Project title:	Narcissus leaf diseases: Forecasting and control of white mould and smoulder
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Location of project:	HRI Kirton and Wellesbourne ADAS Arthur Rickwood Commercial sites in Cornwall, Lincolnshire and Cambridgeshire
Consortium members:	Industrial partners Horticultural Development Council Winchester Growers Ltd Angloflora Ltd F Dring & Sons Ltd Mr W J S Hosking O A Taylor & Sons Ltd Lingarden Ltd F H Bowser Ltd Lords Ground Ltd R H Scrimshaw Ltd Intelligent Micro Design Ltd Academic partners Horticulture Research International ADAS Consulting Ltd
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Key words *Narcissus*, daffodil, bulb, disease forecasting, smoulder, *Botrytis narcissicola*, white mould, *Ramularia vallisumbrosae,* Folicur, Scala, Bavistin DF, Dithane 945, Amistar, Ronilan FL

For accurate reporting, pesticides have generally been referred to by the name of the commercial product. No endorsement is intended of products mentioned, nor criticism of those not mentioned

The following are members of the Consortium for Horticulture LINK project number 188

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CONTENTS

EXECUTIVE SUMMARY

1. Further commercial crops in Cornwall and in eastern England were examined towards the end of their first growing season (June 2000), either by the examination of foliage debris or by monitoring crops in the field (objective 2.2).
   - Dead foliage from all three Cornish sites contained resting bodies of *Ramularia vallisumbrosae*, with 7, 24 and 33% of leaves being affected at the three sites. White mould lesions were present on leaves from the three Cornish sites (with 0.7, 7.0 and 22.3 lesions/plot, respectively, and *Botrytis* was also confirmed on leaves from these sites.
   - Active smoulder lesions were not seen on foliage from the three commercial sites in the east of England (two in Lincolnshire, one in Cambridgeshire), indicating that the commercial fungicide treatments had controlled the disease up to that time. However, smoulder lesions were present on the four crops grown at the experimental sites, which had received no fungicide treatments: there was an average of 45 lesions/plot in the two ADAS Arthur Rickwood crops and an average of 2 lesions/plot in the two HRI Kirton crops.
   - In the second crop year, smoulder was found in all of the seven eastern crops, and white mould on all three of the Cornish crops, but there were no clear correlations between incidence at the end of the first crop year and at the beginning of the second.

2. Further experiments on the germination of resting bodies of *R. vallisumbrosae* were set up in June 2000 at Varfell Farm, Penzance and ADAS Arthur Rickwood (objective 2.3).
   - The germination of resting bodies was first observed on 13 December 2000 for the experiment at Arthur Rickwood, and one week later for the Penzance experiment. Germination continued until late-January 2001.
   - When resting bodies were incubated in damp conditions immediately after recovery, germination occurred earlier. Low temperatures, and, especially, moist conditions, favoured early germination.

3. The monitoring of smoulder and white mould was completed for a third set of second-year commercial narcissus crops (2000-2001), three in Cornwall and three in eastern England (objective 2.4). These crops were not sprayed with fungicide in their second year.
   - White mould lesions appeared in March 2001 at all three Cornish sites, and the number then increased rapidly at Trewaters Farm. At all three sites the foliage was 50% died down by early-May.
   - In the three eastern crops more smoulder primaries were seen at Holbeach St. Marks and Surfleet than at Swaffham Prior Fen. In March-April the numbers of smoulder lesions increased slowly at all three sites, with the highest numbers at Holbeach St. Marks. Rapid loss of green leaf area occurred at all sites in May, starting at the Swaffham Prior Fen site. By mid-May, large numbers of secondary smoulder lesions were seen at Swaffham Prior Fen, and smaller numbers at Holbeach St. Marks.
   - Two crops each at HRI Kirton and ADAS Arthur Rickwood were also monitored for disease. These crops received no fungicides in either year of growth. No white mould was seen. There was a steady increase in the number of smoulder lesions at both sites, and the loss of green leaf surface due to disease occurred at Arthur Rickwood 2-3 weeks earlier than at Kirton.

4. Assessments of the characteristics of a novel leaf wetness sensor are continuing (objective 3.1).

5. Experimental work on temperature, wetness duration and infection was continued (objective 3.2).
   - The presence of damaged leaf tissue was necessary for its infection by *Botrytis narcissicola*. Analysis of data from controlled environment experiments produced a highly significant relationship describing the effect of temperature and wetness duration on infection of narcissus leaves.
   - Controlled environment experiments were also carried out with *Ramularia vallisumbrosae*. The presence of tissue damage was essential for infection by white mould on pre-senescent narcissus leaves. 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 by *R. vallisumbrosae*.
   - In further experiments with *R. vallisumbrosae*, it was shown that phragmospores were the most common spore form produced on white mould lesions. The optimal temperature for phragmospore production was 5 - 15°C. Scolecospores were produced at temperatures of 5 - 10°C. Phragmospores and scolecospores may have different environmental criteria for infection of narcissus leaves.

6. A further series of four fungicide trials, involving second-year crops of cultivars Carlton and Cheerfulness at HRI Kirton and ADAS Arthur Rickwood, were completed in summer 2001 (objective 4.1). Cultivar Carlton was used at either site for experiments into smoulder control, and cv. Cheerfulness for white mould control. No fungicides were applied in the first year of these crops.
   - In trials with cv Carlton, fungicide application was investigated at three phases of growth: shoot emergence (using Ronilan), around flowering (using Folicur) and after flowering (using Scala). There were two applications of fungicides in each phase, and different treatments consisted of applying fungicide in one, two or all three phases. In addition, a six-spray programme of Folicur only was compared.
   - At Arthur Rickwood the application of two fungicide sprays (Ronilan or Folicur) in February and March had no detectable effect on the incidences of primaries or of the proportion bearing sporulating *Botrytis*. In April, when up to four fungicide sprays had been applied, there was a significant difference in the incidence of stems rotting from *Botrytis*: treatments that had received two sprays of Folicur around flowering time had many fewer rotting stems than untreated controls. The total incidence of smoulder lesions on leaves and stems was greatest in untreated plots and was reduced slightly by some of the fungicide programmes. Fungicide treatments had a very noticeable effect on leaf die-back. Four 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 was particularly effective at reducing leaf die-back. At Kirton also, treatments applied around flowering resulted in significant reductions in stem rotting due to *Botrytis*. As at Arthur Rickwood, fungicide treatment had no significant effect of 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, as above.

- Bulb yields at Arthur Rickwood ranged from 46.7 to 61.7 kg per plot, with significant differences between treatments and the greatest yield 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. 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 and two Folicur sprays that gave the greatest yield. The prolongation of green leaf retention and the increase in yield were not closely related.
- In trials with cv Cheerfulness, several fungicide mixtures were investigated, along with Bavistin DF + Dithane 945 at different growth stages (early, conventional and post-flowering). White mould did not develop in these trials, and the effects of treatments on smoulder are described. At Arthur Rickwood, by 8 May, when up to four fungicide sprays had been applied, a high incidence of *Botrytis* stem rotting was recorded and was significantly reduced by fungicide treatments. Fungicide treatment had a very noticeable effect on leaf die-back: two days after the final spray there was 99% die-back in untreated plots and less than 20% in all treated plots. Plots treated with Bavistin + Folicur and Amistar + Folicur were particularly green. Bulb yield at Arthur Rickwood was significantly different between treatments, with Bavistin + Folicur giving a 59% yield increase over untreated plots. At Kirton, foliage dieback was noticeably delayed by most treatments. Bulb yields differed significantly, with least in untreated plots and the greatest yield, a 24% increase, 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.
- The control of white mould was investigated in a crop at Penzance. By 25 April there were marked differences between treatments in both the number of white mould foci and the number of lesions / m of ridge. 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 plots (88.2 lesions / 0.5m) and reduced to 10 lesions or less by all treatments except when the spray programme commenced at first symptoms. Bavistin + Folicur, Bavistin + 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. The three treatments noted above which gave very good control of white mould also resulted in prolonged green leaf retention. 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 0-5 cm 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.

INTRODUCTION

This Annual Report covers all work done on the project between November 2000 and November 2001. The experimental work is presented in the same order as the project objectives, which are listed below.

OBJECTIVES AND TIMETABLE

The aim of the project is to improve understanding of the spread of white mould and smoulder, and develop better control strategies through disease forecasting techniques. This will be achieved through a number of specific scientific objectives and tasks:

1 To produce methods for the reliable production of resting bodies and conidia of *Ramularia vallisumbrosae* and conidia of *Botrytis narcissicola*, using typical pathogenic isolates:
  1.1 Pathogenicity and selection of isolates
  1.2 Production of resting bodies and conidia

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:
  2.1 Disease recognition and assessment
  2.2 Pathogen carry-over from the first year of crops
  2.3 *Ramularia* resting body germination
  2.4 Environmental factors and disease development:
    (a) Monitoring commercial crops
    (b) Monitoring experimental crops
  2.5 Development and validation of precipitation impact sensors

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
  3.2 Effect of leaf wetness and temperature on infection
  3.3 Validation of leaf wetness duration models

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 efficacy:
    (a) Laboratory studies
    (b) Field experiments
  4.2 Formulation of experimental forecasting systems

The interrelationships of the objectives and tasks are shown in Figure 1, and the timetable for the work is summarised in the Figure 2. In the experimental part of this report, the projected work and progress to date is set out for each of the tasks in order.

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

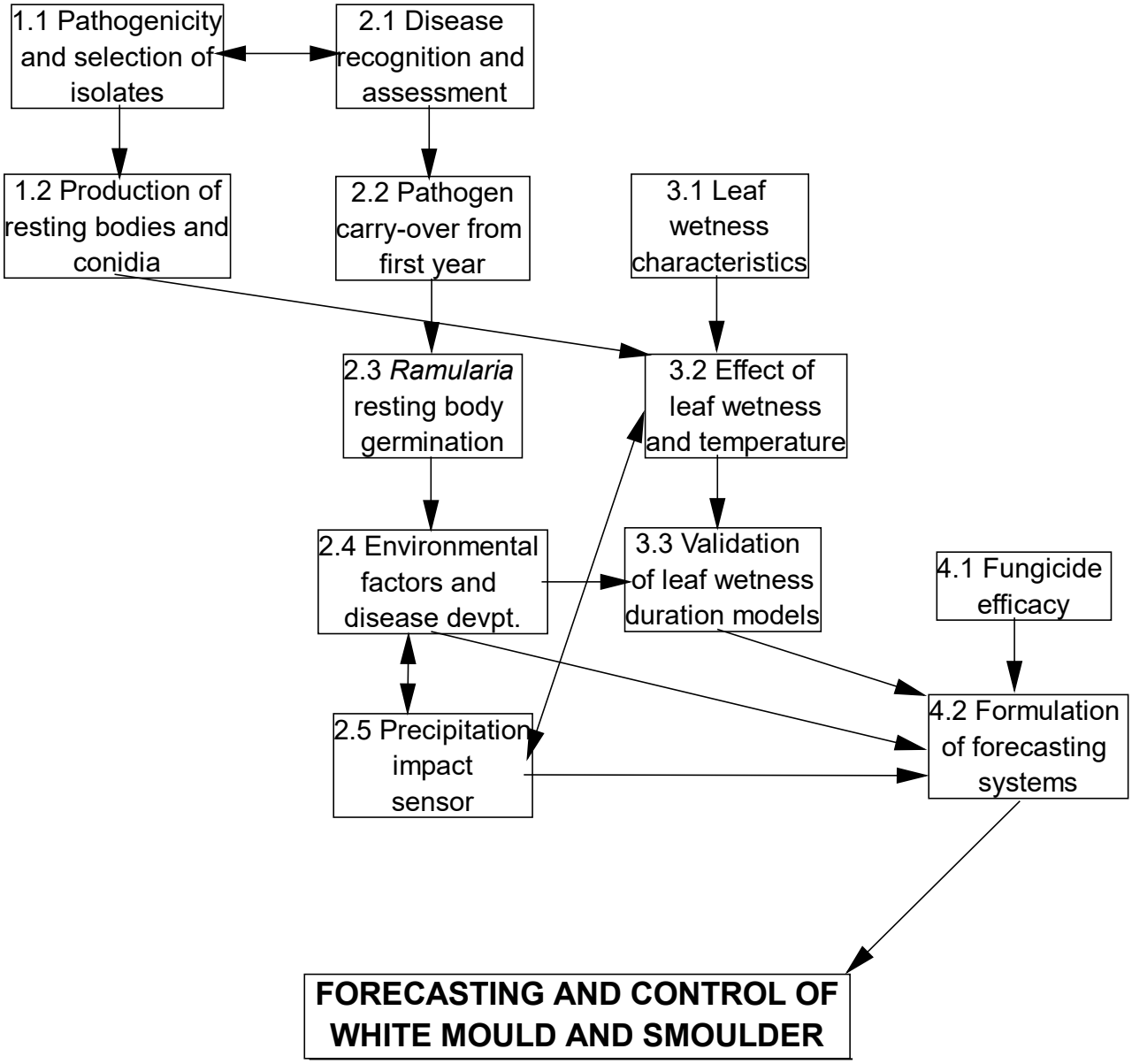


Figure 2. GANTT chart showing timetable for the main practical activities

Task	Activity	1998	1998	1999	1999	1999	1999	2000	2000	2000	2000	2001	2001	2001	2001	2002	2002
		Jul	Oct	Jan	Apr	Jul	Oct	Jan	Apr	Jul	Oct	Jan	Apr	Jul	Oct	Jan	Apr
1.1	Pathogenicity and selection of isolates																
1.2	Production of resting bodies and conidia																
2.1	Disease recognition and assessment																
2.2	Pathogen carry-over from first-year																
2.3	*Ramularia* resting body germination																
2.4	Environmental factors and disease development																
2.5	Precipitation impact sensors																
3.1	Leaf wetness characteristics																
3.2	Effect of leaf wetness duration and temperature																
3.3	Validation of leaf wetness duration models																
4.1	Fungicide efficacy																
4.2	Formulation of fore-casting systems																

EXPERIMENTAL SECTION

1.1 Pathogenicity and selection of isolates

Plan and milestones (taken from the Project Proposal)

Collect a minimum of five samples each of narcissus leaves showing typical symptoms of white mould from crops in Cornwall, and of smoulder from crops in eastern England. Isolate from affected tissues onto potato dextrose agar (PDA) and establish the causal fungi *Ramularia vallisumbrosae* and *Botrytis narcissicola* in culture, free of contaminating micro-organisms. Determine the relative pathogenicity of isolates by inoculating leaves of narcissus cvs Cheerfulness and Carlton (for *R. vallisumbrosae* and *B. narcissicola* respectively) with mycelial plugs of the fungi on agar. Incubate leaves at defined temperatures and high humidity to favour infection. Assess the occurrence and extent of lesions which have developed after 14 days. Describe the variation in pathogenicity. Determine the relative growth, sporulation and resting body production on agar.

Select one isolate of each pathogen, which shows the best combination of growth in culture, sporulation, production of resting bodies and pathogenicity, for use in further studies (task 1.2), keeping the other isolates in reserve. Maintain all isolates on PDA slopes at 4°C for storage.

Milestones

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

All milestones achieved

Progress

All milestones were achieved on time and were reported in the first Annual Report.

1.2 Production of resting bodies and conidia

Plan and milestones (taken from the Project Proposal)

Using the isolates selected in 1.1 (above), attempt culture of *Ramularia vallisumbrosae* and *Botrytis narcissicola* on a range of standard mycological media. Record vigour of growth, sporulation (conidia production) and production of resting bodies. Describe the type of condia produced by *R. vallisumbrosae* on different media. It is anticipated that Medium X (Last and Hanley, 1956) or V8-juice agar will be used for production of *B. narcissicola* conidia. Manipulate factors (e.g., temperature, light and nutrient levels) during growth of fungi to optimise production of conidia and resting bodies. Inoculate pot-grown narcissus cv Cheerfulness with *R. vallisumbrosae*, maintain plants under humid conditions to enhance infection and, after natural leaf senescence, examine leaves for resting bodies of *R. vallisumbrosae*. Compare the time and extent of germination of *R. vallisumbrosae* resting bodies produced *in vitro* and *in planta*. Circulate protocols for the production of conidia to science partners for use in other parts of the project (sections 2.4(b), 3.2 and 4.1(a)).

Milestones

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

Milestones 1.2.1 - 1.2.4 achieved; milestone 1.2.5 deferred

Progress

This work has been described in the First and Second Annual Reports, and is now complete.

2.1 Disease recognition and assessment

Plan and milestones (taken from the Project Proposal)

Unambiguous protocols will be needed for recording the incidence and severity of white mould and smoulder for use in subsequent stages of the project (sections 2.4 and 4.1(b)). As can be seen by the following descriptions, many of the symptoms of white mould and smoulder are rather general, and could potentially be confused with those due to other disorders, such as leaf senescence (either natural, or premature as a result of basal rot or drought). Distinction also has to be made from the darker sporulating lesions of *Stagonospora curtisii*, an occasional pathogen of narcissus crops in this country.

Examine the crops destined for use in section 2.4 of the study (supplemented by other narcissus crops if necessary), refer to standard texts on the diseases of flower-bulb crops, and produce a comprehensive description of the foliar symptoms of white mould and smoulder, accompanied by a photographic key.

For each of the main symptoms (in the case of white mould, for example, foliar lesions, premature leaf senescence and resting bodies in débris), devise a scale of severity of symptoms based on a simple scoring system. For disease incidence, establish an appropriate plot size and sampling routine. Incorporate these factors into a Standard Operating Procedure for disease assessment.

Milestones

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

All milestones achieved

Progress

All milestones were achieved on time and were reported in the first Annual Report.

2.2 Pathogen carry-over from the first year of crops

Plan and milestones (taken from the Project Proposal)

Using the assessment protocols developed in section 2.1, record the incidence and severity of white mould and smoulder in six commercial first-year crops (three in Cornwall, three in eastern England) after flowering and before leaf senescence. A minimum of 100 plants will be examined in each crop.

Re-examine the same six crops during January/February of their second year of growth (part of task 2.4), and record the initial incidence and severity of the two diseases again. Repeat on the six commercial crops used in years 2 and 3. Examine the 18 sets of data to determine if disease occurrence and extent early in the second year of crop growth appear to be related to that recorded at the end of the first year.

Additionally, after senescence, collect samples of senescent foliage and débris from the three Cornish commercial crops and examine these microscopically, and by isolation onto agar if necessary, for evidence of *Ramularia vallisumbrosae* resting bodies. Conduct this exercise twice (in 1999 and 2000) to provide data on the possible carry-over of disease on débris from six first-year crops.

Take this information into account in the development of a spray timing system (section 4.2).

Milestones
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
Milestones 2.2.1 - 2.2.4 achieved

Progress

Examination of crop debris
Samples of dead leaves were collected in June/July from first-year-down commercial crops in Cornwall in 1998, 1999 and 2000, and examined for resting bodies of *R. vallisumbrosae.* Fifty leaves were collected and examined from each site, one every 0.5m. The data from 1998 and 1999 were summarised in earlier Annual Reports.

In early-June 2000, *R. vallisumbrosae* resting bodies were found at all three sites, the incidence of affected leaves being 7% (Newtown), 24% (Fentongollan) and 33% (Tregidgeo). *Botrytis* was also confirmed on leaves from all three sites, being most common at Tregidgeo and Newtown.

Examination of growing crops.
Data relating leaf disease incidence at the end of the first-year-down to that occurring at the start of the second crop year were collected in summer 1999 and spring 2000, and reported in the previous Annual Report. Data for summer 2000 and spring 2001 have now been collected.

Six crops (three each in Cornwall (as above) and in the eastern region) were inspected at crop die down in June/July 2000 and again in 2001 once full emergence had occurred. Details of the sites are given under section 2.4a. Crops growing at the research sites (ADAS Arthur Rickwood and HRI Kirton, see under section 2.4 b) were also examined at these times. The results are shown in Table 2.2.1.

Smoulder was not found in the commercial crops at the end of their first year, suggesting that fungicide treatments had controlled the disease up to that time. However, in the four experimental crops, which remained unsprayed with fungicide and were adjacent to badly affected second-year-down crops, smoulder was readily found. The disease was considerably more serious in crops at ADAS Arthur Rickwood than at HRI Kirton, probably reflecting the early and severe attack of smoulder on adjacent crops at the Cambridgeshire site. Smoulder was found on all seven eastern crops soon after emergence, but there was no clear correlation between incidence at the end of the first crop year and disease levels in the second crop year.

White mould was found only in Cornish crops and occurred at all three sites, with a very high level at Tregidgeo, and the lowest level at Newtown. In the second crop year white mould appeared first at the Trewaters Farm site.

Table 2.2.1 Disease levels in commercial and experimental narcissus crops, cv. Carlton, at the end of their first year (summer 2000) and after emergence in their second year (spring 2001).

Site	Assessment date (day number)		No of smolder lesions/plot[a]		No of white mould lesions/plot[a]	
	End of year 1	Start of year 2	End of Year 1	Start of Year 2	End of Year 1	Start of Year 2
Commercial						
1. Swaffham Prior Fen, Cambs	185	75	0	0.8 (1.53)	0	0
2. Holbeach, Lincs	178	74	0	2.0 (2.05)	0	0
3. Surfleet, Lincs	173	74	0	4.4 (3.08)	0	0
4. Newtown, Cornwall	166	24	0	0	0.7 (1.00)	0
5. Fentogollan, Cornwall	165	24	0	0	7.0 (5.46)	0
6. Tregidgeo/ Trewater, Cornwall	165	24	0	0	22.3 (10.65)	0.04 (0.283)
Experimental						
1. ADAS, Cambs (Cornish stock)	145	46	50.7(2.38)	0.2 (0.09)	0	0
2. ADAS, Cambs (Lincs stock)	145	46	42.1(2.44)	0.3 (0.06)	0	0
3. HRI, Lincs (Cornish Stock)	158	66	2.9 (1.57)	1.7 (1.77)	0	0
4. HRI Lincs (Lincs stock)	158	64	1.5 (1.42)	2.3 (2.16)	0	0

[a] Figures are means for 50 plots each 0.5m in length, with standard errors in parenthesis

2.3 *Ramularia* resting body germination

Plan and milestones (taken from the Project Proposal)

The scientific literature indicates that *Ramularia vallisumbrosae* resting bodies, which develop in senescent narcissus leaves, are the only means of pathogen carry-over: there is no recorded alternative host and no carry-over in bulbs (Gregory, 1939). Moreover, these resting bodies are believed to be relatively short-lived (less than one year). They are thus a weak link in the life-cycle of the pathogen. Information on the time, extent and duration of resting body germination would allow more accurate timing of fungicide sprays to achieve good disease control with minimum fungicide input. Information on environmental factors which may trigger germination may allow white mould risk at the start of a season to be assessed from recorded weather data.

Bury multiple sets of *R. vallisumbrosae* resting bodies at a shallow depth in soil, and place others on the soil, within secured nylon bags at ADAS Arthur Rickwood (use resting bodies either collected from infected leaves or produced *in vitro*, provided that such resting bodies are found to germinate the same as naturally produced ones, see section 1.2). Examine the deposits at regular intervals and recover a proportion of the bags at monthly intervals from November to April; examine the resting bodies for evidence of germination and viability. Record soil temperature and rainfall and examine data to determine if weather variables appear to trigger germination. Take this information into account in the development of a spray timing system (section 4.2).

Milestones

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

Milestones 2.3.1 - 2.3.4 achieved

Progress

We have previously shown that *Ramularia* resting bodies in leaf debris placed on or in soil in summer 1999 started to germinate, producing characteristic long thin spores (scolecospores), around 7 January 2000. White mould in unsprayed crops in Cornwall was first observed around three weeks later. Resting bodies from a common source germinated at the same time in Cambridgeshire and Cornwall. Germination occurred both on samples buried 5 cm deep and on the soil surface. The trigger for the germination of *R. vallisumbrosae* is 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).

Monitoring resting body germination

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 described in the Second Annual Report). Leaves were collected in early-June and stored dry at ADAS Arthur Rickwood from receipt on 5 June 2001. The bags of leaf debris were placed on the soil surface at ADAS Arthur Rickwood, Cambs and Penzance, Cornwall on 19 June 2000.

The occurrence of germination on samples as they were recovered was first observed on 13 December 2000 (Cambs site), and was occurring at both sites one week later (Table 2.3.1). White mould lesions were not observed in commercial crops until early March (see section 2.4). Germination continued until late-January 2001. 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).

Germination of resting bodies at the two sites is shown for 2000-2001 in Figure 2.3.1, and germination in relation to weekly rainfall totals is presented in Figure 2.3.2. At both sites there was relatively high rainfall (>20 mm) in the week preceding the first germination.

Effect of temperature and rainfall on time of germination

In order to try and identify the factor(s) which influence the time of resting body germination, replicate samples of narcissus leaves bearing *Ramularia* were incubated at ADAS Arthur Rickwood 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*. It appears that *R. vallisumbrosae* is adapted to low temperatures.

Figure 2.3.1 Effect of site on germination of *R. vallisumbrosae* sclerotia, 2000-2001.

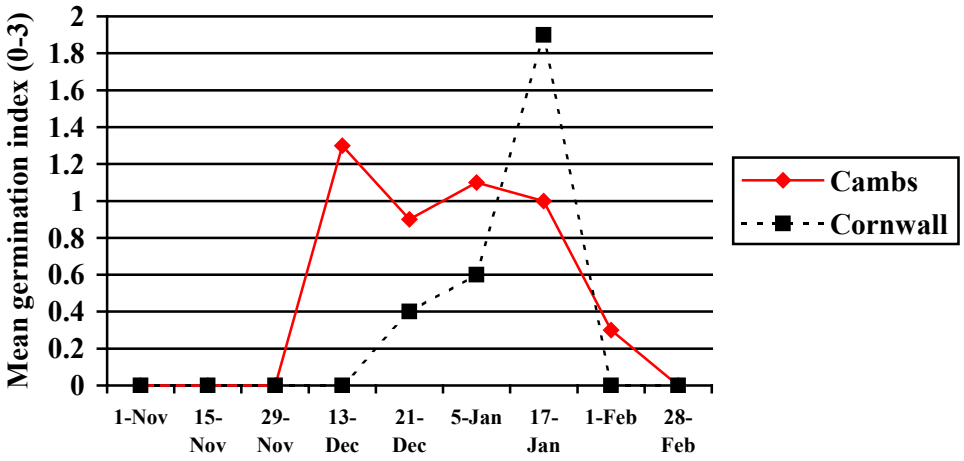
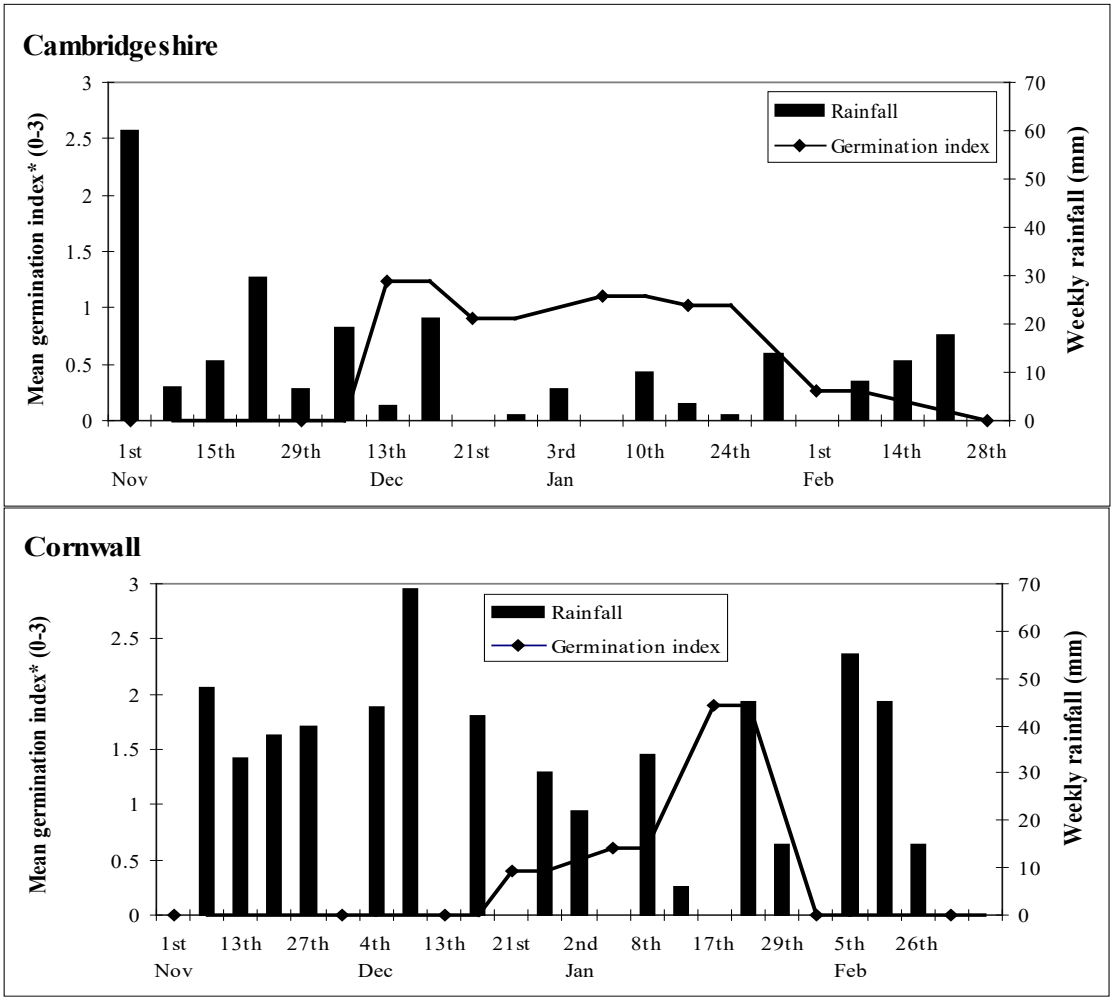


Figure 2.3.2 Germination of *R. vallisumbrosae* resting bodies and rainfall, 2000-2001.



* At sample recovery

Table 2.3.1 Germination of *Ramularia 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	
	Cambs[b]	Cornwall[b]	Cambs	Cornwall
40	-	-	-	-
41	-	-	-	-
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	-	-
12	-	-	-	-

[a]Assessed at time of recovery
[b]Arthur Rickwood, Cambs and Penzance, Cornwall

Table 2.3.2 Germination of *R. vallisumbrosae* resting bodies on narcissus leaf debris in Cambs and Cornwall, 2000-2001

Sample date	Mean germination index (0-3)			
	Cambs		Cornwall	
	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

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

Sample date	Mean number of leaf pieces 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.

2.4 Environmental factors and disease development

Plan and milestones (taken from the Project Proposal)

(a) Monitoring commercial crops In summer 1998, 1999 and 2000, identify commercial narcissus crops suitable for monitoring in the second year of their 'two-year-down' growing cycle (i.e., crops planted in 1997 for monitoring in 1998-99, in 1998 for monitoring in 1999-2000 and in 1999 for monitoring in 2000-2001). In each year, identify three sites in Cornwall and three in eastern England (Lincolnshire and Cambridgeshire); each should be about 0.2 ha in extent and preferably approximately square in area. In selecting crops, use crops and sites which are typical of the geographical area, and take into account the views of those industry partners providing the sites. Mark the selected areas clearly with corner posts, and make arrangement with the owner/manager such that these areas of crop will receive all the normal husbandry operations during their second (monitoring) years (herbicide, fertiliser, flower cropping, etc.) except that no fungicides will be applied. Within each 0.2 ha area, mark a central 0.1 ha area to be used for monitoring purposes (thereby providing a buffer zone so that the monitored area should be free of spray drift).

In autumn, set up an environmental logger centrally in each site to record, at hourly intervals in the growing season (about December to July), soil and air temperatures, leaf wetness, humidity, wind speed, wind direction and rainfall. Download loggers regularly as necessary.

Record the incidence and severity of foliar diseases (white mould and smoulder), using the protocols established under section 2.1, at fortnightly intervals during the second year of crops, from the time of shoot emergence (December to January period) until near-complete foliar senescence or (if earlier) when the crop is lifted (June to July period). When making these records, also note the occurrence of other obvious diseases and disorders (e.g., *Stagonospora curtisii*, 'chocolate spot' or 'physiological rust'), crop damage (e.g., due to frost, hail or flower picking), and any other significant factors, and record the growth stage of the crop (e.g., in bud, flowering, etc.).

Examine disease incidence and severity data, environmental data and other crop information collected, and determine factors (meteorological or otherwise) likely to be important in the occurrence and development of white mould and smoulder (see section 4.2).

(b) Monitoring experimental crops In summer 1998 and 1999, plant two areas of narcissus cv Carlton bulbs at HRI Kirton (Lincolnshire) and two at ADAS Arthur Rickwood (Cambridgeshire). Use these crops to supplement the information collected in (a) above. Each area will be approximately 0.1 ha in extent, will be grown using good local husbandry practices (except that in the second year of each crop (1999-2000 and 2000-01) no fungicides will be applied), and will be provided with facilities for irrigation. After full shoot emergence has taken place in these second crop years (in the January to February period), inoculate one area at each site with *Ramularia vallisumbrosae*, and one with *Botrytis narcissicola*. In the event of a dry spring to summer period, irrigation will be applied to keep the soil around the crop foliage moist. Log environmental data, assess white mould and smoulder incidence and severity, and record crop growth and general comments in a similar way to that described under (a) above. As in (a), use this information to determine factors important in the development of white mould and smoulder. Data from these sites will be especially valuable in the event of unsuitable weather or low disease incidence at the monitored commercial sites in (a).

Milestones

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

Milestones 2.4.1 – 2.4.7 achieved

Progress

(a) Monitoring commercial crops

The methods used for disease monitoring were described in the Second Annual Report, while details of all crops monitored are given in Appendix A. Standard cultural practices were used with these crops, except that no fungicides were applied in the second year of each crop. The results for the first and second sets of crops monitored, 1998-1999 and 1999-2000, have been given in earlier Reports. In the present report full results are given for the third set, 2000-2001. Six commercial crops (cv. Carlton, three in Cornwall and three in eastern England) were monitored at the end of their first crop year and then throughout their second crop year.

2000-2001 The results of disease monitoring near the end of the first crop year are given in Table 2.4.1. In Cornwall, active white mould lesions were found on all three crops, with the largest number at Tregidgeo and the lowest at Newtown (although the most advanced crop from the viewpoint of foliar senescence/loss was Fentongollan). In the east, no active smoulder lesions were found at any of the three commercial sites, although evidence of old lesions on the foliage was seen at Surfleet; by far the most advanced crop in terms of foliar loss was that at Split Drove. On this basis, in 2001 smoulder would be expected to be most severe at Split Drove, and white mould to appear first at Tregidgeo.

Table 2.4.1 Monitoring 2000-2001: disease lesions at the end of the first year of crops in 2000

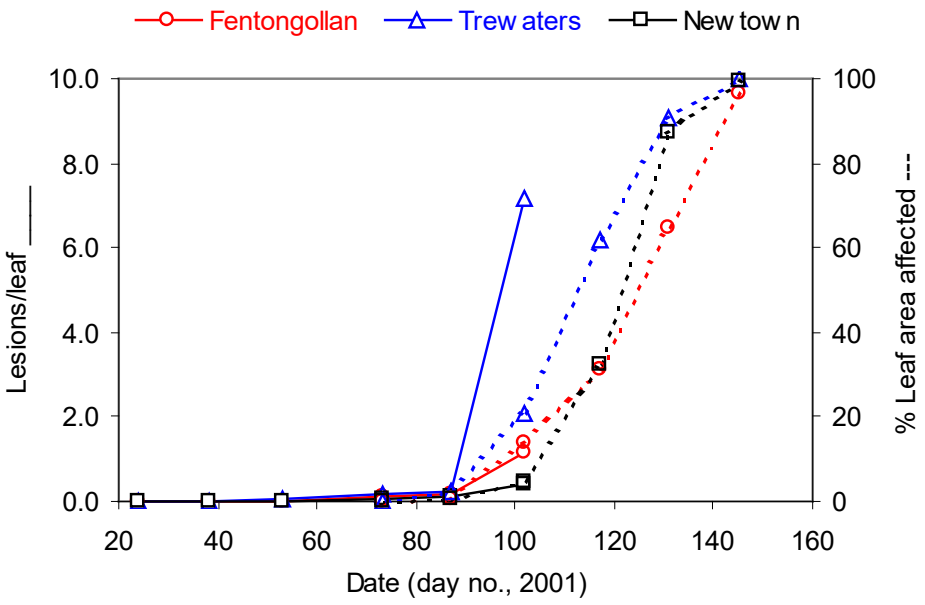
Site	Assessment date (day no.)	Active lesions / plot [a]	% foliar senescence
Split Drove, Swaffham Prior Fen, Cambs.	185	0	23
Black Hovel, Holbeach St. Marks, Lincs.	178	0	4
Surfleet, Lincs	173	0 [b]	8
Newtown, Cornwall	166	0.68 (white mould)	2
Fentongollan, Cornwall	165	7.46 (white mould)	14
Tregidgeo, Cornwall	165	22.24 (white mould)	8

[a] plot size was a 0.5m-length of ridge

[b] some old lesions could be seen on the dead areas of the leaves

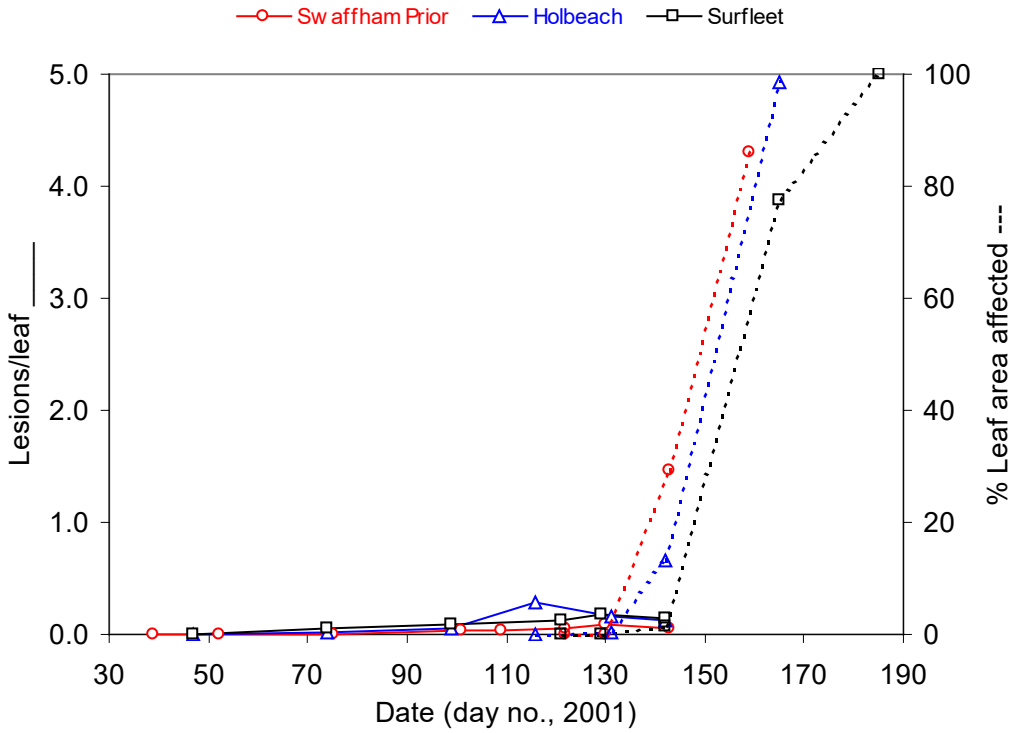
Disease development at these sites in the second year of crops is shown in Figures 2.4.1 for Cornish crops and 2.4.2 for eastern crops. In the Cornish crops few smoulder symptoms were observed, partly because they were masked by other foliar diseases. White mould lesions did not appear until March, increasing at all three sites from the start of April, the increase at Trewaters Farm being dramatic. Thereafter, green leaf area was lost rapidly and at a similar rate at all three sites, with about 50% of green leaf area remaining by early-May. In these crops, the prediction that the Angloflora crop (Tregidgeo/Trewaters Farm) would have the earliest white mould infection was confirmed.

Fig. 2.4.1 White mould in 3 Cornish commercial crops, 2001



In the second-year eastern crops (Fig. 2.4.2) the number of smoulder primaries recorded at the start of the growing season was <0.01 primaries/shoot at Swaffham Prior Fen but much higher (0.04 primaries/shoot) at Holbeach St Marks and Surfleet. 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 St Marks. In May the loss of green leaf area occurred rapidly at all three sites, starting first at the most southerly site (Swaffham Prior Fen). At Swaffham Prior, as recorded in the previous year (see Second Annual Report), there was an explosion in the number of secondary smoulder lesions in mid-May, reaching an estimated average of 2.3 lesions per leaf. Some sporulating secondary lesions were also observed at Holbeach, but not at Surfleet, but distinguishing disease lesions in rapidly senescing leaves is difficult. 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.

Fig. 2.4.2 Smoulder in 3 eastern commercial crops, 2001



(b) Monitoring crops at research sites

The methods used were described in the First and Second Annual Reports. Briefly, in each of 1998 and 1999, two, 2 t lots of bulbs were planted at each of ADAS Arthur Rickwood and HRI Kirton for disease monitoring in the second year of the crops, i.e., starting winter 1999-2000 and 2000-2001. A Cornish stock of cv. Carlton was allocated for monitoring white mould, and a Lincolnshire stock of cv. Carlton for monitoring smoulder. No fungicide sprays were applied in either year of these crops. Details of the bulbs used are given in Appendix B. In 2000-2001 it was not considered necessary to irrigate the crops at Kirton, as had been done in other cases.

The occurrence of smoulder on these crops in 2001 is shown in Figures 2.4.3 and 2.4.4. The crops at Arthur Rickwood showed increasing numbers of smoulder lesions and loss of green leaf area 2 – 3 weeks earlier than those at Kirton, and there were considerably more smoulder lesions at Arthur Rickwood. There were very similar disease levels on bulbs from different sources planted side-by-side at any one site.

Meteorological data

The analysis of meteorological data from all crop monitoring sites is on-going. Some typical examples are shown in Figure 2.4.5. It is interesting to note the contrasting leaf wetness durations between Holbeach and Trethewey in 1999. Durations greater than 20 hours per day occurred commonly at the Cornish site, whereas wetness durations rarely exceeded 10 hours at the Lincolnshire site.

Fig. 2.4.3 Smoulder in crops at Arthur Rickwood, 2001

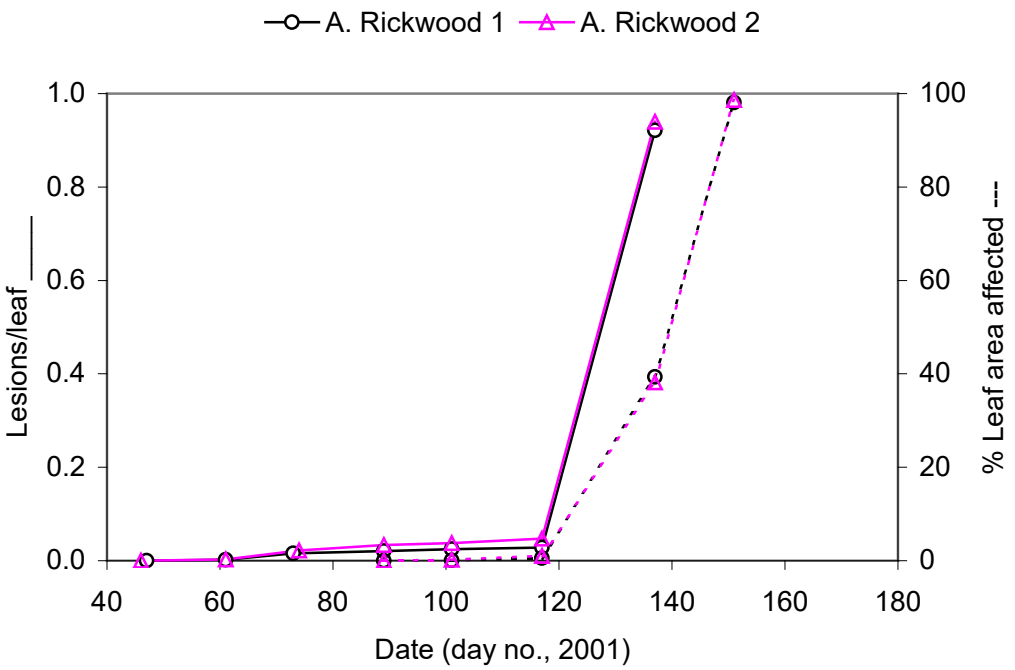


Fig. 2.4.4 Smoulder in crops at Kirton, 2001

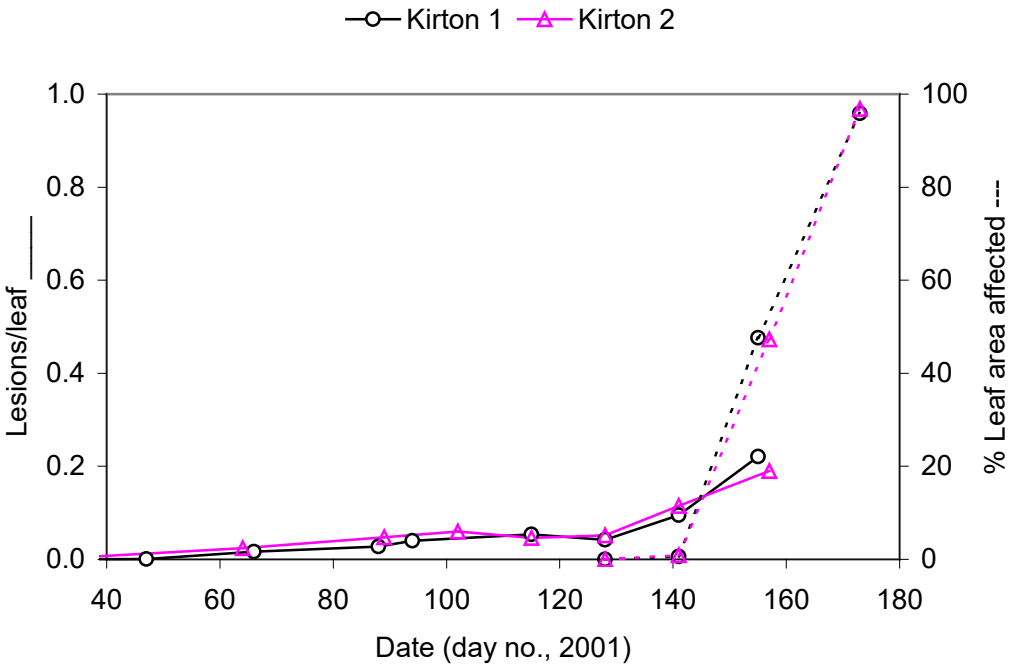
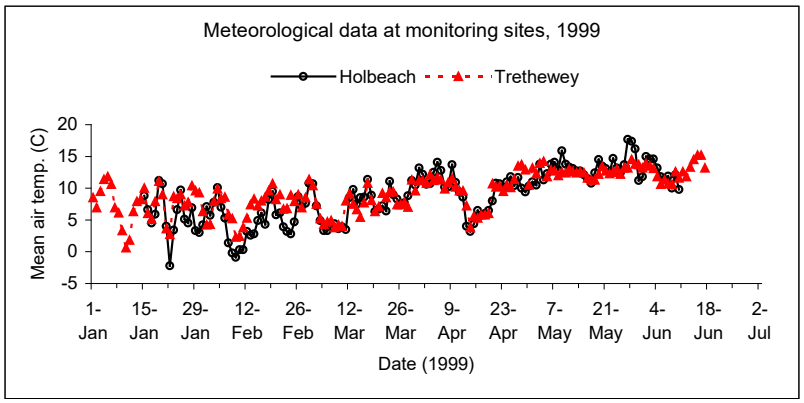
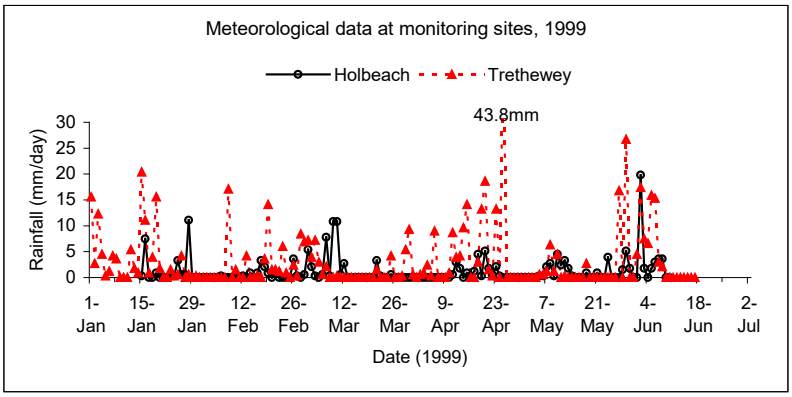
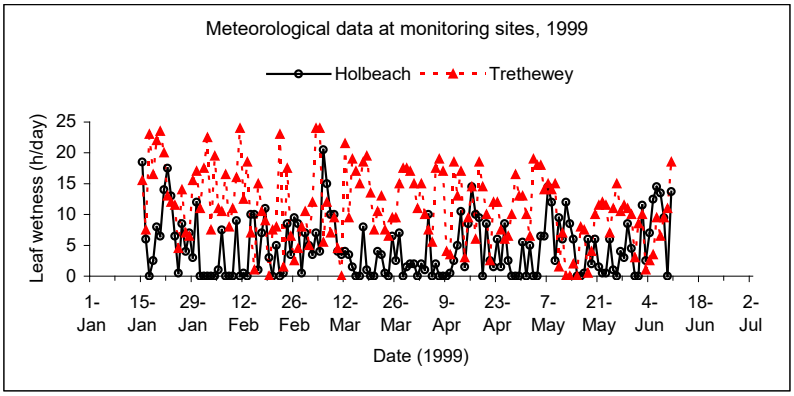
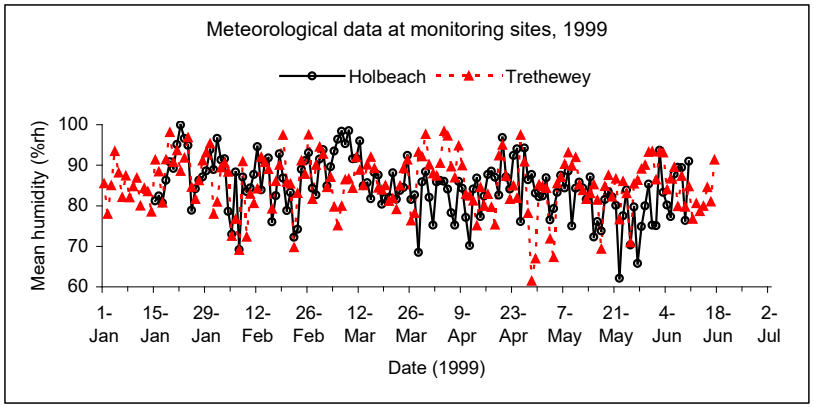


Fig. 2.4.5 Examples of weather data from crop monitoring sites: Trethewey (Cornwall) and Holbeach St. Marks (Lincs.), 1999.









2.5 Development and validation of precipitation impact sensors

Plan and milestones (taken from the Project Proposal)

Conidia of *Ramularia vallisumbrosae* are produced as white spore masses in association with black spherical resting bodies (Forsberg, 1976, p.219). Spores of *R. vallisumbrosae* are splash-dispersed (Gregory, 1939). Rain splash dispersal is potentially a major factor determining the incidence and severity of this pathogen. Measurement of this criterion would be important in forecasting the spread of this disease in the field.

Position experimental precipitation impact sensors, supplied by Aardware Design, within a narcissus crop on a selected site in Cornwall where white mould is being monitored. Use two replicate sensors at a standard height. Take observations during rainfall events of the output from the sensors, and compare this with the degree of rain splash using conventional measurements. In addition, compare the output from the experimental sensors between different rainfall events to ascertain if the rate of rainfall accumulation can be used as an indicator of splash potential. Conduct further tests at one commercial site in Cornwall, using those sensors which give the best approximation of rainsplash from narcissus leaves. At each site log environmental data in a similar way to that described under 2.4(a).

Carry out modifications of the experimental sensors as appropriate in year two, and use the modified sensors for repeat observations taken in years two and three.

Along with data from section 2.4, take this information into account in the development of a spray timing system (see section 4.2).

Milestones
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
Milestone 2.5.1 and 2.5.2 achieved

Progress

The evaluation of precipitation impact sensors was described in the Second Annual Report.

3.1 Leaf wetness characteristics

Plan and milestones (taken from the Project Proposal)

Determine the wetness characteristics of narcissus leaves and stem tissues of varying physiological stages of development under field conditions. At one site in Cornwall where white mould is being monitored, position two standard wetness sensors and two experimental wetness sensors (Aardware Design) in the crops, placing sensors at a standard height above ground and adjacent to narcissus leaf and stem tissues. Position additional experimental sensors of each type at the same locations and adjust the angle of inclination of the sensors. Take observations of the wetness duration of each sensor and the corresponding leaf wetness on the narcissus leaf tissues. At each site log environmental data in a similar way to that described under 2.4(a).

Carry out modifications on the experimental wetness sensors as appropriate, and use the modified sensors in repeat observations taken in years two and three. Compare the responses of the modified sensors to that of sensors used in the first year. Select the leaf wetness sensor output which best represents the wetness characteristics of the narcissus leaf.

Milestones

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

Milestone 3.1.1 achieved

Progress

The evaluation of leaf wetness sensors is continuing. For previous work, see the Second Annual Report.

3.2 Effect of leaf wetness and temperature on infection

Plan and milestones (taken from the Project Proposal)

In controlled environments, investigate the effects of temperature and wetness duration on infection of leaf tissue by conidia of *Ramularia vallisumbrosae* and of leaf and stem tissue of *Botrytis narcissicola*. Carry out replicate experiments and use the data sets obtained to derive models describing the effects of temperature and wetness duration. Develop relationships using statistical modelling techniques describing the effect of temperature and wetness on infection by *R. vallisumbrosae* and *B. narcissicola* on narcissus leaf tissue.

For *B. narcissicola* conidia, investigate infection and the latency of infection (including the duration of latency) on (a) young and old tissues and (b) damaged/wounded and intact tissues. The effect of wound age will also be considered.

Milestones

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

Milestone 3.2.1 achieved

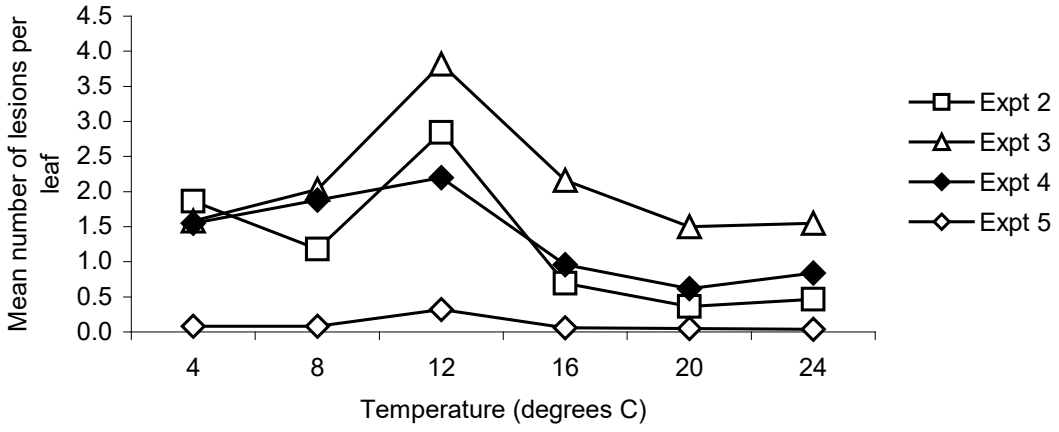
Progress

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

Introduction

Previous work showed that short durations of leaf wetness were sufficient for the infection of narcissus by *B. narcissicicola*, as shown in Figure 3.2.1 (taken from the Second Annual Report).

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



Methods

Probit 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.

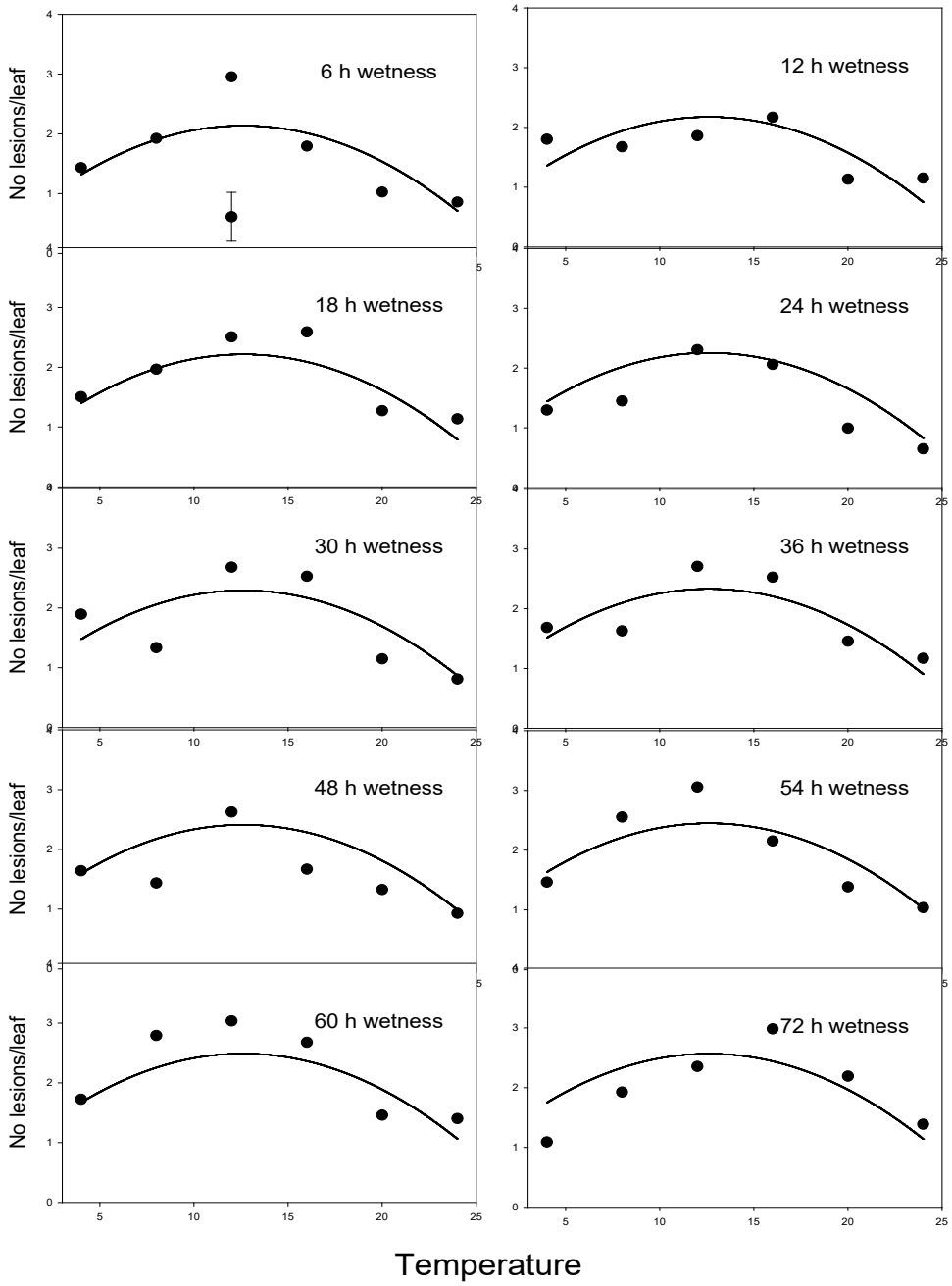
Results

The results showed that the effect of temperature on infection of leaf tissue by smoulder was non-linear (see Fig. 3.2.2). The function which gave the best fit to the line was quadratic. However, the effect of wetness duration on infection of leaf tissue by smoulder was linear (data not presented). A highly significant relationship describing the effect of temperature and wetness duration on infection of narcissus leaves by *B. narcissicicola* was formulated.

Conclusions

- The presence of damage on the leaf tissue was required for infection of pre-senescent narcissus leaves by *B. narcissicicola*.
- Temperature and wetness duration had a highly significant effect on infection of leaves by *B. narcissicicola* in the presence of leaf damage.
- There was a non-linear relationship between infection and temperatures.
- There was a linear relationship between wetness duration and infection.

Figure 3.2.2 Fitted curves for the effect of temperature on infection of narcissus leaves by smoulder



Infection experiments on narcissus cv. Carlton plants with *Ramularia vallisumbrosae* (white mould)

Introduction

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.

Controlled environment experiments have been carried out using inoculum of *Ramularia vallisumbrosae* (white mould) to obtain data from which mathematical models can be derived. Mathematical relationships obtained in this way can be validated under field conditions, and can form the basis of critical timings for fungicide application.

Materials and Methods

Production of plant material
Bulbs of narcissus cv. Carlton were planted, one bulb per pot, in September 2000 in a mixture of 70:30 Fisons F2 compost and sand FP7 pots. They were placed at 5°C in the dark until required. All bulb material was grown at HRI Kirton and transported to HRI Wellesbourne prior to experiments. Approximately 3 days before use, plants were transferred to a glasshouse at a 16/14°C day/night temperature regime. On the day before the plants were inoculated they were placed at high humidity prior to transfer to the controlled environment cabinets.

Production of R. vallisumbrosae *inoculum and inoculation of plants*
Inoculum was obtained from the field by collecting infected leaf material from Cornwall. This was necessary to obtain sufficient amounts of inoculum to conduct controlled environment experiments. Isolates of the pathogen grown on oat meal agar failed to produce sufficient inoculum that was infective on narcissus leaf tissue (experiment 1). Infected leaves containing fresh conidia were washed using sterile distilled water, and collected in a 500 ml Duran flask. Using this method spore suspensions were obtained which routinely gave approximately 10^7 conidia ml^{-1}. Conidia were harvested and the concentration estimated using a haemocytometer. The types of condia were checked in the spore suspension, however in all suspensions 99% of spores were classified as phragmospores. A final inoculum concentration of approximately 5 x 10^5 conidia ml^{-1} was produced in a 1:10 dilution of V8 liquid.

Plants were spray-inoculated using an atomiser. Approximately 2 - 4 ml of inoculum was applied per inoculated pot in all controlled environment experiments. At each temperature plants were maintained under conditions of 98% relative humidity (which resulted 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 timing of experiments and the conditions used are given in Table 3.2.1. After removal of plants from environments all plants were allowed to air dry before labelling and transfer to a glasshouse at a 17/14°C day/night temperature regime. The number of lesions on each leaf of each plant was recorded after a two-

week incubation period in the glasshouse.

Table 3.2.1 Experimental conditions used in white mould infection experiments

Experiment number	Date	Temperatures (°C)	Wetness durations (hours)	Replication (pots / treatment)
1	17 Jan 2001	5, 10, 15, 20, 25, 30°C	1, 3, 6, 12, 18, 24, 30 hrs	4
2	14 Feb 2001	5, 10, 15, 20, 25, 30°C	1, 3, 6, 12, 18, 24, 30 hrs	7
3	26 April 2001	5, 10, 15, 20, 25, 30°C	3, 12, 18, 30 hrs	6
4	30 April 2001	5, 10, 15, 20, 25, 30°C	1, 3, 6, 12, 18, 24, 30 hrs	7
5	9 May 2001	5, 10, 15, 20, 25, 30°C	1, 3, 6, 12,18, 24, 30 hrs	7

Method of simulating plant damage

The leaves of all plants used in controlled environment experiments were modified to simulate 'damage' by rubbing gently with a soft bristle brush to remove surface wax. All leaves on all plants had the brushes drawn over the upper surface of the leaf at least twice.

Results

In the first controlled environment experiment no infection was observed on any treatment, and so there is no data presented. The results of controlled environment experiments 2, 3, 4 and 5 are summarised in Figures 3.2.3 and 3.2.4.

Effect of temperature and wetness duration on infection of narcissus leaves by white mould

Infection was observed on tissues which 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. However, infection occurred over a wide range of temperatures up to 30°C (Fig. 3.2.3). The optimal wetness duration for infection by white mould was after approximately 12 - 18 hours of wetness (Fig. 3.2.4). 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 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.

Figure 3.2.3 Effect of temperature on infection of narcissus leaves by white mould

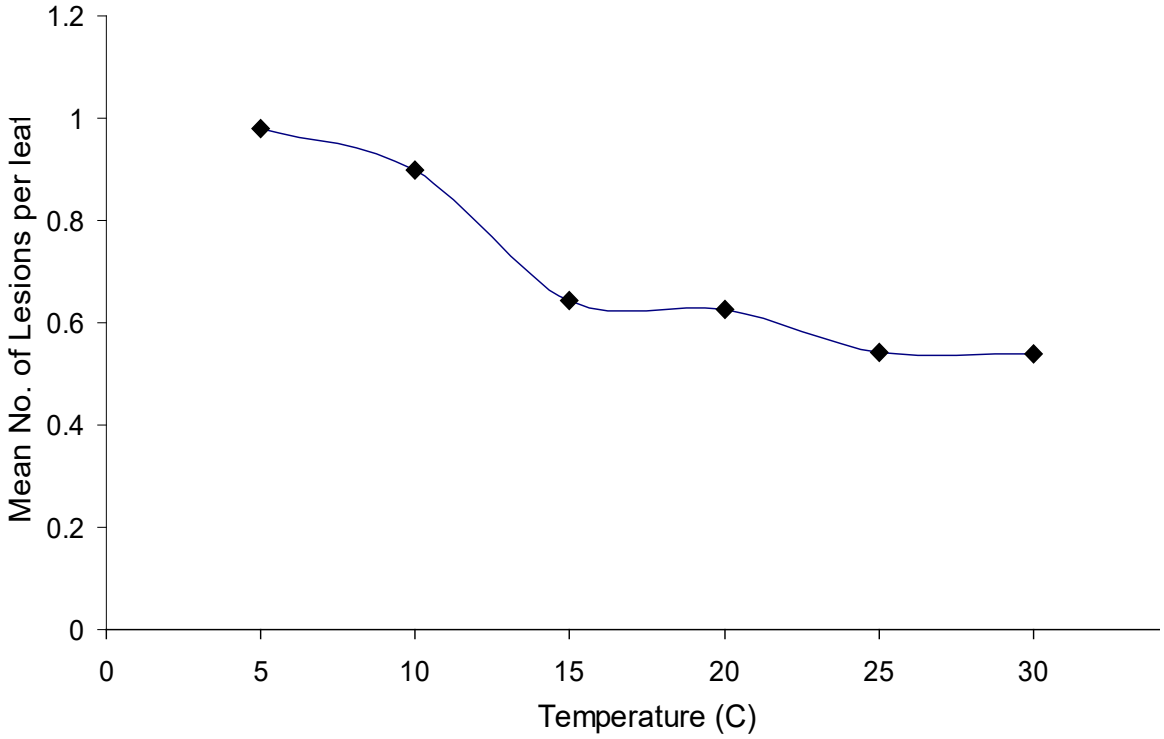
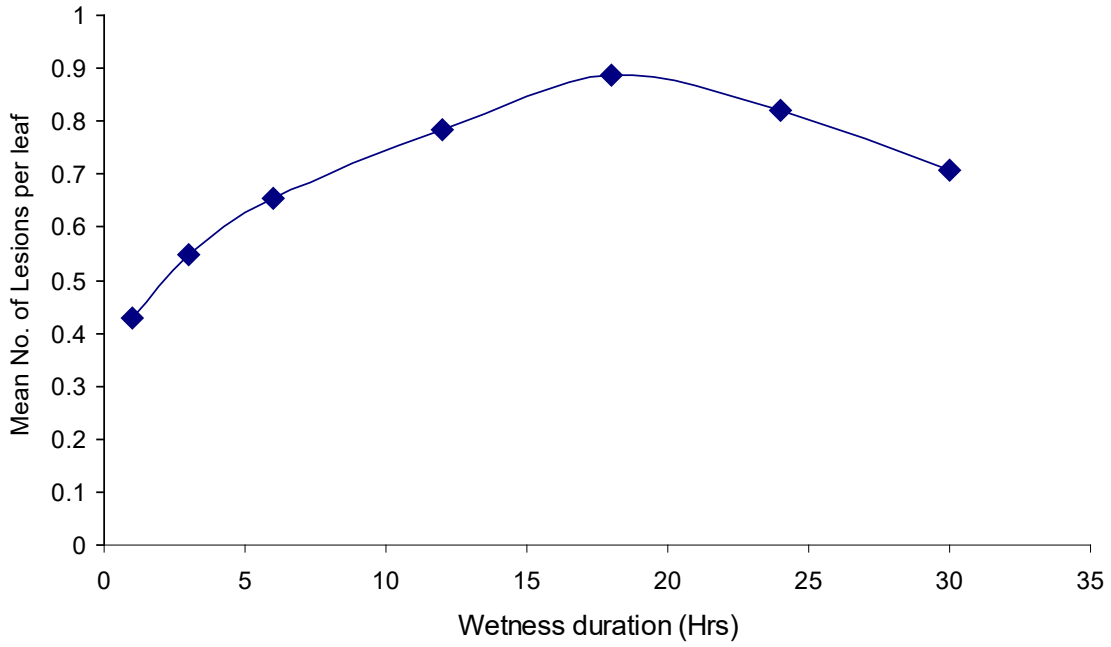


Figure 3.2.4 Effect of wetness duration on infection of narcissus leaves by white mould



Conclusions

- The presence of tissue damage was essential for infection by *R. vallisumbrosae* on pre-senescent narcissus leaves.
- 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*.

Effect of temperature and humidity duration on sporulation by white mould lesions
The effect of temperature and duration of 95% relative humidity was investigated on the time to spore production by white mould lesions.

Selection of R.. vallisumbrosae infected leaves
Typical white mould lesions on narcissus leaves from infection experiments (see above) were selected. Lesions were examined microscopically for the presence or absence of sporulation on the lesions.

Experimental treatments
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 at 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 (i.e. phragmospores, amerospores or scolecospores).

Results

The results shown on Table 3.2.2 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 could not be identified in this experiment.

Table 3.2.2 Effect of temperature and high humidity on sporulation by white mould lesions

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

+ phragmospores: - not present , + small numbers, ++ abundant , +++ high numbers
o scolecospores: - not present , o small numbers, oo abundant , ooo high numbers

Conclusions

- 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.

3.3 Validation of leaf wetness duration models

Milestones (taken from the Project Proposal)

Milestones

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

No milestones due so far

Progress

This work was not due to begin until July 2000.

4.1 Fungicide efficacy

Plan and milestones (taken from the Project Proposal)

(a) Laboratory studies Evaluate selected fungicides currently used for narcissus foliar disease control (e.g. benomyl, chlorothalonil, mancozeb, vinclozolin) and representative products from new fungicide groups (e.g. anilopyrimidines, strobilurins) for their efficacy in controlling *Ramularia vallisumbrosae* and *Botrytis narcissicola* in laboratory tests. It is anticipated this will be done by detached leaf assays using conidial inocula. Apply pathogens to leaves at different timings in relation to fungicide application, in order to determine if any chemicals have a curative effect, i.e. potential for effective use in field crops after infection has occurred.

Select the best fungicide treatments for use in the subsequent years in field crops at experimental sites, first using conventional spray timings (see 4.1(b) below), and then in relation to predictions from the infection models (see section 4.2).

(b)Field experiments In summer 1998, organise narcissus areas for experiments at three sites: at ADAS Arthur Rickwood (Cambridgeshire), at HRI Kirton (Lincolnshire), and on an agreed commercial site in Cornwall.

At ADAS Arthur Rickwood and HRI Kirton, plant areas of approximately 0.1 ha of cv Cheerfulness for investigating the control (by selected fungicide(s)) of white mould, and 0.1 ha of cv Carlton for investigating the control of smoulder. Plant and maintain the areas using typical husbandry practices for the region, except that no routine fungicide treatments will be applied. Mark out each experimental area to form 32 plots, arranged in four randomised blocks containing eight plots (treatments) each. Each plot will be of adequate size and only the central area of each will be recorded, so ensuring that each plot is fully 'guarded' from adjacent treatments. Inoculate the experimental areas with *R. vallisumbrosae* and *B. narcissicola*, respectively, towards the end of the first year's growth, in order to favour disease development in the second (experiment) year. In each case, apply experimental fungicide treatments in the second year of the crop (1999-2000). The fungicide(s) used will depend on the results obtained in part (a) above, and timings of application will be according to a conventional programme, taking into account flower cropping (unless there are relevant early indications from other parts of the study). Apply fungicides via a tractor-mounted sprayer or precision sprayer, as appropriate. Record the incidence and severity of white mould and smoulder, according to the standard protocols, at the start of the programme of fungicide treatments, towards the end of the growing season (before foliage senescence), and approximately mid-way between these dates.

At the commercial site in Cornwall, investigate the control of natural infections of white mould in a susceptible cultivar using one selected fungicide applied at different timings. Otherwise, use the experimental protocol described in the previous paragraph.

Integrate the information on fungicide efficiency and timing into the overall formulation of a spray timing system (section 4.2).

Milestones

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

Milestones 4.1.1 - 4.1.4 achieved

Progress

Previous laboratory and field trials have been reported in the First and Second Annual Reports. The results of additional field trials in 2000-2001, at research sites and in Cornwall, are described below.

Objectives and background

For the trials on cv. Carlton, the objective was to investigate the effect of fungicide sprays, applied at three major growth stages, on disease control, foliage die-back and bulb yield. The three growth stages were:

- Phase I - shoot emergence
- Phase II - around flowering
- 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 was one of several treatments that had then resulted in large increases in bulb yield.

For the trials on cv. Cheerfulness, 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)
- 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.

Materials and Methods

Replicated plots of cvs Carlton (for smoulder trials) and Cheerfulness (for white mould trials) were planted as previously described in September 1999 at ADAS Arthur Rickwood and HRI Kirton, in preparation for fungicide trials in their second year (2000-2001). Details of the bulbs used are as given in Appendix B. Irrigation was applied in dry periods at Arthur Rickwood only. Additionally, a white mould trial was established in a second-year crop of cv. Cheerfulness near Penzance, Cornwall. As in previous years, the plots used at Arthur Rickwood and Kirton comprised 18 and 24m length of ridge, respectively. The plot size at Penzance was 20m length of ridge. In all cases plots were separated by rows of guard plants, the trial areas were surrounded by guard areas, and a randomised block design with three replicate blocks (four replicate plots at Penzance) was used.

No natural infection by white mould was observed on crops at either of the research sites during the first year's growth in 1999-2000, while *Botrytis* occurred on many plants of both varieties at both sites. Plots of cv. Cheerfulness at the two research sites were inoculated in March 2001 by placing leaf debris bearing *R. vallisumbrosae* into guard rows and by introducing green narcissus leaves with white mould lesions into the trials in early-May. At the trial site in Cornwall, infection by white mould occurred naturally. Disease symptoms were recorded at appropriate intervals (see Tables for details).

The smoulder trial treatments were as shown in the following table:

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 wk	+ another 3 wk
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

Ronilan and Folicur were each applied at 1 litre/ha and Scala at 2 litre/ha. Sprays were applied in 250 litre water/ha. For spray dates, see Tables.

The white mould trial 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
9. Untreated

The fungicide 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. For spray dates, see Tables.

Data were analysed by analysis of variance (ANOVA) where suitable, followed by Duncan's Multiple Range Test to separate means. Where the conditions required for ANOVA did not hold, the results were analysed by Friedman's test.

Results

Smoulder trials with cv. Carlton at ADAS Arthur Rickwood and HRI Kirton, 2001

Effects on disease and leaf die-back

At Arthur Rickwood 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 4.1.1). 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 (Table 4.1.2) 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).

Fungicide treatment had a very noticeable effect on leaf die-back (Table 4.1.3 and Fig. 4.1.1). 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.

At Kirton, treatments applied around flowering (phase II) resulted in significant reductions in stem rotting due to *Botrytis* (Table 4.1.1). As at Arthur Rickwood, fungicide treatment had no significant effect of the mean number of smoulder leaf and stem lesions in April (Table 4.1.2), but there was a very marked and highly significant effect on leaf die-back (Table 4.1.3). Treatments 7, 8 and 9 were again particularly effective at reducing foliage die-back.

Effects on bulb yield

At Arthur Rickwood, bulb yields ranged from 46.7 to 61.7 kg per plot, with significant differences between treatments (Table 4.1.4). 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 was associated with the greatest yield (21% greater than untreated plots), although this was not statistically significant.

Effects on bulb rots

The incidence of bulb rots was examined under separate HDC funding (project BOF 41a), and will be reported directly to the HDC.

Table 4.1.1 Effect of fungicide treatment on stem rotting by *Botrytis* in cv. Carlton, April 2001 (flowers picked 28 March at Arthur Rickwood and 4 April at Kirton)

Treatment [a]	Arthur Rickwood (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 (24df)	5.53	2.36

[a] sprays applied by this stage:
Arthur Rickwood - 20 February, 9 March, 22 March, 30 March;
Kirton - 20 February, 14 March, 29 March, 9 April

Table 4.1.2 Effect of fungicide treatment on occurrence of smoulder lesions in narcissus, cv. Carlton, 2001

Treatment	Mean number of lesions per 100 leaves and stems		
	Arthur Rickwood		Kirton
	19 March	19 April	4 April
1. Untreated	2.1	4.9	4.7
2. RR----	1.8	4.7	5.4
3. --FF--	1.2	3.1	5.5
4. ----SS	1.5	4.4	3.9
5. RRFF--	1.7	3.6	4.5
6. RR--SS	1.7	4.1	4.9
7. --FFSS	1.9	4.2	5.2
8. RRFFSS	1.7	3.3	7.1
9. FFFFFF	1.9	3.6	3.8
Significance	NS	NS	NS
SED (24 df)	0.44	0.84	1.88

Table 4.1.3 Effect of fungicide sprays on foliage die-back (%) in narcissus cv. Carlton, 2001

Treatment [a]	Arthur Rickwood		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

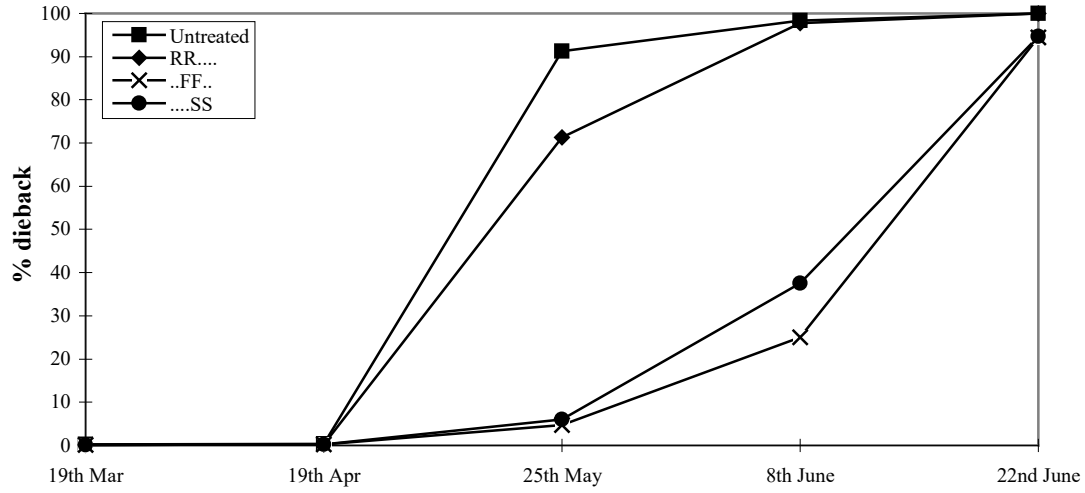
[a] Sprays applied:
Arthur Rickwood – 20 February, 9 March, 22 March, 30 March, 21 April, 11 May;
Kirton - 20 February, 14 March, 29 March, 9 April, 30 April, 21 May
R, Ronilan Fl; F, Folicur; S, Scala
NS, not significant; *, ** and ***, significant at 5%, 1% and 0.1% levels of probability

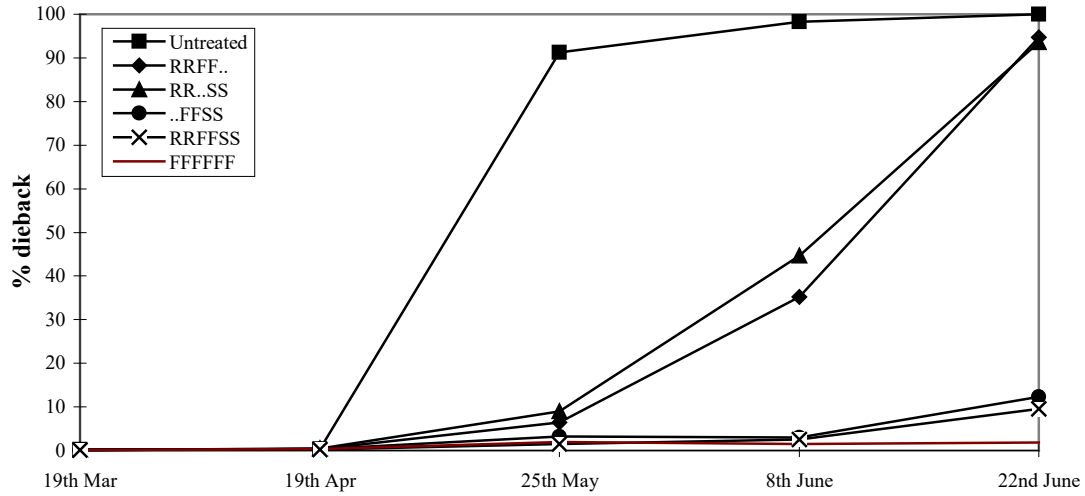
Table 4.1.4 Effect of fungicide sprays on bulb yield[a] of narcissus cv. Carlton, 2001

Treatment	Arthur Rickwood		Kirton	
	Kg/plot	% weight increase	Kg/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 df)	5.24		-	

*** Significant difference between treatments at P<0.001; results in the same column not sharing a common letter are significantly different by Duncan's Mulitple Range test
[a]Yields based on 32.8 kg bulbs planted in 18m-long plots at Arthur Rickwood, and 36.5 kg bulbs planted in 24m-long plots at Kirton (both corresponding to 20t/ha). % weight increase is ((weight increase from planting)/(weight planted))x100.

Figure 4.1.1 Effect of fungicide treatments on leaf die-back, cv. Carlton at ADAS Arthur Rickwood, 2001.





Control of smoulder on cv. Cheerfulness at Arthur Rickwood and Kirton, 2001

Although these trials were inoculated with *R. vallisumbrosae*, and treatments were designed primarily for control of white mould, this disease did not develop. However, natural infection with smoulder occurred at a high level and useful information was gained on the effect of a different range of fungicide treatments, to those evaluated on cv. Carlton, on control of smoulder.

Effects on disease and foliage die back

At Arthur Rickwood, 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 4.1.5), and this was significantly reduced by fungicide treatment. Fungicide treatment had a very noticeable effect on leaf die-back (Table 4.1.6). On 8 June, 2 days after the final spray, there was 99% die-back in untreated plots and less than 20% in all treated plots. Treatments 6 (Bavistin + Folicur) and 8 (Amistar + Folicur) appeared particularly green.

Effects on bulb yield

Bulb yield at Arthur Rickwood was significantly different between treatments, with Bavistin + Folicur giving a 59% yield increase over untreated (Table 4.1.7). At the HRI Kirton site, foliage dieback 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 4.1.5 Effect of fungicide sprays on *Botrytis* stem rot in cv. Cheerfulness at Arthur Rickwood, 2001

Treatment[a]	% 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 df) (between treatments)	5.22
SED (treatments vs control)	4.52

Table 4.1.6 Effect of fungicide sprays on foliage die-back (%) in narcissus cv. Cheerfulness, 2001.

Treatment [a]	Arthur Rickwood		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 df) (between treatments)	4.61	6.91	4.76	2.32
SED (treatments vs control)	5.33	7.98	5.46	2.68

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

Table 4.1.7 Effect of fungicide spray programmes on bulb yield[a] of narcissus cv. Cheerfulness, 2001.

Treatment	Arthur Rickwood		Kirton	
	Kg/plot	% weight increase	Kg/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 df) (between treatments)	4.58		1.65	
SED (treatments vs control)	3.97		1.91	

*** Significant difference between treatments at P<0.001; results in the same column not sharing a common letter are significantly different by Duncan's Mulitple Range test.
[a]Yields based on 32.8 kg bulbs planted in 18m-long plots at Arthur Rickwood, and 36.5 kg bulbs planted in 24m-long plots at Kirton (both corresponding to 20t/ha). % weight increase is ((weight increase from planting)/(weight planted))x100.

Control of white mould on cv. Cheerfulness, Cornwall, 2001

White mould was first observed in the crop in mid-April. At the first assessment on 25 April there were marked differences between treatments in both the number of white mould foci and the number of lesions / m of ridge (Table 4.1.8). The number of foci per plot (20m of ridge) 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 T4 (spray programme commencing at first symptoms; 2 sprays applied). Bavistin + Folicur, Bavistin + Scala and Amistar + Folicur were particularly effective, reducing lesion numbers to 0.8, 3.9 and 0 respectively (Table 4.1.8)

Leaf die-back occurred earlier where white mould was not controlled (Table 4.1.9). The three treatments noted above which gave very good control of white mould also resulted in prolonged green leaf retention.

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.

Table 4.1.8 Effect of fungicide spray programmes on control of white mould cv. Cheerfulness, Cornwall, 2001.

Treatments [a]	25 April		16 May
	Mean no. foci/plot	Mean no. lesions/0.5m	Mean no. lesions / 0.5m
1. Untreated	9.3	11.1	88.2
2. Bavistin + Dithane (0-5cm)	1.3	2.3	10.0
3. Bavistin + Dithane (10-15cm)	2.8	1.8	7.9
4. Bavistin + Dithane (1st symptoms, 23 Apr)	4.6	6.8	33.2
5. Bavistin + Ronilan	1.4	0.4	4.0
6. Bavistin + Folicur	0.1	0	0.8
7. Bavistin + Scala	0.3	0	3.9
8. Amistar + Folicur	0	0	0
Significance	-	-	***
SED (25 df) (between treatments)	-	-	13.68
SED (treatments vs control)			11.85

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

*** Significant difference between treatments at P<0.001

Table 4.1.9 Effect of fungicide spray programmes on leaf die-back - Cornwall, 2001

Treatments	% leaf die-back	
	16 May	30 May
1. Untreated	12.0	74.4
2. Bavistin + Dithane (0-5cm)	0.3	8.3
3. Bavistin + Dithane (10-15cm)	0.2	6.5
4. Bavistin + Dithane (1st symptoms, 23 Apr)	2.3	16.5
5. Bavistin + Ronilan	0.1	5.7
6. Bavistin + Folicur	0.1	1.3
7. Bavistin + Scala	0.1	1.3
8. Amistar + Folicur	0	0.8
Significance	***	***
SED (25 df) (between treatments)	1.25	4.87
SED (treatments vs control)	1.09	4.22

Cost-benefit analysis of fungicide treatments: 1999-2000

The estimated cost of fungicide treatment (excluding application costs) evaluated on second year down cvs Carlton and Cheerfulness in 2000 is given in Tables 4.1.11 – 4.1.12. In the particular circumstances of these plot 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.

Table 4.1.11 Cost-benefit assessment of second-year fungicide treatment on cv. Carlton: 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)
ADAS						
1. Untreated	50.1	34.81	-	-	-	-
2. Benlate + Dithane (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 (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
HRI						
1. Untreated	71.5	39.04	-	-	-	-
2. Benlate + Dithane(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 (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 £2.25/kg; Ronilan £24.50/l; Bravo £3.48/l; Scala £36/l; Amistar £39.20/l; Folicur £17.25/l; Unix £21.50/l.

[b] Cost of application not included.

Planted weight was 2 kg/m^2

Table 4.1.12 Cost-benefit assessment of second-year fungicide treatment on cv. Cheerfulness: 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 6 sprays[a] (£)	Margin[b] over chemical cost (£/ha)
ADAS						
1. Untreated	39.9	27.69	-	-	-	-
2. Benlate + Dithane (0.5+1.5 kg)	52.3	36.31	8.62	3,878	80.25	3,798
3. Bavistin + Dithane (0.5+1.5 kg)	50.2	34.88	7.19	3,234	50.25	3,184
4. Bravo (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
HRI						
1. Untreated	38.4	21.05	-	-	-	-
2. Benlate + Dithane (0.5+1.5 kg)	45.8	25.11	4.06	1,826	66.88	1,759
3. Bavistin + Dithane (0.5+1.5 kg)	47.1	25.82	4.77	2,146	41.88	2,105
4. Bravo (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 £2.25/kg; Bavistin DF £10/kg; Bravo £3.48/l; Scala £36/l; Amistar £39.20/l; Folicur £17.25/l; Stroby £140/kg. 6 sprays applied at ADAS site, 5 at HRI

[b] Cost of application not included.

Planted weight was 2 kg/m^2

4.2 Formulation of experimental forecasting systems

Plan and milestones (taken from the Project Proposal)

Formulate disease forecasting systems using component infection models and other criteria which have been elucidated during the project. Propose how spray timing systems may be modified by other criteria (e.g., cultivar, rain splash, etc), and formulate an integrated forecasting system for grower use. Consider the most appropriate means of developing a user-friendly version of the spray timing system. Formulate a proposal to cover commercial validation of the forecasting system (support for which has been agreed in principle with the HDC).

Milestones

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

Milestone not yet due

Progress

This section of the work is not due to begin until July 2001.

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APPENDIX A Details of commercial crops used for monitoring purposes

1998-1999

Owner and address	Place and grid reference of field	Notes
Lords Ground Ltd Lords Ground Farm Centre Swaffham Prior Fen Cambs CB5 0LA	Field 29 Commissioners Farm, Swaffham Prior Fen TL533674	South edge of a large area of cv Carlton. Some shelter from trees to east. Drainage ditch to south. Peat soil. Grown on 0.90 m ridges.
F H Bowser Ltd Holbeach Lincs PE	Bingham Lodge Holbeach St Marks TF394321	Central part of a large area of Carlton and other cultivars. Grown on 0.90 m ridges.
Winchester Growers Ltd Herdgate Lane Pinchbeck Spalding Lincs PE	Wilson's Field Gosberton TF250296	Central part of a large area of Carlton and other cultivars. Grown on 0.76 m ridges.
R H Scrimshaw & Sons Halvose Farm Halvose Manaccan Cornwall TR12 6LD	Park Beet Home Manaccan SW762257	Site sheltered, with trees on south and east of selected areas. Adjacent crop is narcissus cv Planet. Grown on 0.90 m ridges. Relatively dense, shallow planting
Fentongollan Farm (Mr J Hosking) Tresillian Truro Cornwall TR2 4AQ	Tolskiddy Field Fentongollan SW862433	Sloping site. Relatively low planting density. Grown on 0.76 m ridges. Part of a larger area of cv Carlton to the west. Narcissus crop to east lifted 1998
Angloflora Ltd Penhale Farm Tregony Truro Cornwall TR2 5SH	Trethewey Farm SW915432	Exposed, sloping site. Central part of a large area of cv Carlton. Grown on 0.90 m ridges

1999-2000

Owner and address	Place and grid reference of field	Notes
Lords Ground Ltd Lords Ground Farm Centre Swaffham Prior Fen Cambs CB5 0LA	Highfen Farm Swaffham Prior Fen TL538684	North corner of a large area of cv Carlton. Peat soil. Grown on 0.90 m ridges.
F H Bowser Ltd Holbeach Lincs PE	Bingham Lodge Holbeach St Marks TF396324	Central part of a large area of Carlton and other cultivars. Grown on 0.90 m ridges.
Winchester Growers Ltd Herdgate Lane Pinchbeck Spalding Lincs PE	Sly's Field Gosberton TF268305	Cultivar Standard Value. Central part of a large area of a range of cultivars. Grown on 0.76 m ridges.

R H Scrimshaw & Sons Halvose Farm Halvose Manaccan Cornwall TR12 6LD	Park Beet Home Manaccan SW762257	Same crop as in 1998-1999, grown for a third year.
Fentongollan Farm (Mr J Hosking) Tresillian Truro Cornwall TR2 4AQ	Polsue Manor Farm Tresillian SW857464	Sloping site. Grown on 0.76 m ridges. Part of a larger area of cv Carlton and other varieties to the east.
Angloflora Ltd Penhale Farm Tregony Truro Cornwall TR2 5SH	Woodland Valley 6 Grampound Road SW914510	Sloping site. Central part of a large area of cv Carlton. Grown on 0.90 m ridges

2000-2001

Owner and address	Place and grid reference of field	Notes
Lords Ground Ltd Lords Ground Farm Centre Swaffham Prior Fen Cambs CB5 0LA	Split Drove Swaffham Prior Fen TL551674	Central part of a large area of Carlton and other cultivars. Peat soil. Grown on 0.90 m ridges.
F H Bowser Ltd Holbeach Lincs PE	Black Hovel Holbeach St Marks TF385329	Central part of a large area of Carlton and other cultivars. Grown on 0.90 m ridges.
Winchester Growers Ltd Herdgate Lane Pinchbeck Spalding Lincs PE	Tunnards Field Surfleet TF255275	Central part of a large area of Carlton and other cultivars. Grown on 0.76 m ridges.
R H Scrimshaw & Sons Halvose Farm Halvose Manaccan Cornwall TR12 6LD	Newtown SW750240	South end of a large area of Carlton and other cultivars, grown on 0.90 m ridges.
Fentongollan Farm (Mr J Hosking) Tresillian Truro Cornwall TR2 4AQ	Fentongollan SW862435	Sloping site. Grown on 0.76 m ridges. Part of a larger area of cv Carlton and other varieties.
Angloflora Ltd Penhale Farm Tregony Truro Cornwall TR2 5SH	Tregidgeo SW957475 Trewaters Farm SW849534	Observations at end of first year crop at Tregidgeo, following which the crop was lifted due to a change of plan and observations were moved to Trewaters Farm. Both sloping sites, part of large areas of cv Carlton, grown on 0.90m ridges.

APPENDIX B Source and use of bulb stocks for monitoring and field trials at research sites

1998-2000

Disease	Study type	Cultivar	Supplier	Stock origin	Bulb grade (cm)	Weight (t)	Site planted
White mould	Monitoring	Carlton	Angloflora	Cornwall	16+	2 2	Kirton Arthur Rickwood
White mould	Field trial (see 4.1(b))	Cheer-fulness	Winchester Growers	Cornwall	10-12 and 12-14 (11:5 w/w)*	2 2	Kirton Arthur Rickwood
Smoulder	Monitoring	Carlton	L W van Geest Lingarden	Lincs Lincs	10-12 10-12	2 2	Kirton Arthur Rickwood
Smoulder	Field trial (see 4.1(b))	Carlton	O A Taylor	Lincs	10-12	2 2	Kirton Arthur Rickwood

* before use, the bulbs were mixed in the ratio shown

1999-2001

Disease	Study type	Cultivar	Supplier	Stock origin	Bulb grade (cm)	Weight (t)	Site planted
White mould	Monitoring	Carlton	Angloflora	Cornwall		2 2	Kirton Arthur Rickwood
White mould	Field trial (see 4.1(b))	Cheer-fulness	Winchester Growers	Cornwall		2 2	Kirton Arthur Rickwood
Smoulder	Monitoring	Carlton	F Dring Lingarden	Lincs Lincs		2 2	Kirton Arthur Rickwood
Smoulder	Field trial (see 4.1(b))	Carlton	O A Taylor	Lincs		2 2	Kirton Arthur Rickwood