal) of diseased plants is carried out, all leading to the more rapid spread of pests and diseases. Further, pathogens build up in UK crops because of the 'two-year down' growing system for narcissus used almost universally in the UK – bulbs are planted one year and harvested two years later - and crops are now being grown for three or more years, multiplying these problems. It would not be desirable to reverse any of these husbandry practices, which have all been developed to reduce costs and increase income in an industry where gross margins are small. Compared with the 'one-year-down' system, the two-year growing system produces a good yield of smaller bulbs with more flowers per tonne, and has been proven to be more economical in the UK than the conventional one-year system used in the Netherlands (Rowell, 1986). Consequently, controls for foliar diseases need to be robust enough to cope with these agronomic challenges – for financial reasons changing these practices, even for the sake of pest and disease control, is not an option in the present economic climate.

There has been no recent research in the UK on narcissus white mould, and little on smoulder (see review below), with the exception of the testing of various fungicide spray programmes (mainly carried out in the 1970s and 1980s). At present, bulb growers attempt to control the diseases using a programme of fungicide sprays applied through the growing season. In some cases, up to six or seven sprays may be used per season, often by spraying every 5 to 7 days or as soon as weather conditions are fit for spray operations. These sprays are applied without knowing whether they are given at the most effective times or, indeed, whether they are effective at all. This Horticulture LINK project addressed these questions, and will lead to more effective and rational disease-control strategies through disease forecasting techniques enabling growers to target the most appropriate spray dates. The importance which the UK bulbs industry attaches to this research is evidenced by the fact that the individual industry partners making up the Consortium are considered to represent 70 - 80% of the UK narcissus acreage.

The project

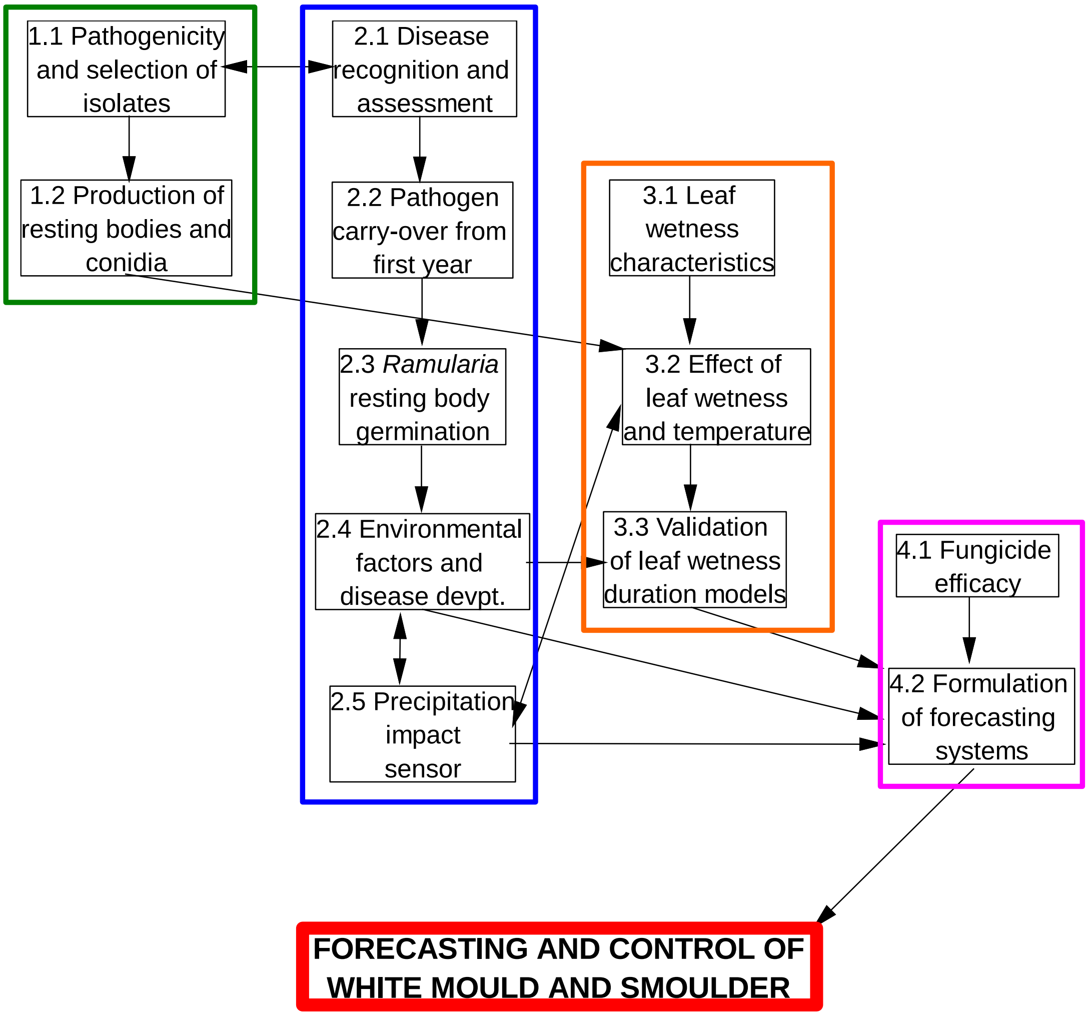
The aim of the project was: **"To improve our understanding of the spread of white mould and smoulder, and develop better control strategies through disease forecasting techniques".** This aim was delivered through four objectives:

1. To produce methods for the reliable production of resting bodies and conidia of *R. vallisumbrosae* and conidia of *B. narcissicola*, using typical pathogenic isolates.

2. To determine the effect of initial inoculum and environmental factors (including rainsplash for *R. vallisumbrosae*) on the epidemiology of white mould and smoulder within second-year daffodil crops.
3. To construct and test models relating tissue wetness duration and temperature to infection of narcissus tissues by *R. vallisumbrosae* and *B. narcissicola*.
4. To use tissue wetness models and other disease development criteria to provide a system for alerting growers to the timing of effective spray applications of appropriate fungicides.

The relationships between these objectives and their subordinate tasks are shown in Figure 1. In planning the project, the likelihood of impediments to achieving individual tasks was considered, and alternatives planned where appropriate: for example, white mould field trials were planned using commercial crops in Cornwall, in case it was not possible to establish the disease at research sites in the east. Full experimental details and results can be found in the four Annual Reports previously issued to Consortium members and the HDC. Unless otherwise stated, narcissus cv. Carlton, one of the two most widely grown varieties in the UK, was used in this project. Details of sites used are given in Appendix 2. The project milestones are listed in Appendix 3.

Figure 1. Flow-chart showing the interdependence of objectives and tasks



Literature review

White mould There appears to be no recent research or review on *R. vallisumbrosae* on narcissus. Although the pathogen is reported to be distributed widely in the bulb growing areas of Western Europe and North America (Moore *et al*., 1979), the disease does not appear in standard Dutch textbooks on daffodil growing (e.g., Bergman *et al*., 1978; van der Zwet *et al*., 1990), and is rare in the Pacific north-west of the USA (Chastagner, personal communication). In the UK, experimental work on the aetiology, symptomatology and control of the disease was published by Gregory in the late 1930s (Gregory, 1936, 1937, 1939, 1940). At this time Bordeaux Mixture was the fungicide used. Large increases in yield, presumed due to control of white mould, were obtained using high-volume sprays of tank-mix zineb (nabam + zinc sulphate) with petroleum oil emulsion (Jenkins & Hawken, 1969; Forsberg, 1976). Field trials on leaf diseases were carried out over the lifetime of Rosewarne Experimental Horticulture Station (EHS) (Cornwall) (1952-1989); these were rarely specific for white mould, and consisted mainly of applying various fungicide formulations (see summaries in Melville, 1980 and Linfield & Hanks, 1994). Disease levels in trials were often low, and the results were concerned as much with green leaf retention and bulb and flower yields as with contributing to the understanding of leaf diseases. However, partly as a result of this work, mancozeb + benomyl became a standard spray treatment, often with three sprays applied between shoot emergence and flower cropping and a fourth post-cropping (Moore *et al*., 1979). Chlorothalonil was also reported to be effective (O.P. Jones, personal communication). Despite these studies, the fungicide sprays used now are manifestly not fully effective in controlling white mould. A striking feature of white mould is that significant areas of crops can become seriously affected within a few days of the first ‘spots’ being seen.

Smoulder Smoulder was also studied in the UK in the 1940s (Gregory, 1941), but, unlike white mould, its epidemiology has also been investigated more recently. Narcissus crops in northern Scotland were studied by Gray (1971), Gray *et al*. (1975), Gray & Shiel (1975, 1987), Dixon (1986), O'Neill & Mansfield (1982) and O'Neill *et al*. (1982). These studies showed the importance of wet conditions in the development of the disease, and the need for damaged leaves and flower stems before infection can take place. Dipping bulbs in a benzimidazole fungicide were shown to increase bulb yields, presumably by controlling smoulder (Gray, 1971; Gray & Shiel, 1975). Several trials on the control of smoulder by fungicide sprays and cultural means were carried out at Kirton EHS (Lincolnshire) over the period 1972-1978, and many of the comments about spray trials at Rosewarne (see above) apply to this work (see review of Linfield & Hanks, 1994). The range of fungicides sprayed on narcissus crops to combat smoulder was derived in part from these trials. Fungicide spray programmes and bulb dips were, however, often ineffective (Gray, 1971; Melville, 1986). The disease affects mainly crops in their second (or subsequent) year, but cultural operations aimed at reducing the carry-over of infective débris from the first year (e.g. re-ridging crops or burning débris) were not always effective (Melville, 1986).

Unlike white mould, smoulder occurs on Dutch narcissus crops, although it is reportedly seldom of economic importance (van der Zwet *et al*., 1990). Recommendations in Holland include starting a spray programme immediately after shoot emergence, using maneb, mancozeb or zineb/maneb repeated a few times at 7 to 10 day intervals, followed by one or two sprays of benomyl, carbendazim, procymidone (a compound never permitted in the UK), iprodione or vinclozolin just before lodging, taking care to wet the soil surface thoroughly. In Dutch narcissus growing, ‘fire’ (*Sclerotinia polyblastis*) and leaf scorch (*Stagonospora curtisii*) appear to be of more concern, the latter sometimes being wrongly identified as smoulder (van der Zwet *et al*., 1990). In the Netherlands narcissus are usually grown as a one-year-down crop. Dutch research interests in *B. narcissicola* have concentrated in its role in neck rot and in 'skin diseases' (largely in dwarf cultivars), and these have been largely concerned with bulb handling and fungicide dip treatments (including hot-water treatment; e.g. van der Weijden, 1989; Vreeburg & Schipper, 1990).

Disease forecasting is being investigated in the UK for field vegetable crops, but has not yet been applied to flower crops. In the Netherlands, work has been done recently on disease forecasting in *Botrytis elliptica* affecting lily crops (Bastiaansen *et al*., 1997) and in *B. tulipae* of tulips, but it was ascertained at the start of this project that there were no plans to extend this work to narcissus (van den Ende, personal communication, 1997).

Establishing protocols for the production of resting bodies and conidia of *R. vallisumbrosae* and conidia of *B. narcissicola* (Objective 1)

Pathogenicity and selection of isolates (Task 1.1)

Introduction

To obtain cultures of *B. narcissicola* and *R. vallisumbrosae* for use in subsequent parts of the project (Tasks 1.2 and 4.1), isolations were made from smoulder and white mould symptoms on affected plants. Isolates of the fungi that grew and sporulated well in culture, and were demonstrably pathogenic to narcissus, were sought.

Isolation of pathogens from narcissus leaves

Narcissus leaves showing suspected disease symptoms were collected from commercial crops in Cornwall, Cambridgeshire and Lincolnshire between February and July 1998. Fungi associated with the symptoms were determined by microscopic examination and by plating onto an agar medium. White mould (*R. vallisumbrosae*) was confirmed in samples from the South-West only, whilst smoulder (*B. narcissicola*) was confirmed in samples from both the South-West and eastern England. *R. vallisumbrosae* was present on oval leaf lesions. It usually appeared as a white sporulating mycelium, sometimes with small black resting bodies present at the edge of lesions. *B. narcissicola* was found in leaf edge lesions, leaf tip lesions, dead leaves and in flower stalks that were rotting from the tip. Grey mould (*Botrytis cinerea*) was found in dead leaves, in flower stalks rotting from the tip, and in rotting flowers. No fungus was consistently isolated from rusty brown leaf flecks (commonly seen on the inner surface of leaf bases and known as ‘physiological rust’) or on pale brown leaf tips, the symptoms of scorch due to *Stagonospora curtisii*. Various other fungi were isolated from leaves, including a *Fusarium* species, a pale brown sterile fungus and a grey, slow-growing sterile fungus.

Narcissus cultivars noted to be affected by smoulder included Standard Value, Carlton, Barrett Browning, Corinthian, Lothario and Winston Churchill, while cultivars Carlton and Cheerfulness were seriously affected by white mould in Cornwall. Ten isolates consistent with *B. narcissicola* and five of *R. vallisumbrosae* were established in clean culture.

Pathogenicity

Detached leaves, detached bulb scales and pot-grown narcissus plants were used in replicated pathogenicity tests. To supply narcissus leaves out-of-season, narcissus bulbs were pot-grown using cold- and warm-retarding techniques. Inocula consisted of spore suspensions or mycelial discs on V8 juice agar (*B. narcissicola*) or oatmeal agar (*R. vallisumbrosae*). Leaves were inoculated both directly and after wounding the surface with a sterile needle. Inoculated material was incubated in damp conditions and mycelial growth and leaf rotting assessed at intervals. The fungi associated with lesions was determined on a sample of leaves by re-isolation.

Inoculation with mycelial plugs on wounded tissue was found to be the most effective procedure for the production of lesions by both *B. narcissicola* and *R. vallisumbrosae.* No tissue rotting occurred with any of the isolates following inoculation of intact or wounded leaves with conidial suspensions in water. All isolates of *B. narcissicola* caused rotting of leaves and bulb scales, with isolate 98/23 affecting both detached and attached leaves more quickly than other isolates (Table 1.1.1). This isolate was chosen for use in further tests. *R. vallisumbrosae* isolates 98/101, 98/102a and 98/120 all caused a high level of infection on detached wounded narcissus leaves (Table 1.1.2). On attached leaves, no lesions appeared until 14-20 days after inoculation. By 37 days after inoculation there was abundant sporulation at most sites.

Table 1.1.1. Pathogenicity of *B. narcissicola* from mycelial inocula on detached narcissus leaves and bulb scales.

Isolate	Mean number of inoculation sites (per 20) developing spreading lesions			
	Intact leaves		Wounded leaves	
	2 days	5 days	2 days	5 days
On leaves				
98/21	0	0	2	19
98/22	0	1	17	20
98/23	0	6*	19	20
98/105	0	1	11	19
98/108	0	0	6	16
Control	0	0	0	5
On bulb scales				
98/21	2	4	2	13
98/22	8	17	12	17
98/23	1	11	8	18
98/105	3	16	10	19
98/108	0	1	0	8
Control	0	0	0	0

*Fleck lesions at point of fungus-leaf contact

Table 1.1.2. Pathogenicity of *R. vallisumbrosae* on detached narcissus leaves.

Isolate	Mean no. of inoculation sites (per 20) developing spreading lesions			
	Intact leaves		Wounded leaves	
	11 day	14 day	11 day	14 day
98/101	0	0	15	19
98/102a	0	0	14	19
98/102b	0	0	9	13
98/120	0	0	13	18

Production of resting bodies and conidia (Task 1.2)

Introduction

To provide sources of spores and resting bodies for use in further experiments, protocols for culturing *B. narcissicola* and *R. vallisumbrosae* were developed and made available to the research group. 'Best practice' protocols were refined as the work progressed.

Culture of fungi

Isolates of *B. narcissicola* and *R. vallisumbrosae* were cultured on tap-water agar (TWA), V8 juice agar (V8), potato dextrose agar (PDA), oatmeal agar (OA), malt extract agar (MEA), Medium X (Last & Hamley, 1956) and daffodil leaf extract agar (DLEA). Cultures were placed either in a non-illuminated incubator at 18°C or under UV-light (12 hour light/dark cycle) at ambient laboratory temperature. Mean daily growth rates were calculated and the extent of sporulation and production of sclerotia or resting bodies were assessed on an increasing scale from 0 to 5.

B. narcissicola

Isolates of *B. narcissicola* generally grew well on PDA and V8-juice agar and produced sclerotia on all media. Conidial production on all media was sparse when plates were incubated in the dark, but was considerably improved when plates were placed under a UV-light/dark cycle. This successfully induced moderate sporulation on DLEA.

R. vallisumbrosae

Isolates of *R. vallisumbrosae* grew considerably more slowly than *B. narcissicola*. Fastest growth was obtained on V8 agar, whilst greatest spore production was on OMA with cultures incubated at in the dark. Resting body production was good on PDA, V8 and OMA.

Potted narcissus plants were inoculated on their leaves with mycelial plugs of *R. vallisumbrosae* and grown in a cool, moist environment for 1 week. White mould lesions developed at most inoculation sites, causing leaf yellowing and collapse. Resting bodies of *R. vallisumbrosae* developed at some inoculation sites, but at others soft rot and secondary fungal infections predominated. To circumvent this difficulty in culturing *Ramularia*, large quantities of narcissus leaves naturally infected by *R. vallisumbrosae* and bearing abundant resting bodies of the fungus were collected from the disease monitoring site at Manaccan and stored for use in Tasks 2.3 and 4.1. This obviated the specific need to produce resting bodies for experimental work by the inoculation of leaves or in culture.

The effect of initial inoculum and environmental factors on the epidemiology of white mould and smoulder in second-year crops (Objective 2)

Disease recognition and assessment (Task 2.1)

Introduction

Diseases of narcissus and their control have previously been reviewed by Bergman *et al*. (1978), Moore *et al*. (1979), Chastagner & Byther (1985), ADAS (1985, 1986) and van der Zwet *et al*. (1990). Smoulder (*B. narcissicola*) has been well described in this literature, but information on white mould (*R. vallisumbrosae*) is old and relatively sparse. Of the three main narcissus producing countries – the UK, Netherlands and USA – white mould appears to be a problem only in the UK, possibly because of the predominance of the two-year-down growing system. The aim of this task was to provide clear descriptions of disease symptoms and a protocol for practical disease assessment.

Crop assessment

Several narcissus crops were examined at the farms of the industrial partners in Cornwall and in the east of England in spring and summer 1998. Disease symptoms were noted and compared with descriptions in the literature, and numerous samples were examined in the laboratory at ADAS Arthur Rickwood for the identification of pathogens and for use in Objective 1.

White mould symptoms

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

Smoulder symptoms

In the early stages of shoot emergence in winter/spring, smoulder appeared as ‘smoulder primaries’, i.e. heavily infested, recently emerged 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 two or more cm in length, bearing grey sporulating material that appeared fluffy under a hand-lens; the lesions were bounded by yellowing areas. One-sided lesions resulted in the leaf bending at this point due to restricted growth. In some cases, leaf lesions spread rapidly late in the growing season, a feature not described in the literature. The leaves sometimes 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. When pulled up, such withered leaves often carried sclerotia or a grey mass of spores at the base. Small (1 - 2 mm diameter) oval or circular black sclerotia could also be found on leaf debris. Smoulder can also cause flower spotting, although this was not observed in the present study. In some cases, laboratory investigations showed that the spores were of grey mould, *B. cinerea*, rather than *B. narcissicola*, but it was not practical to distinguish the two in the field.

Other disease and disorder symptoms

Leaf scorch (*Stagonospora curtisii*) was also found, and mainly affected the leaf tips resulting in a reddish-brown or blackening zone bordered by a yellowing area. The lesions could spread down the leaf, although during the present project they appeared mainly to be restricted to the tips. Black pycnidia may appear in the lesions. Although not seen in the present study, the flowers may also be affected and plants may senesce early. Other disorders needed to be distinguished from the foregoing diseases. ‘Chocolate spot’ consists of dark chocolate brown-coloured spots and streaks on leaves and flower stalks, while ‘physiological rust’ consists of rust-coloured lesions that may be common on leaf and flower stalk surfaces, sometimes serious enough to cause significant disfigurement; both are considered to be physiological disorders. In premature leaf senescence, individual leaves or whole shoots may become yellow and die prematurely, in advance of the bulk of the crop; this is not associated with dark lesions as in the case of smoulder, and may be due to underlying *Fusarium* or other bulb rots, or to drought or root disturbance. Frost on newly emerged shoots may cause death of the leaf tips, often occurring uniformly across a whole crop. Slug damage results in ragged leaf tips. Other damage may occur, such as herbicide damage (general yellowing of foliage, often at row ends where sprayers are turned on) or physical damage from weather (hail or wind) or field operations (such as flower cropping or roguing).

Disease assessment

Assessment protocols were developed for recording the frequency, severity and distribution of white mould and smoulder, based on using a fixed, X-shaped sampling pattern of 50, 0.5m-long sampling areas within a 0.1 ha crop area in which no fungicide sprays were applied. Initially, the frequency of disease symptoms was recorded on a scale from 0 (absent) to 3 (>50% of leaves per sample area affected), but this method was considered insufficiently quantitative for disease modelling purposes when disease spread was rapid. Subsequent monitoring involved recording the numbers of disease lesions per leaf and leaves per plot, and the percentage of leaf area dead or affected by disease. In serious cases of white mould and late-season smoulder, counting the numbers of individual lesions became impractical later in the season. These protocols were successfully used for monitoring smoulder and white mould in Task 2.4.

Pathogen carry-over from the first year of crops (Task 2.2)

Introduction

The purpose of this task was to determine if the occurrence and extent of white mould and smoulder early in the second crop year is related to that recorded in crops or on leaf debris at the end of the first year.

Crop monitoring and disease carry-over

Results from 19 crops examined in the field at senescence and emergence (1999-2000 and 2000-2001) are summarised in Table 2.2.1. These indicated that crops which were unaffected by smoulder at crop senescence often, but not always, showed little or no smoulder at the start of the following season. However, some crops that were apparently healthy at senescence developed moderate levels of smoulder soon after emergence the following season. Crops which are obviously affected by smoulder at crop senescence appear to be at high risk of developing smoulder early the following season. There was no obvious relationship between relative incidence of smoulder at the end of one season and the incidence at the start of the next season.

White mould was confirmed only in Cornwall in these monitoring studies. The disease was found in all crops at the end of the first year of growth and in two out of four crops early in the second year (by day 42). The disease was not observed until early-March in the remaining two crops, and there is thus an increasing probability that infection may have spread into the crop from another location, rather than having been carried-over on site.

Table 2.2.1. Influence of inoculum occurrence at crop senescence on the incidence of smoulder and white mould early in the following season (Cornish crops identified by 'w' and eastern crops by 'e').

Disease and status at crop senescence	Number of crops in each category[a]				
	End of year 1	Start of year 2			
		Nil	Slight	Moderate	Severe
Smoulder - absent	11	5 (w)[b]	2 (e)	4 (e)	0
Smoulder – present	8	0	3 (e)	4 (e)	1 (e)
White mould - absent	14	14 (e)	0	0	0
White mould - present	4	2 (w)	2 (w)	0	0

[a] Slight, <1 lesion/0.5m; moderate, 1-5 lesions; severe, >5 lesions

White mould in leaf debris and disease carry-over

White mould resting bodies were identified in debris from one of the three sites in 1999. All three sites had developed white mould by mid-February 2000, being most severe at the site where the resting bodies had been detected. In summer 2000, white mould resting bodies were found in debris at all three sites, and they had all developed white mould by late-January in 2001.

Conclusions

- Although based on a relatively small data-set collected over two years, it appears that the occurrence of smoulder and (or) white mould early in crop growth is related as much to geographic location of the crop, as to inoculum level the preceding year.

- Crops in the South-West, even where treated with fungicides, are often affected by white mould at the end of year 1, and many (but not all) of these develop white mould early in year 2 (if the crop remains unsprayed).
- Crops in the South-West are not commonly affected by smoulder in either the first or second year.
- Crops in the east which are treated with fungicide in year 1 are often free of obvious smoulder at crop senescence; nevertheless, many of these crops show smoulder symptoms at the start of year 2.
- Crops in the east that were affected by smoulder at the end of year 1 all developed smoulder at the start of year 2; a majority was moderately or severely affected.
- Currently, crops in the east are not commonly affected by white mould in either the first or second year.

***Ramularia* resting body germination (Task 2.3)**

Introduction

When spore production on white mould lesions ceases, and the leaves begin to wither, masses of small black sclerotia or resting bodies, just visible to the unaided eye, are formed. These remain dormant in the leaf trash during summer and autumn but germinate in winter to produce spores which infect newly emerging leaves (Moore *et al*., 1979). Gregory (1939) observed that sclerotia began to sprout in January and February, producing long, thin *Cercosporella*-type spores, termed scolecospores. It has been suggested that the sclerotia of *R. vallisumbrosae* are the sexual stage of an unknown member of the Mycosphaerellaceae family, which instead of developing to become perithecia develop abnormally to become sclerotia.

The objective of this part of the project was to monitor the germination of *R. vallisumbrosae* resting bodies with a view to determining if the time of their germination in the spring could be used as a component of a spray timing system. Factors affecting the timing of their germination are unknown. Factors that have been found to influence germination of sclerotia of other fungi include low temperature (e.g., ergots of *Claviceps purpurea* require chilling), aeration (e.g. *Sclerotium rolfsii*) and moisture (e.g., rainfall promotes carpogenic germination of *Sclerotinia sclerotiorum* sclerotia) (Coley-Smith & Cooke, 1971; Dillard *et al*., 1995; Twengstrom *et al*., 1998).

1999-2000 experiment

Dead narcissus leaves severely affected by white mould and containing abundant resting bodies of *R. vallisumbrosae* were collected from Manaccan in April 1999 and allowed to dry. Small nylon mesh bags were filled with sterilised silver sand and 10 lengths of dried leaf bearing obvious *R. vallisumbrosae* resting bodies. Replicated sets of bags were attached to pegs and laid on the soil surface at Mepal (Cambridgeshire) and Penzance (Cornwall) in June/July 1999. Additionally at Mepal further bags were buried 5 cm deep.

Samples were recovered at monthly intervals starting mid-October 1999. At each sampling time, one bag was selected at random from each of the three rows. Leaf pieces were recovered from the sand by sieving, gently washed to remove remaining sand grains and then examined microscopically for evidence of spore production from the resting bodies. Leaves were also incubated in a humid chamber at laboratory temperature and re-examined after 7 days. Where fungal growth was found associated with resting bodies when examined by low power microscopy, five pieces per sample were examined at high power to determine if spores were characteristic of *Ramularia*.

Leaf pieces that had been buried in the soil were more friable and showed greater degradation than those placed on the soil surface. The recovery and cleaning process was effective and *Ramularia* resting bodies were readily found. Hyphal strands bearing amerospores and/or phragmospores, typical of *R. vallisumbrosae*, were found on occasional buried leaves at the first recovery in mid-October. Clumps of long, thin scolecospores, the type of spore produced by *Ramularia* resting bodies, were first confirmed on 7 January 2000, with peak germination around 1 - 7 February.

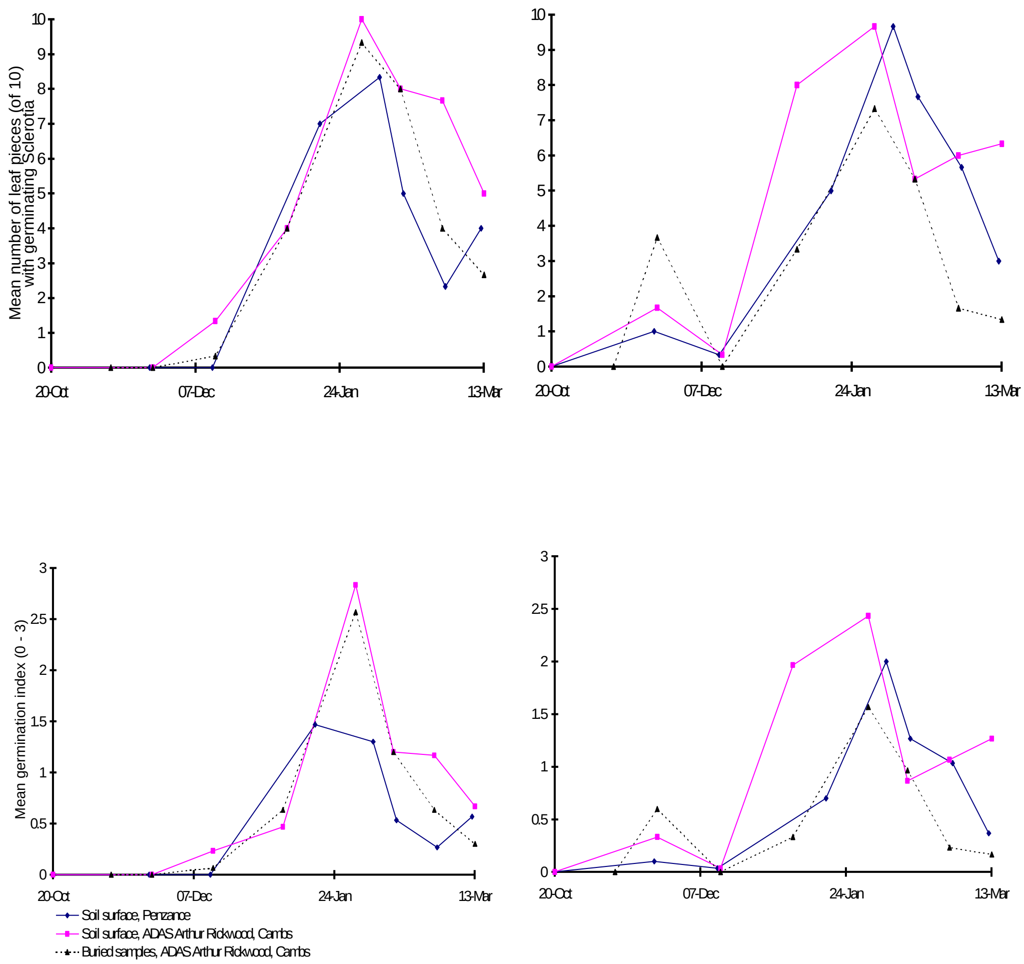
Germination then declined, although spore production was still visible on samples recovered in mid-March (Figure 2.3.1). There was little difference between sites in the time at which germination occurred.

The times of initial and peak germination of *Ramularia* on leaf debris showed no detectable difference at the Cambridgeshire and Cornwall sites. Germination occurred on samples buried at 5 cm as well as that on the surface, indicating light or daylength are unlikely to be trigger factors for germination. A summation of cumulative temperature, rainfall and frost events between 15 August and 31 March for Cambridge and Cornwall was prepared. Although Cornwall was noticeably warmer, wetter and had fewer ground frosts than Cambridge, especially in December, this did not have a detectable influence on the time at which *Ramularia* resting bodies germinated.

White mould was first reported on a narcissus crop at Manaccan on 26 January 2000, on leaf tips of a third year crop, at Tresillian on 9 February and at Truro on 11 February. Our first confirmation of white mould was thus just 19 days after we first confirmed scolecospore production in the South-West.

Figure 2.3.1. Germination of *R. vallisumbrosae* resting bodies on leaf debris, 1999/2000.

On recovery After incubation



2000-2001 experiment

Monitoring was repeated in 2000-2001. Resting bodies in leaf debris collected from a naturally infected crop in Cornwall were prepared in multiple sets of bags as previously described. Leaves were collected in early-June and stored dry at Mepal from receipt on 5 June 2001. The bags of leaf debris were placed on the soil surface at Mepal and Penzance on 19 June 2000.

The occurrence of germination on samples as they were recovered was first observed on 13 December 2000 at Mepal, and was occurring at both sites one week later (Table 2.3.1). Germination continued until late-January 2001. White mould lesions were not observed in commercial crops until early March 2000. When samples were incubated in a damp chamber for one week immediately after recovery, the rate of germination increased and the first detected germination was earlier (29 November 2000), indicating that sclerotia were at the point of germination at this time (Table 2.3.2).

Table 2.3.1. Germination of *R. vallisumbrosae* resting bodies from 1 October to 31 March at two locations, 1999-2000 and 2000-2001.

Week no.	% leaf pieces with germination[a]			
	1999-2000		2000-2001	
	Mepal	Penzance	Mepal	Penzance
42	0	0	-	-
43	-	-	-	-
44	-	-	0	0
45	-	-	-	-
46	-	-	-	-
47	0	0	-	-
48	-	-	0	0
49	-	-	-	-
50	13	0	63	0
51	-	-	50	20
52	-	-	-	-
1	40	-	60	30
2	-	70	-	-
3	-	-	53	30
4	-	-	-	-
5	100	-	16.7	0
6	-	83	-	-
7	80	50	-	-
8	-	-	-	-
9	77	23	0	0
10	-	-	-	-
11	50	40	-	-

[a] Assessed at time of recovery

Table 2.3.2. Germination of *R. vallisumbrosae* resting bodies on narcissus leaf debris at Mepal and Penzance, 2000-2001.

Sample date	Mean germination index (0-3)			
	Mepal		Penzance	
	At recovery	After incubation	At recovery	After incubation
1 Nov	0	0	0	0
29 Nov	0	1.3	0	2.7
13 Dec	1.3	3.0	0	1.4
21 Dec	0.9	2.5	0.4	2.3
5 Jan	1.1	2.3	0.6	1.5
17 Jan	1.0	1.9	1.9	1.7
1 Feb	0.3	0.6	0	0.5
28 Feb	0	0.1	0	0.8

The effect of temperature and moisture on germination

In order to identify the factor(s) which influence the time of resting body germination, replicate samples of narcissus leaves bearing *Ramularia* were incubated at Mepal under different conditions of temperature and moisture.

Temperature

1. At 5ºC in the dark
2. At 10ºC in the dark
3. At 15ºC in the dark

Samples were set up in September 2000 on moist filter paper in glass Petri dishes. Leaves were sprayed with water 2 or 3 times per week to keep the leaves damp.

Moisture

1. Outside on the soil surface, exposed to rainfall.
2. Outside suspended above the soil surface, shielded from rainfall

Samples were set up in July 2000. Leaves were prepared in nylon bags of sterile sand, as for the monitoring experiment.

The results are shown in Table 2.3.3. Exposure of resting bodies to rainfall or soil moisture favoured early germination. With the exception of the observation made on 28 February, no germination occurred on leaves kept dry, though the resting bodies germinated rapidly once placed in a humid incubator. The small amount of germination observed on ‘dry’ treatments on 28 February may have resulted from sufficient absorption of moisture over the previous weeks, while the lack of demonstrable germination in the ‘wet’ treatment at the same date may have been due to germination having already taken place earlier. Low temperature (5ºC) resulted in slightly earlier germination compared with higher temperatures. Elsewhere in this project (see section 3.2) it has been demonstrated that low temperatures (5-10°C) are optimal for infection of narcissus leaves by *R. vallisumbrosae*. These results suggest *R. vallisumbrosae* is adapted to germinate and infect narcissus in low temperatures.

The effect of weather on germination

Germination of resting bodies at the two sites in relation to weekly rainfall total and soil temperature is shown in Figure 2.3.2 and 2.3.3, respectively. At both sites there was relatively high rainfall (>20 mm) in the week preceding first germination. The mean soil temperature declined quite sharply in the period immediately preceding germination, to <10˚C at 30cm depth and <7˚C at 5cm depth.

Table 2.3.3. Influence of temperature and moisture on *R. vallisumbrosae* resting body germination, 2000-2001.

Sample date	Mean number of leaf pieces (per 10) with germination[a]				
	Temperature (ºC)			Moisture	
	5	10	15	Exposed to rain	Dry
10 Oct	0	0	0	-	-
1 Nov	0	0	0	0 (0)	0 (0)
7 Nov	0	0	0	-	-
14 Nov	0	0	0	-	-
21 Nov	0	0	0	-	-
29 Nov	-	-	-	0 (7.0)	0 (1.7)
8 Dec	0	0	0	-	-
13 Dec	0	0	0	6.3 (10.0)	0 (4.7)
22 Dec	10	3	4	5.0 (9.7)	0 (4.0)
3 Jan	10	3	5	6.0 (10.0)	0 (9.0)
9 Jan	10	4	10	-	-
18 Jan	10	7	10	5.3 (8.0)	0 (8.3)
24 Jan	10	7	10	-	-
31 Jan	10	10	10	1.7 (2.3)	0 (9.7)
8 Feb	5	5	8	-	-
13 Feb	5	4	3	-	-
22 Feb	2	2	3	-	-
28 Feb	0	2	6	0 (0.3)	2 (10.0)
7 Mar	0	2	4	-	-

[a] Figures in parenthesis are germination after 7 days' humid incubation at 15ºC

Conclusions

- Although time-consuming and requiring trained staff, a semi-quantitative determination of *R. vallisumbrosae* resting body germination is possible by microscopy of prepared leaf pieces.
- No germination was observed in the autumn period (weeks 42 – 49). Germination was first observed in the winter (weeks 50 – 02), continued in late-January to early-March (weeks 05 – 11) and then ceased, so that germination was observed over a period of several weeks from early-December to mid-March.
- Resting bodies collected from a common source (Cornwall) showed a similar, though not identical, germination pattern when maintained outside in Cambridgeshire and Cornwall.
- Laboratory experiments demonstrated that both moisture and temperature influence germination. No germination occurred when resting bodies were protected from rainfall. Germination at 5˚C was more consistent and slightly earlier than at 10 - 15˚C.
- Monitoring of resting bodies in Cambridgeshire and Cornwall suggested that germination may be preceded by cool, wet weather (soil temperature <10˚C at 30cm depth and >20mm rainfall in the preceding week).
- Further work is required to determine the degree of variation in the time of first germination, from site to site and from year to year, in order to determine its practical usefulness as a component of a spray-timing system. Results obtained to date suggest there is a risk of *R. vallisumbrosae* spores occurring from mid-December onwards.

Figure 2.3.2 Germination of *R. vallisumbrosae* resting bodies and rainfall, 1999-2000 and 2000-2001

Mepal Penzance

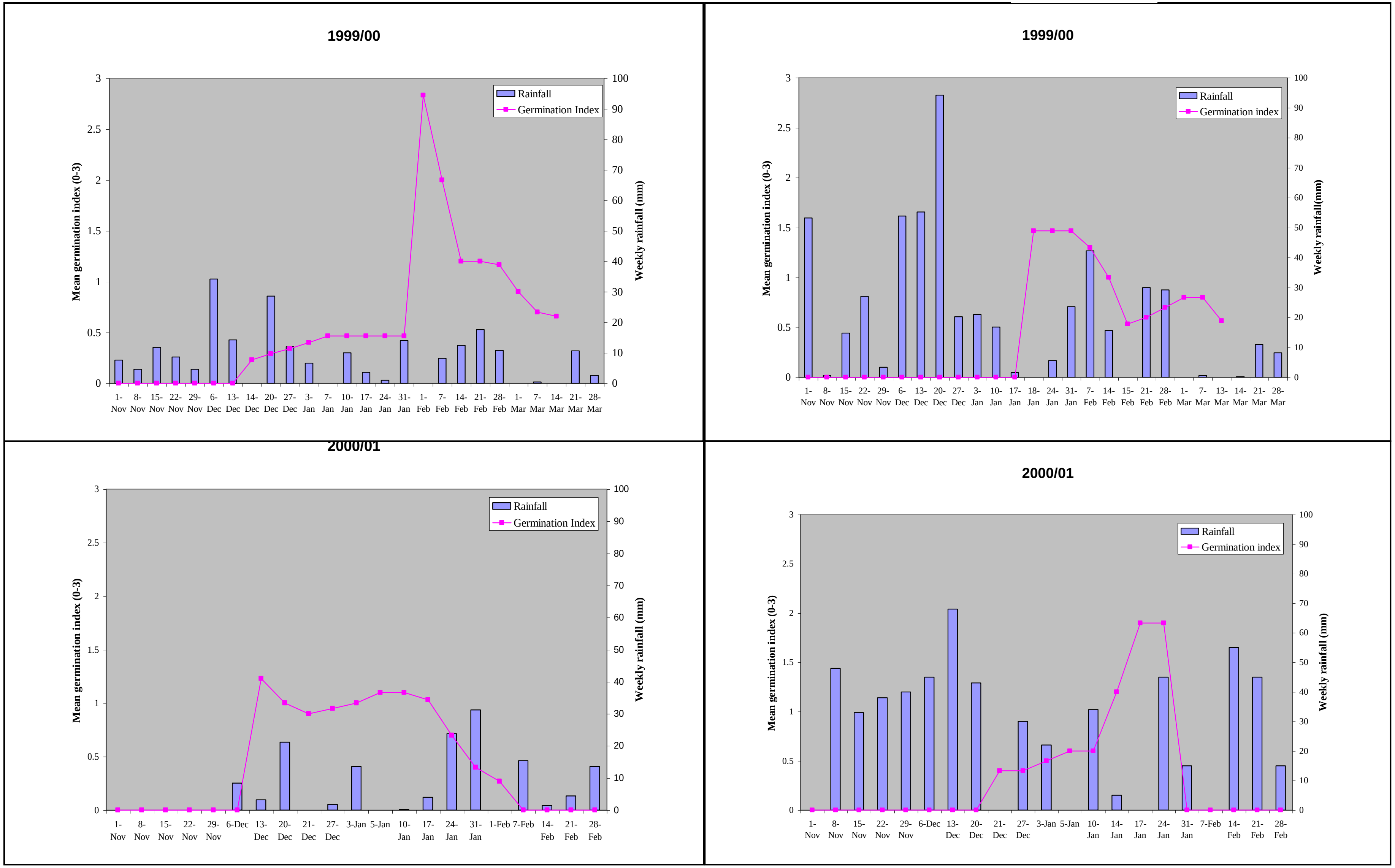
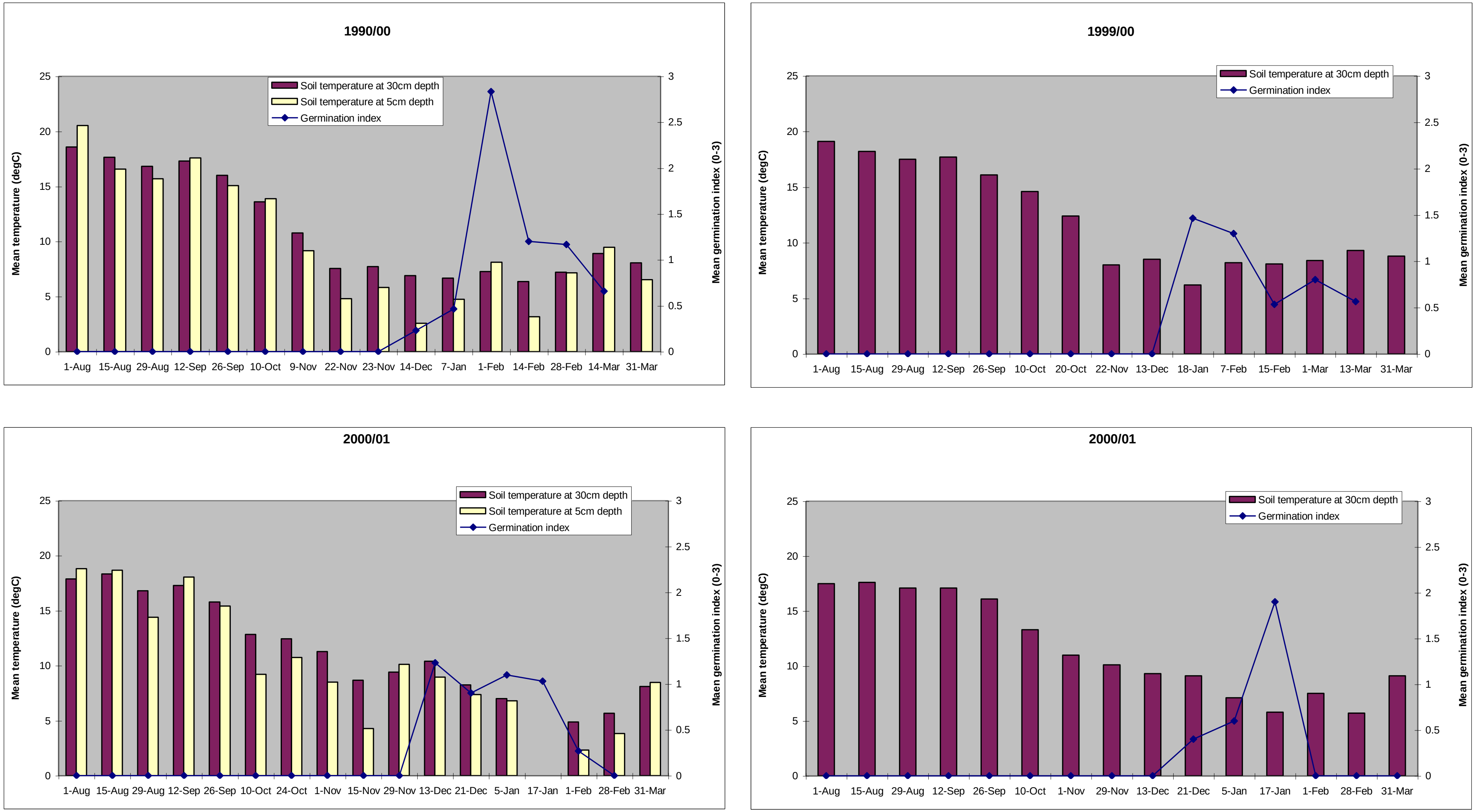


Figure 2.3.3. Germination of *R. vallisumbrosae* resting bodies and temperature, 1999-2000 and 2000-2001

Mepal Penzance



Environmental factors and disease development (Objective 2.4)

Introduction

The core of this project was the formulation of models for smoulder and white mould infection in relation to temperature and leaf wetness. It was recognised, however, that other factors, primarily environmental but also due to cultural or husbandry practices, would influence disease development. Commercial narcissus crops in Cornwall and eastern England were monitored to gain information on the epidemiology of the diseases

Six industrial partners agreed to provide 0.2 ha areas of commercial crops for this exercise. Crops, in their first year, were selected in 1998, 1999 and 2000 for monitoring in their second year (1998-1999, 1999-2000 and 2000-2001), with three sites in Cornwall and three in eastern England each year. No fungicides were applied in the second year of these crops, but, otherwise, normal commercial husbandry was applied. Crops were monitored in the central 0.1 ha of each area so as to leave a buffer zone between the monitored area and the surrounding crop which was sprayed as normal. Smaartlog weather stations were set up in each area. These gave a recording of temperature, humidity, leaf surface wetness, rainfall soil temperature, wind speed and wind direction. Automatic recordings of these parameters were taken every 30 min or as appropriate. Foliar diseases were recorded (see Task 2.1) towards the end of the first growing season and at about 2-week intervals throughout the second growing season. Similar monitoring exercises were carried out in 2000 and 2001 on second-year crops, two at Mepal and two at Kirton, using bulb stocks supplied by the industrial partners. At these sites no fungicides were applied in either crop year, and crops were irrigated in dry periods to encourage disease development. Details of sites are given in Appendix 2.

Commercial crops in Cornwall

In the Cornish crops few smoulder primaries were observed, and any subsequent smoulder symptoms were effectively swamped by those of white mould, so it was not practical to record smoulder throughout the season. Similarly, occasional symptoms of scorch (*Stagonospora curtisii*) were obvious only early in the season.

1998-1999 season In the three Cornish sites, although some dried leaf lesions were seen at the end of the first growing season, these could not be unequivocally identified as being due to white mould. In the second year, white mould symptoms started to increase rapidly at all three sites from mid-February, becoming more rapid at Manaccan than at Tresillian or Truro. However, the subsequent loss of green leaf area was fastest at Truro and became slower at Manaccan (at the latter farm the site was sheltered by trees on two sides) (Figure 2.4.4). Despite the more south-westerly location of the Manaccan site, mean temperatures corresponded very closely at all three sites (Figure 2.4.1). The increase in white mould symptoms appeared to correlate with the general seasonal increase in temperature (Figure 2.4.1). Mean humidity and rainfall (Figure 2.4.2) were also similar at the three sites, but the hours of leaf wetness were noticeably greater early in the growing season at Truro than at the other sites, suggesting a link with the faster loss of green leaf area at Truro (Figure 2.4.3). In this year the incidence of white mould was starting to increase at all three sites by the time of flower cropping (Figure 2.4.4).

1999-2000 season Exceptionally, in this year the crop at Manaccan was kept down for a third year and was monitored again. Active white mould lesions were found at the end of the first growing season at both Truro and Tresillian, with more at the former site; the second-year crop at Manaccan site had already fully died down at this time. In the third-year crop at Manaccan large numbers of white mould lesions developed rapidly, so that by late-March there were >10 lesions per leaf and thereafter it proved impractical to count lesions accurately due to coalescence and leaf death, and at this time some 25% of the distal leaf surface was dead or dying as a result of the numerous white mould lesions. The loss of green leaf continued rapidly, and by late-April virtually 100% of the leaf area was dead. In the second-year crops at Truro and Tresillian, white mould spread much more slowly, reaching 1 to 2 lesions per leaf by late-April. From early-March, the percentage leaf area affected increased more quickly, reaching 10 to 15% by late-April (Figure 2.4.4). As far as could be ascertained, there was no clear correspondence between disease levels and weather at the three sites. In this year flower cropping took place well before the increase in smoulder symptoms (Figure 2.4.4).

2000-2001 season Near the end of the first crop year active white mould lesions were found on all three Cornish crops, with the largest number at Truro and the lowest at Manaccan, although the most advanced crop for foliar senescence was Tresillian. In the second year white mould lesions did not appear until March, and then increased at all three sites from the start of April, but especially so at Truro. Thereafter, green leaf area was lost rapidly and at a similar rate at all three sites, about 50% of green leaf area remaining by early-May (Figures 2.4.4 and 2.4.5). The correspondence between sites for mean temperature (Figure 2.4.5) and rain records was again high, whereas there were differences between the mean humidity (often less humid at Truro; Figure 2.4.6) and the hours of leaf wetness (often wetter at Tresillian, drier at Truro later in the growing season; Figure 2.4.7). In this year flower cropping once again took place well before the increase in smoulder symptoms (Figure 2.4.4).

Commercial crops in eastern England

At the eastern sites smoulder was the only significant fungal foliar disease encountered, although white mould was identified in some other narcissus crops in the area during the course of the study (O'Neill *et al*., 2002; see Appendix 1).

1998-1999 season In monitoring disease near the end of the first crop year the Lincolnshire sites (Holbeach Marsh and Gosberton Marsh) had at least some leaves with spreading smoulder lesions in about 50% of the sampling areas, and the corresponding figure for Swaffham Prior Fen (Cambridgeshire) was over 90%. In the second year, smoulder lesions started to increase at all three sites from mid-February 1999, initially slightly faster at Holbeach Marsh and Swaffham Prior Fen than at Gosberton Marsh, although later in the season the loss of green leaf area was fastest at Gosberton Marsh. The development of late-season smoulder symptoms was very rapid after mid-April (Figures 2.4.8 and 2.4.11). Daily records for mean temperature (Figure 2.4.8) and humidity corresponded very closely for all three sites. In many instances, however, rainfall (Figure 2.4.9) and the hours of leaf wetness (Figure 2.4.10) were greater at Gosberton Marsh site than the other sites, suggesting a link between leaf wetness and smoulder development. The marked difference in rainfall between the sites emphasises the importance of using local meteorological data. In this year the incidence of smoulder was already increasing at all three sites by the time of flower cropping (Figure 2.4.11).

1999-2000 season At the end of the first crop year, active smoulder lesions were found at Swaffham Prior Fen, but not at the Holbeach Marsh and Gosberton Marsh sites. In the second year, the number of smoulder primaries was low, and from late-March the number of smoulder lesions increased very slowly at all three sites, but was <0.2 lesions per leaf by late-April. The percentage of leaf area affected increased only slowly. In the crop at Holbeach Marsh there was then a rapid increase in the number of 'late-season' smoulder lesions, such that the percentage leaf area affected approached 40% in early-May. Increasing loss of leaf area occurred 2 – 3 weeks later at the Gosberton Marsh and Swaffham Prior Fen sites (Figure 2.4.11). Similar epidemics of late-season smoulder were observed on other narcissus crops in the Holbeach area and on crops at Kirton and Mepal, and similar lesions (though in smaller numbers) at the other monitoring sites (Gosberton Marsh and Swaffham Prior Fen). Isolation of fungi from these lesions confirmed *B. narcissicola* from the Holbeach Marsh, Swaffham Prior Fen and other sites. To a large extent, mean temperature and humidity records corresponded well for the three sites, and there were no obvious patterns in rain and surface wetness records. There was no obvious weather factor differentiating the Holbeach Marsh site (with its early and severe late-season smoulder epidemic) from the other two sites. However, the general increase in symptoms corresponded closely with the increasing temperature in late-spring. In this year flower cropping took place well before the increase in smoulder symptoms (Figure 2.4.11).

2000-2001 season No active smoulder lesions were found at any of the three commercial sites towards the end of the first crop year, although evidence of old lesions on the foliage was seen at Gosberton Marsh and by far the most advanced crop in terms of foliar loss was that at Swaffham Prior Fen. In the second-year crops the number of smoulder primaries recorded at the start of the growing season was <0.01 primaries/shoot at Swaffham Prior Fen, but higher (0.04 primaries/shoot) at Holbeach Marsh and Gosberton Marsh. In late-March to April the number of smoulder lesions increased slowly at all three sites, with the highest number (0.3 lesions/leaf) in this period occurring at Holbeach Marsh (Figures 2.4.11 and 2.4.12). In May the loss of green leaf area occurred rapidly at all three sites, starting first at the most southerly site, Swaffham Prior Fen, where there was an increase in the number of late-season smoulder lesions in mid-May, reaching an estimated average of 2.4 lesions per leaf. Some sporulating late-season lesions were also observed at Holbeach Marsh, but not at Gosberton Marsh, although distinguishing disease lesions in rapidly senescing leaves was difficult. There was a high degree of correspondence between mean temperature records across the three sites (Figure 2.4.12), but there were complex differences between the patterns of mean humidity, rainfall (Figure 2.4.13) and hours of leaf wetness (Figure 2.4.14). There was no obvious weather trigger relating to the increase in late-season smoulder, other than the general seasonal increase in temperature. As in previous years, some leaf samples were examined in the laboratory and *B. narcissicola* and (or) *B. cinerea* were isolated from oval leaf lesions. As in the previous year, flower cropping took place well before the increase in smoulder symptoms (Figure 2.4.11).

Disease monitoring at Mepal and Kirton (2000 and 2001)

Few symptoms of smoulder were seen in first year crops. In the second year all crops had few smoulder primaries, and showed a steady increase in lesion numbers and loss of green leaf area. Crops at Mepal were much more severely affected than those at Kirton, with the incidence of symptoms increasing 2 – 3 weeks earlier than at Kirton. Disease levels were very similar on bulb stocks from different sources planted side by side at any one site.

Conclusions

Smoulder

- There were no consistent differences over the three years in smoulder development at the three commercial sites in the east. Nor was the rate of disease development consistent at one site at different stages of the two-year growth cycle.
- Any differences between sites did not appear to be related to differences in temperature and humidity. However, smoulder seemed to increase with the general seasonal rise in temperature. This would account for faster disease development (by 2 – 3 weeks) at Mepal compared with Kirton.
- A greater incidence of rain and wetness appeared associated with more smoulder at Gosberton Marsh in 1998-1999 only. In other cases the relation was either more complex or similar data were obtained at different sites.
- There was no evidence that bulb stocks from different sources developed different levels of smoulder when planted at the same site.
- There was no clear relationship between the date of flower cropping and an increase in smoulder symptoms.

White mould

- There were no consistent differences over the three years in white mould development at the three commercial sites in Cornwall, nor was the rate of disease development consistent at one site at different stages of the two-year growth cycle.
- Any differences between sites did not appear to be related to differences in temperature. However, white mould seemed to increase with the general seasonal rise in temperature.
- The crop at Manaccan in 1998-2000 was unusual in that the site was sheltered on two sides by trees. Compared with the other sites, white mould developed initially more quickly here in the second crop year, but disease development towards the end of the growing season was slower. Kept down for a third year, white mould developed on this crop very quickly.
- Later in the growing season, white mould developed on the Truro crops in 1998-1999 and 2000-2001 more quickly than at other sites. This may have related to higher levels of surface wetness at these sites compared with the others.

Figure 2.4.1. White mould development and temperatures, Cornwall 1999.

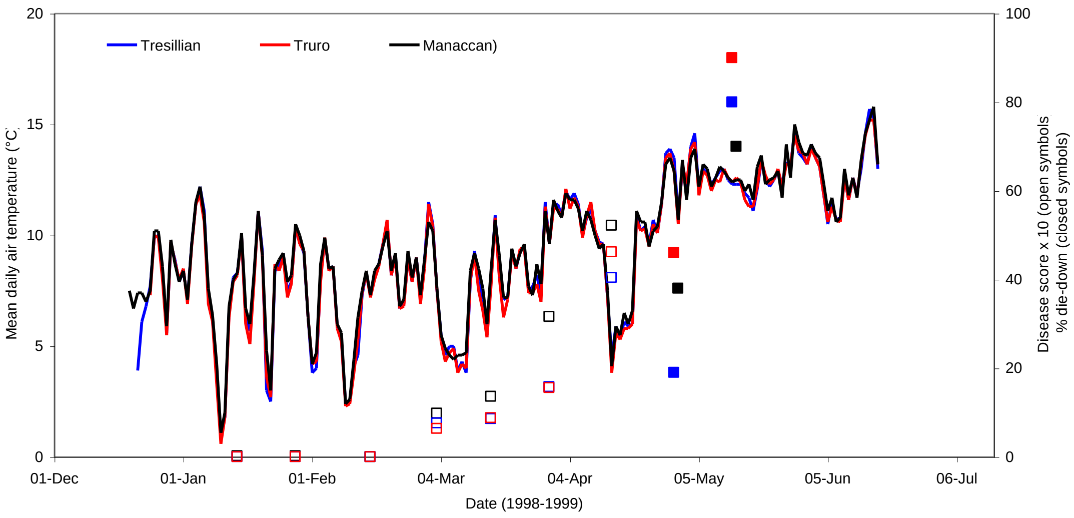


Figure 2.4.2. Rainfall, Cornwall 1999.

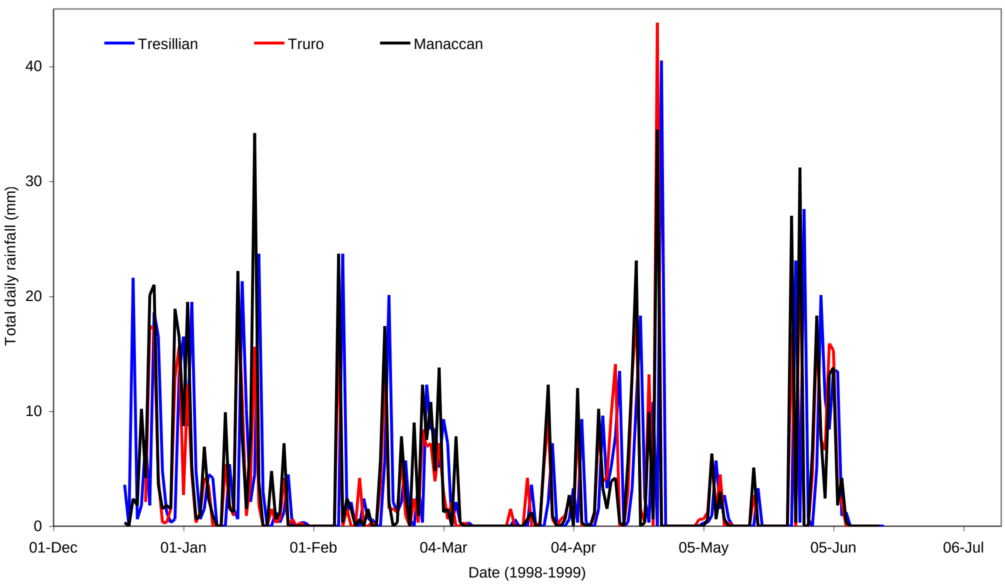


Figure 2.4.3. Surface wetness, Cornwall 1999.

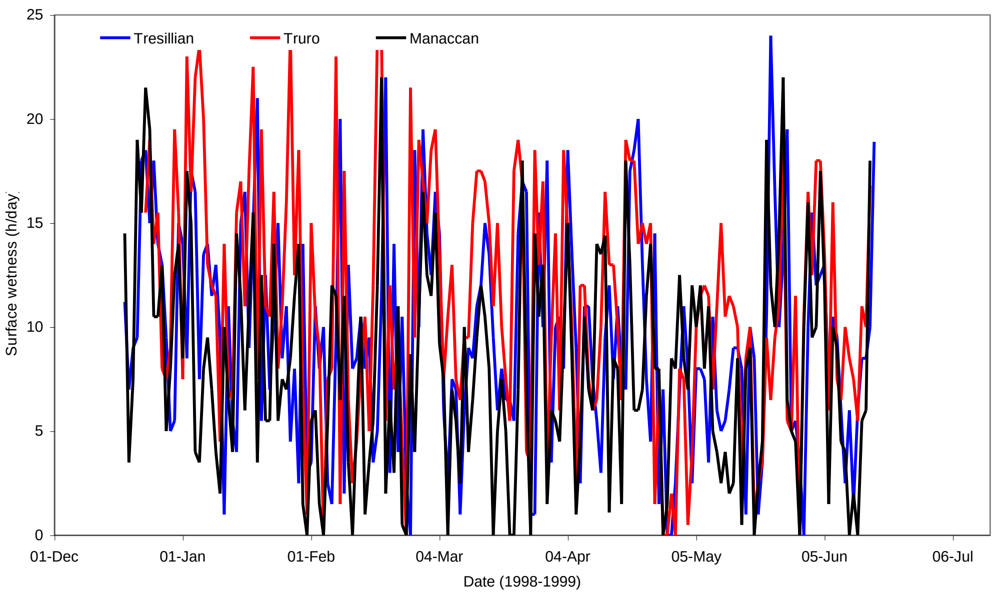
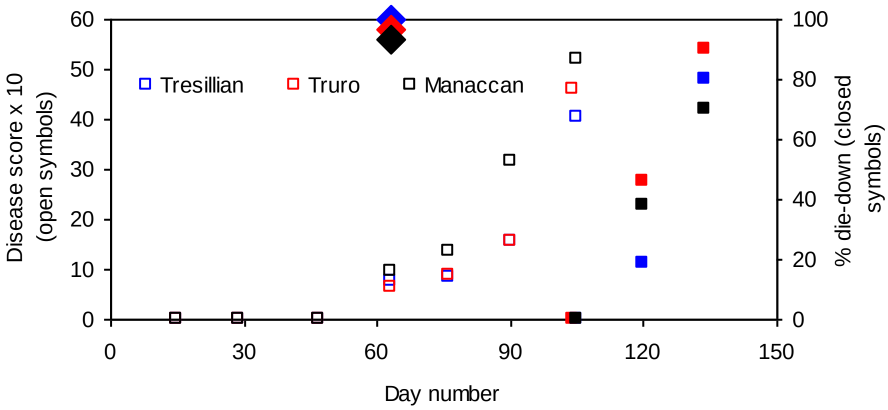
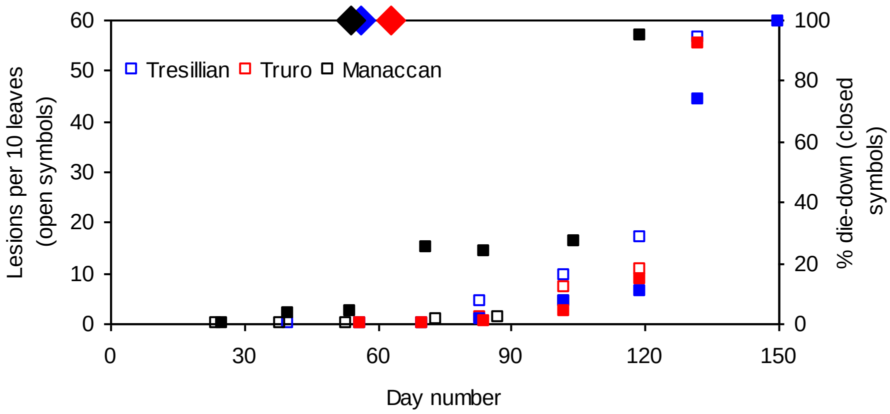


Figure 2.4.4. White mould development in Cornwall, 1999 (top), 2000 (middle) and 2001 (bottom). Diamonds indicate cropping date.



2000



2001

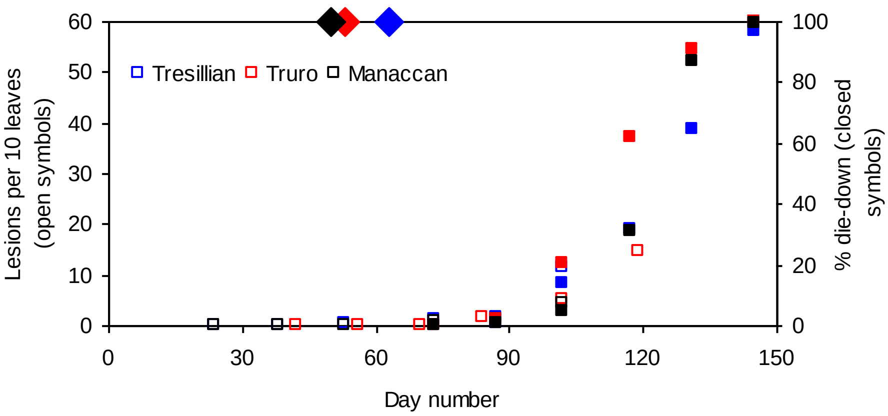


Figure 2.4.5. White mould development and temperatures, Cornwall 2001.

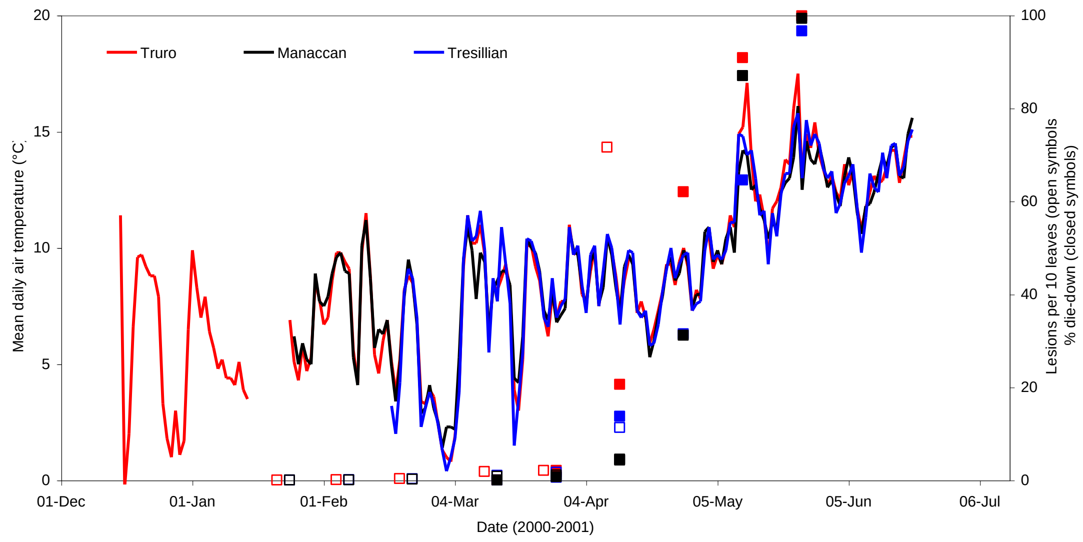


Figure 2.4.6. Relative humidity, Cornwall 2001.

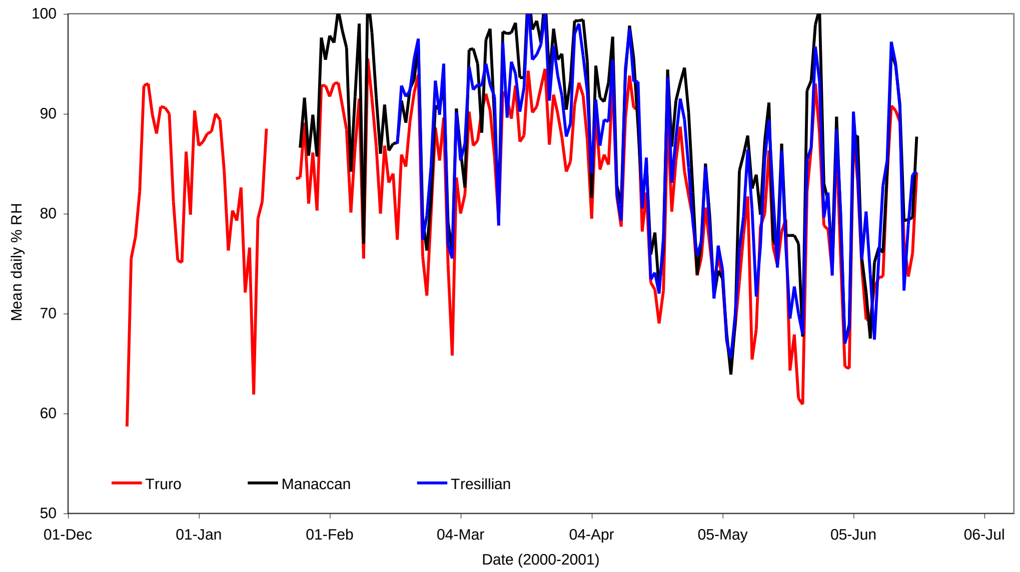


Figure 2.4.7. Surface wetness, Cornwall 2001.

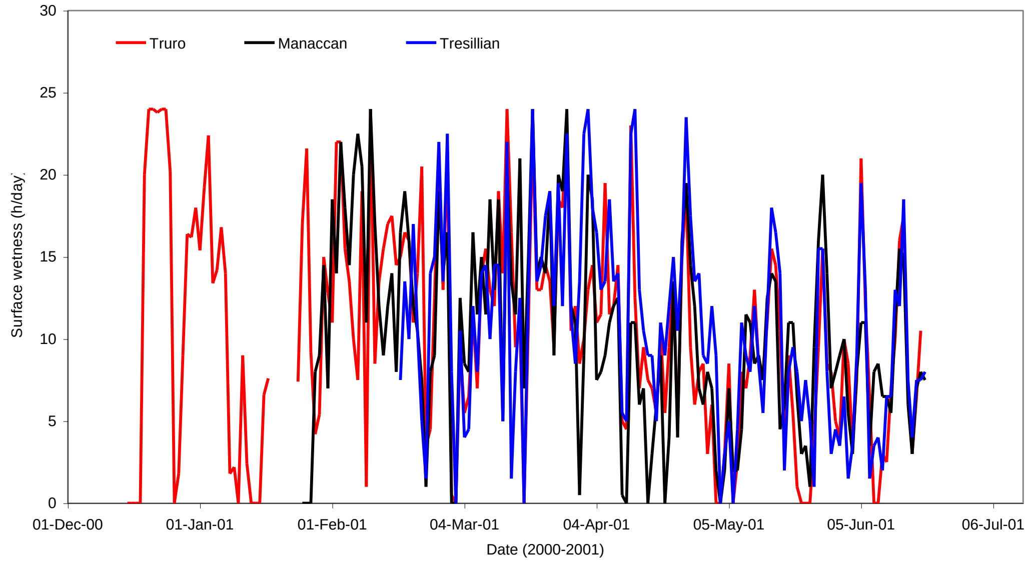


Figure 2.4.8. Smoulder development and temperature, eastern counties 1999.

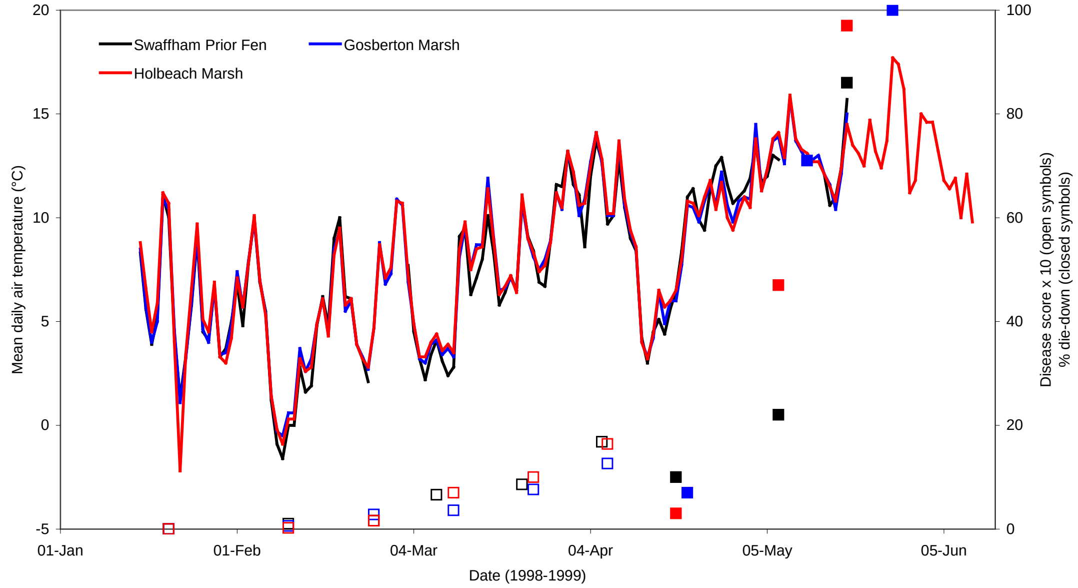


Figure 2.4.9. Rainfall, eastern counties 1999.

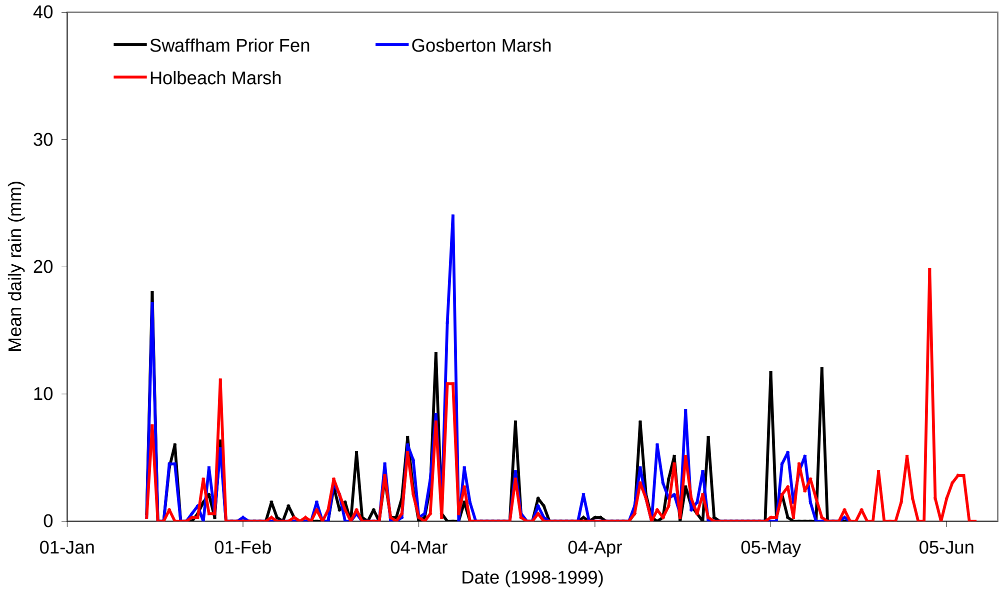


Figure 2.4.10. Surface wetness, eastern counties 1999.

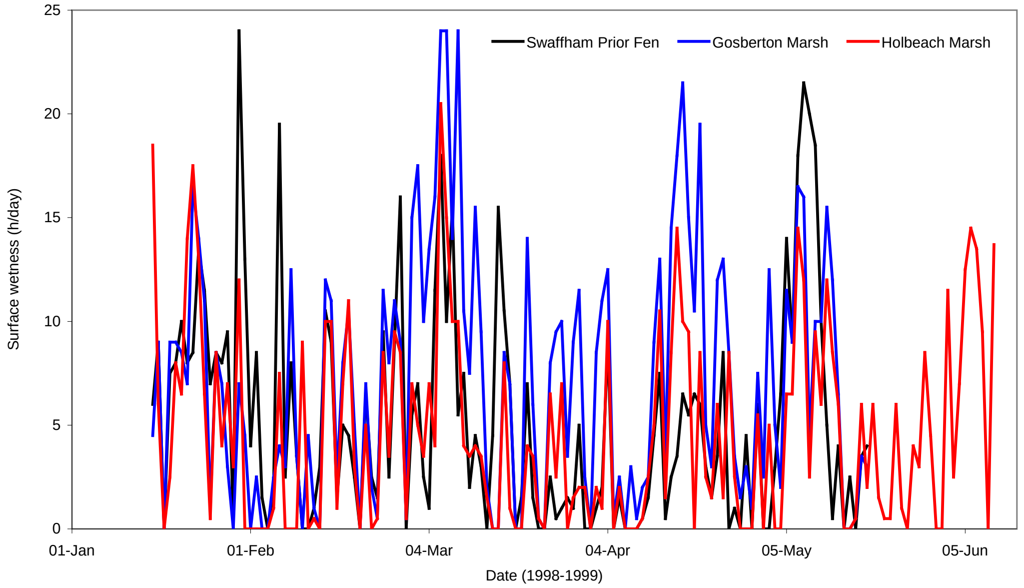
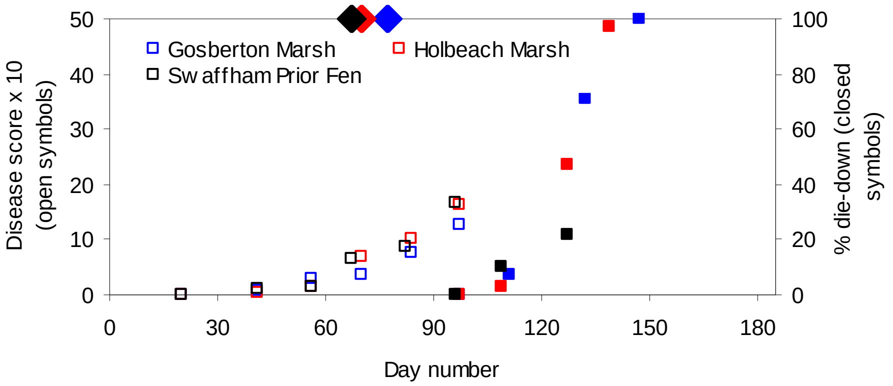
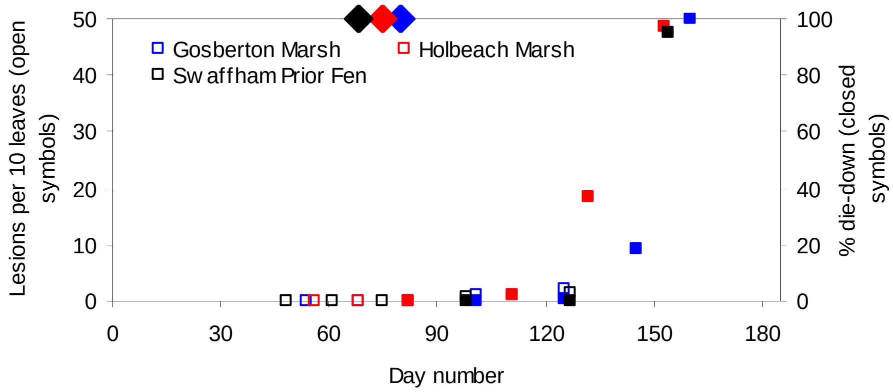


Figure 2.4.11. Smoulder development in eastern counties, 1999 (top), 2000 (middle) and 2001 (bottom). Diamonds indicate cropping date.



2000



2001

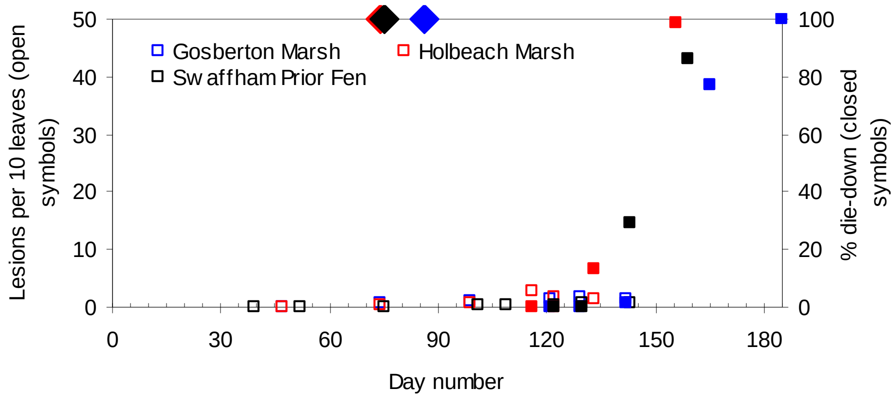


Figure 2.4.12. Smoulder development and temperature, eastern counties 2001

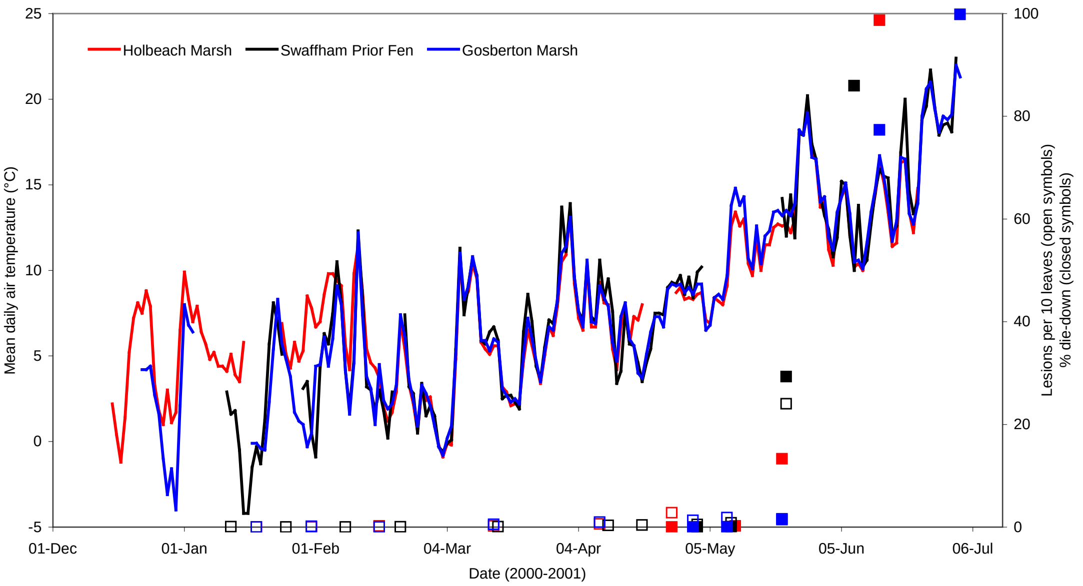


Figure 2.4.13. Rainfall, eastern counties 2001.

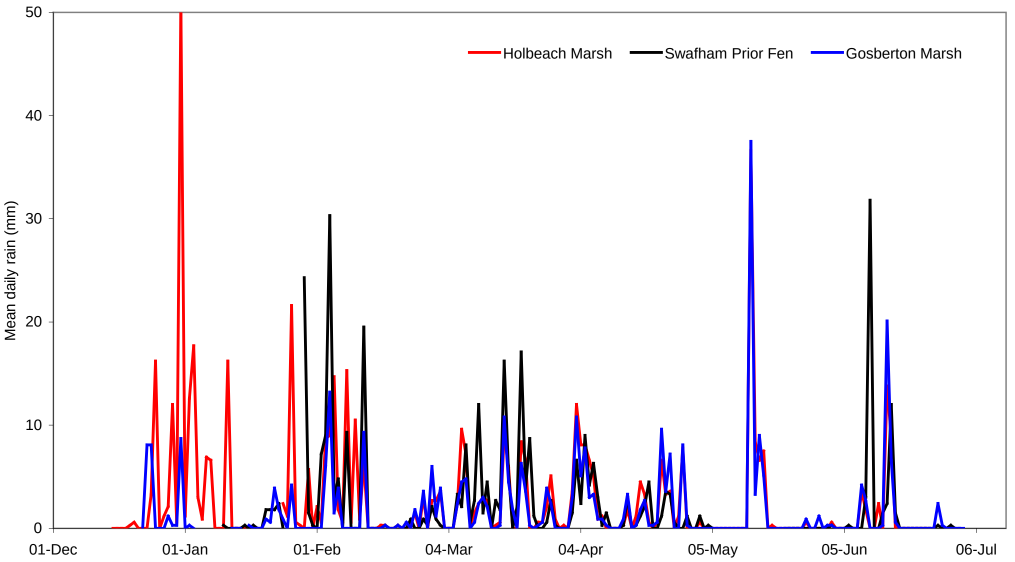
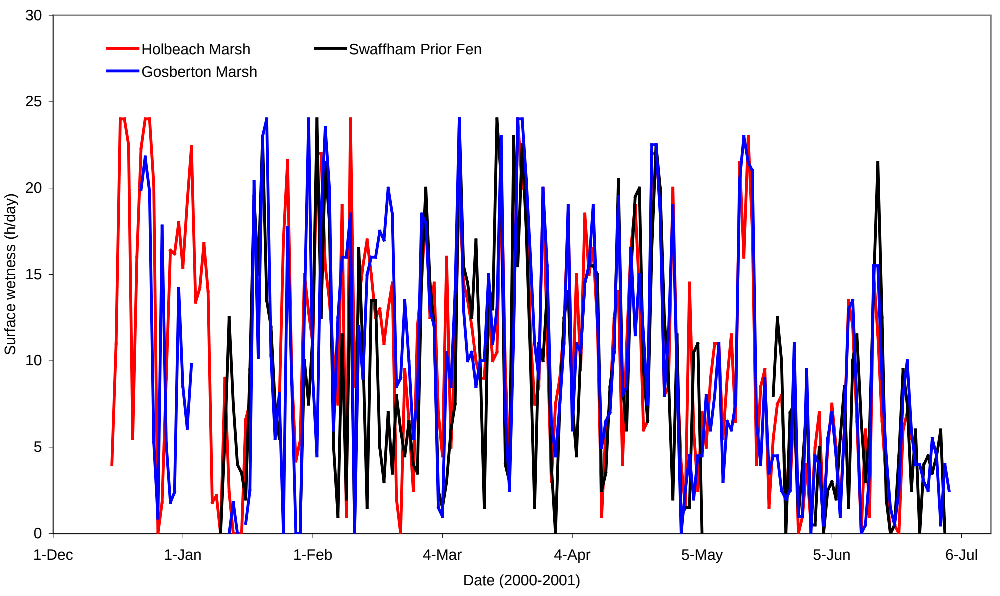


Figure 2.4.14. Surface wetness, eastern counties 2001



Development and validation of precipitation impact sensor (Task 2.5)

Introduction

The addition of a precipitation impact (PI) sensor to environmental monitoring arrays adds a new element to disease epidemiology. Until the development of this sensor it was not possible to quantify the impact energy of rainfall, sleet or hail under field conditions. This facility offers the opportunity of detecting and measuring potentially damaging events within crops. The Aardware Design PI sensor consists of a sealed acoustics chamber with an upward-facing diaphragm. When raindrops impact on the surface of the sensor, pressure pulses (proportional to drop size or impact) are produced on the diaphragm and can be measured in the chamber by an omni-directional microphone. The amplitude of the microphone output is divided into 14 levels (called 'bins'). Previous work demonstrated that, while the upper limit of the sensors output was accurate under UK conditions, the sensitivity at the lower end of the output range was insufficient (Lovell *et al*., 1999, 2002). As *B. narcissicola* infects only damaged tissues, the output at the upper limits of the sensor, where damage would occur, is important. The sensor output could also be used to differentiate between rainfall and hail, since the latter is known to cause significant damage to the crop that could affect the epidemiology of smoulder in particular. Output from the lower end of the scale is also important, in determining the effect of rain-splash on the spread of *R. vallisumbrosae* and *B. narcissicola*. Hence, it may be necessary to optimise the sensor output to measure both types of response.

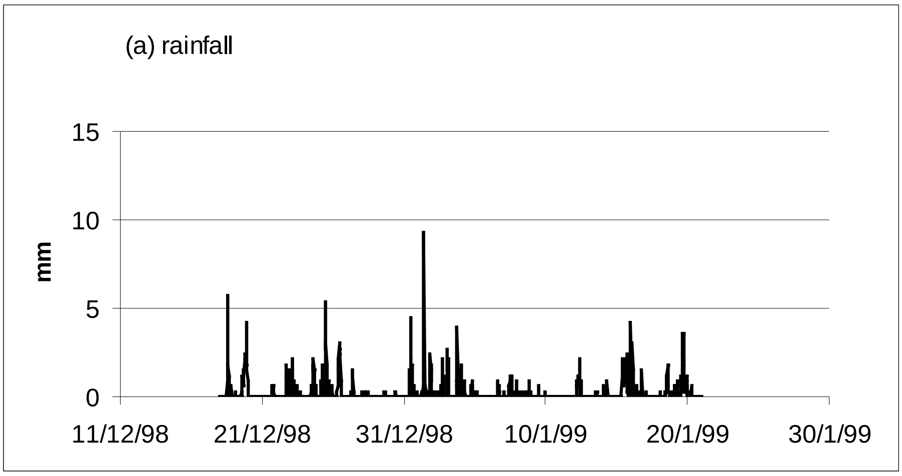
Testing and optimising the PI sensor

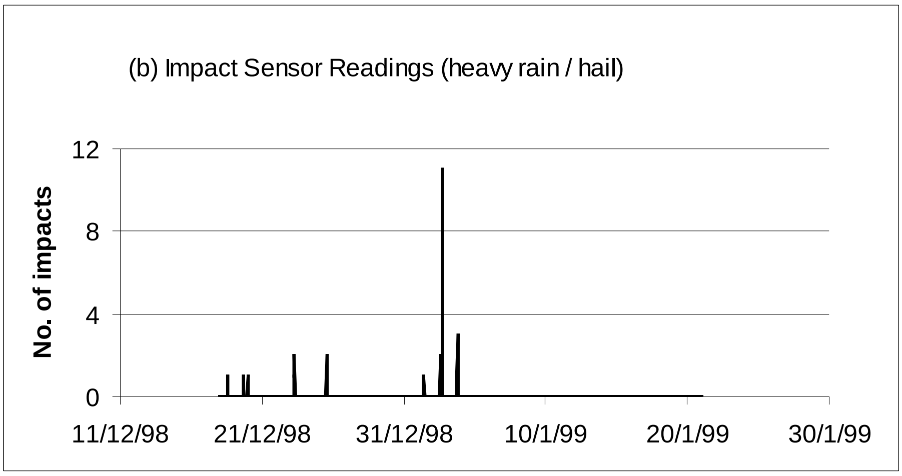
To test the sensor and interpret its output, sensors were placed in crops at Penzance, and another at Kirton. Outputs were monitored over the growing season, along with continuous information on rainfall, temperature, humidity, wind-speed and surface wetness. Some typical results (Figure 2.5.1a) showed the many rainfall events over the December 1998 to January 1999 period. The impact sensor output indicated that only a few of these events were likely to cause significant spread of disease via crop damage. The high impact count recorded on 1 January 1999 corresponded with 2.1 mm of rain in a 30-minute period, indicating the occurrence of hail (Figure 2.5.1b). This event was likely to have caused crop damage, subsequently increasing the likelihood of smoulder and white mould infection. It is possible that the output from the sensor, if validated, could be used to designate when fungicide applications to the crop are necessary, especially when used in conjunction with infection models for smoulder and white mould.

The target area over which raindrops impact the sensor is one factor in the optimisation of the PI sensor. The size of the target area determines the likelihood of raindrop impact during a given sample period. For a target area of 10 cm^2, a single impact equates to 1000 impacts/m^2. The target area of the sensor can be altered by using shields (covers) with apertures of different sizes. These are positioned above the sensor, thereby exposing only part of the response surface to rain. Preliminary tests were carried out, followed by a series of field trials using impact shield aperture sizes of 30 – 80 mm diameter which were compared with a 30 mm diameter reference sensor. The smallest aperture was used on the reference sensor as, theoretically, it is better for the detection of the larger, more damaging impacts.

Preliminary results indicated that larger target areas had higher overall numbers of impacts, but most of these were assigned to low levels ('bins'). Smaller target areas had lower numbers of total impacts, with proportionally more impacts being ascribed to higher levels ('bins'). The results can be explained as due to the higher probability of larger droplets being assigned to low levels ('bins') using large target areas, because with a larger zone of impact available from which impacts are sampled some impacts are further from the microphone. This gives a higher proportion of impacts being assigned to lower levels ('bins'). These results confirm previous findings, although the sampling time used will also affect these results. Results suggested an optimal shield diameter of 30 - 40 mm diameter. This was more likely to mean that many high energy impacts were assigned to the higher levels ('bins'). Further field tests would be required to ascertain a signature (pattern of outputs in different bins) which corresponded to potential leaf surface damage events.

Figure 2.5.1. Rainfall and precipitation impact counts at the Cornish field experiment site for December 1998 - January 1999.





In further tests, different shield diameters were used to optimise signal output at the upper limits of the sensor, important as a diagnostic determinant of crop damage. Each size comparison was made in two successive trials, over the period January to April 2000. Three impact sensors were positioned in the field within a narcissus crop at Kirton. The 30 mm reference sensor was positioned at the top of the crop canopy. A test sensor (30-80 mm) was placed adjacent to the reference sensor and a further test sensor was placed at the base of the foliage. Environmental data (rainfall and leaf wetness) were recorded at the same position as the reference sensor.

The effect of using 30 mm diameter sensor shields on reference and test sensors on the measurement of raindrop impacts at the same point is shown in Figure 2.5.2. There was little difference between the output of the two sensors over the measurement period in the field (Table 2.5.1). (In interpreting these figures, it should be noted that rainfall is collected by a tipping bucket mechanism inside the rain gauge; the measurement of rainfall by the rain gauge is therefore not continuous, but shows as pulses which do not appear to correspond to the PI readings.) This indicates that the positional effect between the two sensors was not significant. Any subsequent trends in the results could be explained only by the differences in impact shield aperture diameters. The percentage of all impacts assigned to bin numbers higher than 1 ranged between 16 and 23%. The effect of a difference in shield aperture diameter of 10 mm (reference and test diameters of 30 and 40 mm) on the measurement of raindrop impacts at the same point is shown in Figure 2.5.3. There was an increase in the numbers of impacts assigned to the bin 1 category on the test sensor (40mm shield). However, the numbers assigned to the higher bin numbers remained similar to the reference sensor (30 mm diameter) (Table 2.5.1). The percentage of impacts assigned to bin numbers higher than 1 was between 28 and 31%.

On 3 March 2000 a shower of hail occurred during the experiment, and a greater range of output was observed. Increasing the difference in shield aperture diameter to 30 mm (30 and 60 mm shields) increased the number of impacts in bin 1 by 33% (Figure 2.5.4). However, the percentage of impacts assigned to the higher bin numbers increased in comparison to the reference sensor (Table 2.5.1). The percentage of impacts assigned to bin numbers higher than 1 was increased by 9% on output from the test sensor. A greater range of impact output was also observed during observations taken on 3 April 2000. Although hail was not specifically noted, it is possible that there may have been hail present in the rainfall event. Increasing the difference in shield aperture diameter to 50 mm (30 and 80 mm shields) dramatically increased the number of impacts in bin 1 by approximately 50% (Figure 2.5.5.). This was likely to have been an underestimation as the maximum output (255) was reached over the sampling period. However, the percentage of impacts assigned to the higher bin numbers increased in comparison to the reference sensor (Table 2.5.1). The percentage of impacts assigned to bin numbers higher than 1 was increased by 17% on the test sensor.

Table 2.5.1. Comparison of total impacts during rainfall of reference and test PI sensors with different shields.

Date	Sensor	Shield diam. (mm)	Impact sensor output							
			Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Bin 6	Total	% (>bin 1)
14 February	Ref.	30	326	54	12	3	1	0	396	18
	Test	30	302	58	18	9	2	0	389	22
21 February	Ref.	30	273	68	29	8	4	0	382	28
	Test	40	455	129	55	13	9	0	661	31
3 March	Ref.	30	108	17	11	4	4	2	146	26
	Test	60	312	94	30	18	18	11	483	35
3 April	Ref.	30	1858	238	91	41	18	18	2264	18
	Test	80	2867	786	292	144	55	25	4169	45

Figure 2.5.2 Comparison of reference and test sensors with 30 mm aperture diameters (14/02/2000)

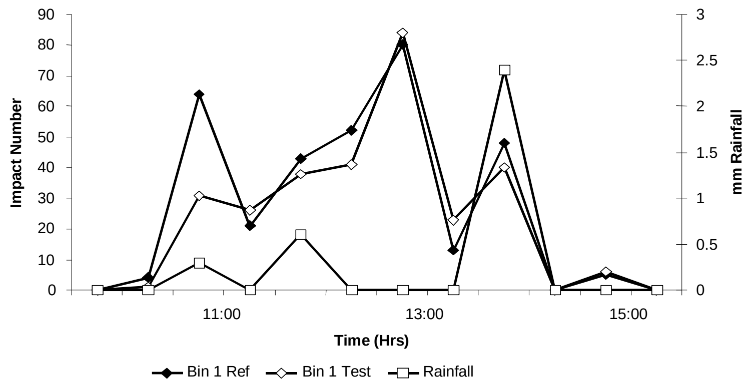


Figure 2.5.3 Comparison of reference (30 mm) and test sensors (40 mm) aperture diameters (21/02/2000)

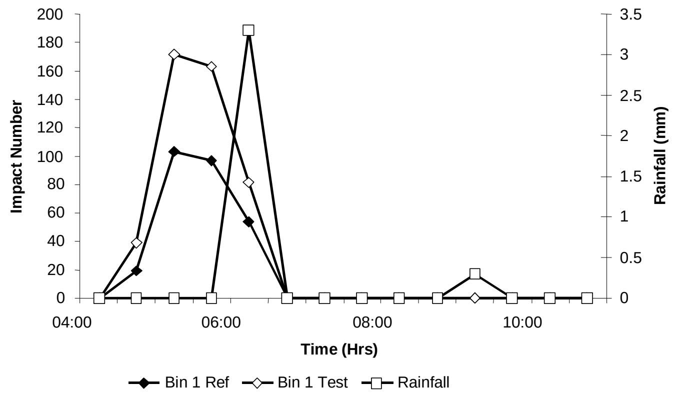


Figure 2.5.4 Comparison of reference (30 mm) and test sensors (60 mm) aperture diameters(03/03/2000)

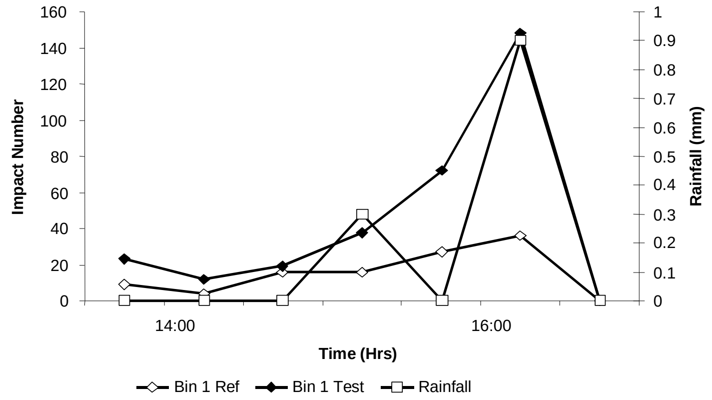
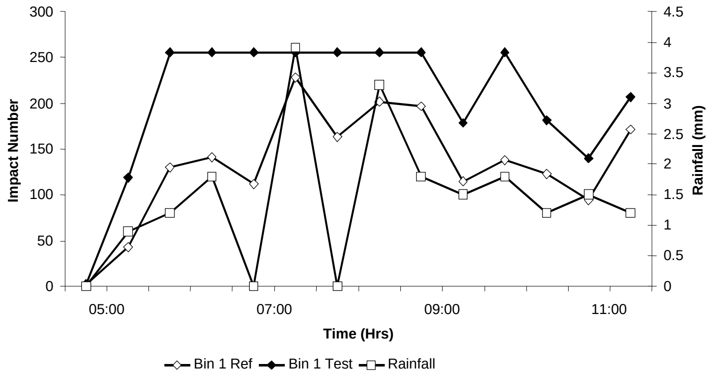


Figure 2.5.5 Comparison of reference (30 mm) and test sensors (80 mm) aperture diameters (03/04/2000)



Effects of rainfall events on *B. narcissicola* development

To determine the effects of precipitation impact on leaf damage and disease infection, pot-grown narcissus plants were placed in the field at Kirton for periods of 72 h during which they were exposed to natural rainfall. Control sets of plants were placed nearby under a clear polythene cover, so that they did not receive any rain. These experiments were carried out over the January to April 2000 period. After this period both exposed and control plants were moved from the field to a cool glasshouse where they were inoculated with *B. narcissicola* and misted for the next 24 hours. Plants were examined for symptoms of smoulder at intervals until leaf senescence.

Some typical symptoms of smoulder (leaf tip, leaf margin and stem end lesions) were seen on the plants throughout these experiments. However, the number of lesions was very low, with typically around 1% of leaves being affected. There was no obvious increase in the number of lesions in 'exposed' plants over those in covered, control plants, and, at these low frequencies, it is unlikely that statistically significant effects could be demonstrated. In two instances when hail was observed in the locality during exposure periods, inoculation with *B. narcissicola* and misting was repeated, but still no signs of infection was elicited. Some other symptoms – mainly rust-like lesions on leaves or stems – were seen very occasionally, and as on further examination these did not reveal *B. narcissicola* they were assumed to be related to incidental damage.

The main results were:

- Increasing impact sensor shield diameter to >40 mm had a significant effect on the numbers of impacts assigned to higher bin numbers, although it was unclear what the optimal level should be to reflect narcissus leaf damage.
- There were no rainfall events during the tests which produced outputs in the higher bins (>9), indicating the absence of hail (although hail was noted at one recording time).
- There was no increase in smoulder levels observed on exposed plants to any of the rain events used in the study, although signs of damage on leaves were recorded in some instances.

Effect of duration of high-impact energy droplets from simulated rainfall on infection of leaves by *B. narcissicola*

Previous experiments using natural rainfall were unable to produce meaningful leaf damage or infection with *Botrytis*. Higher impact rain droplets were needed. Facilities for these tests were made available at the Institute of Arable Crops Research (IACR), Long Ashton, carrying out experiments on 20 September 2000. Tests were carried out using a rainfall simulator operating at 2.5 bar water pressure to produce raindrops, of varying impact energy, that were measured by the impact sensor. Two PI sensors were placed on either side of the area of simulated rainfall. Pot-grown narcissus plants were placed under the rain simulator, either exposed to the 'rainfall' or protected from it by clear polythene film to serve as controls. The experiment was done twice, using plants at two stages of development, either pre-flowering (with flower buds and stiffly upright extending leaves, experiment 1) or post-flowering (with mature but mainly upright foliage, experiment 2).

The rainfall rate delivered in this experiment was 92.4 mm/h, the maximum possible for the equipment. Plants were subjected to two, 13-minute period of rain simulation. Foliage was then surface-dried using a fan at ambient temperatures, and was taken to HRI Wellesbourne on the same day for inoculation using conidial suspensions of *B. narcissicola*. The plants were incubated in a glasshouse and leaves were checked at regular intervals for disease. After three weeks all leaves were scored for the number of *B. narcissicola* lesions. Non-sterilised leaf pieces were removed from necrotic areas surrounding typical lesions on both exposed and control plants, and plated directly on to DLEA before incubation at 20°C. All isolations confirmed the presence of *B. narcissicola*, regardless of treatment. The outputs from the two sensors exposed to rain simulation are given in Table 2.5.2, and results of plant assessments in Table 2.5.3.

Table 2.5.2. Sensor outputs (number of impacts) from rain simulation experiments.

	Experiment 1		Experiment 2	
Bin level	Sensor 1 (right)	Sensor 2 (left)	Sensor 1 (right)	Sensor 2 (left)
4	166	91	143	94
5	187	119	116	115
6	157	90	86	85
7	111	59	69	84
8	95	41	44	44
9	55	29	26	35
10	42	14	15	29
11	33	15	16	17
12	20	10	8	17
13	14	11	13	13
14	39	23	20	33
15	7	2	2	7
16	0	0	0	0

Table 2.5.3. Number of *B. narcissicola* lesions on leaves exposed to simulated rainfall.

Pot number	Number of lesions			
	Experiment 1		Experiment 2	
	Exposed	Control	Exposed	Control
1	0	0	0	0
2	2	0	0	1
3	1	0	2	2
4	0	0	0	1
5	0	0	1	0
6	0	2	0	1
Totals	3	2	3	5

The results showed that exposure to the rain simulation had little effect on the numbers of *B. narcissicola* leaf lesions (Table 2.5.3). There were equal numbers of lesions in plants exposed to or protected from the effects of rain simulation. Output from the impact sensor showed that raindrops giving an output in the bin 14 level were present in the simulation. However the number of these was low in comparison the number of drops giving lower output energies. Consideration of the area of exposure means that relatively few of these bigger droplets would have impacted on leaf tissues. Additionally, it would be unlikely that the same leaf piece would have had more than one impact by these bigger droplets. The output of the simulator was the maximum output possible from this piece of equipment, but it is possible that this was not high enough to give differences between exposed and protected plants. Higher impact energies from droplets can be produced from rain towers.

Effect of duration of high-impact energy droplets from a rain tower on infection of leaves by *B. narcissicola*

The IACR rain tower provided high-energy droplets that were measured by the PI sensor in the higher input categories. The raindrop mass used was 2.53 µg (µl), equating to a diameter of 1.69 mm. The height of the rain tower (drop height to leaf) was 8.650 m. The terminal velocity of droplets was reached at approximately 8.01 m at a speed of 5.94 m/s. Droplets were impacted directly on the PI sensor (without shield) to determine their impact energy. On each of three separate runs, leaves (from pot-grown, post-flowering plants) were placed at the bottom of the tower and fixed horizontally with sticky tape over the impact sensor surface. Droplets were impacted directly on to the leaf surfaces for 5-minute periods. The leaves attached to the sensor were exposed to 32 drops/min with an average of 144 to 154 drops per 5 minute test period. The visual examination of treated leaves showed that the drops produced visible changes in the appearance of the waxy cuticle of the leaf. Unexposed equivalent areas of other leaves in the same pot were selected as untreated controls. Exposed leaf parts and control segments were carefully marked and remained attached to the plants throughout the test. Pots with treated and untreated leaf segments were taken to HRI Wellesbourne and inoculated as described previously with *B. narcissicola* spore suspension on the same day as the experiment and again 6 days later, to ensure that they had been exposed to adequate inoculum. The output from the PI sensor exposed to raindrops in the rain tower is shown in Table 2.5.4 for three individual test runs.

Table 2.5.4. Impact sensor output data from rain tower tests.

Bin	Test 1	Test 2	Test 3
7	1	2	0
8	11	3	3
9	31	33	24
10	18	17	19
11	26	39	43
12	26	24	18
13	11	11	11
14	25	26	36
15	0	0	0
16	0	0	0
Total	149	155	154

Following inoculation, leaves from test 1 were assessed for visible symptoms of infection, and surface changes and necrosis was noted. Control and treated leaf segments from test 2 were surface-sterilised before plating onto DLEA. The control plates all remained free of *Botrytis*, while one leaf piece from the treated leaves was colonised by *Botrytis*, with an indication of sclerotia present. For test 3, half of the leaf pieces were sterilised, and the other half was not sterilised, before plating. All 10 plates with non-sterilised, treated segments developed *Botrytis*, sclerotia being present. None of the plates with sterilised treated segments, nor of the control segments (whether sterilised or not), showed any *Botrytis*.

The results indicated that treating narcissus leaves with raindrops 1.7 mm in diameter enabled *B. narcissicola* to survive on the leaf surface after inoculation. The mechanism of survival is unclear from the experiment. Since most sterilised leaf pieces did not give *Botrytis* after they had been plated, it is possible that *Botrytis* is not surviving on the leaf as a result of colonisation of leaf material. However, this point would require further clarification and on this basis the experiment could be repeated.

The results may indicate that this is a hitherto unknown part of the *Botrytis* life cycle on narcissus and could possibly be implicated in premature leaf die-back, which occurs on some narcissus crops. There is the possibility that the droplets themselves cause micro-wound sites in the epidermis that allow *Botrytis* to survive on the leaf surface until other points of damage occur. This type of colonisation would help smoulder effectively to exploit any more substantial forms of damage that arose in the crop. The results tentatively suggested that the impact sensor could be used to establish damage criteria within narcissus crops. The threshold value on the sensor output at which the mechanism is observed has yet to be established, but this must equate approximately to outputs in the bin 12 – 14 range.

Field observations on the effect of rainfall or hail on crop damage and *B. narcissicola* infection

A field crop of narcissus sited at Mepal was affected by severe rainfall/hail on 25 May 2000. Leaves from the affected crop were sent to HRI Wellesbourne for examination and to ascertain the extent of colonisation by *B. narcissicola*. As a result of this weather, leaves in the crop were flattened, with only the flower stems remaining upright. Two types of damage were observed on the crop. First, small (3-5 mm diameter) brown irregularly shaped areas on the leaves were seen at the bottom of the crop. Secondly, there were smaller white flecks on the upper leaves, with symptoms mostly confined to the sides of leaves and stems facing upwards. *B. narcissicola* colonisation was assessed by plating leaf pieces (either surface-sterilised or not) to agar and incubating. Both types of symptoms were plated, brown necrotic areas from lower leaves and white flecks from upper leaves. The results are given in Table 2.5.5.

Table 2.5.5. Results of isolations from sterile and non-sterile brown and white fleck lesions.

Plate no.	No. of *Botrytis* colonies			
	Brown fleck		White fleck	
	Non-sterile	Sterile	Non-sterile	Sterile
1	0	1	2	0
2	1	0	1	0
3	1	1	2	0
4	1	2	1	0
5	2	1	1	0
6	2	2	0	0
7	0	2	0	0
8	2	0	2	0
9	0	0	0	0
10	0	1	0	0
11	2	0	2	0
12	2	1	1	0
Totals	13	11	12	0

These results indicated that *Botrytis* was present on all types of damage recorded from the field. However, there was a clear difference in the association of *Botrytis* from the different types of symptom. *Botrytis* could be isolated from brown flecks (which were bigger areas of potential damage resulting from the hail/rainfall) which were either sterilised or non-sterilised. However, *Botrytis* could only be isolated from white fleck symptoms that remain non-sterilised and could not be isolated if leaf pieces had been sterilised prior to plating. Further isolations are required from crops affected by hail or heavy rainfall to confirm these results. The evidence suggests that such events cause direct and indirect colonisation of tissues by *B. narcissicola*. In future work, impact sensors should be added to weather stations at field sites to correlate the development of smoulder with the rainfall drop sizes. This might provide further evidence of a relationship between symptomless crop damage and rainfall type.

<u>Effect of frost damage on infection of narcissus tissue by *R. vallisumbrosae*</u>

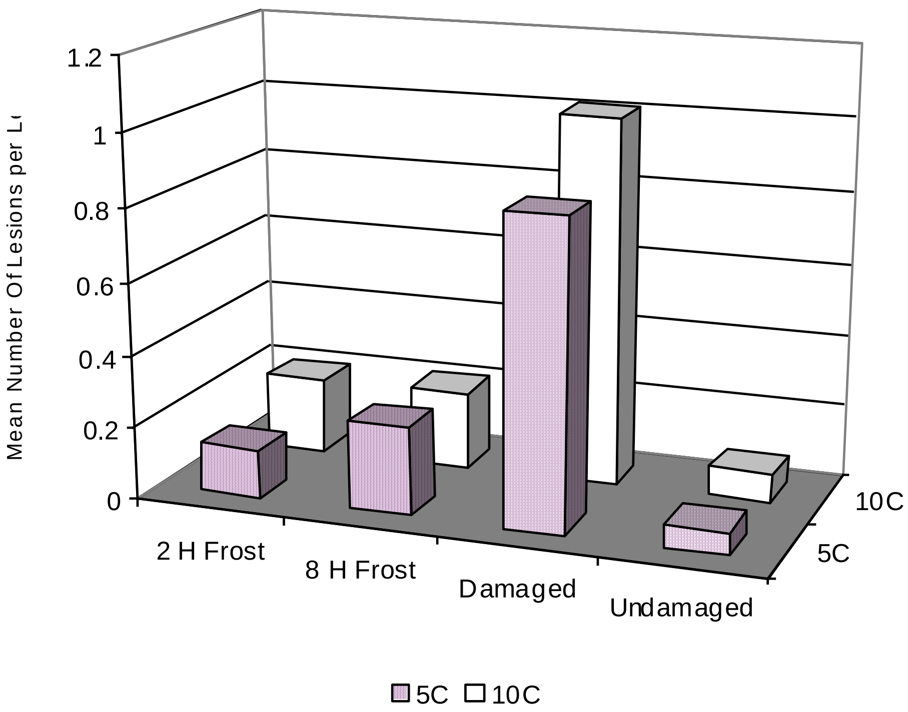
Frost is possibly a major component of tissue injury in narcissus, leading to infection. Individually pot-grown narcissus bulbs were used in controlled environment experiments to investigate this effect.

R. vallisumbrosae inoculum was obtained by collecting infected, sporulating leaf material from Penzance in April 2002. Conidia were washed from leaves with sterile distilled water to produce a spore suspension. In all suspensions 99% of spores were classified as phragmospores (Gregory, 1939). Control plants remained undamaged, while others were subjected to simulated 'damage' by rubbing gently with a soft bristle brush to remove surface wax, leaves having the brushes drawn over the upper surface of the leaf at least twice. In order to simulate frost damage, undamaged plants were placed in controlled environment cabinets at -2°C for 2, 8 or 22 hours. Before inoculation plants were sprayed with distilled water containing wetter, and after all leaves were uniformly wet plants were inoculated with conidial suspension as described above. Plants were placed in controlled environment cabinets at 5 or 10ºC and 99% relative humidity, causing leaf wetness, for 48 hours, before moving to a glasshouse. The number of lesions on each leaf of each plant were recorded after a two-week period in the glasshouse.

Disease on plants exposed for 22 hours at -2°C could not be measured because the tissues had become water soaked and damaged. Many of the leaves were dead and it was therefore difficult to distinguish lesions on them. Other results showed that plants exposed to either 2 or 8 h of frost produced higher levels of white mould infection in comparison to undamaged leaves (Figure 2.5.6). There were very low levels of infection on undamaged leaves, only occasional lesions being produced. Infection on undamaged leaves had not been observed in previous experiments. In comparison, damaged leaves had higher lesion numbers present and these were significantly different from either undamaged or frost exposure treatments. Exposure to frost (-2°C) appeared to cause damage to narcissus leaf tissues and result in enhanced levels of white mould infection. Further experiments would be required to confirm this result. Exposure time at -2°C (2 – 8 hours) did not appear to affect the amount of white mould infection.

The results showed that frost may represent a potential source of damage in narcissus crops. The duration of frost appears to be critical, with durations of over eight hours being extremely detrimental. However 8-hour frost periods are not common in coastal areas where narcissus is grown, indicating that other sources of damage might also be very important. The age of leaf tissues may also play some role in this respect, as younger leaves appear to be more waxy and more rigid than older and longer leaves.

Figure 2.5.6. Mean number of white mould lesions per leaf after frost treatment.



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Models relating tissue wetness duration and temperature to infection of narcissus tissues by *R. vallisumbrosae* and *B. narcissicola* (Objective 3)

Leaf wetness characteristics (Task 3.1)

Introduction

Standard surface wetness sensors from Aardware Design were used throughout the project, but it is possible that the response of this sensor to wetting and drying does not relate to the typical responses of narcissus leaves. A new design of surface wetness sensor was developed by Aardware Design during the course of the project, and was tested under controlled conditions. The original wetness sensor has a large flat responsive surface, whereas the new design has a small circular response surface in a well that collects water from a sloping surface. The sensor was made with different well diameters.

Comparison of sensors

The responses of the two types of wetness sensors were compared in a controlled temperature store operating at 10˚C and 80% relative humidity, with a standard air-flow and fluorescent tubular lamps on for 12 hours per day. Two standard (flat) wetness sensors (one with an unmodified surface, one with a resin-coated surface to modify surface tension characteristics of drops) and five modified (well) sensors (diameters 5, 6, 8, 10 and 12mm) were held by clamps in a horizontal array and connected to a data logger. Pot-grown narcissus plants were used to provide leaves that were also clamped horizontally, adjacent to the sensors, one sample being left undamaged and the other being de-waxed by gently rubbing the leaf surface. The plants used were at flowering stage, and tests were carried out mid-way along the adaxial surface of mature leaves.

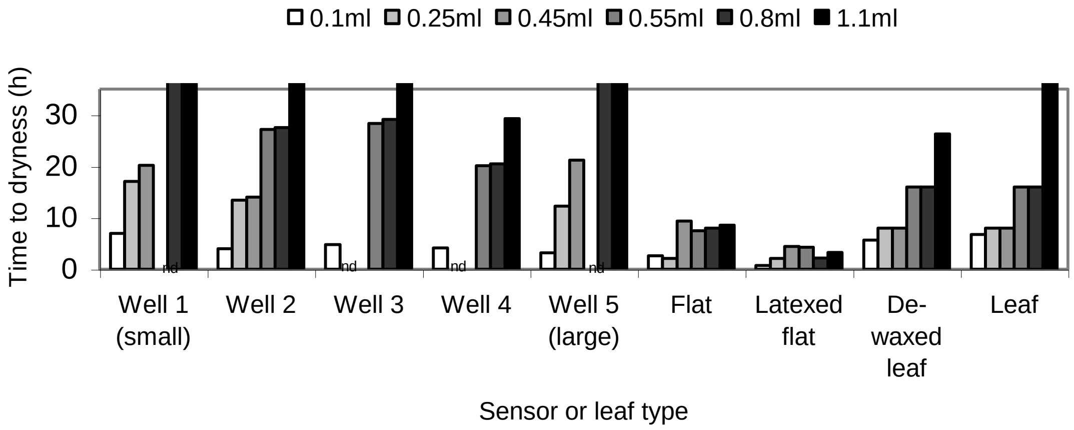
Water drops of different sizes were placed via a micropipette on the central part of the sensor or leaf surface. Drops of volume 0.10, 0.25, 0.45, 0.55, 0.80 and 1.10ml were used in successive runs of the test. The time until complete evaporation had occurred was recorded, which was taken as either when the logger had recorded a consistent dry reading, or, in the case of leaves, by direct observation, up to about 30 hours.

Typical results are shown in Figure 3.1.1. For the smallest drop size (0.1ml) there was little difference in the evaporation time between the seven sensor types and the leaf, either intact or de-waxed. For increasing drop sizes, all well-type sensors showed progressively extending evaporation times, while evaporation time was relatively rapid and uniform in the flat sensors (particularly so when using a latex-covered surface). For leaves, the increase in evaporation time with larger drops was not as great as for the well sensors, except that in the case of the largest drop size (1.10 ml) evaporation time was greatly extended, especially on the undamaged leaf surface.

Conclusions

The results showed that the well-type sensors gave outputs for different drop sizes that corresponded more accurately to that of the leaf. In comparison the flat, conventional wetness sensor gave little difference in the pattern of evaporation of different sized drops. The effect was exacerbated by using a latex coating on the sensor surface. Sensors with well diameters of 5 or 6 mm appeared to match the characteristic drying times of leaves most accurately.

Figure 3.1.1. Evaporation times for different drop sizes on wetness sensors (nd = not determined)



Effect of leaf wetness and temperature on infection (Task 3.2)

Introduction

The effects of environmental conditions on the infection of narcissus by *B. narcissicola* and *R. vallisumbrosae* are not well described, previous R&D having concentrated on disease control. An understanding of these interactions is essential for developing disease forecasting techniques. Experiments were conducted under controlled environment conditions following the determination of optimal inoculation methods.

Preliminary infection experiments with *B. narcissicola*

B. narcissicola was cultured on a number of media to find the most reliable method of conidial production (see also Objective 1). When grown on PDA large numbers of sclerotia were produced, while growth on V8 agar gave abundant mycelium. However, using DLEA resulted in condial production, with cultures producing the highest concentrations of conidia when grown for 7 days. DLEA was used for all subsequent conidial production.

Inoculation methods were evaluated using pot-grown narcissus. This treatment simulated damage that might be caused by weather (e.g., wind or rain) or by the presence of mites. Before inoculation, leaves were damaged using an array of pins, piercing the leaf at three points. Using damaged and non-damaged leaves, several inoculation methods were tested, including squashing mycelial plugs onto the leaf, applying droplets of conidial suspension in water or nutrient medium, and spray application of conidial suspension in water or medium. In some cases plants were 'pre-conditioned' in a polythene bag enclosure for 48 hours before inoculation. DLEA was used for all conidia production. After inoculation, plants were assessed for disease at weekly intervals for 3 weeks or until the leaves had died.

The results confirmed that infection will occur only if inoculations are carried out on damaged leaves, or if conidial inocula are supplemented by nutrients, as previously reported by O'Neill and Mansfield (1982). Mycelial plug inoculation on damaged leaves resulted in the largest number of spreading lesions after 3 weeks, but this method, and the droplet inoculation method (which produced little or no infection) did not relate to field conditions. Subsequent inoculations were, therefore, carried out using sprays of conidial suspensions. Suspensions (10^4 to 10^6 conidia/ml) in water and nutrient medium (1:10 V8 juice) were sprayed onto leaves. Inoculation with suspensions of 10^5 conidia/ml in V8 juice medium gave the largest number of spreading lesions, and this method was used in future experiments. Inoculation with an aqueous suspension of either water or 100 % V8 juice containing 10^6 conidia/ml resulted in fewer active lesions.

Leaf wetness and humidity are important factors determining infection by *B. narcissicola* in the field. The effect of high humidity on infection was investigated under glasshouse conditions by placing a plastic bag over plants for 48 hours before inoculation with conidia in nutrient medium. In these treatments infection was observed only if the bag was left in place until the onset of symptoms, usually after 2 to 3 weeks, with no infection if the bag was removed 24 or 48 hours after inoculation.

Infected leaf material was taken from successful inoculation treatments, and the presence of *B. narcissicola* confirmed after isolation onto PDA.

Controlled environment studies on the effect of temperature and wetness duration on infection of narcissus by conidia of *B. narcissicola*

Airborne fungal pathogens require appropriate environmental conditions, notably wetness, to complete critical stages in their lifecycles. The presence of bound or unbound water is a requirement for disease development by many species of *Botrytis* (Clarkson *et al*., 2000). The relationships between meteorological parameters such as wetness, temperature and humidity and stages in the fungal life cycle can be determined most appropriately from experiments conducted in controlled environments. The data from these experiments can be used to derive mathematical models that summarise the relationship between environmental factors and infection. Validated relationships derived from controlled environment experiments can then be used to form the basis of a forecasting system for timing the applications of fungicides. Any mathematical models need to be robust if they are to be used to determine pathogen responses under fluctuating conditions in the field. Without models that describe rate functions, it would be impossible to assess the likely impact of environmental conditions in the field.

Experiments were carried out using individual, pot-grown narcissus plants cold-stored in batches to produce plants ready from December 1999 onwards. Plants were grown in a glasshouse and placed at high humidity prior to transfer to controlled environment cabinets. In five experiments, sixty or one hundred plants were inoculated at each of 4, 8, 12, 16, 20 and 24°C, damaging leaves and spraying a conidial suspension of *B. narcissicola* as described above. Experiment 1 was not successful, due to the failure of the humidification system in two of the controlled environment cabinets. In Experiments 2 and 3, plants were damaged by using pins which were either drawn along the surface of the epidermis or used to penetrate the epidermis directly at several pre-set points on each leaf. The damage thus caused could be directly exploited by conidia of *B. narcissicola*. Since this was a severe method of simulating damage, the method used was changed in Experiments 4 and 5. In Experiments 4 and 5, soft bristle brushes were used on the leaf to remove surface wax. All leaves on all plants had the brushes drawn over the upper surface of the leaf at least twice. In Experiments 2 and 3, plants were sprayed with distilled water for 0.2 min every 9.8 min to maintain conditions of leaf wetness following inoculation. In Experiments 4 and 5 plants were maintained at a humidity of approximately 96% without leaf wetting. Sample plants were removed from each temperature after 6 - 72 hours and transferred to a glasshouse. The numbers of lesions were recorded after two weeks in the glasshouse.

The results of Experiments 2 – 4 are summarised in Figures 3.2.1 and 3.2.2. A short duration (6 hours) of wetness was sufficient for infection of narcissus by *B. narcissicola*. For substantial infection the presence of free water was required, although some infection could occur at optimal temperatures under conditions of high humidity. The results showed that the optimal temperature for infection was 12˚C, as there was an increase in the numbers of lesions at this temperature after 6 hours wetness duration.

When infection was assessed after 24 hours of wetness, temperatures of 4, 8, 12 and 16˚C gave higher levels of leaf infection in comparison to temperatures of 20 and 24˚C

(Figure 3.2.3). The presence of free water (experiments 2 and 3) in comparison to relative humidities of 96% (experiments 4 and 5) appeared to be more favourable for infection with both 6 and 24 hours of wetness. After 24 hours wetness duration there was little difference in the level of infection on leaves at temperature of 4, 8, 12 and 16˚C.

Figure 3.2.1. Average number of lesions per leaf at 4 - 24 C (6 hours wetness duration)

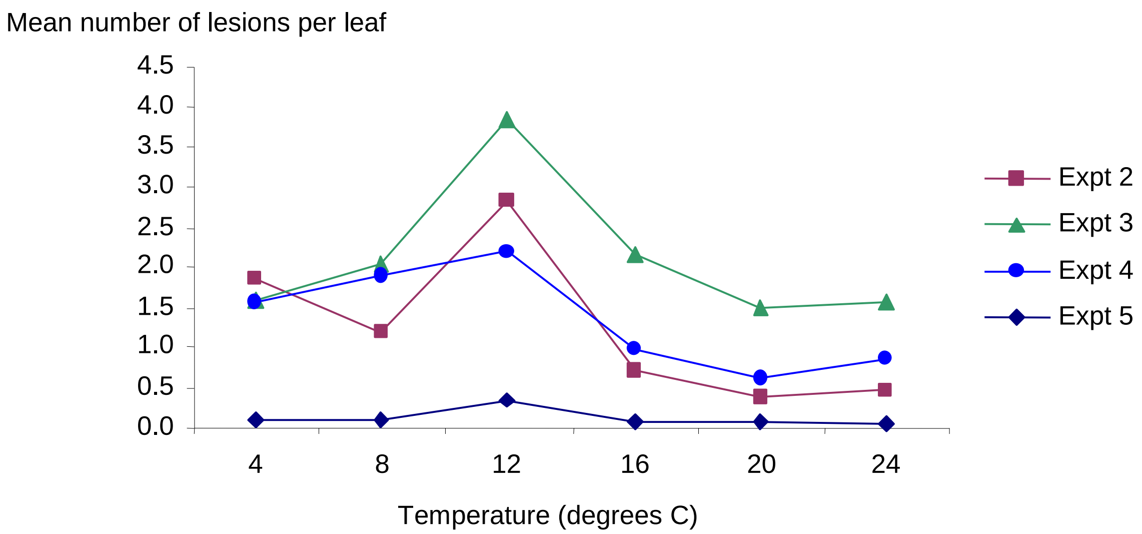
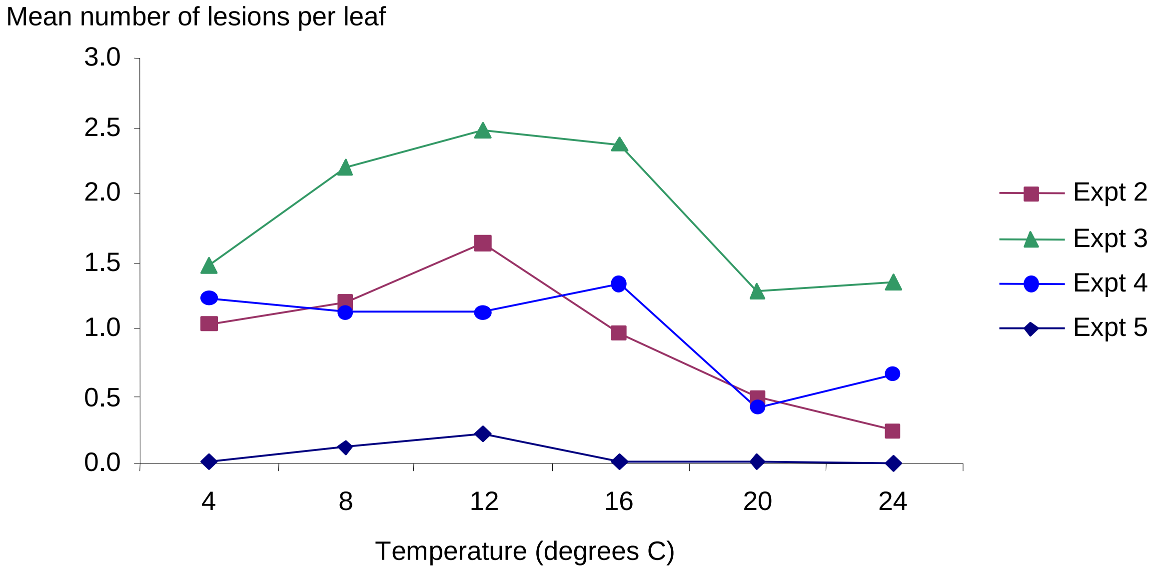


Figure 3.2.2. Average number of lesions per leaf at 4 - 24 C (24 hours wetness duration)



Modelling the effect of temperature and wetness duration on infection by *B. narcissicicola*

Statistical analysis was used to investigate the effect of temperature and leaf wetness on infection of narcissus leaves by smoulder. Analysis of variance was used to determine if the effect of temperature and wetness had a significant effect on infection and to check for significant differences between replicates. No significant differences between replicate experiments were found, and therefore the data from each replicate experiment was pooled before curve-fitting techniques were applied. Linear and non-linear modelling techniques were used to fit relationships to the pooled data.

The results showed that the effect of temperature on infection of leaf tissue by smoulder was non-linear (see Fig. 3.2.6). The function giving the best fit to the line being quadratic. The effect of wetness duration on infection of leaf tissue by smoulder was, however, linear (data not presented). The following relationship was highly significant in describing the effect of temperature and wetness duration on infection of narcissus leaves by *B. narcissicola*:

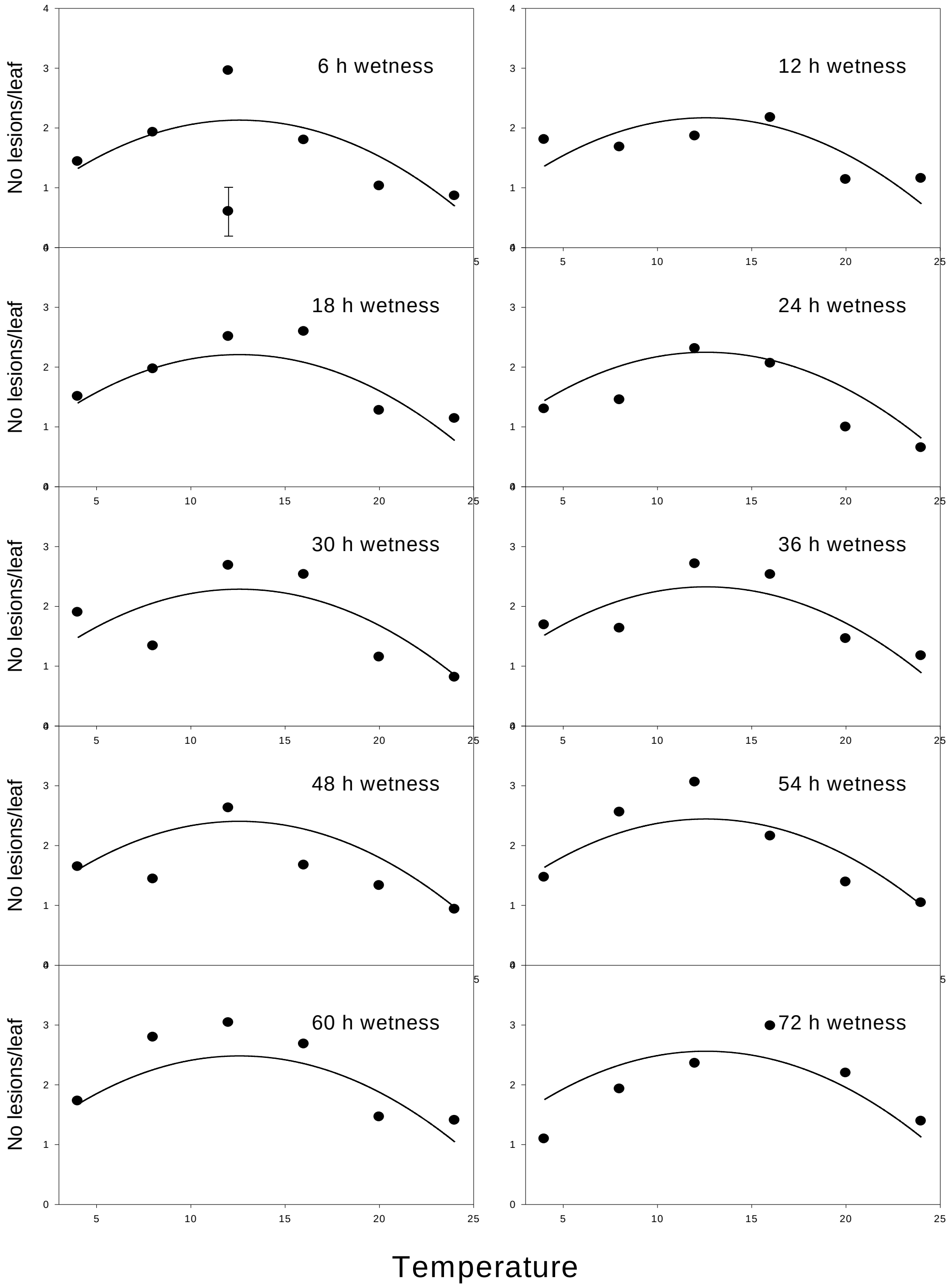
$$\text{Number of smoulder lesions per leaf} = 0.351 + 0.0065w + 0.277t - 0.011t^2$$

where w = leaf wetness duration (hours) and t = temperature (°C), assuming that leaf wetness duration is between 1 and 72 hours and temperature is between 4 and 24°C.

Conclusions on conditions favouring *B. narcissicola* infection

- Leaf damage was required for the infection of narcissus leaves by *B. narcissicola*.
- Optimal inoculation and incubation conditions were spray inoculation with 10^5 conidial/ml suspension in 1:10 V8 juice, followed by humid incubation until lesion appearance.
- The presence of free water was required for infection of narcissus tissues by *B. narcissicola.*
- Temperatures of 12˚C are optimal for infection by *B. narcissicola* at short wetness durations (approximately 6 hours).
- Temperatures of 4 – 16˚C were optimal for infection *B. narcissicola* at relatively long wetness durations (approximately 24 hours).
- Temperature and wetness duration had a highly significant effect on infection of leaves by *B. narcissicola* in the presence of leaf damage.
- There was a non-linear relationship between infection and temperature.
- There was a linear relationship between wetness duration and infection.

Figure 3.2.3. Fitted curves for the effect of temperature on infection of narcissus leaves by smoulder.



<u>Preliminary infection experiments with *R. vallisumbrosae*</u>

R. vallisumbrosae has a number of different types of conidia depending on temperature, humidity, type of available nutrients and life cycle stage (Gregory, 1939). Conidial identification is difficult as the literature states that conidial dimensions vary between specimens, different parts of the same leaf and culture media used to produce spores. No precise dimensions or characteristics are available for direct comparison. Preliminary infection experiments were necessary to determine the most important inoculation conditions, and for this a reliable method for routine conidial production was required. Gregory (1939) established that infection could occur on young uninjured leaves, indicating that mature tissue has a greater resistance to hyphal entry through stomata, following germinating conidia lodging in the leaf cuticle.

Previous studies had indicated that culture media had an important effect on which life cycle stage *R. vallisumbrosae* produced (see Objective 1). The greatest spore production occurred on BA, with some conidia production on DLEA, and these media were used for subsequent conidia production except when conidia were available from naturally infected plants from Tresillian or Penzance.

Preliminary experiments indicated that spray inoculation was the only reliable method of inoculation with *R. vallisumbrosae*. To simulate field conditions and increase infection in the glasshouse, plants were pre-conditioned by placing in plastic bags for 48 hours before inoculation. In early studies inoculated narcissus leaves had been placed in a moist atmosphere (bell jar or sealed test tubes) (Gregory, 1939). The suspension media giving the largest number of active lesions per leaf was 1:10 V8 juice. Inoculation with conidial suspensions (10^6 conidia/ml in water and medium (1:10 V8 juice) were compared. Inoculations were also carried out on intact and damaged leaves. The methods used were similar to those of the previously described *Botrytis* experiments, with the following treatments:

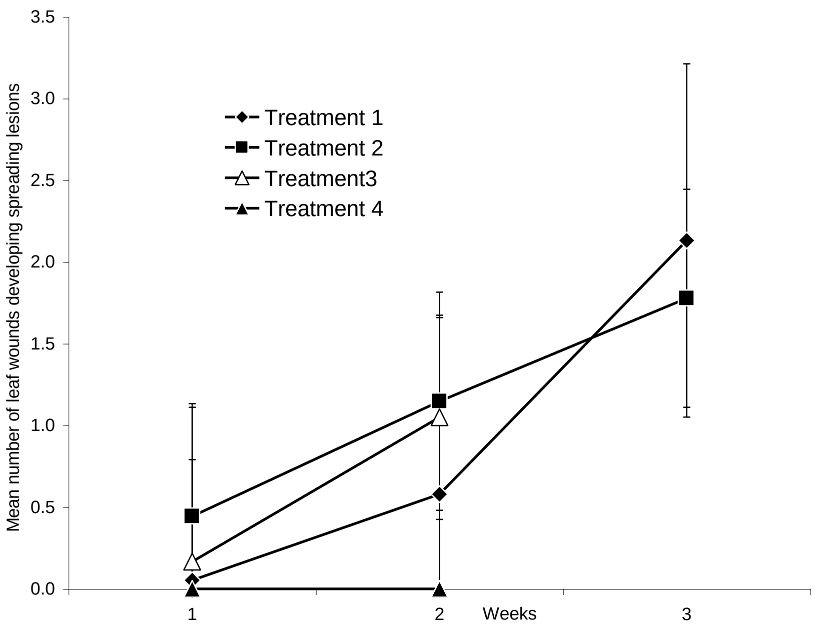
1. Pre-conditioned plants, damaged leaves spray-inoculated with suspension in medium
2. Pre-conditioned plants, damaged leaves spray-inoculated with suspension in water
3. Pre-conditioned plants, non-damaged leaves spray-inoculated with suspension in medium
4. Non-pre-conditioned plants, non-damaged leaves spray-inoculated with suspension in medium

Treatments 1 and 3 compared damaged and undamaged leaves, treatments 1 and 2 compared applying conidia in water or medium, and treatments 3 and 4 compared using pre- and non-pre-conditioned plants.

The results (Figure 3.2.4) indicated that the pathogen could infect undamaged leaves, although a longer period of time was required for lesion spread. Some leaves remained unaffected throughout, even on a plant with infected or sporulating leaves. Plants without disease were still healthy after 3 weeks, the symptoms of natural die-back being different to those of white mould.

Optimal inoculation conditions were shown to be spray-inoculating pre-conditioned plants with 10^6 conidial suspension in 1:10 V8 juice on intact or damaged leaves. High humidity or wetness may be essential for infection to occur because of the loss by evaporation of free water required for conidial germination, and leaf age and tissue status may affect infection, however placing a polythene bag over plants may standardise the responses of all tissue types.

Figure 3.2.4. *R. vallisumbrosae* inoculation methods (see text for treatment descriptions).



<u>Controlled environment studies on the effect of temperature and wetness duration on infection of narcissus by *R. vallisumbrosae*</u>

The relationships between important meteorological parameters, such as wetness, temperature and humidity, and stages in the fungal life-cycle can be determined from experiments conducted under controlled environment conditions. The data from these experiments can be used to derive mathematical models, which summarise the relationship between environmental factors and infection. It is important that viable inoculum is used in these experiments. Several spores types can be found on white mould lesions. Early in the season scolecospores are produced, however the most commonly occurring spore type is the phragmospore, the presence of which on the lesion gives it the characteristic white colour. Amerospores are also produced by this pathogen. The relationship between spore types is not clearly understood, but all three have been observed on white mould lesions in the field. It is possible that each spore type has different requirements for infection of narcissus leaves.

From controlled environment experiments mathematical relationships obtained in this way can be validated under field conditions, and can form the basis of critical timings for fungicide application. Individually potted narcissus bulbs were raised at Kirton and transported to a glasshouse at HRI Wellesbourne approximately 3 days before the start of controlled environment experiments. On the day before the plants were inoculated they were placed at high humidity prior to transfer to the controlled environment cabinets. *R. vallisumbrosae* inoculum was obtained from the field by collecting infected leaf material from Cornwall. This was necessary to obtain sufficient amounts of inoculum since cultured isolates of the pathogen failed to produce sufficient inoculum that was infective on narcissus leaf tissue. Infected leaves with fresh conidia were washed using sterile distilled water to obtain spore suspensions with approximately 10^7 conidia/ml. In all suspensions 99% of spores were classified as phragmospores. A final inoculum concentration of approximately 5 x 10^5 conidia/ml was produced in a 1:10 dilution of V8 liquid. The leaves of all plants used in controlled environment experiments were treated to simulate 'damage' by rubbing gently with a soft bristle brush. All leaves on all plants had the brushes drawn over the upper surface of the leaf at least twice. Plants were spray-inoculated, applying 2 - 4 ml of inoculum per inoculated pot. At each temperature plants were maintained under conditions of 98% relative humidity (resulting in leaf wetness) after inoculation. Five experiments were conducted to investigate the effect of temperature and wetness on infection of narcissus leaves by white mould. The experiments were set up between 17 January and 9 May 2001 under temperatures of 5, 10, 15… 30°C with 1, 3, 6, 12… 30 hours of wetness duration, usually with seven replicates. The number of lesions on each leaf was recorded after a two-week incubation period in the glasshouse. Lesions were examined microscopically for the presence or absence of sporulation.

Sixteen narcissus plants displaying typical white mould lesions were placed at 5, 10, 15, 20, 25 and 30°C under constant 95% relative humidity. Two plants from each environment were removed for treatment 4, 22, 28 and 93 hours after the start of the experiment. Four lesions from each treatment (one from each plant) were selected and marked. Collodion dissolved in ethanol was applied to each selected lesion and allowed to dry. Once dry, the strip of collodion were carefully peeled off the epidermis of the leaf using forceps and placed into a drop of distilled water ('spore-side' uppermost) on a clean, labelled microscope slide, and air-dried. Fresh lesions were selected at each observation time. Slides were stored in the fridge prior to staining with 0.1% trypan blue in lactophenol, and were returned to the fridge for storage when stained. Slides were examined microscopically for the presence or absence of spores, the relative number of spores and spore types.

The results of controlled environment experiments 2, 3, 4 and 5 are summarised in the figures below. Infection was observed on tissues that had been subjected to damage. There was no infection on leaf tissues where the surface layers of wax on the leaf had not been disturbed. White mould infection of narcissus leaf tissues was optimal at temperatures of 5 - 10°C, although it occurred over a wide range of temperatures up to 30°C (Fig. 3.2.5). The optimal wetness duration for infection by white mould was after 12 - 18 hours of wetness (Fig. 3.2.6). Infection could occur after very short periods of leaf surface wetness of just one hour. The results suggest that leaf wetness periods of 12 to 18 hours at temperatures of 5 - 10°C can cause substantial infection by phragmospores of the white mould pathogen. Further work is needed to determine if the other spore types produced by the pathogen are equally infective under similar condition of temperature and wetness duration.

The results shown on Table 3.2.1 indicate the predominant spore type produced on white mould lesions is the phragmospore. The optimal temperature conditions for phragmospore development are approximately 5 - 10°C under conditions of constant 95% relative humidity. However, this spore type can be produced at temperatures of 25°C. Scolecospores were produced on lesions at temperatures of 5 and 10°C. It was not possible in this experiment to quantify the numbers of spores produced precisely. Amerospores were not identified in this experiment.

Figure 3.2.5. Effect of temperature on infection of narcissus leaves by white mould.

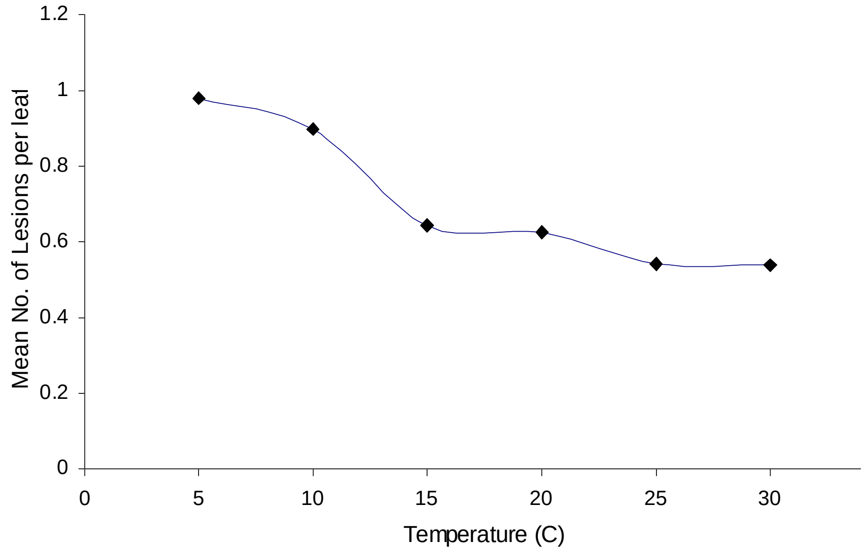


Figure 3.2.6. Effect of wetness duration on infection of leaves by white mould.

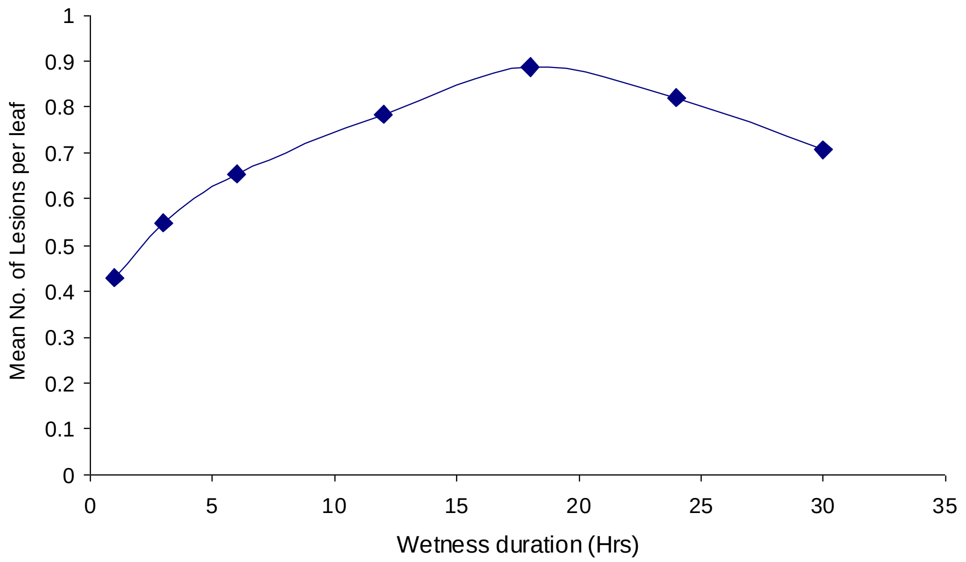


Table 3.2.1. Effect of temperature and humidity duration on sporulation* by white mould lesions.

Temperature	Humidity duration (hours)			
(°C)	4	22	30	96
5	-	-	-	+++ oo
10	-	+	++	+++ ooo
15	-	-	++	+++
20	-	++	++	++
25	-	+	+	+
30	-	-	-	-

* -, spores not present; phragmospores: +, low numbers, ++, abundant , +++, high numbers; scolecospores: oo, abundant , ooo, high numbers

<u>Modelling the effect of temperature and wetness duration on infection of narcissus by *R. vallisumbrosae*</u>

Statistical analysis was used to investigate the effect of temperature and wetness on infection of narcissus leaves by white mould. Analysis of variance was used to determine if the effect of temperature and wetness had a significant effect on infection and to check for significant differences between replicates. No significant differences between replicate experiments were found, therefore the data from each replicate experiment was pooled before curve-fitting techniques were applied. Linear and non-linear modelling techniques were used to fit relationships to the pooled data.

The results show that the effect of temperature on infection of leaf tissue was non-linear (Figures 3.2.7 and 3.2.8). Lesion number was found to be an exponential function of wetness duration with the number of lesions increasing to a maximum, which was described by a logistic function of temperature. The increase in lesion number was significantly described by a logistic function. The following relationship was highly significant in describing the effect of temperature *t* (˚C) and leaf wetness duration *w* (hours) on the number of white mould lesions per leaf *R*:

$$\sqrt{R} = ((0.9509 + F) - (F \times 0.3283^{w})$$

where $F = \left(\dfrac{0.3428}{(1 + \exp(-0.379 \times t - 19.91))} \right)$

and *w* is between 0 and 30 hours and *t* is between 5 and 30ºC.

<u>Conclusions on conditions for *R. vallisumbrosae* infection</u>

- Damage to leaf tissue was required for significant infection of narcissus leaves by white mould**.**
- The presence of free water increased the severity of infection.
- Temperatures of 5 - 10°C are optimal for infection by *R. vallisumbrosae*.
- Wetness durations of 12 - 24 hours are optimal for infection by *R. vallisumbrosae*.
- Temperature and wetness duration had a highly significant effect on infection of immature leaves by white mould.
- There was a logistic relationship between maximum white mould infection and temperature.
- There was an exponential relationship between wetness duration and white mould infection.
- Phragmospores are the most common spore form produced on white mould lesions.
- The optimal temperature for phragmospore production is 5 - 15°C.
- Scolecospores are produced at temperatures of 5 - 10°C.
- Phragmospores and scolecospores may or may not have different environmental criteria for infection of narcissus leaves.

Figure 3.2.7. Fitted curves for the effect of temperature on infection of narcissus leaves by white mould.

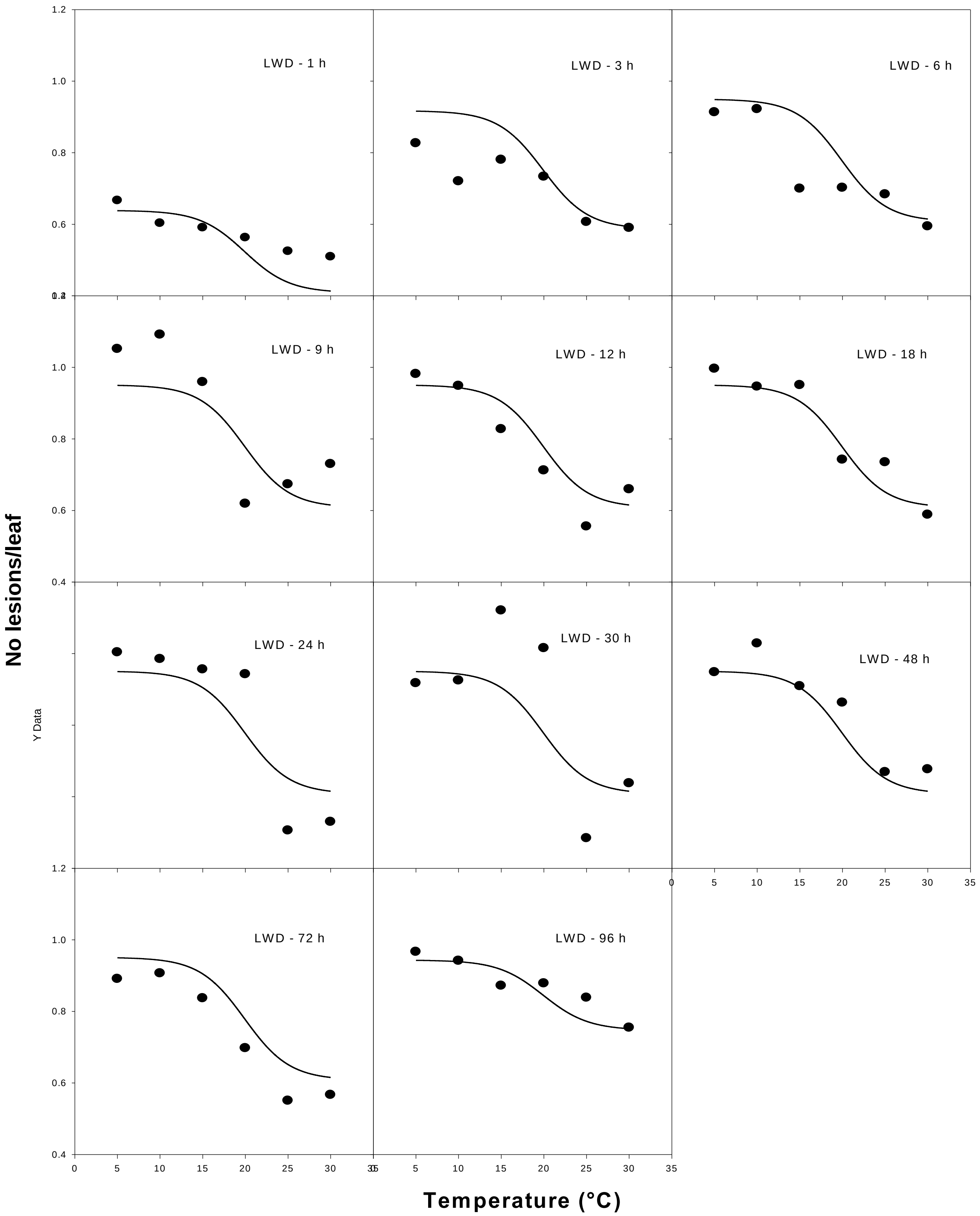
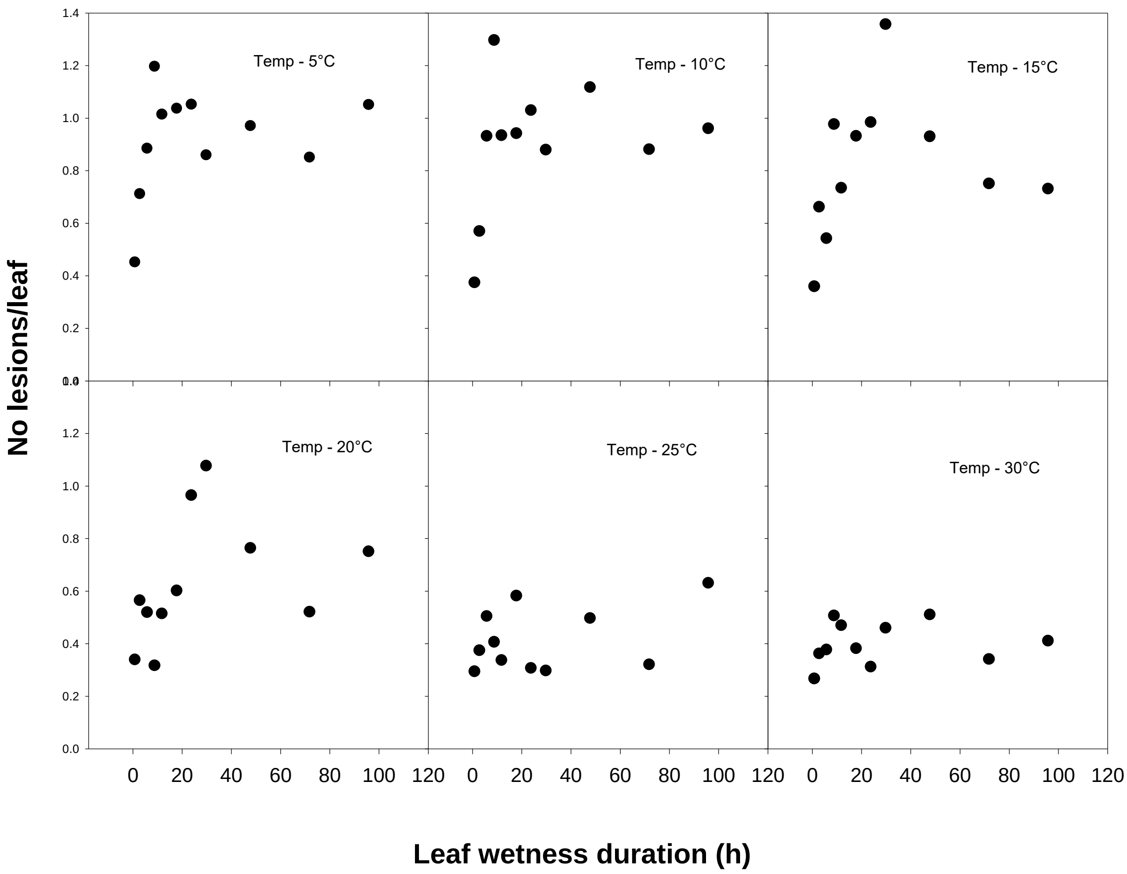


Figure 3.2.8. Fitted curves for the effect of leaf wetness duration on infection of narcissus leaves by white mould.



Validation of leaf wetness duration models (Task 3.3)

Introduction

The relationships for white mould infection (Figure 3.3.1) and smoulder infection (Figure 3.3.2) are presented below in relation to the number of predicted lesions per narcissus leaf. These relationships can be used in conjunction with environmental data from the field (temperature and leaf wetness) to forecast the cumulative lesion numbers and percentage die-back occurring at different narcissus sites. Comparisons of the predicted output from the forecaster (total numbers of predicted lesions) were made against actual observations from the disease development trials. The predicted numbers of lesions per crop were compared with (a) % die-back and (b) the cumulative lesion number observed at each site in eastern England (for smoulder) and Cornwall (for white mould). Comparisons were also made between the predicted total cumulative lesion number after frost periods and observed % die-back and cumulative lesion number. Frost periods were designated as occurring after periods when the air temperature dropped below 0^{o}C.

Figure 3.3.1. Contour plots of the effect of temperature and wetness duration on no. of lesions per leaf of white mould

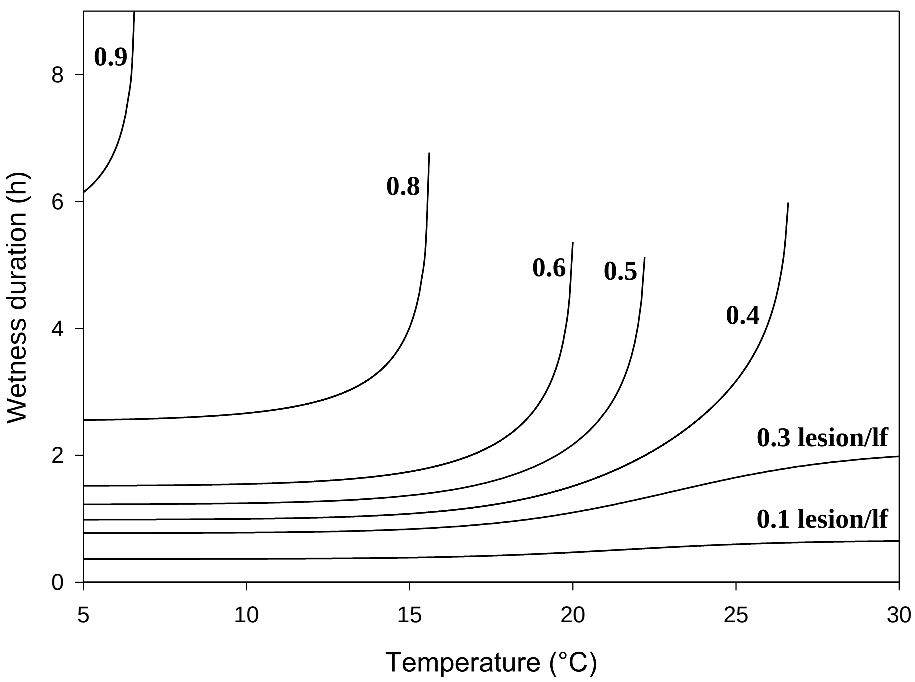
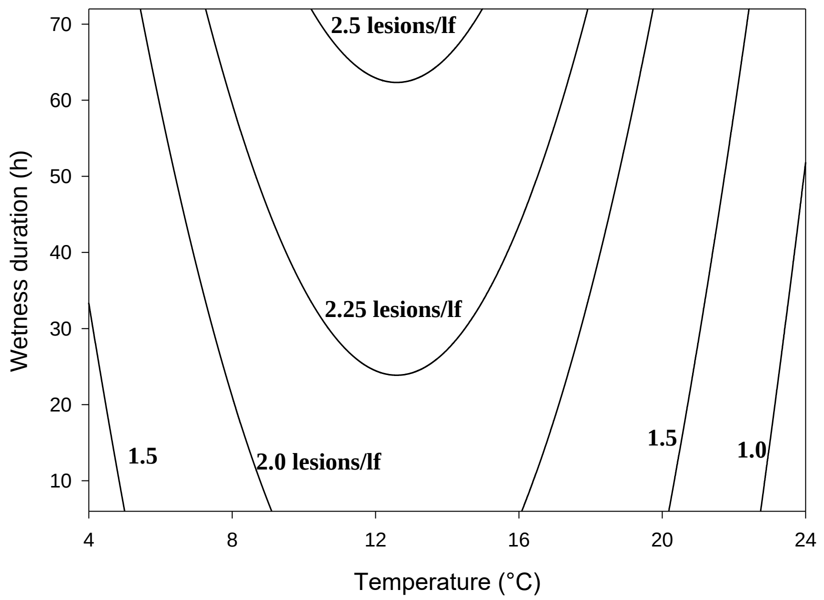


Figure 3.3.2. Contour plot of effects of temperature and wetness duration on no. lesions per leaf of smoulder.



<u>Tests of model predictions for smoulder using disease monitoring data</u>

There was a strong relationship (r^2 = 0.7145) between the observed % die-back in crops in Lincolnshire in 1998/1999 and 2000/2001 and the cumulative predicted total number of lesions (Figure 3.3.3) using the relationship described in Task 3.2. Information from three monitoring sites (Holbeach Marsh, Gosberton Marsh and Swaffham Prior Fen) in 1998/1999 and 2000/2001 was used in the analysis. Crop die-back was observed when the predicted cumulative lesion number increased to over 200 lesions. This suggests that a *B. narcissicola* developmental threshold is required for die-back to be observed in narcissus crops infected with the pathogen. Further data would be required to confirm this relationship. However, there was a poor relationship (r^2 = 0.2294) between the cumulative predicted total number of lesions and the observed number of smoulder lesions (Figure 3.3.4).

Figure 3.3.3. Observed % die-back and predicted cumulative lesion number

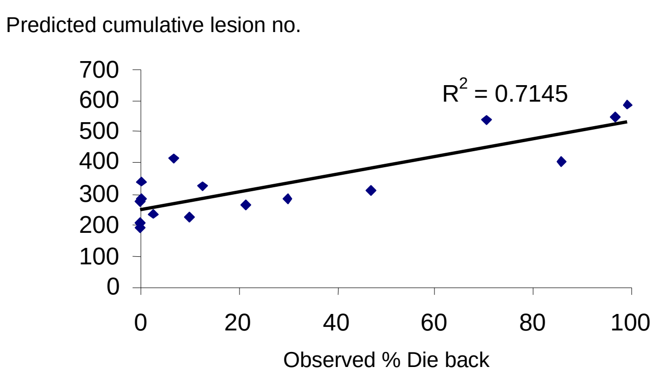
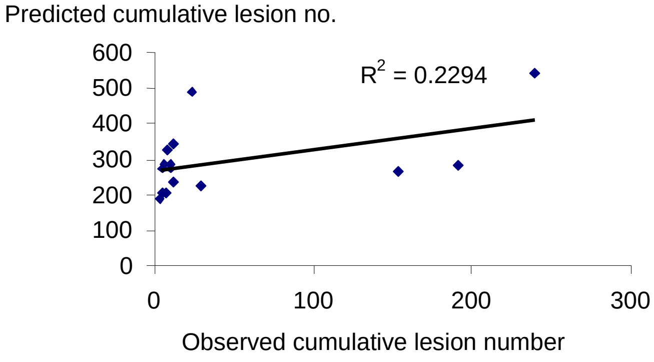


Figure 3.3.4. Observed cumulative lesion number and predicted cumulative lesion number



The assumption that frost periods are required for successful infection of narcissus tissues by *B. narcissicola*, due to the damage caused, changed the relationship between predicted cumulative lesion number and observed % die-back and lesion number. If frost periods were assumed to be necessary for successful infection, there was no relationship ($r^2 = 0.2805$) between the observed % die-back in crops in Lincolnshire in 1998/1999 and 2000/2001 and the cumulative predicted total number of lesions (Figure 3.3.5). However, there was strong negative relationship ($r^2 = 0.6526$) between the cumulative predicted number of lesions and the observed number of smoulder lesions (Figure 3.3.6) after frost periods.

Figure 3.3.5. Observed % die- back and predicted cumulative lesion number after frost periods

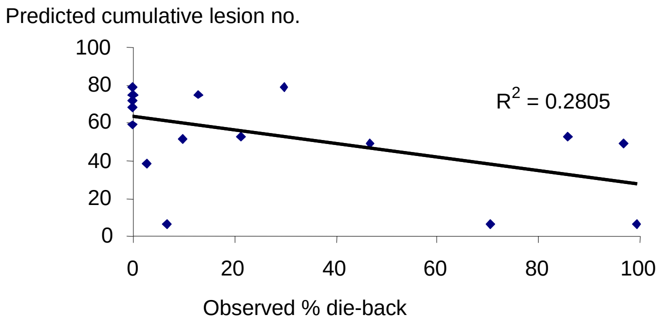
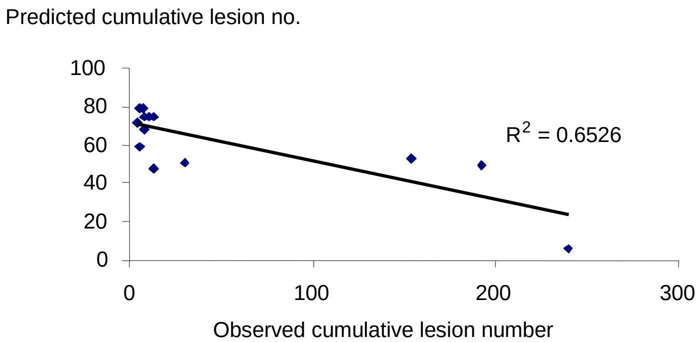


Figure 3.3.6. Observed cumulative lesion number and predicted cumulative lesion number after frost periods



Tests of model predictions for white mould using disease monitoring data

There was a positive relationship ($r^2 = 0.4907$) between the observed % die-back in crops in Cornwall in 1998/1999 (at Tresillian, Manaccan and Truro), and the cumulative predicted total number of lesions (Figure 3.3.7) using the model described in Task 3.2. However, there was no relationship ($r^2 = 0.1100$) between predicted white mould lesion number and observed lesion number (Figure 3.3.8). The relationships are based on a limited amount of disease development data for each disease. Further work would be required to test these relationships and complete their evaluation.

Figure 3.3.7. Observed % die-back and predicted cumulative lesion number for white mould

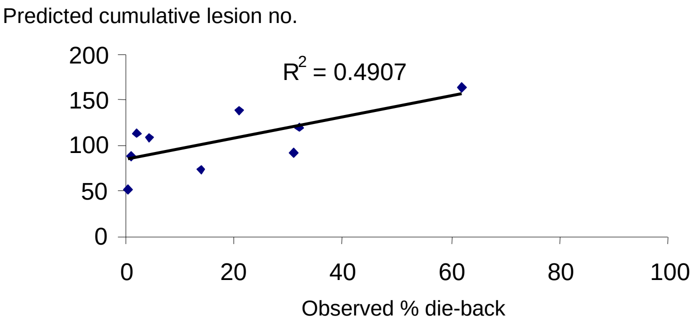
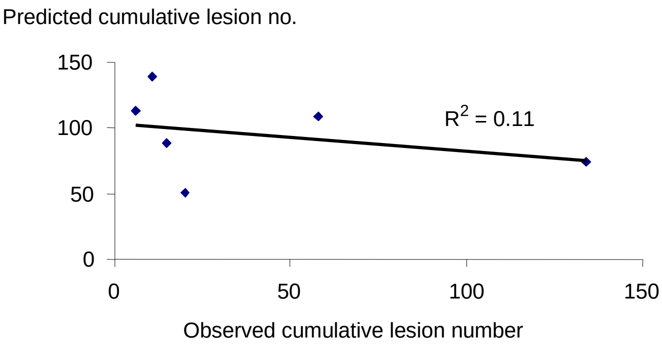


Figure 3.3.8. Observed cumulative white mould lesion number and predicted cumulative lesion number



Conclusions

- With smoulder in particular, the predicted numbers of lesions was less than expected, perhaps because there was in these cases insufficient leaf damage. Further improvements in the accuracy of the models could be obtained by using predictions only from the time that disease was first observed in the crop.
- While the predicted number of lesions did not relate to the observed numbers of lesions, it was strongly related to percentage leaf die-back. This discrepancy may be due to the practical difficulties of accurately counting lesion numbers when these are increasing rapidly and coalescing; percentage die-back, however, is easy to estimate. The results suggest a threshold cumulative level of infection by both white mould and smoulder is required before die-back is observed, which could be used as a predictive tool.
- For smoulder, if disease infection periods after frost were used to determine cumulative predicted lesion numbers, there was no relationship between predicted lesion number and observed percentage die-back, and a negative relationship

between predicted lesion number and observed lesion number. Thus, the effect of frost on lesion numbers for smoulder appeared to be negative despite its potential to cause leaf damage, perhaps because frost is damaging to *B. narcissicola* propagules.

- The resistance to disease of early and late varieties of narcissus could potentially be compared by using predicted percentage leaf die-back.
- Further data could allow locality-specific forecasts to be determined.

Effective spray applications (Objective 4)

Fungicide efficacy (Task 4.1)

Introduction

There appears to have been no published work on the efficacy of fungicide treatments against narcissus smoulder and white mould for over 20 years. Benzimidazole (MBC) fungicides were found to be effective against smoulder in the 1970s (Gray & Shiel, 1975), and some newer fungicides were subsequently incorporated into spray programmes. When this project started, a variety of fungicides was used for control of the disease, including chlorothalonil and vinclozolin. Against white mould, dithiocarbamate fungicides were found to give some control (Gregory, 1940; Jenkins & Hawken, 1969). Subsequently, a mixture of benomyl and mancozeb was found to give improved control, and has remained the standard treatment for white mould until this project, while chlorothalonil has also been shown to be effective (O. Jones, personal communication). The number of fungicide sprays now applied for control of foliar diseases annually ranges up to about seven, generally with more sprays applied in the South-West.

With the recent introduction of new anilinopyrimidine (Forster & Staub, 1996), triazole (Stehmann & de Waard, 1996) and strobilurin or QoI fungicides (Godwin *et al*., 1992), there are prospects for improved control of both diseases, possibly with a reduced number of sprays. In initial laboratory and glasshouse studies, fungicides from these novel groups were evaluated in comparison with the current standard fungicides for their efficacy against *B. narcissicola* and *R. vallisumbrosae*. The most promising chemicals from the laboratory studies were taken forward for evaluation in field trials, in which they were applied both in conventional programmes and using timings designed to provide effective control with fewer spray treatments.

Field trials were carried out over two years at Mepal and Kirton, looking at the control of each pathogen. In smoulder trials the protocol relied on natural crop infections, whereas in white mould trials crops were artificially inoculated with disease material, since white mould does not generally occur in the east. In the first set of field trials, the objective was to compare individual products applied as programmes of five to six sprays each. In the second set of trials, the importance of fungicide application was investigated at different growth stages. As white mould did not occur in these trials, despite repeated inoculation, further field trials were conducted on known white mould-susceptible commercial crops of cv. Cheerfulness (or Yellow Cheerfulness) growing near Penzance.

Laboratory studies using leaf assays

Fungicides were screened in the laboratory using leaf assays. Attached leaves of narcissus, lain horizontally, were sprayed at high volume with test fungicides, wound-inoculated with agar plugs of *B. narcissicola* or *R. vallisumbrosae* cultures (a minimum of two inoculation sites on each of ten leaves), and incubated under suitable lit, humid conditions. Fungicides were applied either one day before inoculation (protectant sprays) or 2 days after inoculation (curative sprays). The fungicides tested, in spring 1999 and 2000, are tabulated below. The number and area of spreading lesions were then assessed at intervals.

1999 Product (a.i.) and rate of product per litre		2000 Product (a.i.) and rate of product per litre	
Untreated control	-	Untreated control	-
Ronilan (50% vinclozolin)	1.0 ml	Plover (250 g/l difenoconazole)	0.25 ml
Bravo 500 (500 g/litre chlorothalonil)	3.0 ml	Folicur (250 g/l tebuconaole)	1.0 ml
Benlate (50% w/w benomyl),	0.5 g	Opus (125 g / l epoxiconazole)	1.0 ml
Dithane 945 (80% w/w mancozeb),	1.5 g	Punch C (125 g / l carbendazim + 250 g/l flusilazole)	0.8 ml
Amistar (25% azoxystrobin)	1.0 ml	Compass (167 g / l iprodione + 167 g / litre thiophanate-methyl)	3.0 ml
Stroby WG (50% kresoxim-methyl)	0.625 g	Bravocarb (100 g / l carbendazim + 450 g/l chlorothalonil)	2.0 ml
Frupica (50% mepanipyrim)	0.8 g	Bavistin + Dithane 945 (50% w/w carbendazim + 80% w/w mancozeb)	0.5 + 1.5 g
Scala (400 g/litre pyrimethanil)	2.0 ml		
Shirlan (50% fluazinam)	1.0 ml		
Unix (50% cyprodinil)	0.67 g		
Benlate + Dithane 945	0.5 + 1.5 g		
Bavistin DF (50% w/w carbendazim)	1.1 g		

Fungicide activity against *B. narcissicola* in leaf assays

All of the fungicides tested reduced lesion size when applied as protectant sprays. Ronilan, Scala and Unix (experiment 1) and Folicur, Opus and Punch C (experiment 2) were particularly effective, resulting in >90% reduction in lesion area, compared with lesion development on untreated leaves, at 8 to 10 days after inoculation (Table 4.1.1). The fungicides were generally less effective when applied as curative sprays 2 days after inoculation, and several treatments gave little or no control. However, five treatments (Amistar, Folicur, Ronilan, Scala and Unix) resulted in a reduction of lesion area of > 60% (Table 4.1.1). In experiment 1, Benlate and Dithane 945 applied alone each gave only slight control, but the mixture showed good protectant activity, suggesting a synergistic effect. These experiments demonstrated, for the first time, good activity against *B. narcissicola* by anilinopyrimidine fungicides (Frupica, Scala and Unix) and triazole fungicides (Folicur, Opus and Plover) and some activity by the strobilurin fungicide Amistar. Based on the results of these laboratory and glasshouse studies the fungicides shown in Table 4.1.3 were taken forward to field trials.

Fungicide activity against *R. vallisumbrosa* in leaf assays

Amistar, Benlate + Dithane 945 and Scala were all very effective, completely preventing the establishment of *R. vallisumbrosae* when applied as protectant sprays Table 4.1.2). Ronilan and Dithane 945 were ineffective, while other treatments were intermediate. The same three fungicides were the most effective treatments against *R. vallisumbrosae* when applied 2 days after inoculation, while Bravo, Stroby WG, Unix, Frupica and Shirlan appeared only slightly less effective (Table 4.1.2). These results demonstrate, for the first time, activity of anilinopyrimidine (Scala) and strobilurin fungicides (Amistar) against *R. vallisumbrosae*, and confirm the good activity of Benlate + Dithane 945. Based on the results of these laboratory and glasshouse studies the fungicides shown in Table 4.1.3 were taken forward to field trials.

Table 4.1.1. Evaluation of fungicides in attached leaf assay for control of *B. narcissicola*.

Product	Mean % reduction in lesion area*	
	Protectant spray	Curative spray
Experiment 1		
Amistar	30	76
Bavistin DF	26	26
Benlate	17	13
Benlate + Dithane 945	75	33
Bravo 500	75	35
Dithane 945	15	0
Frupica	70	35
Ronilan	100	75
Scala	100	66
Shirlan	80	44
Stroby	15	12
Unix	95	64
Experiment 2		
Bavistin + Dithane 945	60	0
Benlate + Dithane 945	22	22
Bravocarb	60	0
Compass	85	10
Folicur	96	65
Opus	95	7
Plover	78	51
Punch C	97	23

*Assessed 8 – 10 days after inoculation.

Table 4.1.2. Evaluation of fungicides in attached leaf assay for control of *R. vallisumbrosae*.

Product	Mean % reduction in lesion area*	
	Protectant spray	Curative spray
Amistar	100	100
Bavistin	87	54
Benlate	90	42
Benlate + Dithane 945	100	100
Bravo 500	62	66
Dithane 945	0	0
Frupica	5	72
Ronilan	0	35
Scala	100	100
Shirlan	12	95
Stroby	65	95
Unix	33	82

*Assessed 8 – 10 days after inoculation.

Table 4.1.3. Summary of activity of selected fungicides, determined in laboratory and glasshouse studies, against *B. narcissicola* and *R. vallisumbrosae*.

Fungicide group and product	Activity*	
	B. narcissicola	*R. vallisumbrosae*
MBC + dithiocarbamate		
Benlate + Dithane 945	****	*****
Bavistin DF + Dithane 945	***	NT
Phthalonitrile		
Bravo 500	****	****
Dicarboximide		
Ronilan	*****	0
Triazole		
Folicur	*****	NT
QoI		
Amistar	**	*****
Stroby WG	*	****
Anilinopyrimidene		
Scala	*****	*****
Unix	*****	**

*Reduction in lesion size when used as protectant sprays: 0, nil; *, 1-20%; **, 21-40%; ***, 41-60%; ****, 61-80%; *****, 81-100%; NT, not tested.

Field trials at Mepal and Kirton

Each field trial was carried out twice, in parallel experiments at Mepal and Kirton. Bulbs (supplied by the industrial partners) were planted at each site in summer 1998 and 1999, and treatments were applied in the second year of crops (2000 and 2001). In each case bulbs of a Cornish stock of cv. Cheerfulness were planted for trials on white mould, and bulbs of a Lincolnshire stock of cv. Carlton for trials on smoulder.

Each of the trials consisted of nine treatment plots arranged in four randomised blocks, with guard plants and double replication of the untreated controls. Cultural procedures followed good commercial practice, except that (1) no routine fungicides were applied in either year of the crop, (2) flowers were picked in both crop years, and (3) irrigation was applied (to encourage the development of disease).

Botrytis occurred on many plants of both varieties after flowering in the first crop year. However, as expected, no natural infection by white mould was seen. To increase the overall likelihood of disease occurring in the second crop year, plots were uniformly inoculated with diseased narcissus tissues and pathogen cultures. Plots of cv. Carlton and Cheerfulness were inoculated after flowering in 1999 with mycelial plugs of *B. narcissicola* and *R. vallisumbrosae*, respectively. Plots of cv. Cheerfulness were further inoculated (four times in all at intervals to March 2000) with diseased leaf débris or fungal cultures. Similar procedures were used in 2000-2001 for inoculating the white mould trials. Despite inoculation, no white mould was observed in any of these trials, although the trials were fully utilised for gaining further data on the control of smoulder (for convenience, these are still referred to as 'white mould trials').

Each fungicide treatment was applied on five or six occasions at 2 to 3 week intervals, using a spray rate of 1000 litre/ha in 2000 and 250 litre/ha in 2001. For spray dates, see tables of results. The trials were irrigated with 5 mm of water, initially once then increasing to 2 or 3 times a week when the crop was dry. However, no irrigation was applied when 5 mm of rain had occurred in the previous 2 or 3 days, nor for 48 hours after fungicide applications. Trials were assessed for smoulder at intervals, counting disease primaries, leaf lesions and (after flower picking) stems rotting from the open end, and estimating the percentage leaf area affected by disease and the remaining percentage green leaf area. Where necessary, plant samples were examined microscopically and by isolation to determine the cause of rots.

Smoulder trials in 2000

In addition to untreated control plots, the following fungicide treatments were used (concentration of products in ml or g per litre): Benlate (0.5 g) + Dithane 945 (1.5 g), Ronilan (1 ml), Bravo 500 (3.0 ml), Scala (2.0 ml), Amistar (1.0 ml), Folicur (1.0 ml) and Unix (0.67 g).

Results at Mepal Fungicide treatment had no significant effect on the number of smoulder primaries, which ranged from 0.07 to 0.13 per 100 shoots on 8 February 2000, or on the number of leaf lesions. However, by 13 April, shortly after flower picking and following three spray applications, there were large differences between treatments (Table 4.1.4). The number of rotting stem ends was significantly reduced by Ronilan, Scala, Folicur, Unix and Amistar. Benlate + Dithane 945 and Bravo 500 were ineffective. The cause of stem rotting was determined as *B. narcissicola*.

Table 4.1.4. Comparison of fungicides for control of smoulder on cv. Carlton at Mepal in 2000.

Treatment†	Mean no. primaries / 100 shoots	Mean no. *Botrytis* lesions / 100			
		Leaves			Stems
	8 February	8 February	7 March	13 April	13 April
1. Untreated	0.10	0.04	0.69	1.43	2.97
2. Benlate + Dithane 945	0.07	0.06	1.16	2.13	2.62
3. Ronilan	0.13	0.04	1.12	1.49	0.39
4. Bravo 500	0.08	0.04	1.06	2.09	3.20
5. Scala	0.07	0.06	0.98	1.60	0.44
6. Amistar	0.12	0.08	0.93	1.46	0.89
7. Folicur	0.11	0.05	0.99	1.69	0.52
8. Unix	0.13	0.08	1.12	1.88	0.63
Significance	NS	NS	NS	NS	***
SED (25 d.f.)					
between trts	0.041	0.032	0.328	0.370	0.590
vs control	0.036	0.028	0.284	0.321	0.511

In this and subsequent Tables, ***, ** and * indicates significant at the 0.1, 1.0 and 5.0% levels of probability, respectively; NS, not significant.
†Sprays applied: 17 February, 13 March and 31 March.

A large increase in the number of smoulder leaf lesions occurred in the late-April to early-May period. These showed as distinctive, sharply defined elongated oval lesions, grey-brown in colour, usually towards the middle of the leaf length and often on the bend of a leaf. *B. narcissicola* was consistently isolated from this symptom. With time, there was associated leaf yellowing and withering, and by 5 May the untreated plots were clearly showing more die-back caused by smoulder than all other plots. Occasionally, similar lesions also occurred on flower stems. Leaf die-back was noticeably delayed by Ronilan, Scala, Amistar, Folicur, Unix and Benlate + Dithane 945, and to a lesser extent by Bravo 500 (Table 4.1.5).

Table 4.1.5 Effect of fungicides on leaf die-back in cv. Carlton at Mepal and Kirton in 2000

Treatment†	% leaf area die-back	
	Mepal (19 May)	Kirton (6 June)
1. Untreated	72.7	80.4
2. Benlate + Dithane 945	9.6	16.3
3. Ronilan	3.2	11.3
4. Bravo 500	32.9	13.8
5. Scala	1.0	10.0
6. Amistar	10.0	16.3
7. Folicur	0.9	8.8
8. Unix	4.3	13.8
Significance (7 d.f.)‡	<0.001	<0.001

†Sprays applied: Mepal, 17 February, 13 March, 31 March, 19 April, 9 May and 21 May; Kirton, 17 February, 10 March, 20 March, 31 March, 19 April and 22 May
‡Friedman's analysis

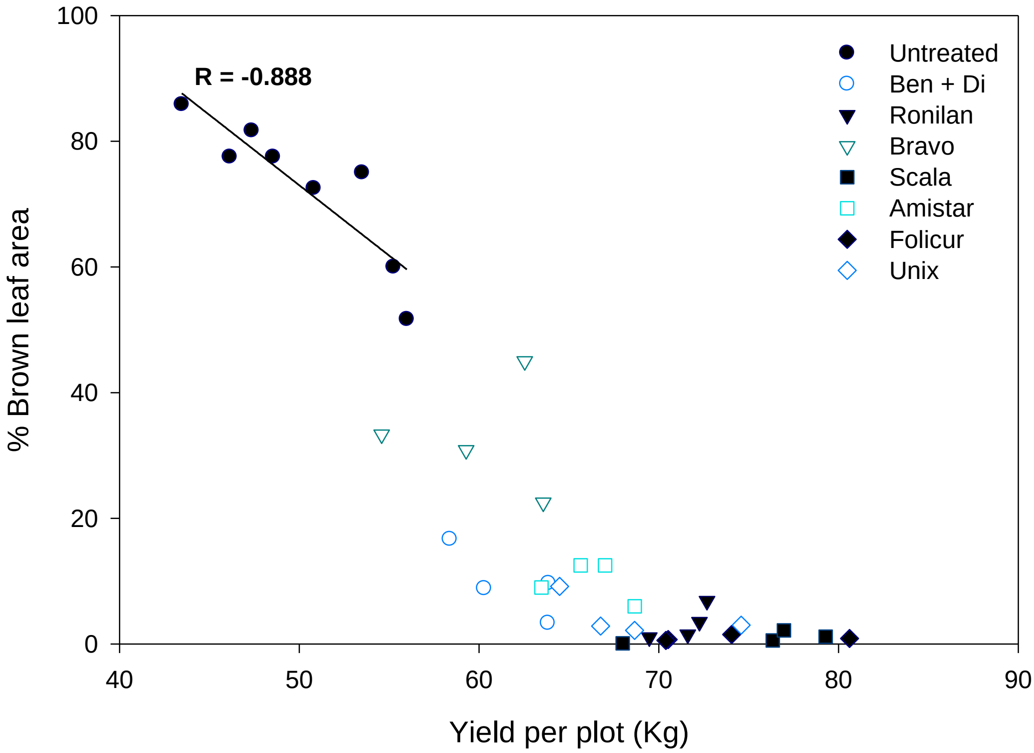
All fungicide treatments resulted in greater bulb yields, compared with controls (Table 4.1.6). Folicur and Scala, the two treatments which resulted in the greatest green leaf retention, resulted in the largest yields (48 and 50% over the control, respectively). The yield of untreated controls (3.13 kg/m) was increased by Scala to 4.70 kg/m. There was a clear negative association between leaf area die-back and yield (r = -0.888; P = 0.005, 6 d.f.) (Fig. 4.1.4).

Table 4.1.6. Effect of fungicides on bulb yield of cv. Carlton at Mepal and Kirton in 2000.

Treatment[a]	Mepal			Kirton	
	Kg per 16m plot		Relative yield	Kg per 24m plot	Relative yield
1. Untreated	50.1	a	100	71.2	100
2. Benlate+Dithane	61.6	bc	123	74.0	104
3. Ronilan	71.5	ef	143	76.3	107
4. Bravo 500	60.0	b	120	77.0	108
5. Scala	75.2	f	150	75.6	106
6. Amistar	66.2	cd	132	75.6	106
7. Folicur	73.9	f	148	74.3	104
8. Unix	68.6	de	137	79.9	112
Significance	***		-	NS	-
SED (25 df)					
between treatments	2.73			3.53	
vs control	2.37			3.06	

Results in the same column not sharing a common letter are significantly different by Duncan's Multiple Range Test
[a] sprays applied: see footnote to Table 4.1.5

Figure 4.1.4. Plot of narcissus bulb yield versus % leaf die-back at 19 May 2000 (data for cv. Carlton at Mepal, 2000).



Results at Kirton As at Mepal, fungicide treatment had not reduced the number of primary symptoms when the crop was assessed in February 2000. By 15 April, the disease had increased considerably with over 13 lesions per 100 leaves in untreated plots, and there were significant differences between treatments (Table 4.1.7). The disease was greatly reduced by Ronilan and Folicur, and moderately reduced by Bravo 500, Scala and Unix. Amistar had little effect, whilst Benlate + Dithane 945 mix was ineffective.

Table 4.1.7. Comparison of fungicides for control of smoulder on cv. Carlton at Kirton in 2000.

Treatment*	Mean no. *Botrytis* lesions / 100 leaves and stems	
	9 February	15 April
1. Untreated	0.89	13.74
2. Benlate+Dithane 945	1.09	13.51
3. Ronilan	1.14	6.90
4. Bravo 500	0.86	8.51
5. Scala	0.89	8.13
6. Amistar	0.86	11.67
7. Folicur	0.91	6.17
8. Unix	1.20	9.52
Significance	NS	***
SED (25 df)		
between trts	0.172	1.859
vs control	0.149	1.610

*Sprays applied: 17 February, 10 March, 20 March and 31 March.

At this site, rapid leaf die-back due to smoulder occurred approximately 3 weeks later than at Mepal. All fungicide treatments prolonged green leaf retention, with Folicur being most effective. The yield of fungicide-treated plots was greater than that of untreated controls, but these increases were smaller than recorded at Mepal and not statistically significant (Table 4.1.2). Yield of the untreated was 2.97 kg/m, and the greatest yield was following treatment with Unix (3.33 kg/m).

White mould trials, 2000

The treatments applied were (rates of products in g or ml per litre): Benlate (0.5 g) + Dithane 945 (1.5 g), Bavistin DF (0.5 g) + Dithane 945 (1.5 g), Bravo 500 (3.0 ml), Scala (2.0 ml), Amistar (1.0 ml), Folicur (1.0 ml) and Stroby WG (0.625 g).

No white mould developed at either site, despite the repeated inoculation of crops with *R. vallisumbrosae*. However, severe attacks of smoulder developed following natural infection by *B. narcissicola.* At Kirton, when assessed on 6 April 2000, none of the treatments had resulted in a large reduction in smoulder. At the Mepal site, assessed on 4 May, the incidence of smoulder on both leaves and stems appeared to be reduced by all treatments, although differences were not statistically significant.

At both sites fungicide treatment subsequently had a significant effect on smoulder levels (Table 4.1.8). At Mepal, stem rotting was greatest in the untreated controls and least following treatment with Amistar. At Kirton the incidence of leaf lesions was greatest in the untreated controls and least following treatment with Stroby WG. The effect of fungicide treatment on green leaf retention was strikingly similar at the two sites, with Folicur and Stroby WG resulting in prolonged green leaf retention.

Table 4.1.8. Effect of fungicides on control of smoulder and green leaf retention on cv. Cheerfulness at Mepal and Kirton in 2000.

Treatment[a]	Mepal		Kirton	
	No. smoulder lesions per 100 stems (4 May)	% leaf area die-back (2 June)	No smoulder leasions per 100 leaves (17 May)	% leaf area die-back (6 June)
1. Untreated	1.10	96.4	14.9	99.9
2. Benlate + Dithane	0.70	82.5	4.9	71.3
3. Bavistin + Dithane	0.32	81.3	5.3	73.8
4. Bravo 500	0.57	78.8	6.9	66.3
5. Scala	0.40	86.3	11.1	95.8
6. Amistar	0.27	85.0	9.3	97.0
7. Folicur	0.38	58.3	3.8	11.3
8. Stroby WG	0.84	57.5	2.6	12.5
Significance[b]	0.047	0.001	0.001	0.001
SED between trts	-	-	2.25	-
vs control	-	-	1.95	-

[a] sprays applied: Mepal, 13 March, 31 March, 19 April, 9 May, 20 May and 7 June; Kirton, 13 March, 31 March, 19 April, 15 May and 31 May

[b] statistically significant differences between treatments according to Friedman's test (7 d.f.), or by analysis of variance (smoulder data at Kirton, 35 d.f.).

All fungicide treatments resulted in yield increases, compared with untreated controls (Table 4.1.9). Folicur resulted in the highest yield (3.94 kg/m) at Mepal, and Stroby WG (2.15 kg/m) at Kirton. The effects of Benlate + Dithane and Bavistin + Dithane were similar. Amistar had relatively little effect on green leaf retention, and resulted in a relatively small yield increase at both sites. Bravo 500 had noticeable effects on green leaf retention, ranking third in efficacy after Folicur and Stroby WG at both sites.

Table 4.1.9. Effect of fungicides on bulb yield of cv. Cheerfulness at Mepal and Kirton in 2000.

Treatment[a]	Mepal			Kirton		
	Kg / 16m plot		Relative yield	Kg / 24m plot		Relative yield
1. Untreated	39.9	a	100	38.4	a	100
2. Benlate + Dithane	52.3	cd	131	45.8	cd	119
3. Bavistin + Dithane	50.2	bcd	126	47.1	d	123
4. Bravo 500	54.0	d	135	46.7	d	122
5. Scala	47.7	bc	120	42.3	b	110
6. Amistar	44.6	ab	112	43.2	bc	112
7. Folicur	63.0	e	158	50.3	e	131
8. Stroby WG	61.5	e	154	51.7	e	135
Significance	***			***		
SED (25 df)						
between treatments	2.99			1.54		
vs control	2.59			1.34		

[a] sprays applied: Mepal, 13 March, 31 March, 19 April, 9 May, 20 May and 4 June; Kirton, 13 March, 31 March, 19 April, 15 May and 31 May

<u>Smoulder trials at Mepal and Kirton, 2001</u>

The objective in these trials was to investigate the effect of fungicides, applied at three key growth stages, on disease control, foliage die-back and bulb yield. The three growth stages were: Phase I, shoot emergence; Phase II, around flowering; and Phase III, after flowering. Two fungicide sprays were applied during each of the three phases. All combinations of phase I, II and III treatments were tested, resulting in totals of two, four or six sprays per treatment. The fungicides used at each phase were chosen from different mode-of-action groups, to reduce the risk of selecting fungicide-resistant pathotypes. Additionally, a six-spray programme of Folicur was evaluated to provide continuity with the trials of 1999-2000, this being one of several treatments that had then resulted in large increases in bulb yield. Ronilan and Folicur were each used at 1 litre/ha, and Scala at 2 litre/ha. The treatments were:

Treatment		Phase I		Phase II		Phase III	
		Spray 1	Spray 2	Spray 1	Spray 2	Spray 1	Spray 2
	Growth stage	Shoots 10-15 cm tall	+ 2 weeks	In bud	1 day after picking	3 weeks later	+ another 3 weeks
1.	Untreated	-	-	-	-	-	-
2.	I	Ronilan	Ronilan	-	-	-	-
3.	II	-	-	Folicur	Folicur	-	-
4.	III	-	-	-	-	Scala	Scala
5.	I+II	Ronilan	Ronilan	Folicur	Folicur	-	-
6.	I+III	Ronilan	Ronilan	-	-	Scala	Scala
7.	II+III	-	-	Folicur	Folicur	Scala	Scala
8.	I+II+III	Ronilan	Ronilan	Folicur	Folicur	Scala	Scala
9	I+II+III	Folicur	Folicur	Folicur	Folicur	Folicur	Folicur

At Mepal, the incidence of primaries on 19 March 2001 ranged from 5.0 to 8.0 per plot. The application of two fungicide sprays (either Ronilan or Folicur) on 20 February and 9 March had no detectable effect on the incidences of primaries or of the proportion bearing sporulating *Botrytis*. By 19 April, when up to four fungicide sprays had been applied, there was a significant difference in the incidence of stems rotting from *Botrytis* (Table 4.1.10). Treatments that had received two sprays of Folicur, on 22 March (bud stage) and 30 March (2 days after picking), had 37 - 46% stems with rotting, compared with 98 - 100% in untreated plots. The total incidence of smoulder lesions on leaves and stems in March was greatest in untreated plots and appeared to be reduced slightly by some of the fungicide programmes, although treated plots were not statistically different then or at a later assessment (19 April).

Table 4.1.10. Effect of fungicide treatment on stem rotting by *Botrytis* in cv. Carlton at Mepal and Kirton in 2001.

Treatment [a]	Mepal (19 April) % stems rotting	Kirton (24 April) Mean number of stems rotting per 0.5m sub-plot
1. Untreated	100.0	6.5
2. RR----	98.5	4.8
3. --FF--	40.5	1.0
4. ----SS	100.0	9.8
5. RRFF--	45.5	1.3
6. RR--SS	97.5	3.8
7. --FFSS	36.5	0.3
8. RRFFSS	43.5	1.0
9. FFFFFF	46.0	0.3
Significance	***	***
SED (24d.f.)	5.53	2.36

[a] sprays applied: Mepal, 20 February, 9 March, 22 March and 30 March; Kirton, 20 February, 14 March, 29 March and 9 April.

Fungicide treatment had a very noticeable effect on leaf die-back (Table 4.1.11 and Fig. 4.1.5). On 8 June, 4 weeks after the final spray, leaf die-back was 98% in untreated plots and less than 50% in all other treatments which had received two mid-season and/or two late-season sprays. The combination of two mid-season and two late-season sprays (treatments 7, 8 and 9) was particularly effective at reducing leaf die-back.

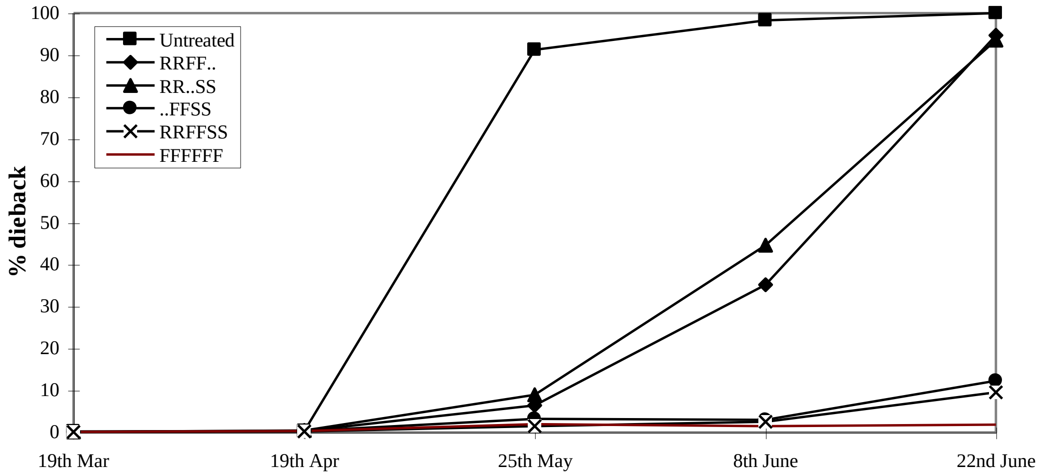
Table 4.1.11. Effect of fungicides on % foliage die-back in cv. Carlton at Mepal and Kirton in 2001.

Treatment [a]	Mepal		Kirton	
	8 June	22 June	7 June	26 June
1. Untreated	98.3	100.0	92.5	100.0
2. RR----	97.7	100.0	75.0	99.9
3. --FF--	25.0	94.5	1.0	87.9
4. ----SS	37.6	94.7	12.5	97.6
5. RRFF--	35.2	94.8	1.8	80.1
6. RR--SS	44.7	93.6	14.0	96.1
7. --FFSS	3.0	12.3	1.8	69.1
8. RRFFSS	2.5	9.5	2.5	60.8
9. FFFFFF	1.5	1.8	0.8	68.9
Significance	***	***	***	***
SED (24 df)	9.04	1.98	5.76	5.52

[a] Sprays applied: Mepal, 20 February, 9 March, 22 March, 30 March, 21 April and 11 May; Kirton, 20 February, 14 March, 29 March, 9 April, 30 April and 21 May.

At Kirton, treatments applied around flowering (phase II) resulted in significant reductions in stem rotting due to *Botrytis* (Table 4.1.10). As at Mepal, fungicide treatment had no significant effect on the mean number of smoulder leaf and stem lesions in April, but there was a very marked and highly significant effect on leaf die-back (Table 4.1.11). Treatments 7, 8 and 9 were again particularly effective at reducing foliage die-back.

Figure 4.1.5. Effect of fungicides on leaf die-back in cv. Carlton at Mepal in 2001.



At Mepal, bulb yields ranged from 46.7 to 61.7 kg per plot, with significant differences between treatments (Table 4.1.12). The greatest yield (65.8 kg, a 39% increase over untreated plots) resulted from using a four-spray programme, consisting of two Ronilan sprays during emergence and two sprays of Folicur around flower picking. The two six-spray programmes were also very effective at increasing yield, by 25 and 31%, respectively, compared with untreated plots. The two-spray programmes and two of the four-spray programmes had little or no effect on yield.

At Kirton, it was also the four-spray programme of two Ronilan sprays (during emergence) and two Folicur sprays (around flower picking) that were associated with the greatest yield (21% greater than untreated plots), although this was not statistically significant.

Table 4.1.12. Effect of fungicide sprays on bulb yield[a] of cv. Carlton at Mepal and Kirton in 2001.

Treatment	Mepal		Kirton	
	Kg/16m plot	% weight increase†	Kg/24m plot	% weight increase†
1. Untreated	47.2 a	44.1	57.2	56.8
2. RR----	46.7 a	42.6	58.9	61.5
3. --FF--	50.5 ab	54.2	60.5	65.8
4. ----SS	51.0 ab	55.7	61.9	69.7
5. RRFF--	65.8 c	100.9	66.0	80.9
6. RR--SS	47.4 a	44.7	64.9	77.9
7. --FFSS	49.4 a	50.8	64.6	77.1
8. RRFFSS	58.8 abc	79.5	65.7	80.1
9. FFFFFF	61.7 bc	88.3	63.8	74.9
Significance	***	-	NS	-
SED (24 d.f.)	5.24		-	

†% weight increase = ((weight increase from planting)/(weight planted))x100.

White mould trials at Mepal and Kirton, 2001

For the white mould trials the objectives were to investigate the effect of (a) selected fungicide mixtures and (b) commencing a programme of Bavistin DF + Dithane 945 at three different growth stages: early (at 0-5cm shoot length), conventional (at 10-15cm), and late (after flowering). The fungicide mixtures chosen were designed to provide control of both smoulder and white mould. When used as a mixture of two products, each fungicide was used at half its normal recommended rate (Amistar, 0.5 litre/ha; Bavistin DF, 0.5 kg/ha; Dithane 945, 1.5 kg/ha; Folicur, 0.5 litre/ha; Ronilan FL, 0.5 litre/ha and Scala, 1.0 litre/ha) in 250 litre water /ha.

As expected from previous results, no white mould symptoms were observed on crops at either trial site during the first year's growth in 2000, while *Botrytis* occurred on many plants of both varieties at both sites. At Mepal, smoulder was first observed on 16 March, although the incidence of primaries on 11 April remained low, ranging from 0 to 0.8 per plot (16 m of ridge), with no significant differences between treatments. By 8 May, when up to four fungicide sprays had been applied, a high incidence of *Botrytis* stem rotting was recorded (Table 4.1.13), and this was significantly reduced by fungicide treatment. Fungicide treatment had a very noticeable effect on leaf die-back (Table 4.1.14). On 8 June, 2 days after the final spray, there was 99% die-back in untreated plots and less than 20% in treated plots. Treatments 6 (Bavistin + Folicur) and 8 (Amistar + Folicur) were particularly green.

Table 4.1.13. Effect of fungicide sprays on *Botrytis* stem rot in cv. Cheerfulness at Mepal in 2001.

Treatment	% stems rotting (8 May)
1. Untreated	46.5
2. Bavistin + Dithane (0-5cm)	28.5
3. Bavistin + Dithane (10-15cm)	28.0
4. Bavistin + Dithane (after flowering)	34.2
5. Bavistin + Ronilan	24.5
6. Bavistin + Folicur	20.5
7. Bavistin + Scala	22.0
8. Amistar + Folicur	24.0
Significance	***
SED (25 d.f.)	
between treatments	5.22
treatments vs control	4.52

Table 4.1.14. Effect of fungicide sprays on % foliage die-back in cv. Cheerfulness in 2001.

Treatment [a]	Mepal		Kirton	
	8 June	22 June	7 June	2 July
1. Untreated	98.7	100.0	97.1	100.0
2. Bavistin + Dithane (0-5cm)	4.5	29.4	23.0	80.4
3. Bavistin + Dithane (10-15cm)	3.2	16.3	33.0	82.9
4. Bavistin + Dithane (after flowering)	4.0	28.5	25.3	84.6
5. Bavistin + Ronilan	5.0	34.0	40.7	93.5
6. Bavistin + Folicur	1.3	1.3	20.3	77.4
7. Bavistin + Scala	19.8	80.2	25.5	91.5
8. Amistar + Folicur	1.0	1.2	16.3	71.0
Significance	***	***	***	***
SED (25 d.f.)				
between treatments	4.61	6.91	4.76	2.32
treatments vs control	5.33	7.98	5.46	2.68

[a] Sprays applied: Mepal, 20 February (treatment 2), 9 April, 21 April, 8 May, 22 May and 6 June; Kirton, 20 February (treatment 2), 14 March, 9 April, 24 April, 9 May, 21 May and 5 June.

Bulb yield at Mepal was significantly different between treatments, with Bavistin DF + Folicur giving a 59% yield increase over untreated (Table 4.1.15). At Kirton, foliage die-back was noticeably delayed by most treatments. Bulb yields differed significantly with least in untreated plots and the greatest yield, a 24% increase, occurred following treatment with Amistar + Folicur. At both trial sites, Bavistin DF + Ronilan Fl was the least effective fungicide treatment, giving the smallest yield increase over untreated plots, though still a significant increase.

Table 4.1.15. Effect of fungicide spray programmes on bulb yield[a] of cv. Cheerfulness in 2001.

Treatment	Mepal		Kirton	
	Kg/16m plot	% weight increase	Kg/24m plot	% weight increase
1. Untreated	30.8 a	-6.0	39.0 a	6.9
2. Bavistin + Dithane (0-5cm)	44.4 ab	35.5	47.0 bc	28.8
3. Bavistin + Dithane (10-15cm)	40.8 ab	24.5	45.9 bc	25.8
4. Bavistin + Dithane (after flowering)	47.2 ab	44.1	47.0 bc	28.8
5. Bavistin + Ronilan	38.3 ab	16.9	44.3 b	21.4
6. Bavistin + Folicur	49.0 b	49.6	45.7 bc	25.3
7. Bavistin + Scala	39.6 ab	20.9	47.3 bc	29.7
8. Amistar + Folicur	46.2 ab	41.0	48.3 c	32.4
Significance	***		***	
SED (25 d.f.)				
between treatments	4.58		1.65	
treatments vs control	3.97		1.91	

White mould field trials in Cornwall

Trials were carried out in the second crop year of crops of cv. Yellow Cheerfulness (cv. Cheerfulness in 2000 planting) planted at Penzance in 1997, 1999 and 2000 (treatments in 1999, 2001 and 2002, respectively). In each year an area of the crop of 0.2 ha was allocated, and this area received no routine fungicide sprays in its second year; apart from this, the grower applied normal husbandry practices. All trials were arranged with four replicate plots of six treatments in a randomised block design.

1999 trial The objective was to determine the effectiveness of a standard fungicide mixture (Benlate + Dithane 945) applied at different spray timings. Of importance were fungicide sprays applied in the early spring, when resting bodies of *R. vallisumbrosae* are reported to germinate. The fungicide used was a mix of Benlate and Dithane 945 (applied as 0.5 kg/ha Benlate plus 1.5 kg/ha Dithane 945 in 1000 litres water/ha). The treatment timings were:

1. Untreated control
2. Fungicide spray applied every 10 days (or as soon as practicable) from foliage 10 - 15 cm tall stage until 4 weeks after flowering
3. As treatment 2, but first spray omitted
4. As treatment 2, but first two sprays omitted
5. One spray only, at leaves 10-15 cm tall stage
6. Two sprays, at 10-15 cm tall stage and 10 days later.

White mould, smoulder and scorch (*Stagonospora curtisii*) all occurred naturally in the trial. White mould was first confirmed in the trial area by 4 March 1999, although a total of only 11 lesions were recorded at that date. At an assessment on 8 April, the severity of white mould was greatest in the untreated plots and considerably less where plants had been sprayed with Benlate + Dithane three and four times (Table 4.1.16). Application of one or two sprays when the leaves were 10-15 cm tall was insufficient to provide control through to April. A final assessment was made on 7 May 1999, after flowering, when the percentage leaf area that was brown was estimated. There were some very clear differences between the treatments: three of the spray programmes resulted in very good control of white mould (Table 4.1.16), with statistically highly significant (P<0.001) differences between treatments.

Table 4.1.16. Comparison of Benlate + Dithane 945 spray programmes for control of white mould on cv. Yellow Cheerfulness in Cornwall in 1999.

No. of sprays*	Timing of sprays 27/2	11/3	22/3	1/4	13/4	Mean number white mould lesions (8 April)	Mean % leaf area dead (7 May)
1. Nil	-	-	-	-	-	23.8	73.7
2. Five	+	+	+	+	+	2.5	4.4
3. Four	-	+	+	+	+	3.2	5.0
4. Three	-	-	+	+	+	4.0	6.3
5. One	+	-	-	-	-	18.0	83.7
6. Two	+	+	-	-	-	16.8	56.2
Significance (15 d.f.)						0.16	<0.001
SED						6.712	4.60

*Benlate + Dithane 945

The severity of smoulder on 8 April was greatest in untreated plots and appeared to be reduced slightly by all treatments, although differences were not statistically significant. None of the fungicide treatments reduced leaf scorch. There was no evidence from this trial that early sprays in February and March, when *Ramularia* resting bodies are germinating, were critical in providing good disease control. A three-spray programme commencing in late-March, 18 days after white mould was confirmed, was as effective as a five-spray programme starting one month earlier.

2001 trial The objective was to investigate the effect of fungicide mixtures selected, on the basis of the primary fungicide screen, as ones likely to give good control of both white mould and smoulder. Additionally, the effect of commencing a protectant programme of Bavistin DF + Dithane 945 at three different growth stages was evaluated. The treatments were:

1. Untreated
2. Bavistin DF + Dithane 945 every 2-3 weeks starting at shoots 0-5cm long
3. Bavistin DF + Dithane 945 every 2-3 weeks from shoots at 10-15cm stage
4. Bavistin DF + Dithane every 2-3 weeks starting at first symptom of white mould (or immediately after flower picking, if no white mould present)
5. Bavistin DF + Ronilan FL every 2-3 weeks from 10-15cm stage
6. Bavistin DF + Folicur every 2-3 weeks from 10-15cm stage
7. Bavistin DF + Scala every 2-3 weeks from 10-15cm stage
8. Amistar + Folicur every 2-3 weeks from 10-15cm stage

The rates used were: Amistar, 0.5 litre/ha; Bavistin DF, 0.5 kg/ha; Dithane 945, 1.5 kg/ha; Folicur, 0.5 litre/ha; Ronilan Fl, 0.5 litre/ha; Scala, 1.0 litre/ha. Sprays were applied in 250 litres water/ha.

White mould was first observed in the crop in mid-April. At the first assessment on 25 April 2001 there were marked differences between treatments in both the number of white mould foci and the number of lesions/m of ridge (Table 4.1.17). The number of foci per plot ranged from nil (Amistar + Folicur) to 9.3 (untreated), with up to 11.1 white mould lesions/0.5 m. When assessed on 16 May, 2 weeks after the final spray, there were large differences between treatments. White mould was greatest in the untreated (88.2 lesions/0.5m) and reduced to 10 lesions or less by all treatments except treatment 4 (spray programme commencing at first symptoms; two sprays applied). Bavistin DF + Folicur, Bavistin DF + Scala and Amistar + Folicur were particularly effective, reducing lesion numbers to 0.8, 3.9 and 0 respectively.

Leaf die-back occurred earlier where white mould was not controlled (Table 4.1.17). The three treatments noted above which gave very good control of white mould also resulted in prolonged green leaf retention.

Table 4.1.17. Effect of fungicide spray programmes on control of white mould and leaf die-back on cv. Yellow Cheerfulness, Cornwall, 2001.

Treatments [a]	Mean no. lesions / 0.5m (16 May)	% leaf die-back	
		16 May	30 May
1. Untreated	88.2	12.0	74.4
2. Bavistin + Dithane 945 (0-5cm)	10.0	0.3	8.3
3. Bavistin + Dithane 945 (10-15cm)	7.9	0.2	6.5
4. Bavistin + Dithane 945 (1st symptoms, 23 April)	33.2	2.3	16.5
5. Bavistin + Ronilan	4.0	0.1	5.7
6. Bavistin + Folicur	0.8	0.1	1.3
7. Bavistin + Scala	3.9	0.1	1.3
8. Amistar + Folicur	0	0	0.8
Significance	***	***	***
SED (25 d.f.)			
between treatments	13.68	1.25	4.87
treatments vs control	11.85	1.09	4.22

[a] Sprays applied: 16 February (treatment 2 only), 2 March, 16 March 31 March, 16 April, 23 April, 7 May

In this trial, when white mould did not occur until mid April, there was no benefit in starting the spray programme when shoots were at 0-5cm compared with the more conventional first spray applied at 10-15 cm. Although commencing the spray programme at first symptoms of white mould resulted in a saving of four fungicide applications, it produced an inferior level of disease control and green leaf retention. Two of the novel fungicide mixtures tested (Amistar + Folicur and Bavistin DF + Folicur) gave very good disease control and appear to be an improvement on the current standard treatment (Bavistin DF + Dithane 945).

2002 trial The objective of the trial was to determine if the spray interval could be extended from at least 2 to 3 weeks, and the total number of sprays thereby reduced, without losing effective control of this potentially epidemic disease. The current grower standard treatment for white mould (Bavistin DF + Dithane 945) was compared with a new treatment (Amistar + Folicur) devised in this project. The treatments were:

1. Untreated control
2. Bavistin DF + Dithane 945 every 10-14 days from shoots at 10-15 cm
3. Amistar + Folicur every 10-14 days from shoots at 10-15 cm
4. Bavistin DF + Dithane 945 every 21-28 days from shoots at 10-15 cm
5. Amistar + Folicur every 21-28 days from shoots at 10-15 cm
6. Bavistin DF + Dithane 945; one spray at 5-10 cm, then every 14 days starting when white mould was confirmed in the crop
7. Amistar + Folicur; one spray at 5-10 cm, then every 14 days starting when white mould was confirmed in the crop
8. Bavistin DF + Dithane 945: every 14 days from confirmation of white mould in the crop.
9. Amistar + Folicur: every 14 days from confirmation of white mould in the crop

The total number of sprays applied ranged from three to six (Table 4.1.18). The final spray was applied in early May, after which the foliage flopped and it was impractical to achieve good spray cover. The rates of application were: Bavistin DF, 0.5 kg/ha; Dithane 945, 1.5 kg/ha; Amistar, 0.5 l/ha; and Folicur, 0.5 l/ha. Sprays were applied at 250 l/ha.

Table 4.1.18. Spray programmes evaluated for control of white mould in Cornwall in 2002

Treatment (spray interval)		Spray dates						
		6 Feb	21 Feb	7 Mar	24 Mar	5 Apr	18 Apr	3 May
1.	Untreated	-	-	-	-	-	-	-
2.	Bavistin + Dithane (10-14d)	-	□	□	□	□	□	□
3.	Amistar + Folicur (10-14d)	-	□	□	□	□	□	□
4.	Bavistin + Dithane (21-28d)	-	□	-	□	-	□	-
5.	Amistar + Folicur (21-28d)	-	□	-	□	-	□	-
6.	Bavistin + Dithane (1 + first symptoms)	□	-	-	-	□	□	□
7.	Amistar + Folicur (1 + first symptoms)	□	-	-	-	□	□	□
8.	Bavistin + Dithane (first symptoms)	-	-	-	-	□	□	□
9.	Amistar + Folicur (first symptoms)	-	-	-	-	□	□	□

White mould did not occur in the trial until early April, at flower cropping, approximately a month later than in 1999 (when first occurrence of the disease was on 4 March). The disease progressed steadily resulting in about 25 lesions/0.5m length of ridge in untreated plots by 1 May (Table 4.1.19). Fungicide treatments significantly reduced disease development, with Amistar + Folicur every 14 days being the most effective. Bavistin DF + Dithane 945 applied every 14 days from first symptoms, or one spray on 6 February followed by sprays from first symptoms (5 April), were considerably less effective than other treatments. In all of the paired comparisons, Amistar + Folicur resulted in less white mould than Bavistin DF + Dithane 945. Amistar + Folicur applied every 21-28 days (resulting in a total of 3 sprays) was as effective as Bavistin DF + Dithane 945 applied every 14 days (a total of 6 sprays). The application of a preventative spray on 6 February followed by none until first symptoms gave no advantage over a spray programmes starting at first symptoms, probably because of the long time interval (8 weeks) between the first two sprays.

These results indicated that a reduction in spray numbers can be achieved by using Amistar + Folicur rather than Bavistin DF + Dithane 945. Amistar + Folicur applied every 21-28 days was not statistically inferior in disease control to the same fungicide treatment applied every 14 days, and achieved a saving of three sprays.

Amistar + Folicur applied at first symptoms and then every 14 days resulted in control similar to that of Amistar + Folicur applied as a routine every 21-28 days, and significantly better than the equivalent programme of Bavistin DF + Dithane 945. These results indicate that if white mould occurs in a crop before any fungicides have been applied, out of the treatments tested Amistar + Folicur is the best choice to treat established disease.

Table 4.1.19. Effect of fungicide spray programmes on control of white mould in cv. Cheerfulness in 2002.

Treatment (spray interval)		Mean no. foci per plot				Mean no. affected leaves/m			
		11 April		1 May		11 April		1 May	
1.	Untreated	7.5	d	96.5	f	4.5		49.2	c
2.	Bav + Dit (10-14d)	3.0	abc	48.7	cd	2.8		10.5	a
3.	Ami + Fol (10-14d)	0.3	a	8.3	a	0.3		2.5	a
4.	Bav + Dit (21-28d)	4.8	bcd	52.2	d	3.3		14.0	a
5.	Ami + Fol (21-28d)	0.8	ab	23.0	ab	1.0		7.8	a
6.	Bav + Dit (1 + FS)	2.5	abc	80.5	ef	3.5		30.3	b
7.	Ami + Fol (1 + FS)	1.5	abc	25.7	ab	1.5		11.0	a
8.	Bav + Dit (FS)	4.5	bcd	74.0	e	4.0		35.2	b
9.	Ami + Fol (FS)	5.0	cd	33.7	bc	3.8		14.0	a
	Significance (24 d.f.)	0.011		<0.001		0.056		<0.001	
	SED	1.82		3.88		1.37		6.19	

All fungicide treatments significantly delayed leaf die-back (Table 4.1.20). On 6 June, 33 days after the final spray, all leaf area was dead in untreated plots compared with less than 10% in treatments 3 and 9, both of which received three late season sprays of Amistar + Folicur. The Amistar + Folicur treatments consistently resulted in greater green leaf retention (less leaf die-back) than the comparable Bavistin DF + Dithane 945 treatments.

Three sprays of Amistar + Folicur applied late in the season (5 April, 18 April and 3 May) resulted in greater green leaf retention that three sprays of the same fungicide mixture applied at extended intervals from earlier in crop growth (21 February, 24 March and 18 April). However, three sprays of Bavistin DF + Dithane 945 did not significantly increase green leaf retention when applied late in the season (from first symptoms of white mould), or when applied earlier in the season at extended intervals. This difference between the two fungicide mixtures is probably due, at least in part, to their differing efficacy in controlling established white mould.

Table 4.1.20. Effect of fungicide spray programmes on leaf die-back associated with white mould on cv. Cheerfulness in 2002

Treatment		Mean % leaf area brown*						
		1 May		21 May		6 June		
1.	Untreated	7.0	(15.3) d	86	e	100	g
2.	Bavistin + Dithane (10-14d)	2.0	(8.1) b	16	b	88	d
3.	Amistar + Folicur (10-14d)	1.0	(5.7) a	5	a	9	a
4.	Bavistin + Dithane (21-28d)	2.3	(8.6) b	39	a	98	fg
5.	Amistar + Folicur (21-28d)	1.0	(5.7) a	11	a	28	c
6.	Bavistin + Dithane (1 + FS)	4.8	(12.5) c	26	c	94	ef
7.	Amistar + Folicur (1 + FS)	1.8	(7.5) b	5	a	18	b
8.	Bavistin + Dithane (FS)	4.3	(11.9) c	26	c	91	e
9.	Amistar + Folicur (FS)	1.8	(7.5) b	5	a	7	a
	Significance (24 df)		(<0.001)	<0.001		<0.001	
	SED		(0.73)	2.63		2.19	

*Angular transformed values are shown in parenthesis.

Label restrictions and good plant protection practice

When considering use of Amistar and Folicur in a spray programme it should be noted that the maximum permitted total doses are: Amistar, 3.0 litres/ha (following the recommendation for use on wheat) and Folicur 3.0 litres/ha l/ha (following the recommendation for use on leeks), equating to a maximum of six sprays of either product at 0.5 litre/ha. Such intensive use of either of these fungicides should be avoided because of the risk of selecting fungicide-resistant pathotypes of *B. narcissicola* and *R. vallisumbrosae*. It should also be noted that the latest permitted spray application for Folicur is 14 days before harvest (e.g. following the leek recommendation), or 7 days if a maximum total dose of 0.75 litres/ha is applied (e.g. SOLA 1624/02, for use on baby leaf brassica). The latest permitted spray application for Amistar is 14 days before harvest (e.g. SOLA 1041/01, for use on celery). Where a SOLA recommendation is used, a copy of the SOLA must be obtained and the conditions of use specified on the SOLA document must be followed. Where an extrapolation is made under the Long Term Arrangements for Extension of Use (2002), the specific restrictions for extension of use must be followed. Both of these uses are entirely at the grower's own commercial risk.

Cost-benefit analysis of treatments for smoulder in eastern England

The estimated cost of fungicide treatment (excluding application costs) evaluated on second-year-down cv. Carlton and Cheerfulness in 2000 is given in Tables 4.1.21 – 4.1.22. In the particular circumstances of these trials, with high levels of secondary smoulder and associated early foliage senescence, and assuming a return of £300/tonne and £450/tonne for the two varieties, respectively, all the treatments resulted in a positive margin over the cost of fungicides. Even with narcissus bulb prices having fallen considerably since the start of the project – say to an average of £250 per tonne in 2002 – there is a clear financial benefit of treatment. Bulb prices are likely to continue to be cyclical.

Table 4.1.21. Cost-benefit assessment of second-year fungicide treatment on cv. Carlton in 1999 – 2000.

Treatment (rate/ha)	Mean plot weight (kg)	Yield per ha (t)	Increase in yield/ha (t)	Value of increase (£) at £300/t	Cost/ha of 6 sprays[a] (£)	Margin[b] over chemical cost (£/ha)
Mepal						
1. Untreated	50.1	34.81	-	-	-	-
2. Benlate + Dithane 945 (0.5+1.5 kg)	61.6	42.77	7.96	2,388	80.25	2,307
3. Ronilan (1 l)	71.5	49.66	14.85	4,454	147.00	4,307
4. Bravo 500 (3 l)	60.0	41.67	6.85	2,056	62.64	1,994
5. Scala (2 l)	75.2	52.19	17.38	5,213	432.00	4,781
6. Amistar (1 l)	66.2	45.97	11.16	3,348	235.20	3,113
7. Folicur (1 l)	73.9	51.32	16.51	4,952	103.50	4,849
8. Unix (0.67 kg)	68.6	47.65	12.84	3,852	86.43	3,766
Kirton						
1. Untreated	71.5	39.04	-	-	-	-
2. Benlate + Dithane 945 (0.5+1.5 kg)	74.0	40.57	1.54	461	66.88	394
3. Ronilan (11)	76.3	41.83	2.80	839	122.50	716
4. Bravo 500 (31)	77.0	42.22	3.18	954	52.20	902
5. Scala (21)	75.6	41.45	2.41	724	360.00	364
6. Amistar (11)	75.6	41.45	2.41	724	196.00	528
7. Folicur (11)	74.3	40.74	1.70	510	86.25	424
8. Unix (0.67 kg)	79.9	43.81	4.77	1,431	72.03	1,359

[a] Products costed at: Benlate £20/kg; Dithane 945 £2.25/kg; Ronilan £24.50/litre; Bravo 500 £3.48/litre; Scala £36/litre; Amistar £39.20/litre; Folicur £17.25/litre; Unix £21.50/litre.

[b] Cost of application not included.

Planted weight was 2 kg/m^2 (20 t/ha)

Table 4.1.22. Cost-benefit assessment of second-year fungicide treatment on cv. Cheerfulness in 1999 – 2000.

Treatment (rate/ha)	Mean plot weight (kg)	Yield per ha (t)	Increase in yield/ha (t)	Value of increase (£) at £450/t	Cost/ha of six sprays[a] (£)	Margin[b] over chemical cost (£/ha)
Mepal						
1. Untreated	39.9	27.69	-	-	-	-
2. Benlate + Dithane 945 (0.5+1.5 kg)	52.3	36.31	8.62	3,878	80.25	3,798
3. Bavistin + Dithane 945 (0.5+1.5 kg)	50.2	34.88	7.19	3,234	50.25	3,184
4. Bravo 500 (3 l)	54.0	37.51	9.83	4,422	62.64	4,359
5. Scala (2 l)	47.7	33.10	5.41	2,434	432.00	2,002
6. Amistar (1 l)	44.6	30.98	3.29	1,481	235.20	1,246
7. Folicur (1 l)	63.0	43.77	16.08	7,238	103.50	7,134
8. Stroby (0.625 kg)	61.5	42.71	15.02	6,759	525.00	6,234
Kirton						
1. Untreated	38.4	21.05	-	-	-	-
2. Benlate + Dithane 945 (0.5+1.5 kg)	45.8	25.11	4.06	1,826	66.88	1,759
3. Bavistin + Dithane 945 (0.5+1.5 kg)	47.1	25.82	4.77	2,146	41.88	2,105
4. Bravo 500 (31)	46.7	25.60	4.55	2,048	52.20	1,996
5. Scala (21)	42.3	23.19	2.14	962	360.00	602
6. Amistar (11)	43.2	23.68	2.63	1,184	196.00	988
7. Folicur (11)	50.3	27.58	6.52	2,936	86.25	2,850
8. Stroby (0.625 kg)	51.7	28.34	7.29	3,281	437.50	2,844

[a] Products costed at: Benlate £20/kg; Dithane 945 £2.25/kg; Bavistin DF £10/kg; Bravo 500 £3.48/litre; Scala £36/litre; Amistar £39.20/litre; Folicur £17.25/litre; Stroby £140/kg. Six sprays applied at ADAS site, five at HRI

[b] Cost of application not included.

Planted weight was 2 kg/m^2 (20 t/ha)

Conclusions: Smoulder control

- Fungicide treatment during shoot emergence did not result in significant reductions in the incidence of smoulder primary symptoms.
- Fungicide products demonstrated to give good control of subsequent smoulder development were Folicur, Ronilan, Scala and Unix. Bravo 500 was good at one site but relatively poor at a second site. Results with Amistar were inconsistent. No significant reduction in smoulder was observed following treatment with Benlate + Dithane 945.
- A large increase in smoulder symptoms can occur after flower picking. These include oval, grey-brown lesions on leaves (especially at points where the leaves bend) and rotting of flower stalks from the open stem end after flower picking. Fungicide treatment at bud stage and 2 d after flower-picking reduced stem rotting by 50-60%.
- Several fungicide treatments, and particularly Folicur, resulted in prolonged green leaf retention.
- All fungicide treatments resulted in increased bulb yields, compared with untreated plots, with a positive margin over chemical costs. In 2000, large yield increases were recorded at Mepal following treatment with Ronilan, Scala and Folicur (on cv. Carlton) and Folicur and Stroby (on cv. Cheerfulness). At Kirton, large yield increases were recorded following treatment with Bravo 500 and Unix (on cv. Carlton) and Folicur and Stroby WG (on cv. Cheerfulness).
- Not unexpectedly, mid- and late-season sprays were more effective than early sprays at delaying leaf die-back.
- At both Mepal and Kirton, the greatest bulb yield increase in cv. Carlton in 2001 resulted from a four-spray programme, consisting of two Ronilan sprays around emergence and two Folicur sprays around flower picking.
- On cv. Cheerfulness in 2001, all of seven fungicide programmes reduced leaf die-back and increased bulb yield. Bavistin DF + Folicur and Amistar + Folicur gave the best reduction of leaf die-back. At Mepal, these treatments and also Bavistin + Dithane 945 gave bulb yield increases of >40% over planted weight. At Kirton, Amistar + Folicur gave the greatest yield, an increase of 32% over planted weight.

Conclusions: White mould control

- White mould occurs commonly in unsprayed crops of narcissus in West Cornwall. The disease is rare in Eastern region, even in unsprayed crops, but was recorded in crops around Holbeach Marsh, Lincs in 2000 and 2001. The disease can progress very quickly to cause premature crop die-back.
- Bavistin DF + Dithane 945, and Benlate + Dithane 945, applied at about 14 day intervals, were effective in controlling white mould in naturally infected crops in Cornwall.
- A single spray of Benlate + Dithane 945, applied to cv. Yellow Cheerfulness in late February 1999, gave no control of white mould.
- A three-spray programme of Benlate + Dithane 945 in 1999 (22 March, 1 April and 13 April) was as effective as a five-spray programme commencing on 27 February. The first spray of the three-spray programme was applied when white mould was still at a very low level.

- In 2001, six-spray programmes of Amistar + Folicur and Bavistin DF + Folicur, applied at 14 day intervals from 2 March to cv. Yellow Cheerfulness, gave excellent control of white mould, and both mixtures appeared more effective than Bavistin DF + Dithane 945.
- In 2002 on cv. Cheerfulness, programmes of Amistar + Folicur were consistently better than equivalent programmes of Bavistin DF + Dithane 945 in controlling white mould and preventing premature leaf die-back.
- Amistar + Folicur applied at 28 d intervals from 21 February was as effective as Bavistin DF + Dithane 945 applied at 14 day intervals from the same date, resulting in a reduction in number of sprays from six to three without loss of disease control.
- A programme of three sprays of Amistar + Folicur, applied at 14 d intervals from the first appearance of white mould in the trial, gave significantly better control of white mould than an equivalent programme of Bavistin DF + Dithane 945. The latter gave only a slight reduction in white mould.

Formulation of experimental forecast systems (Task 4.2)

The following factors, relevant to formulating a disease forecasting system, were studied in the project:

Disease carry-over
The carry-over of disease from one year to the next is a problem in the two-year-down (or longer) growing of narcissus. However, Task 2.2 showed that there was no clear relationship between the level of smoulder or white mould infection in the first crop year and that developing in the second crop year. This conclusion may explain the dubious and variable findings of earlier field experiments in which the removal of leaf debris from narcissus crops at the end of the first year has been ineffective in reducing fungal foliar disease levels in the subsequent year (see Introduction). It was concluded that disease carry-over from the first to the second crop year was not a major factor where a fungicide programme was in use. However, where crops are grown-on for longer than a two-year cycle, additional fungicide sprays in the subsequent year or years would be a logical precaution.

Germination of R. vallisumbrosae *resting bodies*
The resting bodies pass the bulb's 'dormant' season in soil and debris, not in the bulb, hence the germination of these resting bodies was considered in Task 2.3 of this project. Germination was found to occur between mid-December and early-March, irrespective of locality, and required suitable conditions in the preceding week, namely soil temperatures of <10˚C and rainfall of >20mm. In determining fungicide spray dates for narcissus crops at risk from white mould, the occurrence of these soil temperature and moisture conditions at this period of the year would signal the need for a further fungicide spray.

Crop damage through weather
Task 2.5 of the project involved the study of PI sensors as a means of studying the severity of rain events that would spread fungal foliar diseases. However, it was evident that a more important factor was the necessity for leaf damage to occur before pathogen infection (as opposed to surface contamination or colonisation) could take place. In smoulder, the need for damage appears obligate, whereas some infection of white mould occurs even without damage, although damage was clearly advantageous to infection. Typically, artificial infection with either of the pathogens studied required the leaf surface to be damaged by pin-pricks or brushing off the surface wax layer. Hail is the obvious type of adverse weather that is well known to cause leaf damage, and the occurrence of hail on narcissus crops should suggest applying a fungicide spray as soon as practical. Heavy rain is also likely to cause significant crop damage, and, indeed, experimental work in this project showed that relatively few water drops acting at one point at terminal velocity quickly cause the loss of surface wax. Damage due to frost after shoot emergence has long been considered a possible factor in smoulder infection, but factoring frost into the smoulder infection model did not endorse this view. In the case of white mould, a relatively weak effect of frost on infection was shown experimentally. Many other types of weather damage could probably be considered signals for fungicide application.

Flower cropping
While physical damage through flower cropping and other field operations would

logically be expected to predispose narcissus to fungal foliar infections, no clear correlation between the two was evident in this project (Task 2.4).

Environmental factors – surface wetness and temperature
Controlled environment experiments showed a clear need for appropriate surface (leaf) wetness durations and temperatures for smoulder or white mould infection to take place (Tasks 3.2 and 3.3).

Other environmental factors
While the meteorological data examined in Task 2.4 suggested few direct correlations between weather and disease levels. However, these findings confirmed the importance of surface wetness and temperature. A consistent finding in the monitoring of white mould and weather factors in Cornwall was the importance of periods of surface wetness, shown in particular in the early and moderately severe infections at the Truro sites. In smoulder studies in the east, the strongest factor obviously increasing the development of late-season smoulder is the general increase in temperatures from late spring.

The following were studied as aids to a disease forecasting system:

Weather sensors
PI sensors (Task 2.5) and surface wetness sensors (Task 3.1) have been studied and should be added to weather stations monitoring narcissus crops. The new design of wetness sensor gave good results when compared to actual leaf wetness duration on leaves. Using several sensors of varying dimensions may provide useful information on the % wetness of leaves over the whole crop. Further investigations on this aspect are required as this would be a more accurate approach to the assessment of wetness durations in crops.

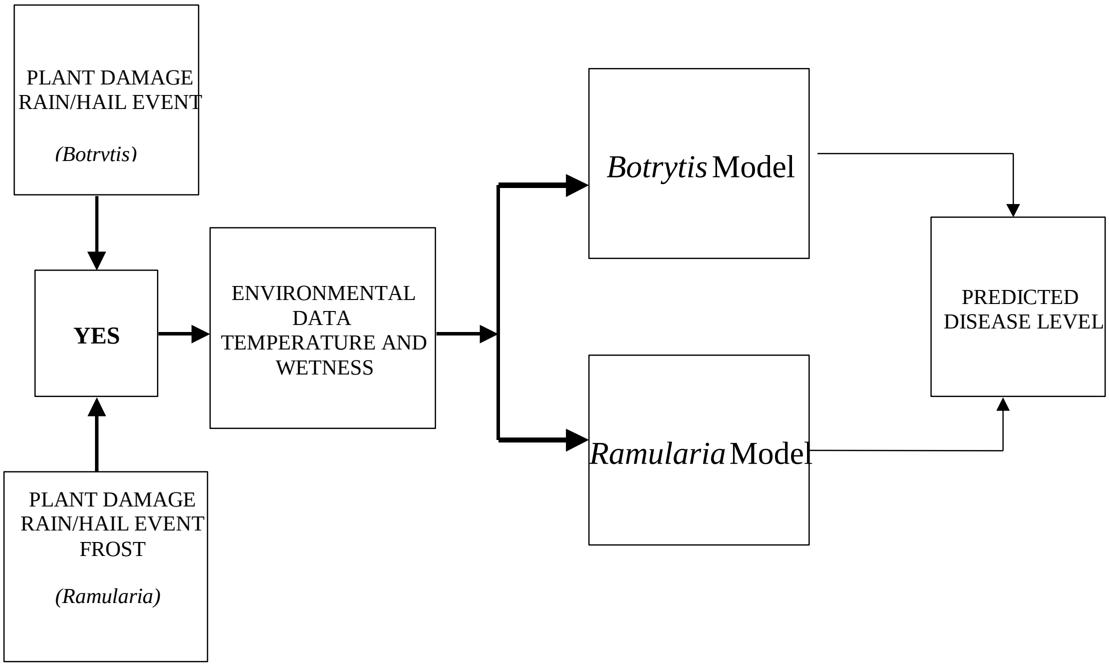
Fungicides
Newer fungicides and novel spray programmes were investigated in the project (Task 4.1) to complement the proposed spray system. There is little point having a spray timing system if only out-dated fungicides are available. The use of Amistar + Folicur appears to be particularly relevant for the South-West, enabling effective control of white mould with a reduced number of sprays, including treating recently established disease.

Based on these findings, a formulation of the narcissus foliar disease forecasting system (or Spray Timing System) is shown in Figure 4.2.1. The system is envisaged as operating in three stages:

- Environmental sensors would be used to detect events in the field (principally hail or heavy rain) that would cause damage to the crop. Damage appears essential for infection by smoulder, and highly advantageous for infection by white mould. This would trigger the next stage of the system.
- Environmental conditions would be monitored to predict the amount of smoulder and white mould that would arise, using the models developed in the project to relate surface wetness duration, temperature and the number of smoulder or white mould lesions. This would trigger a ‘spray alert’ once the predicted lesion number exceeded a critical limit.

- In borderline spray cases, other predisposing conditions would be used to reinforce or downgrade spray alerts. These factors would include crop durations longer than two years, suitable conditions for the germination of *R. vallisumbrosae* resting bodies, frost and flower cropping or other physical damage.

Figure 4.2.1. Formulation of disease forecasting system for smoulder and white mould.



Review

Principal findings

The project set out "To improve our understanding of the spread of white mould and smoulder, and develop better control strategies through disease forecasting techniques". This has been achieved:

- In the 'enabling' part of the project (Tasks 1.1, 1.2 and 2.1) knowledge of the biology of *R. vallisumbrosae* and *B. narcissicola* was gained, covering the pathogenicity of isolates, culture methods for resting body (sclerotia) and conidia production, and disease recognition. This information contributes to the knowledge base for any further research on these pathogens.
- Further information on the biology of *R. vallisumbrosae* and *B. narcissicola* was gained in Tasks 2.2 and 2.3, including pathogen carry-over (in bulbs, soil and crop debris) from the first-year of narcissus crops to subsequent years, and the conditions needed for germination of *R. vallisumbrosae* resting bodies.
- Information on the epidemiology of white mould and smoulder on narcissus crops was obtained in Task 2.4.
- The use of surface wetness (SW) and precipitation impact (PI) sensors as useful tools in narcissus crop/disease modelling was tested and verified (Tasks 2.5 and 3.1).
- Information was gained on the efficacy of currently used and novel fungicides, and of appropriate spray programmes, for the control of white mould and smoulder (Task 4.1). Good disease control can be achieved with some novel fungicides, even using fewer application, giving useful financial benefits. Spray dates should be defined by appropriate conditions, not by the start of shoot emergence and regular spray intervals.
- The effects of wetness duration and temperature on infection and disease development were studied. Predictive mathematical models describing these responses were proposed and partly validated (Tasks 3.2 and 3.3).
- The formulation of a Spray Timing System (STS) using these mathematical models, with subsidiary information on pathogen carry-over, resting body germination and other environmental effects, was proposed (Task 4.2).
- The importance of crop damage as a pre-requisite for infection with *R. vallisumbrosae* or *B. narcissicola* was also established during this project, opening up an important strategy for disease monitoring.
- During the course of the work it was established that *R. vallisumbrosae* is present on some narcissus crops in Lincolnshire, where it had not been previously confirmed.
- During the work 'late-season smoulder' symptoms due to *B. narcissicola* were reported; these had not been previously described and may have been mistaken for premature leaf senescence.
- This project is the first known in which a disease forecasting system has been developed for the control of foliar diseases of flower crops in the UK.

Technology transfer

Achieved

- Following twice-yearly meetings of the Project Consortium, the individual industry partners (representing 70 - 80% of the UK narcissus area) were able to make use of information arising from the project to modify their fungicide spray programme for the control of foliar diseases. The project showed that significant increases in bulb yield and disease control could be achieved through using novel fungicides, even using perhaps half of the number of sprays used previously. These findings have already been put into practice by members of the Consortium.

- General information about the project was made available through articles in HDC News and the Agriculture Link newsletter and presentations at meetings for bulb growers, subject to safeguarding the interests of the industrial members of the Consortium.

In preparation

- Further articles in the HDC News, the Agriculture Link newsletter, the commercial press (‘Grower’) and presentations at meetings for bulb growers.
- Papers for refereed scientific journals.
- HDC Fact-sheets on the control of foliar diseases of narcissus (incorporating older information as well as new findings).

<u>Exploitation</u>

Once the proposed infection models have been further validated, these are envisaged as the key components of a STS for narcissus crops (tentatively named ‘BulbSaver’). Using the STS, the occurrence of appropriate combinations of crop damage, temperature and surface wetness would trigger a warning that crops should receive a fungicide spray. It is envisaged that crops would be sprayed as soon as practical after the STS alerts growers to the need to do so, subject to a minimum interval between sprays and any other restrictions imposed on their use. To extract the most value from the STS, the recommended fungicides would include Amistar, Bavistin DF + Dithane 945, Folicur, Ronilan and Scala, while the number of sprays each year would not exceed four.

In discussions between researchers and industry partners at the commencement of the project, it was considered that the development of the project’s findings would be achieved by a further, relatively small, industry-funded project taking two years. During the project, discussion also took place about the preferred means of delivering the forecast to growers or their advisors. The industry partners made it clear that they would prefer the development of a simple ‘alert’ system, operating rather like the HDC narcissus fly forecast, rather than a more complex web-, PC- or MORPH-based system.

The development project would aim to:

- Validate the infection models. Although partial validation of the models has already been carried out using meteorological and disease monitoring data collected during the project, further validation is needed to test both models, and refine if necessary, preferably involving data from narcissus crops of other cultivars and at other locations. During this validation, the key environmental sensors would measure air temperature, surface wetness and precipitation impact.
- Validate the STS. This would include:
  - Confirm levels of PI and frost that are critical for causing damage to narcissus tissues.
  - Demonstrate practical use in the field of PI and SW sensors.

- Confirm the critical numbers of predicted lesions needed for spraying to be advised.
- Design a delivery system for the STS. The preferred mode would be a simple system of alerting growers and consultants – “Spray now” - delivered by automated fax or email systems. The alert would include current advice on the appropriate fungicides to use at each spray occasion, and the likely number of sprays that would be required. Up-to-date information is particularly important at a time when the availability of horticultural pesticides is posing serious problems for the industry.
- The delivery system would be tested and refined if necessary. Testing could be carried out by the industrial partners, in the second year of the development project.
- Workshops to provide training in the use of the system.

Proposals for a ‘product development and technology transfer’ project are being prepared for submission to Cornwall Horticulture Enterprises Ltd under the EU ‘Objective 1’ scheme for Cornwall and the Isles of Scilly. Discussions are under way with the HDC, growers and others to secure industry funding.

Benefits of project

The potential economic benefit of the project for the UK bulbs industry was estimated at the outset of the project as £2,177k *per annum*. This was made up of reduced losses in bulb and flower yield, and through savings due to reduced pesticide use (costs of pesticides and application):

	Benefits (£k saved *per annum*)	
	Cornwall	Eastern England
Improved yields through disease reduction	490	1,300
Reduced costs through reduced fungicide applications	193	194
	683	1,494
	2,177	

The price of narcissus bulbs is notoriously cyclical, and the above estimates were prepared when the wholesale price of commonplace narcissus cultivars was £400 - £500 per tonne. At the time the project was completed, prices were in a trough, averaging (across varieties) about 50% of these values, producing the following savings:

	Benefits (£k saved *per annum*)	
	Cornwall	Eastern England
Improved yields through disease reduction	245	650
Reduced costs through reduced fungicide applications	193	194
	438	844
	1285	

Although of course less favourable in the present circumstances, the annual financial benefits still well outweigh the one-off cost of the project (£523k, of which cash contributions were £371k). Naturally, there are also non-quantifiable or difficult-to-quantify benefits, including environmental ones, some of which are listed in the table on the next page, along with suggested indices for monitoring them.

Area	Benefit	Evidence
Science and technology	• Improved knowledge of the biology and epidemiology of the pathogens *R. vallisumbrosae* and *B. narcissicola*.	Publication of refereed papers. Further scientific advances
	• Improved strategies for foliar disease control based on an enhanced understanding of the relationships between environmental factors and the infection, development and spread of the pathogens causing white mould and smoulder.	Reduction in foliar diseases over time
	• Understanding of the importance of crop damage due to adverse weather	Damage-related control measures for narcissus foliar diseases, and adoption for other crops
	• Knowledge of PI and SW sensors in relation to foliar diseases	Increased use of PI and SW sensors
Industry	• Prediction of optimal times for applying fungicides to the narcissus crop. More economical, rational and targeted use of the fungicides most effective against foliar diseases, resulting in better disease control and improved bulb and flower quality. As one or both of these diseases affects all areas of the UK where narcissus are grown, the models will be relevant to the whole of the UK bulb industry.	Fewer fungicide sprays, evidenced by Pesticide Use Survey
	• Economic benefits of reducing pesticide costs and making savings in the labour required for pesticide application.	Reduced costs, evidenced by Pesticide Use Survey
	• A more favourable pesticide audit, enabling producers to maintain market share. The infection models would be 'justification' tools for spray application.	Fungicides applied only when justified, evidenced by successful sales to multiple retailers
	• Awareness of potential for white mould infections in eastern England	Survey crops in eastern England. White mould not a problem here
	• Awareness of late-season smoulder symptoms	Take remedial action. Improved yields
	• Incidental improved control of neck rot, a disorder also involving *B. narcissicola*.	Less neck rot problems. Low incidence of *B. narcissicola* in neck rot studies
Retailer and consumer	• There is consumer interest in a wider range of narcissus types and cultivars, so better and more economical disease control should enable growers to exploit varieties in demand but prone to fungal diseases.	More varied products on sale.
	• More environmentally friendly commercial production methods should provide added appeal.	Improved sales. Positive consumer attitudes
Environment	• There will be environmental benefits from a reduction in the heavy programme of fungicide sprays used at present with narcissus crops.	Less pesticides used, evidenced by Pesticide Use Survey
H&S	• Worker safety would be improved through picking of less heavily sprayed crops.	Less pesticides used around cropping time. No adverse responses recorded.
Rural development	• In Cornwall the advent of effective control of white mould would enable more marginal (e.g., higher and wetter) sites to be used for narcissus growing. Continuing serious losses due to white mould could cause some Cornish growers to leave narcissus production, and the loss of employment would have a serious effect on the already depressed local economy. When grown for flower as well as bulb production, narcissus are considered to have one of the highest rates for employment per hectare of field-grown crops.	Maintenance of UK bulbs industry, particularly in south-west England. Continued requirement for labour.

<u>Science aspects</u>

Biology and epidemiology of R. vallisumbrosae

The conditions needed for spore germination and growth (temperature and leaf surface wetness) have been defined. *R. vallisumbrosae* is capable of infecting narcissus leaves that are undamaged, but leaf damage has been shown to markedly enhance infection. Frost damage was shown to enhance infection, but much less so than physical damage, perhaps because the mild frosts experienced in Cornwall result in relatively little disruption of the leaf structure. Unlike *B. narcissicola*, *R. vallisumbrosae* spores do not appear to require a ready source of nutrients to infect narcissus. Since the spores and resting bodies of the fungus are relatively short-lived, information on sources of inoculum and patterns of spread (including, in the relatively heterogeneous topography of the Cornish bulb-growing areas, the effects of field boundaries and gateways on distribution) is desirable. Although the project has contributed to the practical epidemiology of white mould, several aspects of the biology of the pathogen remain incompletely known, for example the life-cycle and relationships between spore types.

Biology and epidemiology of B. narcissicola

As well as the appropriate conditions for spore germination and growth (temperature and leaf surface wetness), the infection of narcissus leaves by the smoulder pathogen almost always appears to require a damaged leaf surface and a source of nutrients. Frost, often supposed to be a source of leaf damage that can leads to infection by smoulder, appeared to have a relatively weak effect. Hence, in the cases of both *R. vallisumbrosae* and *B. narcissicola*, physical crop damage from hail and flower cropping are probably the most important factors in infection.

Leaf damage and infection

The project has well illustrated the significance of a damaged plant surface for fungal infection to occur. The definition of the critical level of such damage is likely to be a complex issue and a general one in predicting the development of foliar pathogens.

Effectiveness of newer fungicides

A mixture of Amistar + Folicur was found to be very effective in controlling white mould, with significant advantages over Bavistin DF + Dithane 945. This may, possibly, result from synergism between the two chemicals (evidence was found of synergism between Bavistin DF and Dithane 945), from the prolonged green leaf retention especially notable with Folicur, or from the different modes of action of the fungicides. Although there was no evidence of resistance in *R. vallisumbrosae* to Bavistin DF + Dithane 945, with repeated use a reduced sensitivity to this fungicide mixture cannot be discounted.

For smoulder, new fungicides from the anilinopyrimidine and strobilurin groups were found to give good control of the disease. These fungicides have been previously demonstrated as active against the closely related grey mould (*Botrytis cinerea*). The interrelationship of smoulder development, leaf die-back and bulb yield warrants further study. Although the programme of four sprays during and after flowering gave the greatest green leaf retention, a programme of four sprays during emergence and flowering gave the largest increases in bulb yield.

Climate change

Climate change is now recognised to be a global concern, and will impact on UK farm operations (Hossell *et al*., 2001). Weather-based disease predictors, as developed for smoulder and white mould in this project, are particularly valuable in considering the effects of climate change on the future impact of pests and diseases on crops. In the case of narcissus in the UK, some of the major pests and diseases are operating at the apparent limits of their range. Thus, white mould and large narcissus fly have been considered to be relevant only to the Cornish bulb industry, but first narcissus fly, and now white mould, have been found in the eastern counties. At present, the Scottish bulb-growing has been considered favourable for narcissus because the cooler conditions do not favour *Fusarium* bulb rots or aphid virus vectors.

Another aspect is that increasingly unsettled weather in the UK may result in the availability of fewer ‘spray days’, already a rarity in the South-West in many years. Based on the UKCIP98 Medium High scenario of climate change, winter and late autumn are predicted to be wetter than at present, with an increase in precipitation of 9 to 13% by the 2050s. Increasing winter rainfall may increase the likelihood of white mould epidemics occurring in eastern England as well as in the South-West. For an arable crop farm located in Cambridgeshire, it is predicted that available work days (i.e., days when ground conditions allow normal tractor-mounted operations, such as crop spraying) for weeks 1 – 12 (the period when narcissus fungicides are most used) will decline from an average of 24.5 days (1961-1990 baseline) to 18.5 and 17.5 days by the 2020s and 2050s, respectively (Harris & Hossell, 2002). In the South-West, available work days for weeks 1 – 12 will remain at less than 1 per week to the 2050s. Hence, there will continue to be only snatched opportunities for crop spraying, and the fewer of these you need because there is a better product or programme, the more likely it will be to achieve reasonable control of diseases. The Amistar + Folicur treatment identified in the project, which provides control of white mould when applied at 21 – 28 day intervals, will be a significant advantage over Benlate + Dithane 945, as the available work days in winter decline.

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Appendix 1. Report of white mould in eastern England

Plant Pathology (2002) **51**, 400

NEW DISEASE REPORT

First report of white mould (*Ramularia vallisumbrosae*) on daffodils (*Narcissus*) in eastern England

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White mould (*Ramularia vallisumbrosae*) is a common and damaging foliar disease on daffodils (*Narcissus* cultivars) in the south-west of England that in some years causes epidemics in commercial *Narcissus* crops in Cornwall and the Isles of Scilly. Outside of this region the disease has previously been identified in Warwickshire, central England (Anonymous, 1929), on Anglesey, Wales (Baker, 1972), and in southern Scotland (Dennis & Foister, 1942). However, there appears to be no previous confirmation of the disease in eastern England (J.B. Briggs, E. Roberts, A. Inman, personal communication) where *Narcissus* has been grown on a large scale for over 100 years (Dobbs, 1983).

White mould was identified in May 2001 on *Narcissus* cv. Carlton in Holbeach Marsh, Lincolnshire, in a crop being monitored for foliar diseases at *c.* 14-day intervals from January. Lesions occurred on leaves and stems and typically were pale brown, oval, *c.* 10–30 × 5–10 mm in size and contained numerous minute black, sclerotium-like bodies. Microscopic examination revealed amerospores and phragmospores characteristic of *R. vallisumbrosae* (Moore, 1979). The disease was subsequently confirmed in crops of *Narcissus* cvs Spellbinder and Dutch Master on other farms in Lincolnshire (Holbeach Marsh and Kirton). Plant Health inspectors reported similar symptoms affecting various cultivars on several farms in the Spalding, Holbeach and Kirton area in 2000 and 2001 (E. Roberts, personal communication), however, the pathogen was not confirmed in these cases. In Cornwall, sporulating lesions of white mould are often seen in February before flowering, whereas in these newly reported instances in Lincolnshire, the lesions were seen after flowering, from May onwards.

Although there is commercial interchange of bulbs between south-west and eastern England, the major bulb-growing regions in the UK, the disease is not believed to be bulb-borne (Moore, 1979). In spring, no white mould was observed on plants grown from severely affected *Narcissus* cv. Carlton bulbs, harvested in July 1999 in Cornwall and grown under humid glasshouse conditions or in a polythene tunnel at Kirton (Lincolnshire) and Mepal (Cambridgeshire), respectively. This report suggests that there may now be an increased risk in eastern England, where *c.* 60% of UK *Narcissus* are grown, of outbreaks of this potentially epidemic disease with consequent early foliage die-down and associated reductions in bulb yield.

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Appendix 2. List of field sites

The table shows the locations used for field experiments and crop monitoring (two-year-down crops of cv. Carlton, unless otherwise stated).

Location	Field name and grid reference (years refer to monitoring or treatment years)		
	1998-1999	1999-2000	2000-2001
Swaffham Prior Fen Cambs	Field 29 TL533674	Highfen Farm TL538684	Split Drove TL551674
Holbeach Marsh Lincs	TF394321	TF396324	Black Hovel TF385329
Gosberton Marsh Lincs	Wilson's Field TF250296	Sly's Field[1] TF268305	Tunnards Field TF255275
Manaccan Cornwall	Park Beet Home[2] SW762257	Park Beet Home[2,3] SW762257	Newtown SW750240
Tresillian Cornwall	Tolskiddy Field SW862433	Polsue Manor Farm SW857464	Fentongollan SW862435
Truro Cornwall	Trethewey Farm SW915432	Woodland Valley 6 SW914510	Tregidgeo[4] SW957475 Trewaters Farm SW849534
ADAS Arthur Rickwood, Mepal Cambs[5]	-	House Ground TL442824	House Ground TL442824
HRI, Kirton Lincs[5]	-	40 Acres TF295395	40 Acres TF295395
	1998-1999	2000-2001	2001-2002
Penzance Cornwall[6]	Nancledra SW498354	Nancledra SW495348	Cucurrian SW510350

[1] Cv. Standard Value
[2] Sheltered site
[3] Third-year-down crop
[4] Observations of first-year crop at Tregidgeo but crop then lifted, observations moved to Trewaters Farm.
[5] Cv. Carlton and Cheerfulness
[6] Cv. Yellow Cheerfulness (1998-1999 and 2000-2001) or Cheerfulness (2001-2002)

Appendix 3. List of project milestones

All milestones were achieved, as detailed in the prior Annual Reports, with the exception of 1.2.5 for which an explanation is given below.

1.1.1 Five samples of affected leaves for each disease collected by December 1998
1.1.2 Isolates established in clean culture by December 1998
1.1.3 Pathogenicity tests completed by June 1999
1.1.4 Isolate selection completed by June 1999
1.1.5 Stock cultures of *R. vallisumbrosae* and *B. narcissicola* established by June 1999

1.2.1 Growth of *R. vallisumbrosae* and *B. narcissicola* on standard mycological media recorded by December 1998
1.2.2 Optimisation studies on fungal growth completed by June 1999
1.2.3 Standard protocols for conidia production circulated to science partners by June 1999
1.2.4 Results of *R. vallisumbrosae* resting body production on leaves summarised by December 1999
1.2.5 Report on germination of resting bodies *in vitro* and *in planta* completed by June 2000
This milestone was considered unnecessary to achieve, since collection of infected narcissus leaves from commercial crops proved a more effective way of obtaining *Ramularia* resting bodies in quantity.

2.1.1 Definitive descriptions of the foliar symptoms of white mould and smoulder produced by December 1998
2.1.2 Scoring system for white mould and smoulder disease severity established by December 1998
2.1.3 Sampling procedures for scoring incidence of white mould and smoulder symptoms circulated by December 1998
2.1.4 Standard protocols for assessing the incidence and severity of smoulder and white mould symptoms circulated by December 1998

2.2.1 Disease levels at end of first year crops (first set) recorded by December 1999
2.2.2 Disease levels at start of second year crops (first set) recorded by June 2000
2.2.3 Disease levels at end of first year crops (second set) recorded by December 2000
2.2.4 Disease levels at start of second year crops (second set) recorded by June 2001
2.2.5 Report on influence of pathogen carryover completed by December 2001

2.3.1 First set of resting bodies buried by December 1999
2.3.2 Examination of recovered resting bodies (first set) completed by June 2000
2.3.3 Second set of resting bodies buried by December 2000
2.3.4 Examination of recovered resting bodies (second set) completed by June 2001
2.3.5 Report on *R. vallisumbrosae* resting body germination completed by December 2001

2.4.1 Commercial sites for monitoring in 1998-99 identified by September 1998
2.4.2 Experimental plots at research sites for disease inoculation and monitoring in 1999-2000 set up by September 1998
2.4.3 Commercial sites for monitoring in 1999-2000 identified by September 1999
2.4.4 Experimental plots at research sites for disease inoculation and monitoring in 2000-01 set up by September 1999
2.4.5 Meteorological, disease, crop and other data from the commercial sites monitored in 1998-99 summarised by December 1999
2.4.6 Commercial sites for monitoring in 2000-01 identified by September 2000

2.4.7 Meteorological and other data from the commercial and research sites monitored in 1999-2000 summarised by December 2000
2.4.8 Meteorological and other data from the commercial and research sites monitored in 2000-01 summarised by December 2001
2.4.9 Report on the relationships between environmental, crop and other factors and the occurrence and development of white mould and smoulder prepared by June 2002

2.5.1 Sites for investigation of rain-splash sensors established by March 1999
2.5.2 Data from precipitation impact sensors summarised by March 2001
2.5.3 Report on the measurement of rainfall splash from narcissus leaves prepared by June 2002

3.1.1 Sites for investigation of leaf wetness sensors established by March 1999
3.1.2 Data on wetness characteristics of narcissus leaves and wetness sensors summarised by March 2001
3.1.3 Report of the wetness characteristics of narcissus leaves and all wetness sensors prepared by June 2002

3.2.1 Report on the relationship of temperature and wetness duration to the infection of narcissus tissues by conidia of *B. narcissicola* prepared by March 2001
3.2.2 Report on the relationship of temperature and wetness duration to the infection of narcissus leaves by conidia of *R. vallisumbrosae* prepared by June 2002

3.3.1 Report on the experiments to test the validity of leaf wetness duration models for infection by *R. vallis-umbrosae* and *B. narcissicola* by June 2002

4.1.1 Plots for field experiments planted by December 1998
4.1.2 Fungicide efficacy on inoculated detached leaves determined by December 1999
4.1.3 Treatments for field experiments selected by December 1999
4.1.4 Report on fungicide field experiments completed by December 2000

4.2.1 Draft proposal for further work on field testing of narcissus foliar disease forecasting system produced by June 2002

