HDC Project BOF 41
MAFF Project CSA 4716
Second Annual Report (May 2000)

NARCISSUS LEAF DISEASES: FORECASTING AND CONTROL OF WHITE MOULD AND SMOULDER

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Project title:	**Narcissus leaf diseases: forecasting and control of white mould and smoulder**
Report:	**Second Annual Report (May 2000)**
Previous reports:	**First Annual Report (April 1999)** **Six-monthly Report (November 1999)**
HDC Project number:	**BOF 41**
MAFF Project reference:	**CSA 4716**
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Location:	**HRI Kirton and Wellesbourne, ADAS Arthur Rickwood, and commercial sites in Cornwall, Lincolnshire and Cambridgeshire**
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Date commenced:	**July 1998**
Date completion due:	**June 2002**
Keywords:	***Narcissus*, daffodil, bulb, disease forecasting, smoulder, *Botrytis narcissicola*, white mould, *Ramularia vallisumbrosae***

CONTENTS

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.

EXECUTIVE SUMMARY

1. Isolates of *Botrytis narcissicola* and *Ramularia vallisumbrosae* were grown on a range of media and under different conditions to optimise the production of conidia (objective 1.2). Protocols were developed for conidia production for use in further work (objectives 2.3 and 4.1). A supply of resting bodies of *R. vallisumbrosae* was obtained from naturally infested leaves, obviating the specific need to produce resting bodies by the inoculation of leaves or in culture.

2. Commercial crops in Cornwall and in eastern England were examined towards the end of their first growing season, either by the examination of dead leaves or by monitoring crops in the field (objective 2.2). Foliage at all three Cornish sites showed white mould lesions, and dead foliage from the worst affected site (Manaccan) contained abundant resting bodies of *R. vallisumbrosae*. Active smoulder lesions were seen on foliage from one of the three commercial sites in the east of England, as well as on three of the four crops growing at experimental sites (ADAS Arthur Rickwood, Cambs, and HRI Kirton, Lincs). As further information is obtained the data will be examined to determine if disease levels at the end of the first year of cropping can be incorporated into a disease forecasting scheme. Initial results indicated that crops which are affected by smoulder or white mould at the end of their first year develop the disease early in the second year, but the lack of evident smoulder or white mould at the end of the first year does not necessarily indicate that crops will be free of the disease at the start of their second year.

3. Resting bodies of *R. vallisumbrosae* were placed on the soil surface or buried 5 cm deep in May 1999 at Varfell Farm, Penzance and ADAS Arthur Rickwood (objective 2.3). Resting bodies were recovered and examined at monthly intervals, beginning October 1999. Some hyphal strands, with spores characteristic of *R. vallisumbrosae*, were found at this date. Scolecospores were first confirmed on 7 January 2000, with peak germination occurring on 1-7 February, declining thereafter. There was little difference between the two sites in the times of germination. This information will be examined as a possible component of a spray timing system.

4. The monitoring of smoulder and white mould was continued in second-year commercial narcissus crops, three in Cornwall and three in eastern England (objective 2.4). These crops were not sprayed with fungicide in their second year. Crops at HRI Kirton and ADAS Arthur Rickwood were similarly monitored for disease; these crops received no fungicides in either year of growth, and were irrigated in the spring of the second year in order to provide conditions favourable to disease development. Later in the project, these observations will be used to test disease models.

1999 White mould lesions increased in all three Cornish commercial crops from early February, but one site (Manaccan) was much more seriously affected than the others and foliage died down early at this site. In the three eastern commercial crops, the incidence of smoulder lesions increased by about the same rate in each, from late January onwards, and late-season smoulder lesions were widespread. Weather data logged for these sites illustrated the milder, wetter climate in Cornwall compared with the east, and also showed marked differences between sites in the same region in terms of rainfall.

2000 Monitoring is continuing in five new second-year commercial crops and at the Manaccan site, which is being retained for a third year. The number of smoulder primaries at the start of the second (or third) year was consistently low at all sites. In the crop at Manaccan the number of white mould lesions increased rapidly (>10 per leaf by late-March), accompanied by the rapid loss of green leaf area, such that the crop was effectively defoliated by late-April. Crops at the other two Cornish sites were also affected by white mould: by late-April there were 1 – 2 lesions per leaf, and the percentage of leaf area dead or dying reached 15 – 20%. Occasional smoulder and leaf scorch (*Stagonospora curtisii)* symptoms were swamped by white mould. In the three eastern commercial crops, the number of smoulder lesions increased slowly at first, with <0.2 lesions per leaf in mid-April. In one crop (Holbeach St Marks) the number of lesions then increased rapidly, such that by early-May 40% of the leaf area was affected. At present, similar effects have been seen on other crops in the east. *B. narcissicola* has been confirmed in these lesions at two of the monitored sites and at other sites.

The two crop areas monitored at each of the experimental sites showed a steady increase in the number of smoulder lesions. By late-April the crops at ADAS Arthur Rickwood were much more severely affected (5 – 10% leaf area affected) than the crops at Kirton (<2%).

5. Preliminary evaluations were carried out with a precipitation impact (PI) sensor (objective 2.5). The use of different shield size was investigated for optimising sensor output, and examples of sensor outputs are presented. Increasing the effective diameter of the PI sensor had a significant effect on the number of rain impacts assigned to higher 'bin' numbers (higher energy drops). The rainfall events that occurred in these experiments were insufficient to damage leaves of exposed narcissus plants and allow successful infection by *B. narcissicola*.

6. Experiment protocols were developed in readiness for work on the leaf wetness characteristics of narcissus tissue (objective 3.1). A novel design of wetness sensor has been received and is undergoing tests in the laboratory before being deployed in the field.

7. Preliminary experiments were carried out on the infection of narcissus leaves with *B. narcissicola* and *R. vallisumbrosae* conidia, to study the effect of leaf wetness and temperature on infection (objective 3.2). Infection of narcissus leaves by *B. narcissicola* required leaf damage. Optimal inoculation and incubation conditions were established for both pathogens, these including high humidity. In a series of controlled environment (CE) experiments, narcissus plants were inoculated with *B. narcissicola* conidia following leaf damage (produced by using pins or a bristle brush across the leaf surface). The inoculated plants were placed in CE cabinets at 4 - 24˚C and either sprayed to maintain leaf wetness or maintained at 96% relative humidity without leaf wetting. At intervals (6 – 72 hours) samples of plants were returned to a glasshouse and the number of smoulder lesions produced was recorded 2 weeks later. Tissue damage and the presence of free water were required for successful infection. At short wetness durations (about 6 hours) temperatures of 12˚C were optimal for infection by *B. narcissicola*; at longer wetness durations (about 24 hours) a wider range of temperatures (4 - 16˚C) was effective.

8. Laboratory studies were completed using attached leaf bioassays to screen fungicides for their protective and curative effectiveness against *B. narcissicola* and *R. vallisumbrosae* (objective 4.1(a). Used against *B. narcissicola*, Ronilan, Bravo 500, Benlate + Dithane, Scala, Unix, Shirlan, Plover, Folicur, Opus, Punch C and Compass gave good control as preventive treatments. Used as curative treatments (2 days after inoculation), none of the fungicides was as

effective as when used as preventives, although Ronilan, Scala, Unix, Plover and Folicur gave some control. Used against *R. vallisumbrosae*, Benlate + Dithane, Amistar and Scala prevented disease establishment when used as protective sprays. Benlate + Dithane, Amistar and Scala were the most effective when used as curative treatments, with Bravo 500, Stroby, Unix, Shirlan and an experimental fungicide being only slightly less effective.

9. Using a commercial Cornish crop of variety Yellow Cheerfulness, spray programmes of Benlate + Dithane 945 were applied (objective 4.1(b)). A 'full ' programme consisted of spraying at about 10 day-intervals once the shoots were 10-15 cm high, and other treatments consisted of missing the first one or two sprays, or applying the first one or two sprays only; in a control, no fungicide was applied. The spray programme that commenced on 22 March (18 days after white mould was confirmed) was as effective in preventing leaf senescence as the full programme that commenced on 27 February, before the disease was seen.

10. Fungicides were also tested for their effectiveness in field trials with second-year crops at Kirton and ADAS Arthur Rickwood in spring 2000 (objective 4.1(c)). Cultivar Carlton was used at either site for experiments into smoulder control, and cv. Cheerfulness at either site for white mould control. To encourage disease development, no fungicides were applied in the first year of these crops, they were irrigated in dry periods in the spring of the second year, and they were inoculated (using a variety of methods) with *B. narcissicola* or *R. vallisumbrosae* material, respectively. Fungicides were applied at about 2 week intervals once shoots were 10 – 15cm high (currently five sprays have been applied to cv. Carlton and three to cv. Cheerfulness).

In the trial with cv. Carlton at ADAS Arthur Rickwood, the number of rotting stem ends (caused by *B. narcissicola*) had been reduced by mid-April by sprays of Ronilan, Scala, Folicur, Unix and Amistar). There was a large increase in the number of smoulder lesions in April, and by early-May the untreated control plots clearly showed more dieback of the foliage than any treated plots. In the equivalent trial at Kirton, smoulder levels were most reduced (by mid-April) by Ronilan and Folicur. In the white mould trials with cv. Cheerfulness (a late season variety), no white mould has been seen, despite the repeated inoculation of the crops, but the effects of treatments on smoulder were recorded. So far, there have been no significant differences between the fungicide treatments on the level of smoulder.

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INTRODUCTION

This second Annual Report covers all work done on the project between July 1999 and May 2000. It therefore includes information included in the previous six-monthly report (November 1999). The experimental work is presented in the same order as the project objectives, which are reproduced below for convenient reference.

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

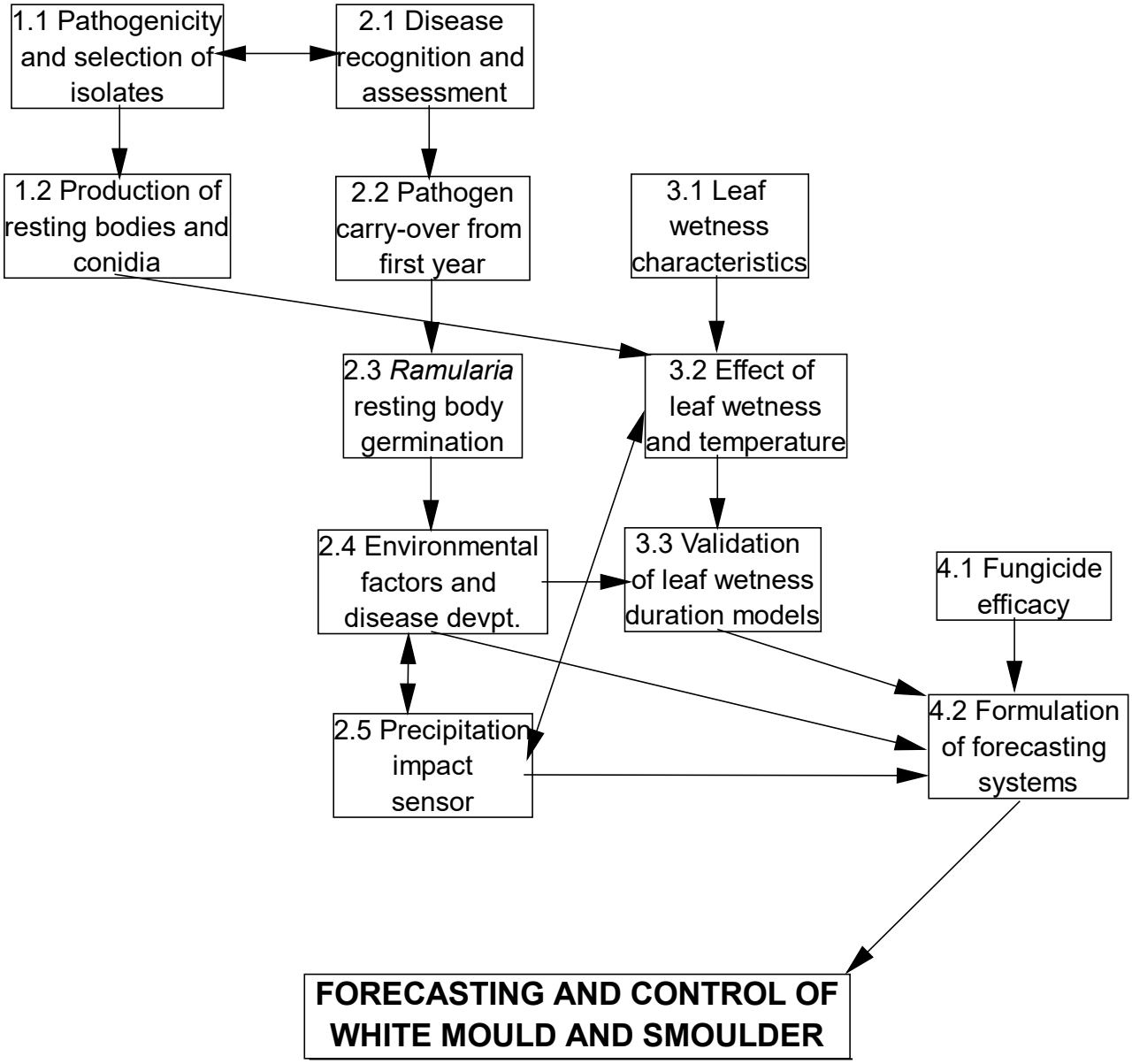


Figure 2. GANTT chart showing timetable for the main practical activities

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 (see below)

Progress

This work is now complete. Progress since the first Annual Report is summarised below.

Isolates of *B. narcissicola* and *R. vallisumbrosae* were grown on a range of agar media and under different incubation conditions to optimise methods for producing conidia and sclerotia.

B. narcissicola grew well on PDA and V8-juice agars. Sporulation was poor when isolates were grown in the dark at 20°C and improved by incubation in a UV light unit. Cultures were exposed to cycling 12 hour periods of UV light and 12 hour periods of dark, which successfully induced moderate sporulation on narcissus leaf extract agar.

R. vallisumbrosae grew very slowly on all agar media. Spore production was greatest on OMA, with cultures incubated at 18°C in the dark. Resting body production was good on PDA, V8 and OMA.

Protocols for culturing *B. narcissicola* and *R. vallisumbrosae* were prepared (see Appendix A) and distributed to the research group. In the light of experience subsequently gained at HRI during culturing these species (see objective 3.2), 'best practice' protocols will be established as the work progresses.

Potted plants of cv Carlton were inoculated on their leaves with mycelial plugs of *R. vallisumbrosae*, enclosed loosely in a polythene bag for 1 week, and stood in a cool greenhouse. White mould lesions developed at most inoculation sites, causing leaf yellowing and collapse. Resting bodies of *R. vallisumbrosae* developed at some inoculation sites, but at others soft rot and secondary fungal infections predominated, and no resting bodies were

observed. However, large quantities of narcissus leaves naturally infected by *R. vallisumbrosae* and bearing abundant resting bodies of the fungus were found at one of the disease monitoring sites in Cornwall (Manaccan). Several sacks of dry, dead leaves were collected in April 1999 and stored ready for use in objective 2.3 (*Ramularia* resting body germination) and objective 4.1 (fungicide efficacy trials). This obviated the specific need to produce resting bodies for experimental work by the inoculation of leaves or in culture; therefore, it is proposed to defer further work leading to milestones 1.2.4 and 1.2.5, although this could be re-started if the need arises.

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 and 2.2.2 achieved

Progress

Examination of crops debris
Samples of dead leaves were collected in mid-June 1999 from first-year-down commercial crops (two sites) and a second-year-down crop (one site) in Cornwall and examined for resting bodies of *R. vallisumbrosae*. Five sets of ten leaves, collected at random, were examined from each site. No evidence of white mould lesions, or of resting bodies of *R. vallisumbrosae*, were found on the samples from Polsue Manor or Grampound Road. However, from Manaccan, the second-year-down crop, all leaves showed obvious dried white mould lesions and contained abundant resting bodies of the fungus.

Examination of growing crops
All six crops due to be monitored in 1999-2000 (three each in Cornwall (as above) and in the eastern region) were inspected in mid-June/early-July 1999. Five were in their first year of growth and one (as above) was in its second year. Details of the sites are given under section 2.4(a). The second-year Cornish crop (at Manaccan) was already seriously affected by white mould, and active white mould lesions were seen on the other two Cornish crops (Polsue Manor and Grampound Road). Active smoulder lesions were seen on the Cambridgeshire crop (Swaffham Prior Fen) but no evidence of smoulder was seen at the two Lincolnshire sites (Gosberton and Holbeach St Marks). Crops growing at research sites (ADAS Arthur Rickwood and HRI Kirton, see under section 2.4(b) were also examined at this time: three of the four crops showed active smoulder lesions.

Disease carry-over

All six commercial crops and the four experimental crops were examined at end of their first year in mid-June/early July 1999 were again examined for disease from shoot emergence in spring 2000. As further information accrues, the data will be examined to determine if disease levels at the end of the first year of cropping can be incorporated into a disease forecasting scheme (Task 4.2). The results are shown in Table 2.2.1. Initial results indicated that crops which are affected by smoulder or white mould at the end of their first year develop the disease early in the second year; but lack of evident smoulder or white mould at the end of the first year does not necessarily indicate that crops will be free of the disease at the start of their second year.

Table 2.2.1 Disease levels in commercial and experimental narcissus crops, cv. Carlton, at end of first year (summer 1999) and start of second year (spring 2000)

	Site	Assessment dates (day no.)		No. of smoulder lesions / plot[b]		No. of white mould lesions / plot[b]	
		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	176	48	2.32	0.64	0	0
2.	Holbeach, Lincs	188	56	0	1.10	0	0
3.	Gosberton, Lincs (cv Standard Value)	182	54	0	0.48	0	0
4.	Manaccan, Cornwall	167	25	nd[a]	0	nd[a]	3.16
5.	Polsue Manor, Cornwall	166	40	0	0	0.44	0.12
6.	Grampound Road, Cornwall	167	42	0	0	0.10	0.10
	Experimental						
1.	ADAS, Cambs (stock ex Cornwall)	167	52	1.40	5.20	0	0
2.	ADAS, Cambs (stock ex Lincs)	167	52	2.56	5.00	0	0
3.	HRI, Lincs (stock ex Cornwall)	186	54	0.50	1.50	0	0
4.	HRI, Lincs (stock ex Lincs)	186	53	0	1.72	0	0

[a] Not determined, crop was in its second year and already badly affected by white mould.

[b] Plot size was 0.5 m length of ridge.

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 and 2.3.2 achieved

Progress

Introduction
The objective of this part of the project is to monitor the germination of *R. vallisumbrosae* resting bodies with a view to using the time of their germination in the spring as a component of a spray timing system.

Methods
Dead leaves of cv. Carlton severely affected by white mould and containing abundant resting bodies of *R. vallisumbrosae* were collected in Cornwall (Manaccan) in April 1999 and allowed to dry. Small bags (15 x 15 cm) were made from nylon mesh (75 µm mesh). Each bag was filled with 20 g sterilised silver sand and 10, 1 cm lengths of dried narcissus leaf bearing obvious *R. vallisumbrosae* resting bodies. Multiple sets of bags were attached to pegs and laid on the soil surface at ADAS Arthur Rickwood, Cambridgeshire and at Varfell Farm (Winchester Growers Ltd), Penzance, Cornwall in June/July 1999. Additionally, at the ADAS site a further set of bags was buried at 5 cm depth. Bags were arranged in three replicate rows.

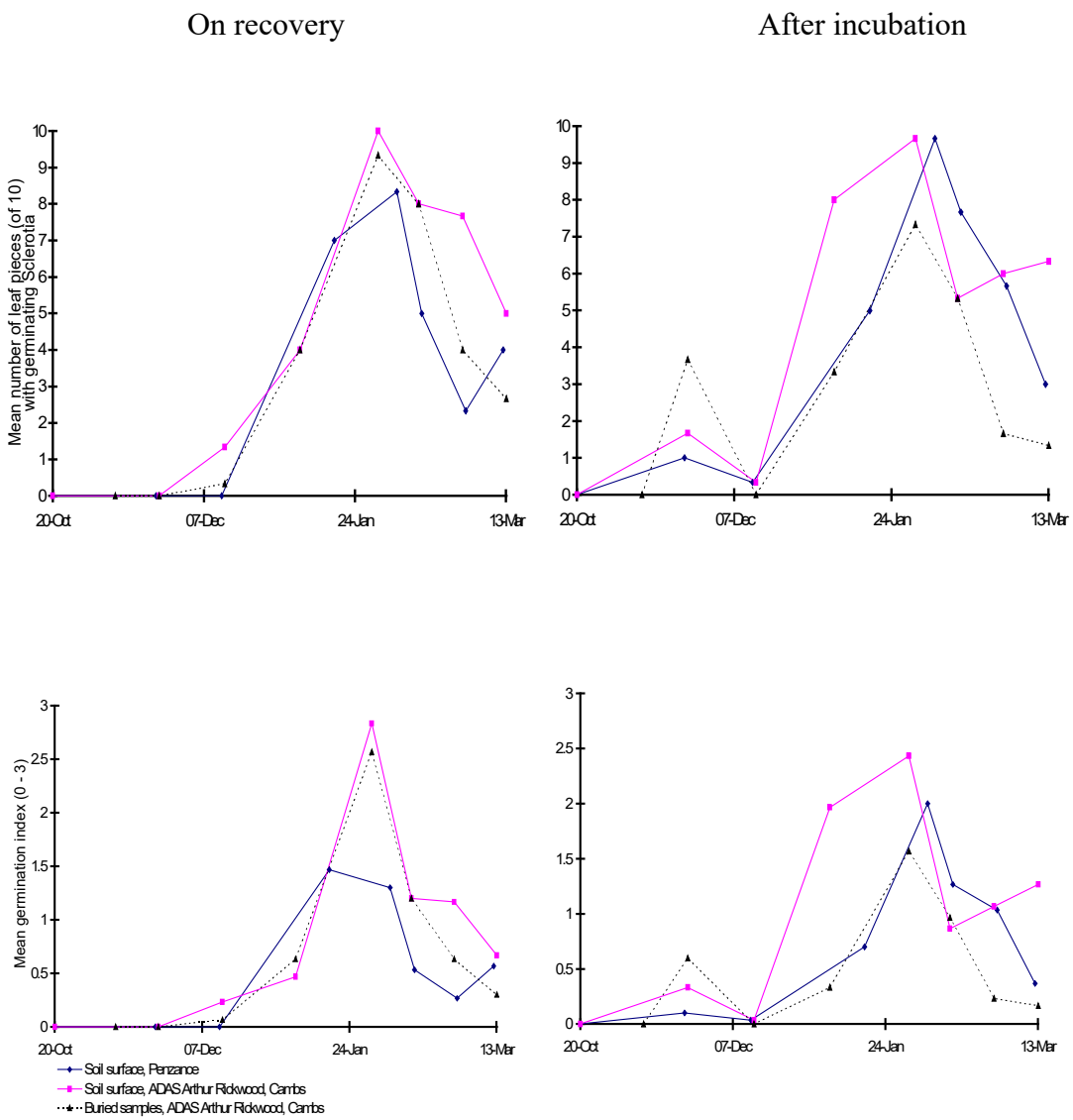
Samples were recovered at monthly intervals, starting mid-October 1999. At each sampling time, one bag was selected at random from each of the three rows. Leaf pieces were recovered from the sand by sieving, gently washed to remove remaining sand grains and then examined microscopically for evidence of spore production from the resting bodies. Leaves were also incubated in a humid chamber at laboratory temperature and re-examined after 7

days. Where fungal growth was found associated with resting bodies when examined by low power microscopy, five pieces per sample were examined at high power to determine if spores were characteristic of *Ramularia*.

Results

Leaf pieces which had been buried in the soil were more friable and showed greater degradation than those placed on the soil surface. The recovery and cleaning process was effective and *Ramularia* resting bodies were readily found. Hyphal strands bearing amerospores and/or phragmospores, typical of *R. vallisumbrosae,* were found on occasional buried leaves at the first recovery in mid-October. Scolecospores were first confirmed on 7 January, with peak germination around 1 - 7 February. Germination then declined, although spore production was still visible on samples recovered in mid-March (Figure 2.3.1). There was little difference between sites in the time at which germination occurred.

Figure 2.3.1 Germination of *Ramularia* resting bodies on narcissus leaf debris - 1999/2000

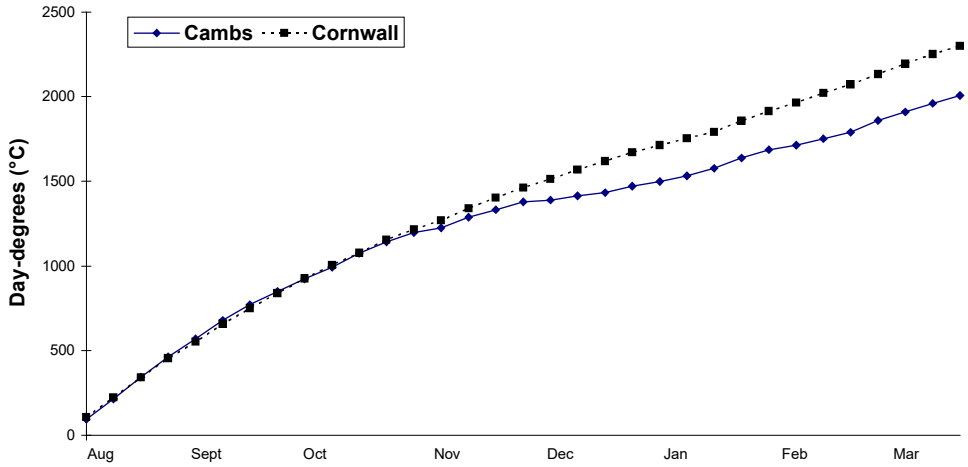


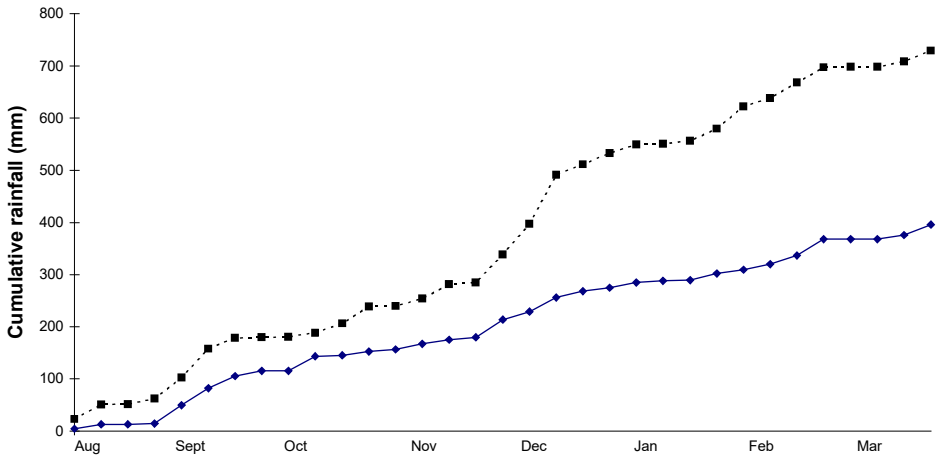
Discussion

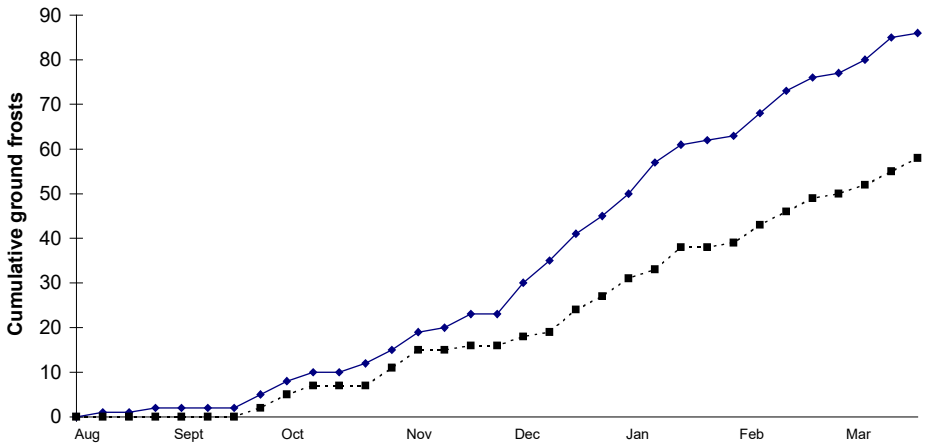
The times of initial and peak germination of *Ramularia* on leaf debris showed no detectable difference at the Cambs and Cornwall sites. Germination occurred on samples buried at 5 cm as well as that on the surface, indicating light or daylength are unlikely to be trigger factors for germination. A summation of cumulative temperature, rainfall and frost events between 15 August and 31 March for Cambs and Cornwall is shown in Figure 2.3.2. Although Cornwall was noticeably warmer, wetter and had fewer ground frosts than Cambs, especially in December, this did not have a detectable influence on the time at which *Ramularia* resting bodies germinated. Timing of *Ramularia* germination will be examined further in 2000/01.

White mould was first reported on narcissus at Manaccan on 26 January, on leaf tips of a third year crop, at Tresillian on 9 February and at Grampound Road on 11 February. The first confirmation of white mould was thus 19 days after the first confirmation of scolecospore production.

Figure 2.3.2 Weather preceeding peak *Ramularia* resting body germination - 1999/2000







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 – 2.4.5 achieved

Progress

(a) Monitoring commercial crops

Materials and methods

1998-1999 Disease monitoring was carried out on second year commercial crops, three in Cornwall (primarily to study white mould) and three in eastern England (primarily to study smoulder). The procedures used were fully described in the first Annual Report.

During the course of the work it was decided that the monitoring methods being used were probably insufficiently quantitative for disease modelling purposes when disease was spreading rapidly, as was observed with white mould in one crop in Cornwall and with the appearance of late-season smoulder lesions in crops in the east. The original procedure had involved scoring a variety of disease symptoms (on a 0-3 scale) in 50 sample areas of each crop. Towards the end of the growing season, therefore, for each of the 50 sampling areas, the total number of distinct disease lesions (smoulder or white mould) on leaves and stems was counted, and the percentage of foliage which was dead or dying through disease was estimated. In addition, on the first of these occasions, the total number of leaves and stems was recorded in each sampling area. Results were then expressed as the average number of lesions per 'leaf' (for convenience the number of lesions on leaves and stems were included together, unless specified differently).

1999-2000 For the second year of the project, similar procedures were followed, monitoring six commercial crops in 1999-2000. As the crop at one of the Cornish sites, Manaccan, had a particularly serious attack of white mould and was being grown-on by the owner for a third year, it was decided that it would be useful to follow this crop for a further year, rather than setting up a new site. For one commercial site in Lincolnshire (Gosberton), no crops of cv Carlton were available, and cv Standard Value was used instead (from observations at Holbeach St Marks in 1998-1999, this variety was known to be susceptible to smoulder). The revised disease assessment protocol was used: early in the growing season, the number of smoulder primaries and of shoots were counted for each sampling area; once the shoots had grown to reveal individual leaves, and then for as long as practical, the number of 'leaves' (leaves plus stems) and the number of smoulder and white mould lesions were counted; and throughout the season the percentage of leaf area affected by disease was recorded.

Details of crops used in both years are given in Appendix B.

Results and discussion

1998-1999 The results of monitoring commercial crops in 1998-1999 were summarised, up to early-April 1999, in the first Annual Report. Observations for the year having been

completed, these are presented in full below.

In monitoring disease near the end of the first growing season (in 1998), the two sites in Lincolnshire had about 50% of the sampling areas with at least some leaves with spreading smoulder lesions, and the corresponding figure for the Cambridgeshire site was over 90%. In the three Cornish sites, although some dried leaf lesions were seen, these could not be unequivocally identified as being due to white mould.

The findings obtained in the second growing season, 1998-1999 (for both the 'standard' monitoring in the earlier part of the growing season, and the 'revised' monitoring in the later part of the season) are tabulated in Tables 2.4.1 – 2.4.6 for the six sites. Scores for white mould in Cornwall and for smoulder in eastern England are summarised in Figures 2.4.1 and 2.4.2, respectively.

For Cornwall, Figure 2.4.1 shows the much greater development of white mould at Manaccan than at the other two sites (Fentongollan and Trethewey). As the method of recording disease was altered late in the growing season, when much of the foliage at Manaccan was already seriously affected by white mould, lesions were difficult to count accurately on the foliage late in the season in this crop: consequently, the lower-than-expected counts of white mould lesions at this site (1.98 lesions per leaf; Table 2.4.1) should be interpreted with this in mind. At Fentongollan and Trethewey 1.83 and 2.73 lesions per leaf were recorded (Tables 2.4.2 and 2.4.3).

For eastern England, Figure 2.4.2 shows that smoulder initially developed somewhat faster at Holbeach St Marks and Swaffham Prior Fen than at Gosberton. By the final recording date, however, there were 1.20, 1.34 and 1.67 smoulder lesions per leaf for the three sites, respectively. Late-season smoulder lesion and foliage senescence results for the eastern sites are summarised in Figures 2.4.3 and 2.4.4, and these show the rapid spread of the disease.

Key meteorological data for these sites are shown in Figures 2.4.5 and 2.4.6 (average temperatures) and 2.4.7 and 2.4.8 (rainfall). These figures show clearly the milder winter temperatures and higher rainfall of the Cornish sites, compared with the eastern sites. The marked differences in rainfall events between the three eastern sites is noteworthy (Figure 2.4.8), emphasising the importance of local meteorological data. Further analysis of weather data is being continued, and will be related to disease development. These observations will be used to test disease models developed later in the project.

Table 2.4.1. Disease incidence in 1998-1999 for commercial crop of cv Carlton monitored at Manaccan, Cornwall

Symptom	Date (day number)									
	1998	1999								
	177	15	29	47	63	76	90	105	120[c]	134[d]
Classic white mould lesions - score[a]	0	0.02	0.02	0	0.98	1.12	1.72	2.38	-	-
Dying leaves (<50%) (white mould) – score	0	0	0	0	0	0.24	1.30	2.04	-	-
Dying leaves (>50%) (white mould) – score	0.05[b]	0	0	0	0	0	0.14	0.70	-	-
Whole leaves dead to base - score	1.55[b]	0	0	0	0	0	0	0.10	-	-
Smoulder lesions - score	0	0	0	0.04	0	0	0.02	0.04	-	-
Stagonospora lesions - score	0	0	0	0.56	0.30	0.36	0.60	0.42	-	-
Rust-like lesions or flecks – score	0.50	0	0	0	0.22	0.10	0.76	0.82	-	-
Chocolate spot – score	0	0	0	0	0	0.98	1.36	1.16	-	-
Leaf tip scorch – score	1.4	0.30	0.30	0.10	0	0	0	0	-	-
Slug damage - score	0	0	0	0.06	0	0	0	0	-	-
Leaves – number per plot	-	-	-	-	-	-	-	-	19.4	-
White mould lesions – number per leaf	-	-	-	-	-	-	-	-	1.98	-
Smoulder lesions – number per leaf	-	-	-	-	-	-	-	-	0.07	-
Foliage die-back - %	-	-	-	-	-	-	-	-	38	70

[a] scores are on a scale of 0 – 3 for 50 sub-samples per crop
[b] presumed white rot
[c] based on 'point' samples
[d] overall crop assessment

Table 2.4.2. Disease incidence in 1998-1999 for commercial crop of cv Carlton monitored at Fentongollan, Cornwall

Symptom	Date (day number)									
	1998	1999								
	177	15	29	47	63	76	90	105	120[c]	134[d]
Classic white mould lesions - score[a]	0.58	0	0	0	0.76	0.86	0.96	1.94	-	-
Dying leaves (<50%) (white mould) – score	0.10[b]	0	0	0	0	0	0.40	1.46	-	-
Dying leaves (>50%) (white mould) – score	1.44[b]	0	0	0	0	0	0.22	0.60	-	-
Whole leaves dead to base - score	2.10[b]	0	0	0	0	0	0	0.04	-	-
Smoulder lesions - score	0	0	0	0	0	0	0	0.02	-	-
Stagonospora lesions - score	0	0	0	0.26	0.70	0.62	1.02	1.00	-	-
Rust-like lesions or flecks – score	0.33	0	0	0.02	0.06	0.20	0.32	0.72	-	-
Chocolate spot – score	0	0.08	0	0	0	0.22	0.86	1.16	-	-
Leaf tip scorch – score	0	0.48	0.34	0.52	0.08	0.04	0	0	-	-
Slug damage - score	0	0	0.24	0.40	0.06	0	0	0	-	-
Leaves – number per plot	-	-	-	-	-	-	-	-	16.7	-
White mould lesions – number per leaf	-	-	-	-	-	-	-	-	1.83	-
Smoulder lesions – number per leaf	-	-	-	-	-	-	-	-	0.04	-
Foliage die-back - %	-	-	-	-	-	-	-	-	19	80

[a] scores are on a scale of 0 – 3 for 50 sub-samples per crop
[b] presumed white rot
[c] based on 'point' samples
[d] overall crop assessment

Table 2.4.3. Disease incidence in 1998-1999 for commercial crop of cv Carlton monitored at Trethewey, Cornwall

Symptom	Date (day number)									
	1998	1999								
	177	15	29	47	63	76	90	105	120[c]	134[d]
Classic white mould lesions - score[a]	0	0	0	0	0.64	0.88	1.06	2.26	-	-
Dying leaves (<50%) (white mould) – score	1.03[b]	0	0	0	0	0	0.50	2.02	-	-
Dying leaves (>50%) (white mould) – score	1.00[b]	0	0	0	0	0	0	0.34	-	-
Whole leaves dead to base - score	1.73[b]	0	0	0	0	0	0	0	-	-
Smoulder lesions - score	0	0	0	0	0	0.10	0.06	0.04	-	-
Stagonospora lesions - score	0	0	0	0.02	0.50	0.38	1.02	0.86	-	-
Rust-like lesions or flecks – score	0.18	0	0	0.02	0.16	0.50	0.78	1.26	-	-
Chocolate spot – score	0	0	0	0	0	0.14	0.26	1.10	-	-
Leaf tip scorch – score	0	0	0.02	0.06	0.26	0.12	0	0	-	-
Slug damage - score	0	0	0.26	0.52	0.14	0.06	0	0	-	-
Leaves – number per plot	-	-	-	-	-	-	-	-	13.2	-
White mould lesions – number per leaf	-	-	-	-	-	-	-	-	2.73	-
Smoulder lesions – number per leaf	-	-	-	-	-	-	-	-	0.08	-
Foliage die-back - %	-	-	-	-	-	-	-	-	46	90

[a] scores are on a scale of 0 – 3 for 50 sub-samples per crop
[b] presumed white rot
[c] based on 'point' samples
[d] overall crop assessment

Table 2.4.4. Disease incidence in 1998-1999 for commercial crop of cv Carlton monitored at Gosberton, Lincs

Symptom	Date (day number)									
	1998	1999								
	155	20	41	56	70	84	97	111[b]	132[b]	147[c]
Smoulder primaries - score[a]	0	0	0	0	0	0	0	-	-	-
Smoulder leaf lesions – score	0	0	0.06	0.28	0.36	0.76	1.26	-	-	-
Advanced smoulder leaf lesions – score	0.50	0	0	0	0	0	0	-	-	-
Dead leaves (smoulder) – score	0.83	0	0	0	0	0	0	-	-	-
Smoulder lesions on cut stalk end – score	0.68	0	0	0	0	0	0	-	-	-
Rust-like lesions or flecks – score	0	0	0	0.02	0	1.20	1.20	-	-	-
Chocolate spot – score	0.38	0	0	0	0	0	0	-	-	-
Leaf tip scorch – score	0.23	0	0	0	0	0	0	-	-	-
Early senescence (individual leaves) - score	0.15	0	0	0	0.02	0.02	0.24	-	-	-
Leaves – number per plot	-	-	-	-	-	-	-	143.5	-	-
Smoulder lesions – number per leaf	-	-	-	-	-	-	-	0.17	1.67	-
Foliage die-back - %	-	-	-	-	-	-	-	7	71	100

[a] scores are on a scale of 0 – 3 for 50 sub-samples per crop
[b] based on 'point' samples
[c] overall crop assessment

Table 2.4.5. Disease incidence in 1998-1999 for commercial crop of cv Carlton monitored at Holbeach St. Marks, Lincs

Symptom	Date (day number)									
	1998	1999								
	155	20	41	56	70	84	97	109[b]	127[b]	139[c]
Smoulder primaries - score[a]	0	0	0	0	0	0	0	-	-	-
Smoulder leaf lesions – score	0	0	0.02	0.16	0.70	0.94	1.56	-	-	-
Advanced smoulder leaf lesions – score	0.55	0	0	0	0	0	0	-	-	-
Dead leaves (smoulder) – score	0.90	0	0	0	0	0.06	0.08	-	-	-
Smoulder lesions on cut stalk end – score	0.20	0	0	0	0	0	0.06	-	-	-
Rust-like lesions or flecks – score	0.10	0	0	0	0	0.42	0.84	-	-	-
Chocolate spot – score	0.48	0	0	0	0	0.02	0.04	-	-	-
Leaf tip scorch – score	0.20	0	0	0	0	0.04	0	-	-	-
Early senescence (individual leaves) - score	0.38	0	0	0	0.08	0.08	0.30	-	-	-
Leaves – number per plot	-	-	-	-	-	-	-	160.6	-	-
Smoulder lesions – number per leaf	-	-	-	-	-	-	-	0.08	1.20	-
Foliage die-back - %	-	-	-	-	-	-	-	3	47	97

[a] scores are on a scale of 0 – 3 for 50 sub-samples per crop
[b] based on 'point' samples
[c] overall crop assessment

Table 2.4.6. Disease incidence in 1998-1999 for commercial crop of cv Carlton monitored at Swaffham Prior Fen, Cambs

Symptom	Date (day number)									
	1998	1999								
	162	20	41	56	67	82	96	109[b]	127[b]	139[c]
Smoulder primaries - score[a]	0	0	0	0	0	0	0	-	-	-
Smoulder leaf lesions – score	0	0	0.10	0.16	0.66	0.86	1.62	-	-	-
Advanced smoulder leaf lesions – score	1.18	0	0	0	0	0	0.02	-	-	-
Dead leaves (smoulder) – score	0.68	0	0	0	0	0	0.04	-	-	-
Smoulder lesions on cut stalk end – score	0.58	0	0	0	0	0.02	0.06	-	-	-
Rust-like lesions or flecks – score	0.33	0	0	0.02	0.30	1.14	1.76	-	-	-
Chocolate spot – score	0	0	0	0	0	0	0.00	-	-	-
Leaf tip scorch – score	0.78	0	0	0.06	0.06	0	0.02	-	-	-
Early senescence (individual leaves) - score	0.35	0	0	0	0.02	0	0.20	-	-	-
Leaves – number per plot	-	-	-	-	-	-	-	114.9	-	-
Smoulder lesions – number per leaf	-	-	-	-	-	-	-	0.26	1.34	-
Foliage die-back - %	-	-	-	-	-	-	-	10	22	86

[a] scores are on a scale of 0 – 3 for 50 sub-samples per crop
[b] based on 'point' samples
[c] overall crop assessment

Figure 2.4.1 Frequency of white mould symptoms at three Cornish sites in early 1999.
Symptoms: 1 = lesions, 2 = dying leaves (<50% dead), 3 = dying leaves (>50% dead)

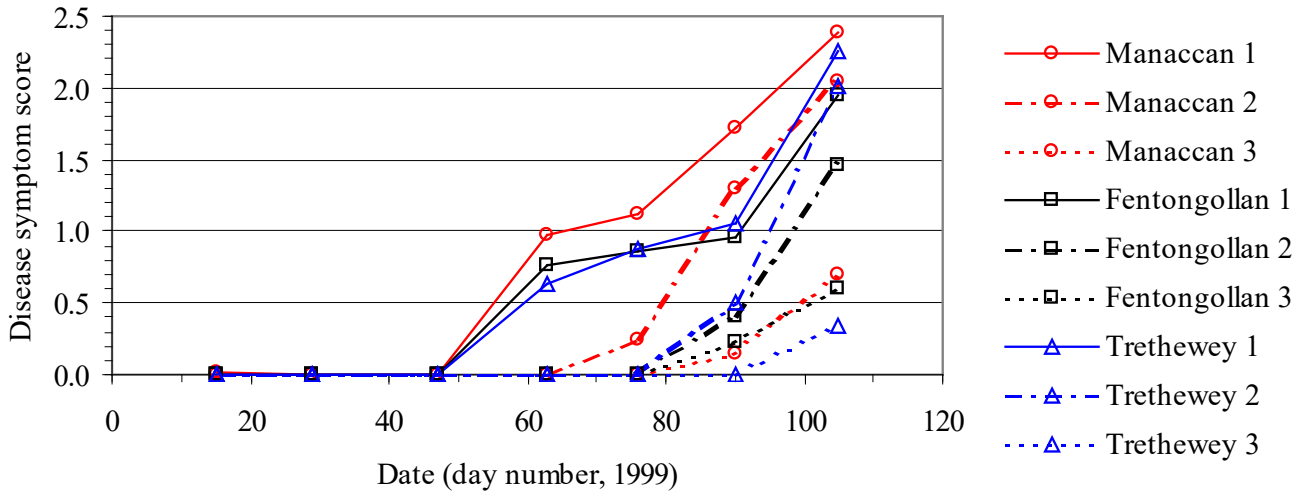


Figure 2.4.2 Frequency of smoulder symptoms in eastern crops in early 1999.
Symptoms: 1 = leaf lesions, 2 = dying leaves, 3 = cut stem lesions

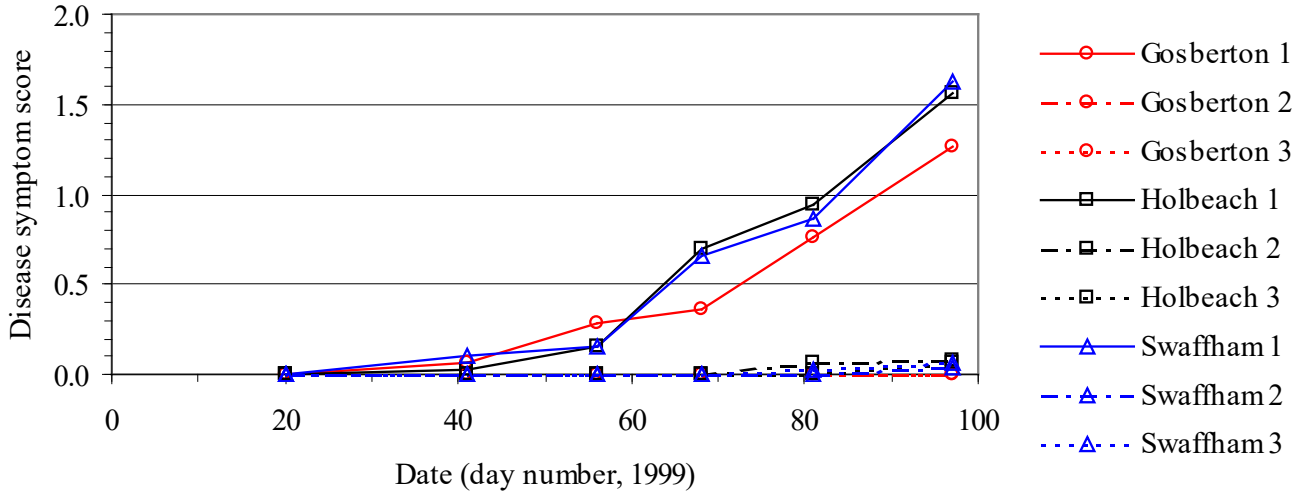


Figure 2.4.3 Number of smoulder lesions per leaf at three eastern sites in 1999

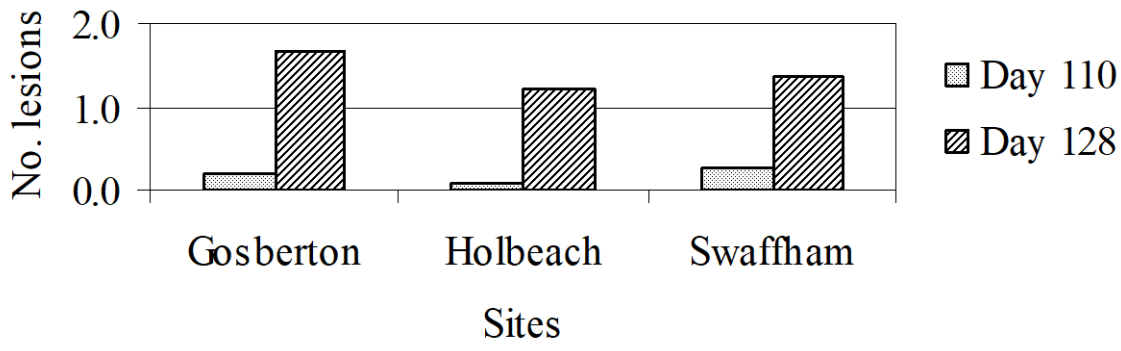


Figure 2.4.4 Percentage leaf die-back at three eastern sites in 1999

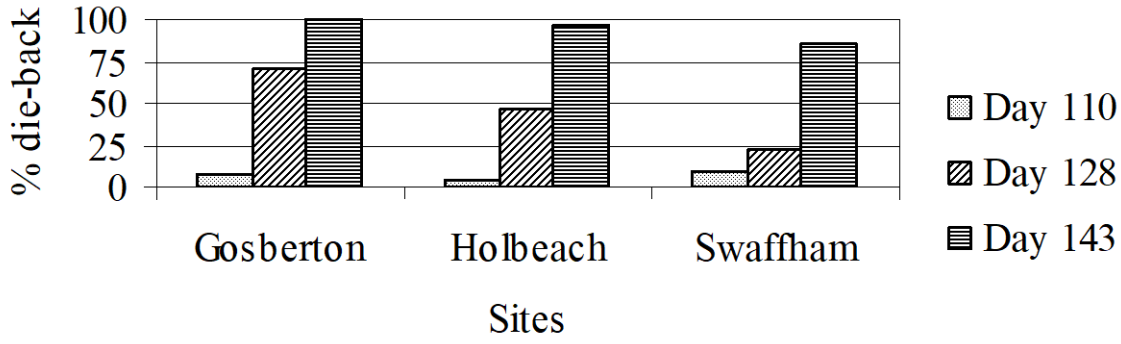
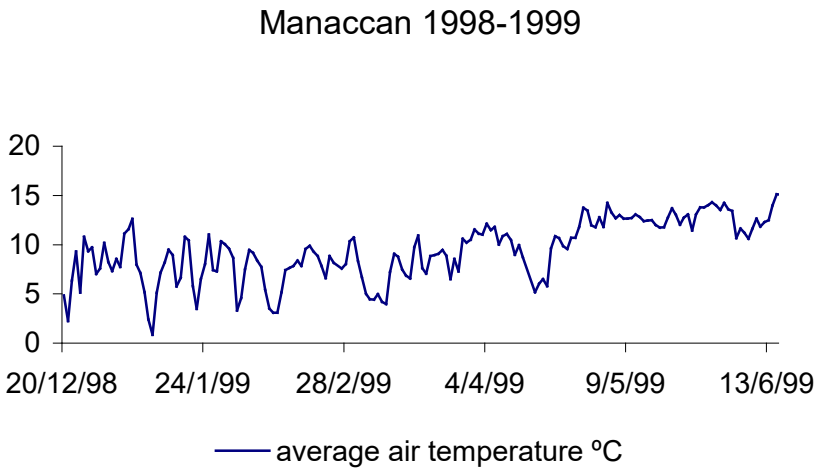
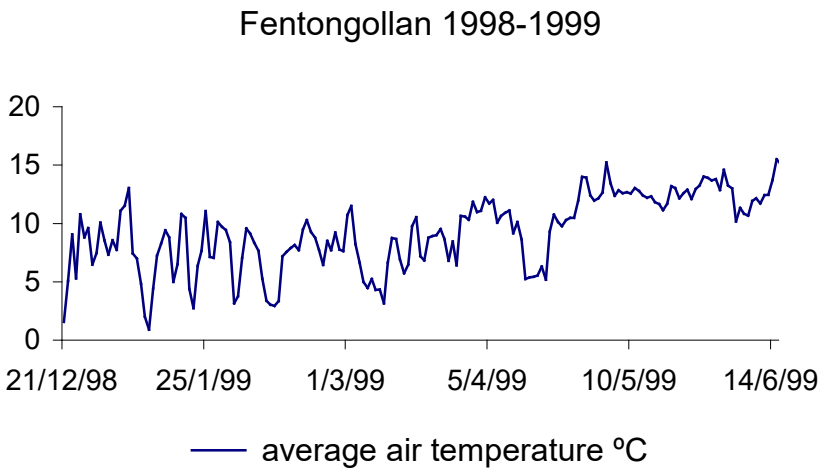


Figure 2.4.5 Average temperatures at the three Cornish sites in 1998-1999





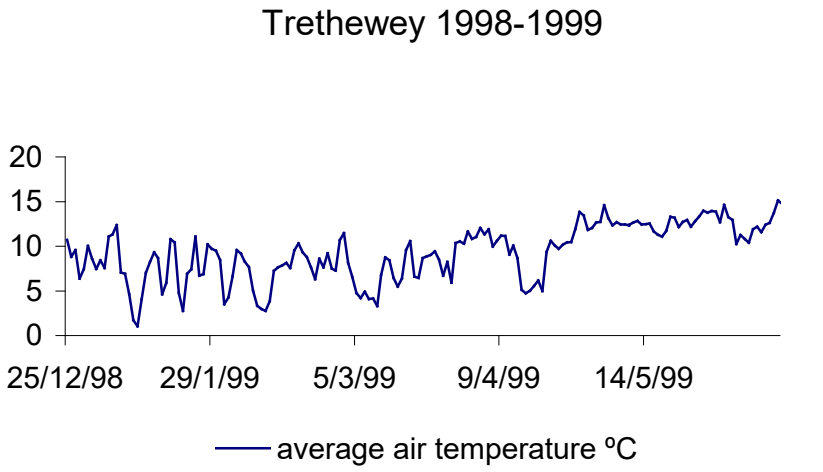
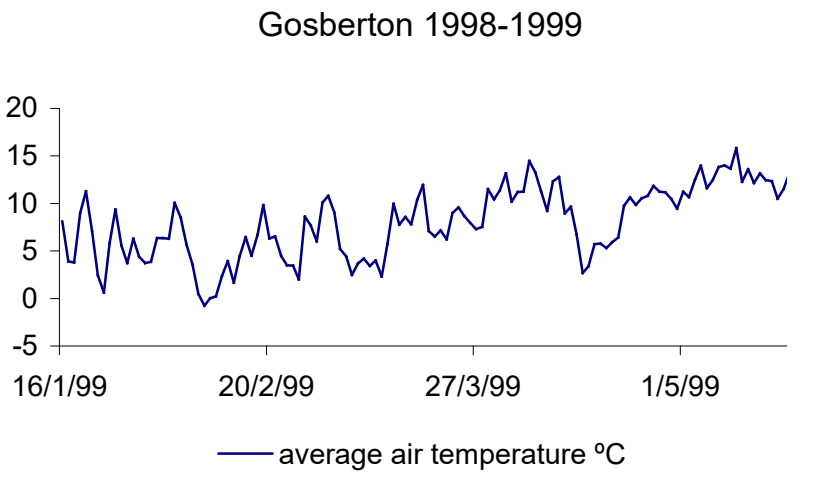
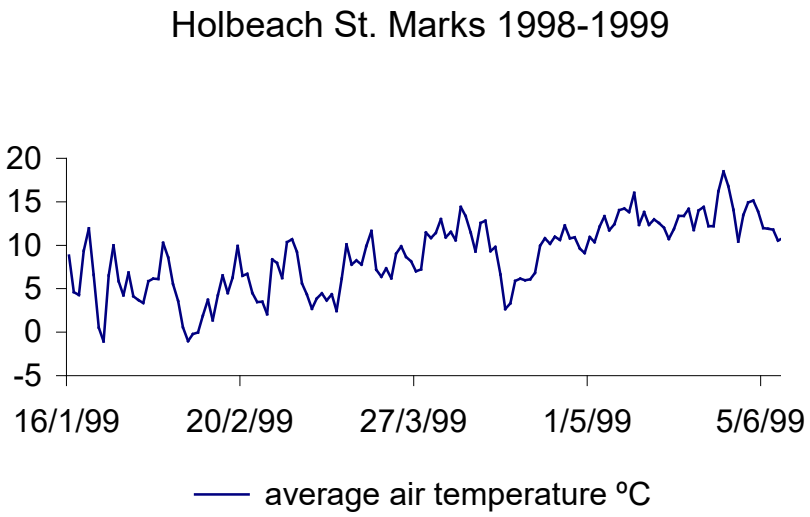


Figure 2.4.6 Average temperatures at the three eastern sites in 1998-1999





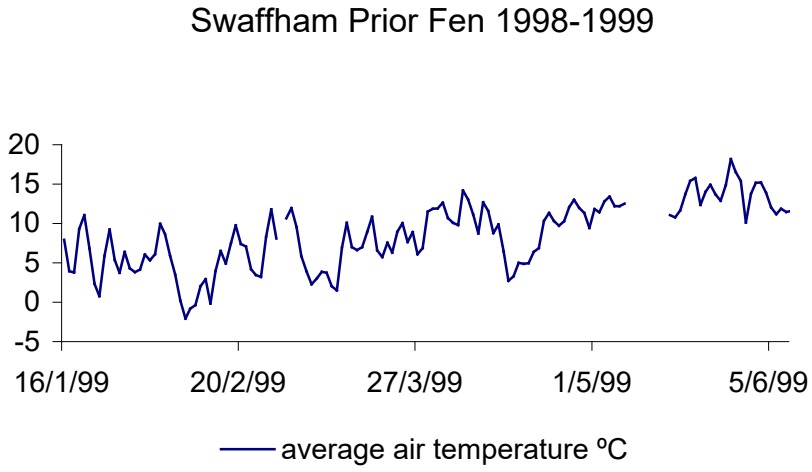
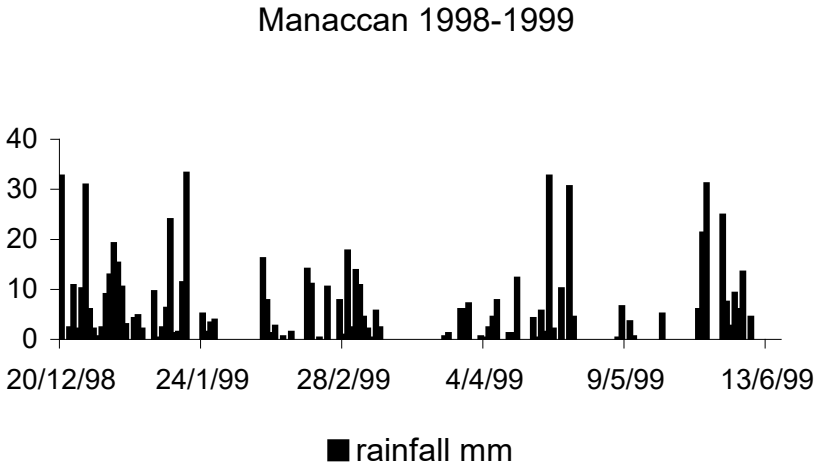
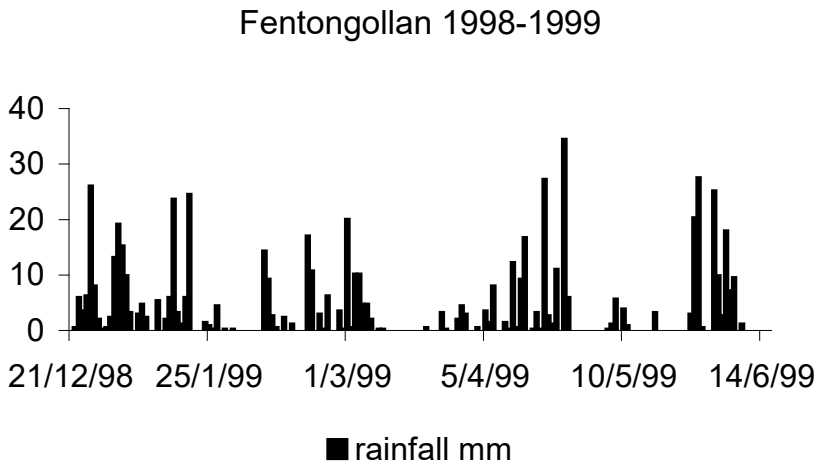


Figure 2.4.7 Rainfall at the three Cornish sites in 1998-1999





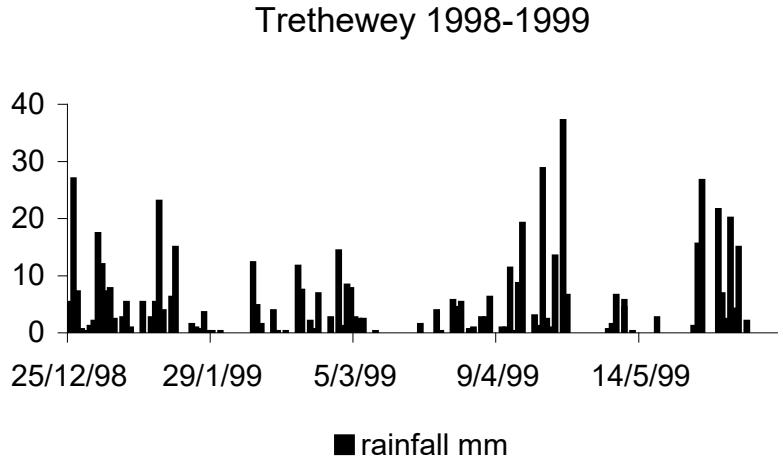
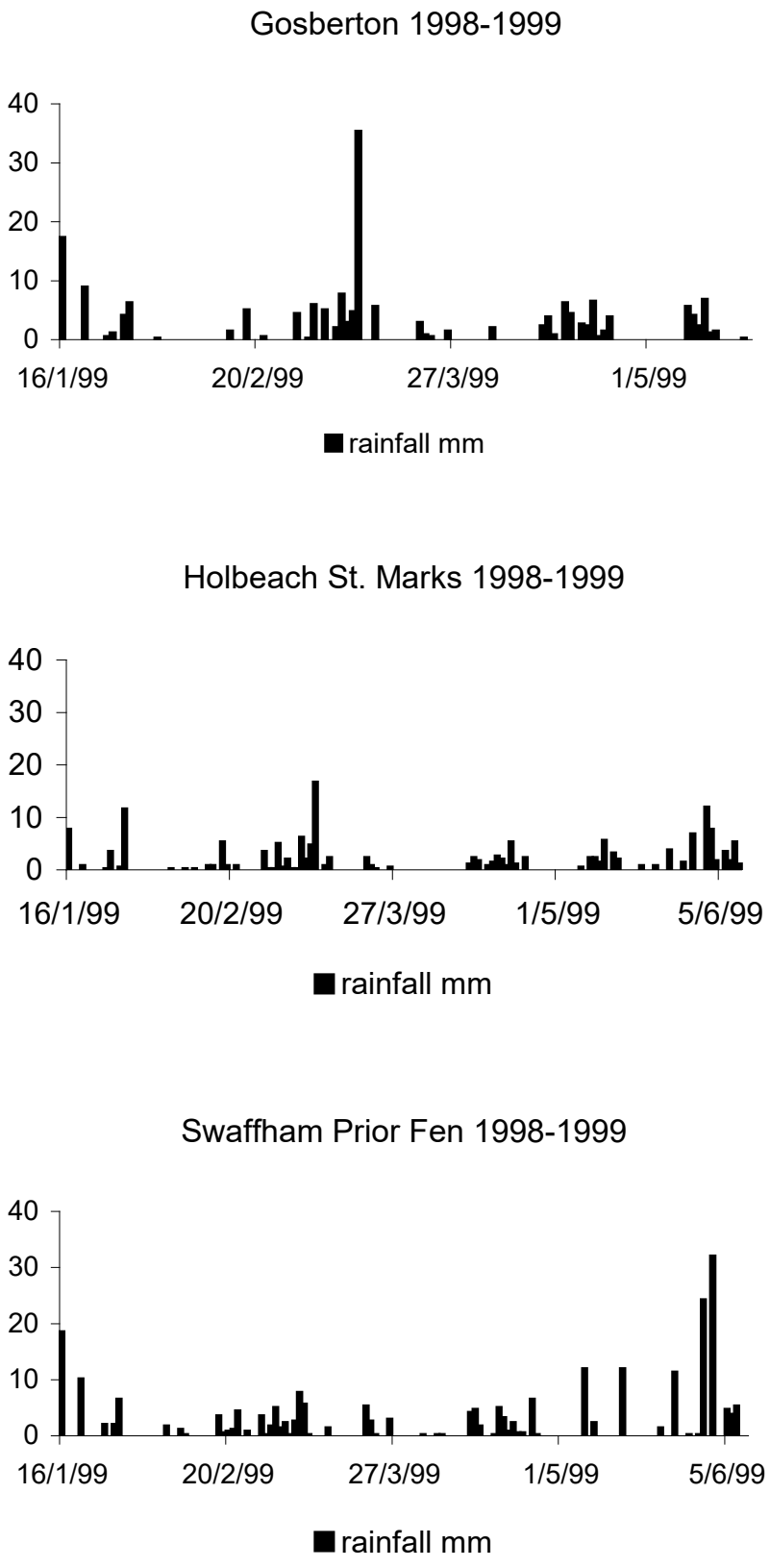


Figure 2.4.8 Rainfall at the three eastern sites in 1998-1999



1999-2000 For the second set of crops (monitored 1999-2000), the results of disease monitoring near the end of the 1998-1999 growing season are given in Table 2.4.7. Active white mould lesions were found in both the first-year crops (with more in the Grampound Road site than at the Polsue Manor site) (the second-year crop at the Manaccan site had already fully died down at this time). In the east, active smoulder lesions were found at the Swaffham Prior site (Cambridgeshire), but not at the two Lincolnshire sites. This information will be used to determine whether lesion numbers at the end of the first growing season relate to disease levels observed in the second: on this basis, smoulder would be expected to appear first at Gosberton and white mould first at Manaccan. The crop at Swaffham Prior also showed a significant number of yellowing leaves, presumed to be symptoms of base rot.

Table 2.4.7. Disease lesions at the end of the first year of crops in 1999 (end of second year at Manaccan)

Site	Assessment date (day no.)	Active lesions per plot	Other symptoms (no. per plot)	% foliar senescence
Swaffham Prior Fen, Cambs.	176	2.32 (smoulder)	5.46 (yellowing leaves, base rot?)	17
Holbeach St. Marks, Lincs.	188	0	-	55
Gosberton, Lincs	182	0	-	88
Manaccan, Cornwall	167	nd[a]	-	100
Polsue Manor, Cornwall	166	0.04[b] (white mould)	-	18
Grampound Road, Cornwall	167	0.10 (white mould)	-	18

[a] not determined, crop already seriously affected by white mould
[b] plus 0.40 old (inactive) white mould lesions per plot

Disease development at the six sites in the second year of crops, up to the time of writing, is shown in Figures 2.4.9 – 2.4.11 (Cornish crops) and 2.4.12 – 2.4.14 (eastern crops).

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

In the third-year crop at Manaccan, large numbers of white mould lesions developed rapidly, so that by late-March there were >10 lesions per leaf and thereafter it proved impractical to count lesions (figure 2.4.9). By the same time, some 25% of the distal leaf surface was dead or dying as a result of the numerous coalescing white mould lesions. The loss of green leaf occurred

rapidly: by late-April virtually 100% of the leaf area was dead. In the crops at Grampound Road and Tresillian (Figures 2.4.10 and 2.4.11), white mould spread much more slowly, reaching 1 – 2 lesions per leaf by late-April. From early-March, the percentage leaf area affected increased quickly, reaching 15 – 20% by late-April.

In the eastern crops (Figures 2.4.12 – 2.4.14) the number of smoulder primaries was also generally low. From late-March the number of smoulder lesions increased slowly at all three sites, but was still <0.2 lesions per leaf in late-April, with the percentage of leaf area affected increasing only slowly at this time. In the crop at Holbeach St Marks, there was then a rapid increase in the number of smoulder lesions, such that the percentage leaf area affected approached 40% in early-May. Similar epidemics of late-season smoulder have been observed on other narcissus crops in the Holbeach area and on crops at HRI Kirton and ADAS Arthur Rickwood, and similar lesions (though in smaller numbers) have been seen at the other monitoring sites (Gosberton and Swaffham Prior Fen). Isolation of fungi from these lesions is ongoing, and *B. narcissicola* has been confirmed from the Holbeach St Marks, Swaffham Prior Fen and other sites.

Figure 2.4.9 White mould at Manaccan, 2000

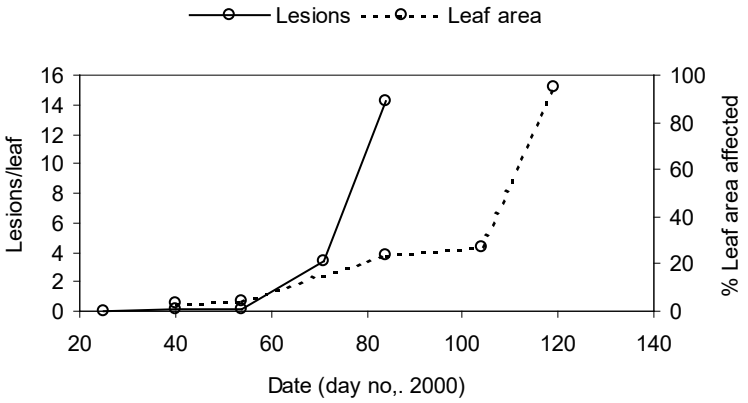


Figure 2.4.10 White mould at Grampound Road, 2000

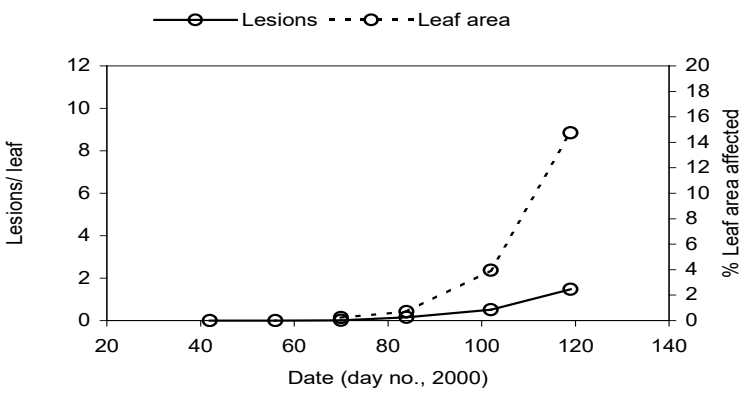


Figure 2.4.11 White mould at Tresillian, 2000

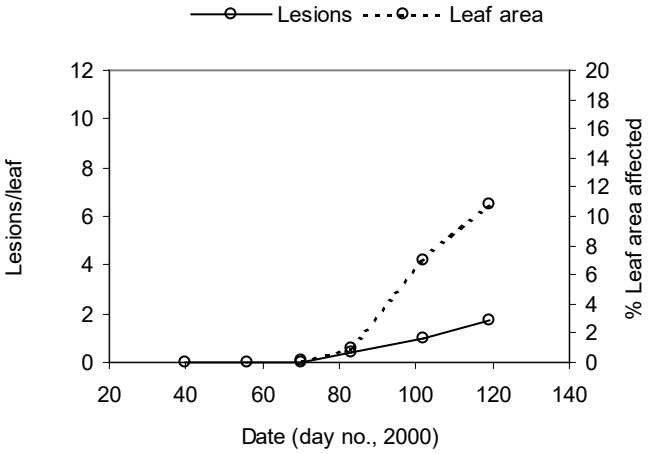


Figure 2.4.12 Smoulder at Gosberton, 2000

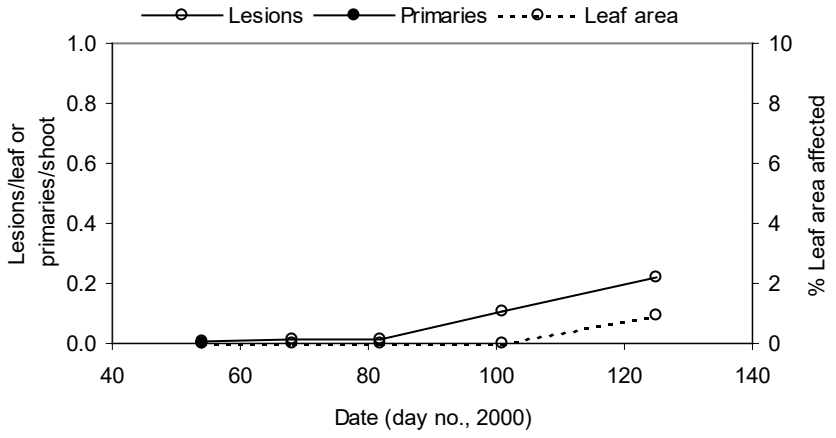


Figure 2.4.13 Smoulder at Holbeach St Marks, 2000

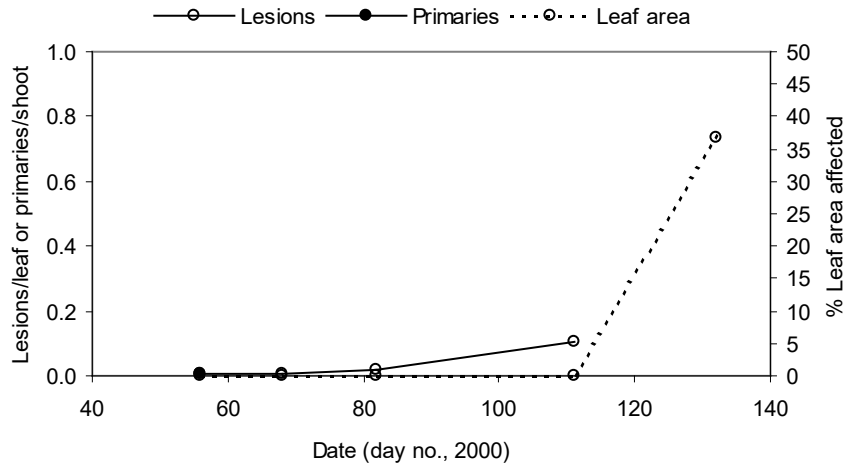
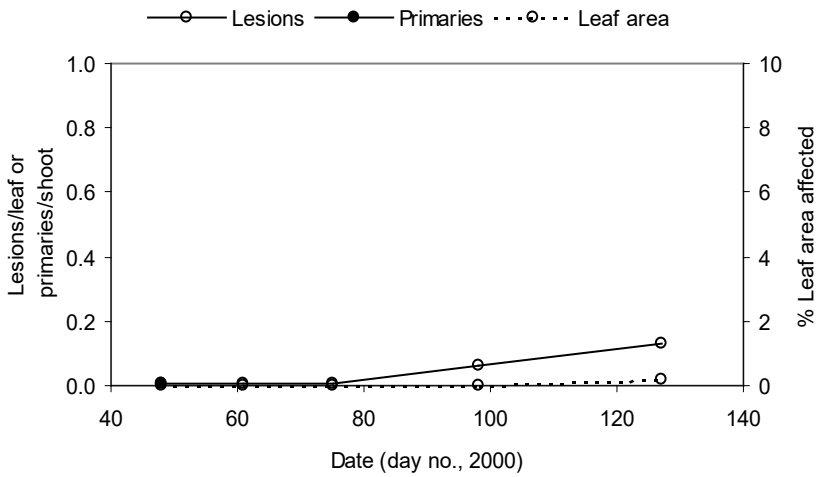


Figure 2.4.14 Smoulder at Swaffham Prior Fen, 2000



(b) Monitoring crops at research sites

Materials and methods

In each of 1998 and 1999 four, 2 t lots of bulbs were planted at each of ADAS Arthur Rickwood and HRI Kirton for disease monitoring and field experiments in the second year of the crops, i.e., starting 1999-2000 (see section 4.1(b) for information on the field experiments). The bulbs planted were as follows:

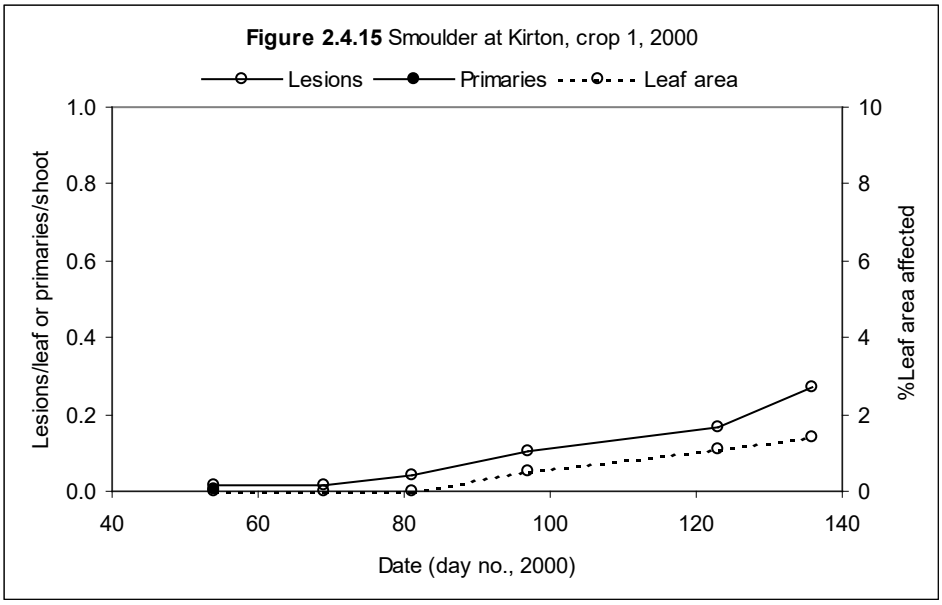
- for monitoring white mould - Cornish stock of cv Carlton
- for monitoring smoulder - Lincolnshire stock of cv Carlton
- for field trials on white mould - Cornish stock of cv Cheerfulness
- for field trials on smoulder - Lincolnshire stock of cv Carlton

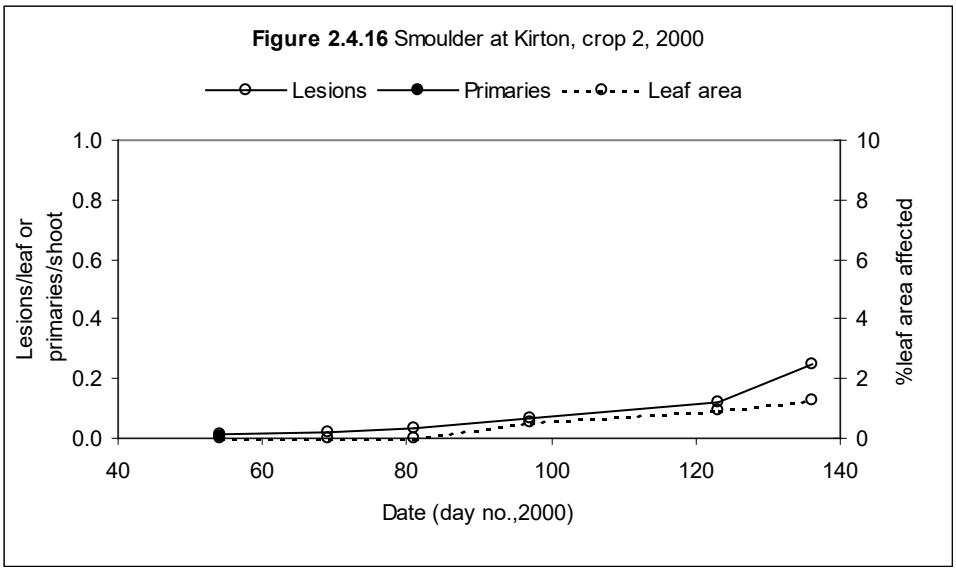
No fungicide sprays were applied in either year of these crops. Further information was given in the first Annual Report. Details of all bulbs used are given in Appendix C. These crops were irrigated in their second year, as described for the fungicide experiments in section 4.1(c).

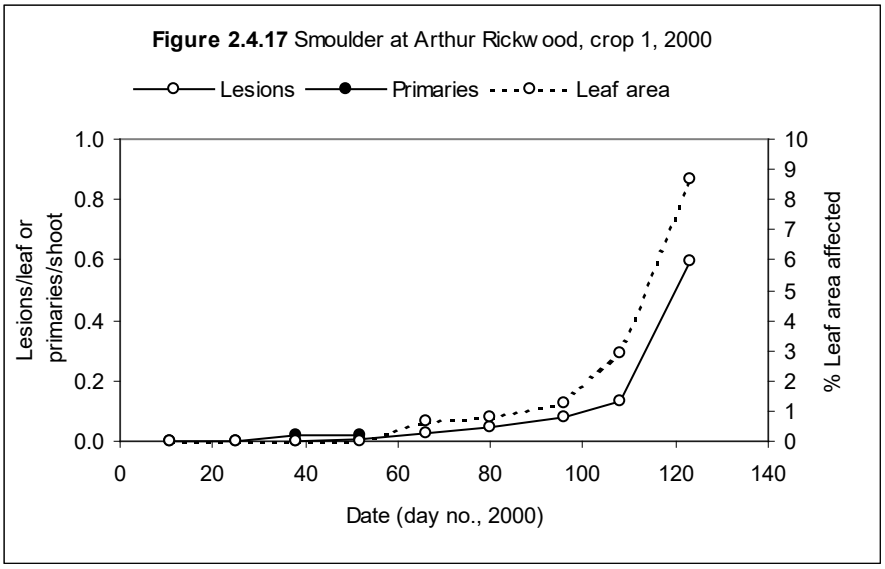
Results and discussion

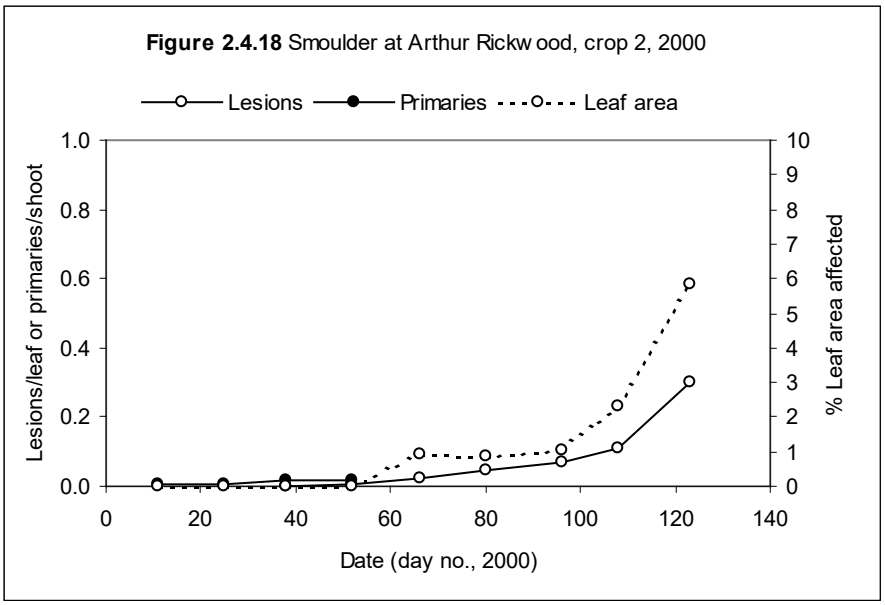
1998-2000 The experiment plots of cv Carlton planted in September 1998 at ADAS Arthur Rickwood and HRI Kirton grew well with no evidence of white mould and no typical primary symptoms of smoulder. At both sites, however, some *Botrytis* developed on senescent leaves or stems after flowering, and crop growth and the incidence of *Botrytis* was assessed in June/July 1999 for 50 x 0.5 m-long sample areas in each crop. At ADAS Arthur Rickwood there was an average of 1.4 and 2.6 affected 'leaves' (leaves plus stems) per 0.5m plot for the Cornish and Lincolnshire stocks, respectively. At HRI Kirton the incidence of smoulder lesions was very low in the Cornish stock (0.5 lesions per 0.5m) and no lesions were detected in the Lincolnshire stock.

The occurrence of smoulder on these crops is shown in Figures 2.4.15 – 16 (Kirton) and 2.4.17 – 18 (Arthur Rickwood). All four crops showed a low number of smoulder primaries, and a steady increase in lesion numbers and in loss of green leaf area. By late-April the two crops at Arthur Rickwood were much more severely affected than those at Kirton. At this time <2% of leaf area was affected at Kirton but 5-10% at Arthur Rickwood. The reasons for this difference are being investigated.









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 achieved

Progress

Impact Sensor Validation

Experiment 1 Preliminary experiment on the effect of impact shield aperture size on measurement of raindrop impact (determination of reference sensor settings)

Introduction
Raindrop impacts produce a series of electrical pulses which can be monitored by the impact sensor. Air pressure pulses produced by raindrops impacting onto a diaphragm are detected using an omni-directional microphone within the sensor. The amplitude of the microphone output can be divided into 14 levels ('bins'). Previous work demonstrated that the upper limit of the sensors output was accurate under UK conditions. However, the sensitivity at the lower end of the output range was not sufficient (Lovell *et al*., 1999). As *Botrytis narcissicola* infects only damaged tissues, the output from the sensor at the lower end of the scale may be less important in comparison with the upper limits of the sensor output. However, output from the lower end of the output scale will be important in determining the effect of rain-splash by *Ramularia vallisumbrosae* (and *B. narcissicola*). Therefore it maybe necessary to optimise the sensor output to measure each type of response.

Materials and methods
Target area over which impacts are measured is one determinant in the optimisation of the sensor. The size of the target area of the impact sensor determines the likelihood of raindrop

impact during a given sampling period. For a target area of 10 cm ^{2} a single impact equates to 1000 impacts/m^2. Preliminary experiments investigated the usage of different shield diameters to optimise the signal output at the upper limits of the sensor output, since this could be then used as a diagnostic determinant of narcissus tissue damage.

Results

The results (Figure 2.5.1) show the output for two separate rain events, each with the same amount of rainfall, using a wide target area of 80 mm diameter (Figure 2.5.1a) and a small target area of 30 mm diameter (Figure 2.5.1b). The results indicate that larger target areas had higher overall numbers of impacts, but most of these were assigned to low bin numbers. Smaller target areas had lower numbers of total impacts with larger numbers of impacts being ascribed to higher bin numbers. The results can be explained due to the higher probability of larger droplets being assigned to small bin numbers using large target areas (caused by the higher zone of impaction available from which impacts are sampled). These results confirm previous findings, although the sampling time used will also affect these results. However, it is unclear if there were any differences in the two separate rain events used in these experiments.

Note: Rainfall is collected by a tipping bucket mechanism inside the rain gauge. The measurement of rainfall by the rain gauge is therefore not continuous but shows as pulses. Therefore it is possible to have impact events when the output from the rain gauge appears low.

Conclusions

- Greater aperture diameter of shield gave higher overall numbers of impacts, however most of these were assigned to low bin numbers.

- Smaller aperture diameter of shield gave lower numbers of total impacts, with larger numbers of impacts being ascribed to higher bin numbers.

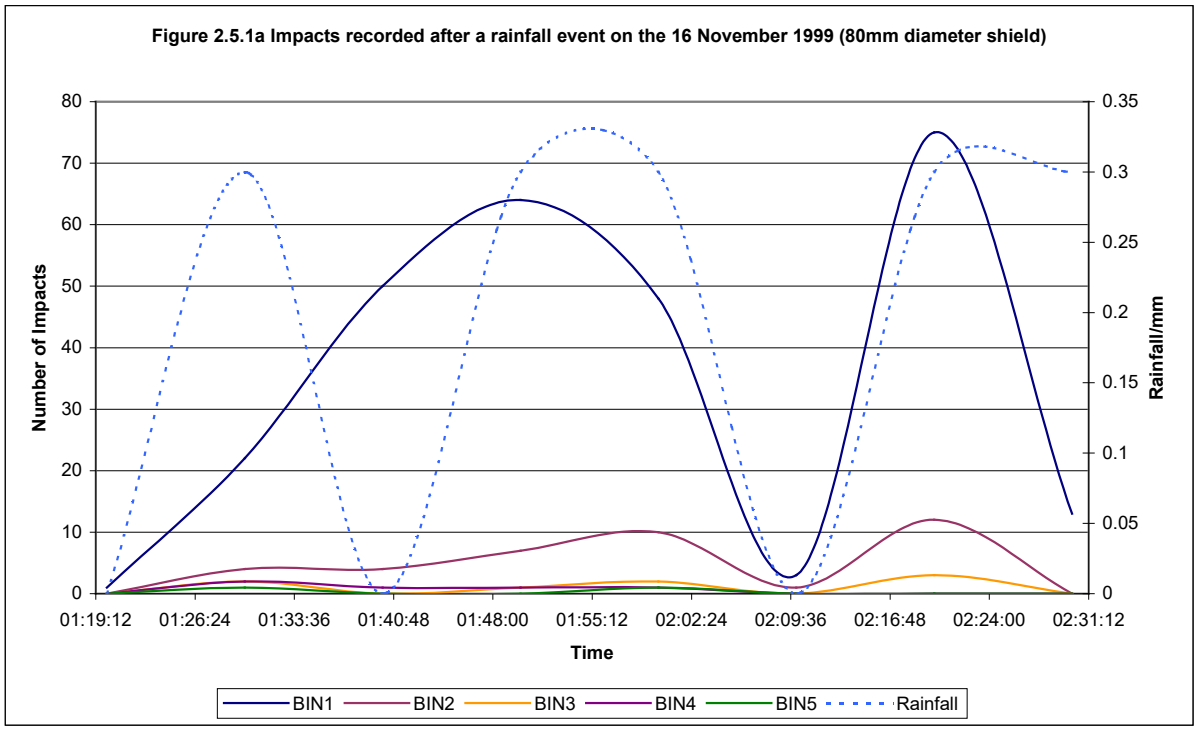
- The optimal settings for determining rain-splash events could not be determined from these tests.

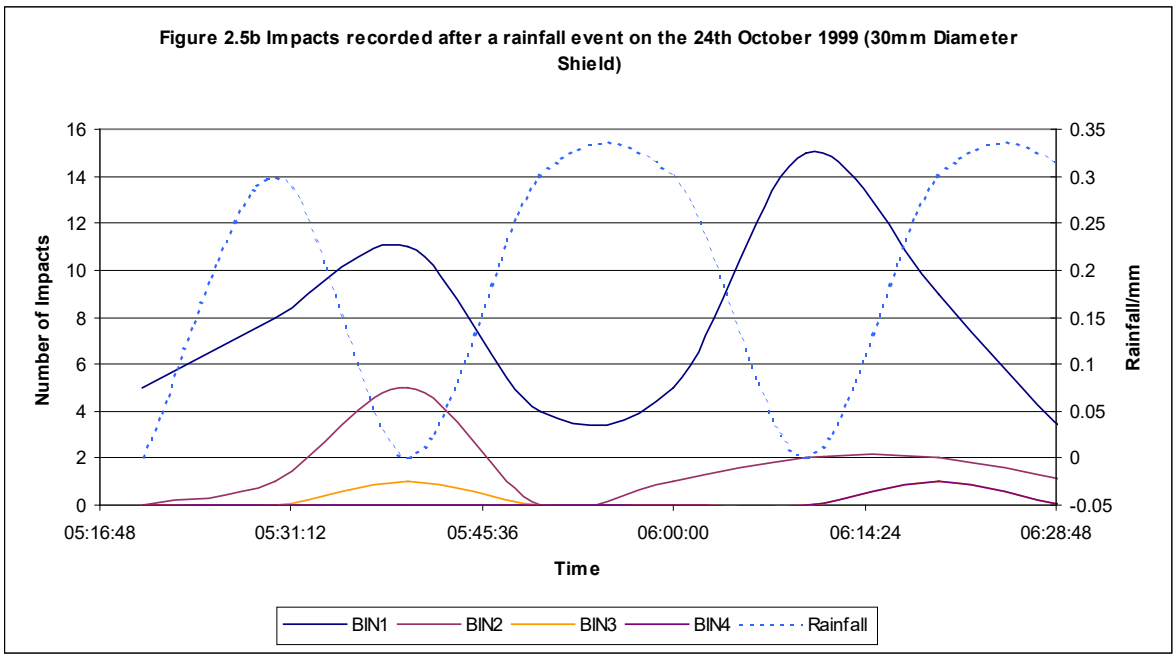
Experiment 2 The effect of impact shield aperture size on measurement of raindrop impaction

Introduction

The precipitation impact sensor (PI sensor) comprises of a sealed acoustics chamber with an upward facing diaphragm. When raindrops of varying size impact on the surface of the sensor pressure pulses are produced on the diaphragm which can be measured within the chamber by an omni-directional microphone. The amplitude of the microphone output signal is discriminated into one of 14 levels ('bins'). Bin one was set to detect droplets > 1.4 mm. The output of the sensor is affected by (1) the sample period and (2) the target area on which raindrops can impact.

Figure 2.5.1





Materials and methods

Aperture sizes used The target area of the impact sensor can be altered by using shields with apertures. These are positioned above the sensor response surface and therefore expose only part of the sensor response surface to raindrop impaction. The impact shield aperture sizes were compared in the field in a series of tests as listed in Table 2.5.1. It was decided that the reference sensors should have the smallest aperture size (30 mm diameter), as theoretically better for the detection of larger more damaging droplet impacts.

Table 2.5.1 Schedule of tests of impact shield aperture size

Date	Aperture diameter (test sensor)	Aperture diameter (reference sensor)
4, 7 February 2000	0	0
11, 14 February 2000	30	30
18, 21 February 2000	40	30
25 February, 2 March 2000	50	30
3, 6 March 2000	60	30
10, 16 March 2000	70	30
31 March, 3 April 2000	80	30

Tests of impact sensor shield aperture size and plant damage

Three impact sensors were positioned in the field within a crop of narcissus cv. Carlton. One sensor acted as a reference as above, and was positioned at the top of the crop canopy. A second, test sensor was placed adjacent to the reference sensor and 30-80 mm aperture sizes were tested (see Table 2.5.1). Another test sensor was placed at the bottom of the foliage. As splash dispersal occurs mainly at the base of the crop this sensor could be used to ascertain the effect of aperture diameter on the potential measurement of splash dispersal within the crop. Environmental data (rainfall and leaf wetness) were recorded at the same position as the reference sensor.

Bulbs cv. Carlton (12/14cm grade) were placed in an un-lit cold store (9˚C) in batches from 3 August to 21 September 1999. Six weeks after the start of cold storage, bulbs were planted, ten bulbs per 4 litre pot, in medium grade sphagnum peat, and were returned to the 9˚C cold store. The cold store temperature was lowered to 5˚C on 1 December 1999 and to 1˚C on 1 January 2000. This produced plants ready for use in experiments in batches from December 1999 onwards. Any diseased plants were not used.

Ten pots of narcissus plants were placed during each rainfall event adjacent to the impact sensors. A further 10 pots were placed nearby, as controls, under a clear polythene cover so that they did not receive raindrop impacts. After a period of 72 hours in the field both exposed and control plants were removed from the field to a glasshouse (heated to 3˚C, ventilated at 15˚C). All plants were inoculated with *B. narcissicola* (as described previously) and were misted for the next 24 hours. Flowers were removed at anthesis because decaying flowers could encourage disease. The plants were examined for symptoms of smoulder at intervals until leaf senescence. Any symptoms of *B. narcissicola* were compared with the profile of impact events.

Results

Effect of impact sensor shield aperture diameter

(i) Reference and test shield diameter 30mm

The effect of impact sensor shield aperture diameter on measurement of raindrop impacts at the same point is shown in Figure 2.5.2 (bin 1). There was no difference between the output of the two sensors over the measurement period in the field (Table 2.5.2). This indicates that the positional effect between the two sensors was not significant. Any subsequent trends in the results could be explained only by the differences in impact shield aperture diameters. The percentage of impacts assigned to bin numbers higher than 1 ranged between 16 and 23 % (Table 2.5.2).

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

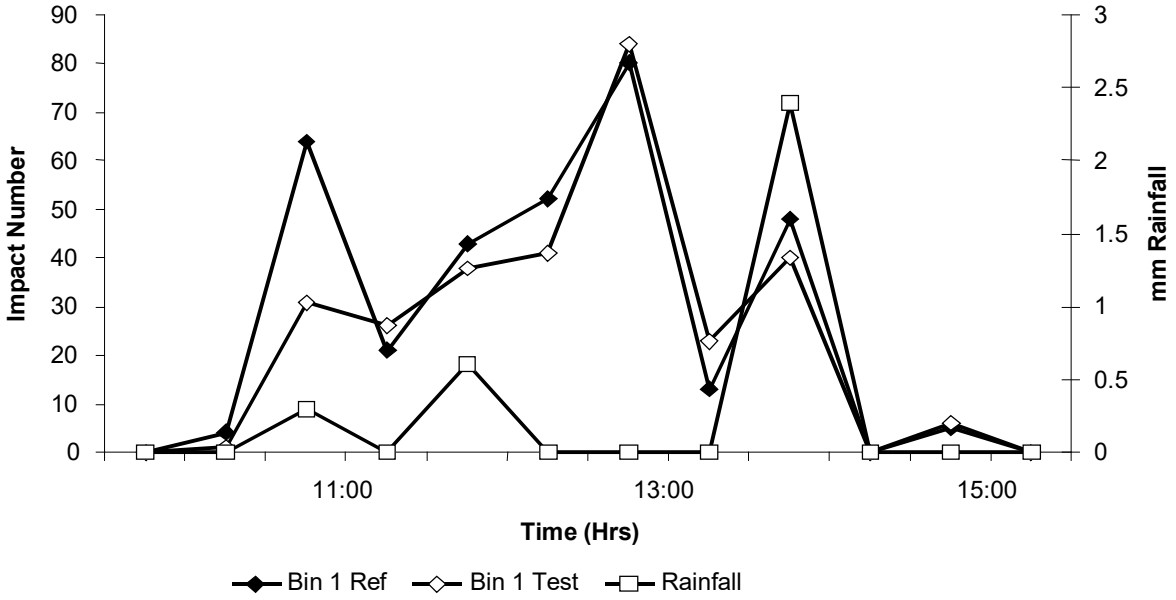


Table 2.5.2 Comparison of total impacts during rainfall (14 February 2000) of two impact sensors with equivalent shield diameters.

Sensor	Aperture size	Impact Sensor Output						
		Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Total	% (> Bin 1)
Reference	30	326	54	12	3	1	396	18
Test	30	302	58	18	9	2	389	22

(ii) Reference shield diameter 30mm / test shield diameter 40 mm

The effect of a difference in shield aperture diameter of 10 mm on the measurement of raindrop impacts at the same point is shown in Figure 2.5.3 (bin 1). There was an increase in the numbers of impacts assigned to the bin 1 category on the test sensor (40mm shield aperture). However, the numbers assigned to the higher bin numbers remained similar to the reference sensor (Table 2.5.3). The percentage of impacts assigned to bin numbers higher than 1 was between 28 and 31 % (Table 2.5.3).

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

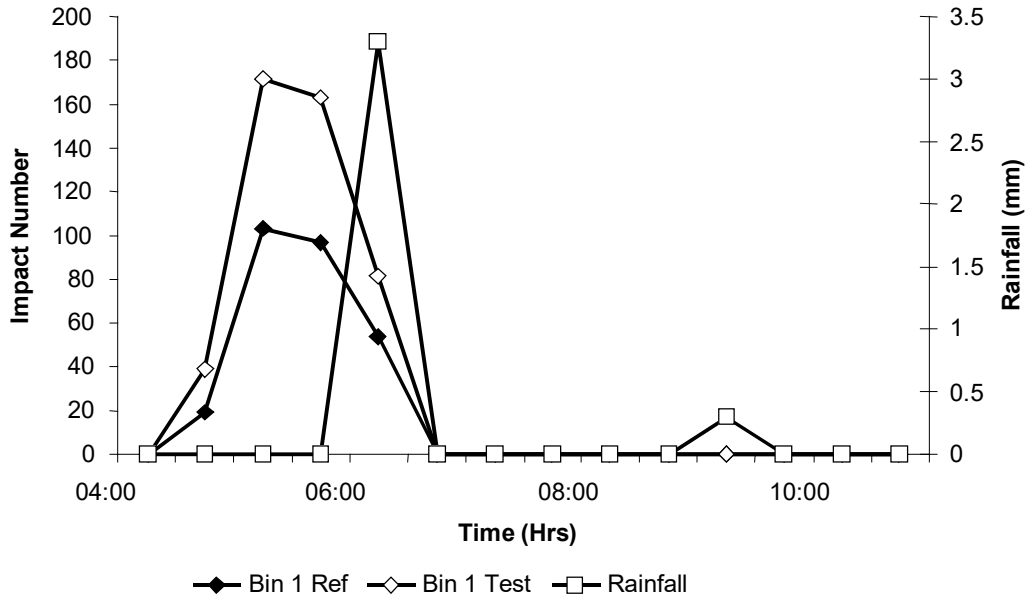


Table 2.5.3 Comparison of total impacts during rainfall (21 February 2000) of two impact sensors with shield diameters of 30 and 40 mm.

Sensor	Aperture size	Impact Sensor Output						
		Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Total	% (> Bin1)
Reference	30	273	68	29	8	4	382	28
Test	40	455	129	55	13	9	661	31

(iii) Reference shield diameter 30mm / test shield diameter 60 mm

On 3 March 2000 a shower of hail was noted at the observation site. A greater range of impact output was observed at this observation time. Increasing the difference in shield aperture diameter to 30 mm increased the number of impacts in bin 1 by 33 % (Figure 2.5.4, bin 1). However the percentage of impacts assigned to the higher Bin numbers increased in comparison to the reference sensor (Table 2.5.4). The percentage of impacts assigned to bin numbers higher than 1 was increased by 9 % on output from the test sensor (Table 2.5.4).

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

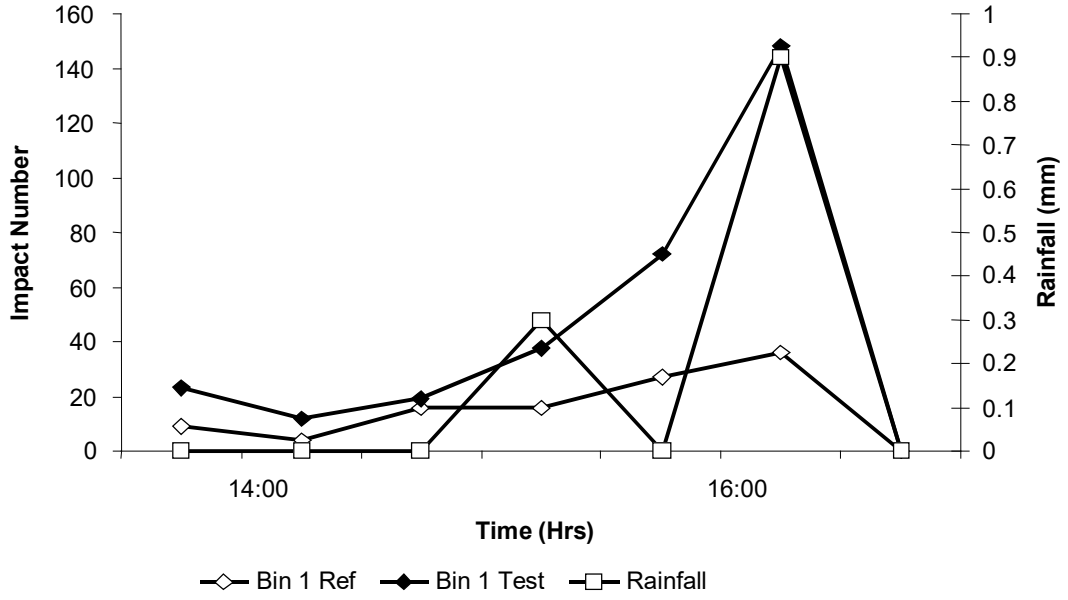


Table 2.5.4 Comparison of total impacts during rainfall (03/03/2000) of two impact sensors with shield diameters of 30 and 60 mm.

Sensor	Aperture size	Impact Sensor Output							
		Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Bin 6	Total	% (> Bin1)
Reference	30	108	17	11	4	4	2	146	26
Test	60	312	94	30	18	18	11	483	35

(iv) Reference shield diameter 30mm / test shield diameter 80 mm

A greater range of impact output was observed during observations taken on the 3 April 2000. Although hail was not noted on this day it is likely that there may have been hail present in the rainfall event. Increasing the difference in shield aperture diameter to 50 mm dramatically increased the number of impacts in bin 1 by approximately 50 %. Figure 2.5.5 (bin 1). This was likely to have been an underestimation as the maximum output (255) was reached over the sampling period. However the % of impacts assigned to the higher bin numbers increased in comparison to the reference sensor (Table 2.5.5). The percentage of impacts assigned to bin numbers higher than 1 was increased by 17 % on the test sensor (Table 2.5.5).

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

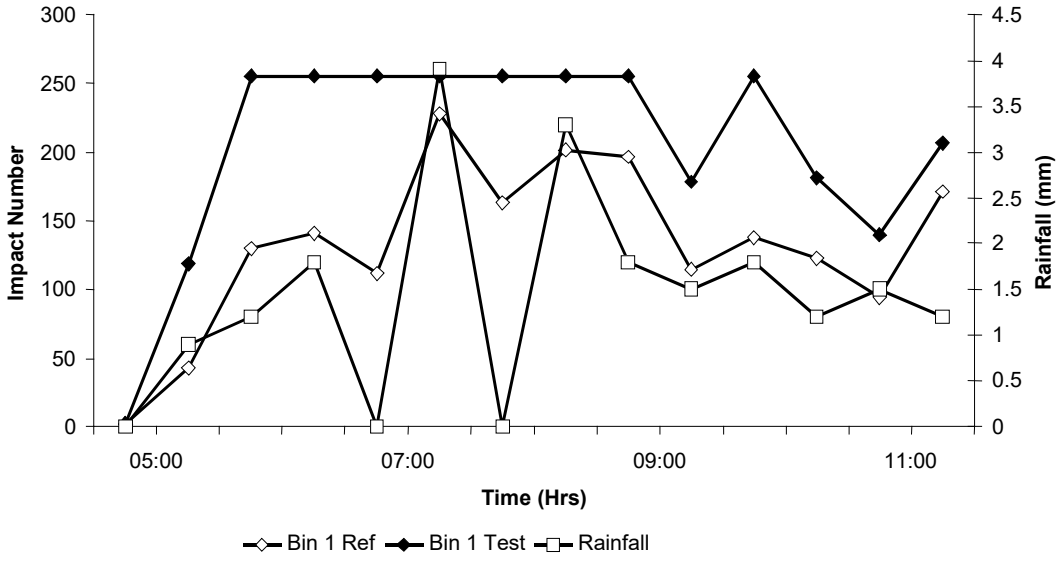


Table 2.5.5 Comparison of total impacts during rainfall (3 April 2000) of two impact sensors with shield diameters of 30 and 80 mm.

Sensor	Aperture size	Impact Sensor Output							
		Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Bin 6	Total	% (> Bin1)
Reference	30	1858	238	91	41	18	18	2264	18
Test	60	2867	786	292	144	55	25	4169	45

Effects of rainfall events on disease inoculation

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

Conclusions

- Increasing impact sensor shield diameter had a significant effect on the numbers of impacts assigned to higher bin numbers, although it was unclear what the optimal level should be to reflect narcissus leaf damage. Shield diameters of 40 mm were not observed to contribute to this effect (further data maybe required to confirm this).

- Further field data is required to determine fully the effect of shield size on impact sensor output.

- There were no rainfall events used in the tests which produced outputs in the high bin numbers > 9 indicating the absence of hail (although hail was noted at one recording time).

- There was no increase in smoulder levels observed on exposed plants to any of the rain events used in the study, although signs of damage on leaves were recorded in some instances.

- Laboratory studies are required using plants to simulate the production of varying levels of damage on narcissus leaf tissues. This could then be matched to sensor outputs. These plants should then be inoculated to determine what impact sensor output will result in higher smoulder susceptibility.

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.

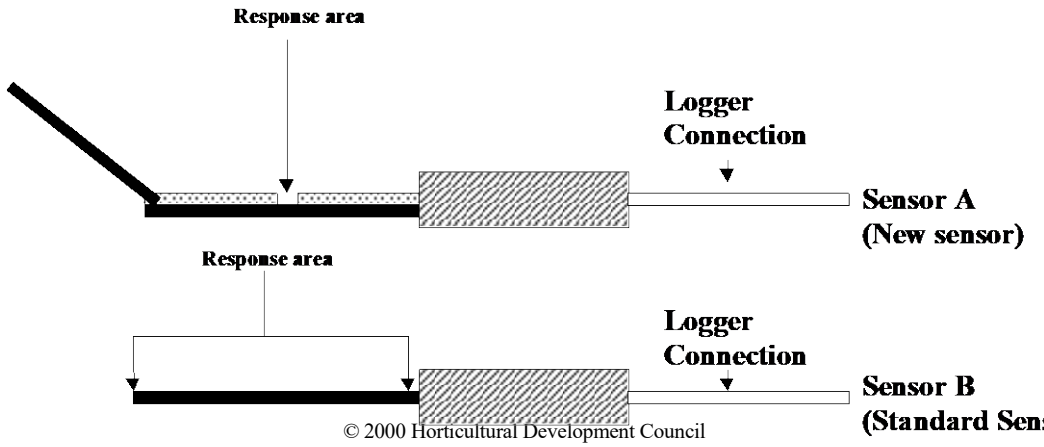
Milestones
3.1.1 Sites for investigation of leaf wetness sensors established by March 1999
3.1.2 Data on wetness characteristics of narcissus leaves and wetness sensors summarised by March 2001
3.1.3 Report of the wetness characteristics of narcissus leaves and all wetness sensors prepared by June 2002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

Progress

New wetness sensors have been received from Aardware Design and these are undergoing tests in the laboratory before being deployed in the field. A comparison of the new leaf wetness sensor and the standard leaf wetness sensor is shown in Figure 3.1.1.

Future work
The responses of the two wetness sensors will be compared alone and in combination with the changes in drop size distributions of water droplets on narcissus leaves after rainfall events. Using estimates from both sensors for example it maybe possible to relate outputs to actual changes in percentage wetness coverage on leaves at the point of measurement. Experimental protocols have been developed in readiness for this work (Appendix D).

Figure 3.1.1 Comparison of Wetness Sensor Design



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

No milestones due so far

Progress

Preliminary infection experiments with Botrytis narcissicola *on narcissus cv. Carlton*

Introduction

The effect of interactions of environmental conditions on *B. narcissicola* infection is unknown. To investigate these interactions under controlled environmental conditions standard inoculation methods are required. A range of inoculation methods was investigated based upon published papers and recommendations from Dr Tim O'Neill. It was necessary to derive the optimal inoculation conditions if larger scale experiments were to be carried out, then mathematical models describing the effect of the interaction of environmental conditions on infection by *B. narcissicola* can be described.

Prior to inoculation experiments, a reliable method for conidial production by *B. narcissicola* in culture was also required.

Methods of conidial production used for *Botrytis narcissicola*

Based on previous studies different types of culture media produced different responses by *B. narcissicola*. The following culture media were used to investigate the optimal production of *B. narcissicola* conidia:

- CAN (Calcium Nitrate Agar)
- PDA(Potato Dextrose Agar)
- V8 juice agar
- DEA (Daffodil leaf extract agar).

All cultures were incubated at 18-20°C with a 12 hour UV light/12 hour dark cycle.

Results

When *B. narcissicola* was grown on PDA large numbers of sclerotia were produced. However

growth on V8 agar gave abundant mycelium. Both CNA and DEA resulted in condial production. Daffodil leaf extract agar produced the highest conidial concentrations when cultures were grown for 7 days. Daffodil leaf extract agar grown cultures were used for all subsequent conidial production. Harvested culture plates routinely gave approximately 10^6 conidia ml^{-1}.

Methods of narcissus inoculation with *Botrytis narcissicola*

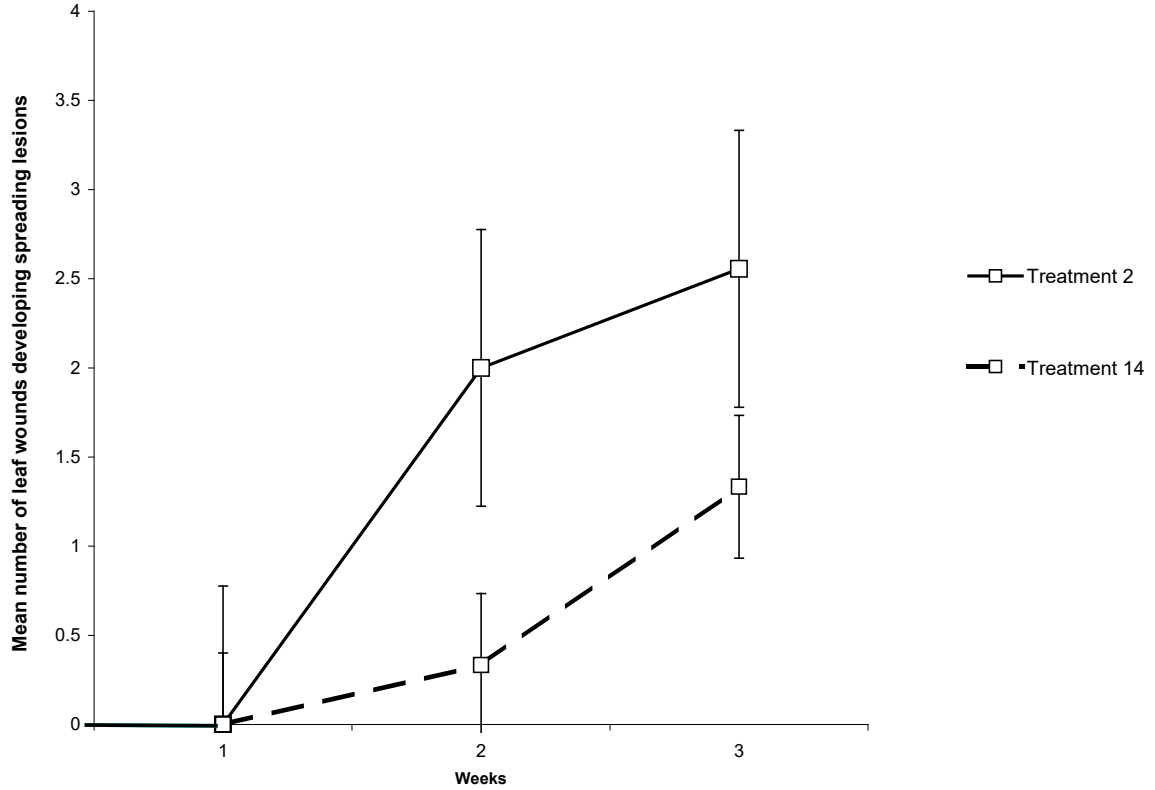
Narcissus pots containing between 6 and 10 healthy plants were greened up at 15°C and treatments started when the majority of plants had fully flowered. Plants were inoculated using a range of treatments as listed in Table 3.2.1. After inoculation plants were assessed for disease once a week, for 3 weeks or until the all leaf material had died.

Damage to the narcissus leaves was inflicted using pin-pricks arranged as ten pins mounted on a cork. The mounted pins were then used to pierce the leaf at three points on the leaf. This treatment simulated damage under field conditions, which can be caused by weather (i.e. wind, rain damage) or by the presence of mites.

Table 3.2.1 Inoculation Methods Used

Treatments	Presence/absence of spreading lesions
1. Mycelial plugs (from V8 juice agar cultures) were squashed onto intact leaves and left for 24hrs in a mist tent before being returned to 15°c glasshouse.	Absence
2. 5mm mycelial plugs were squashed on to damaged leaves and treated as in 1.	Presence
3. Intact leaves were inoculated with 20µl droplets containing 10^4 conidial suspension in water. Non-absorbent cotton wool was taped to the leaf to prevent droplet runoff and treated as in 1.	Absence
4. As 3 but inoculating damaged leaves. Leaves were damaged using a pin with a cork underneath the leaf to prevent tearing.	Absence
5. Intact leaves were inoculated with 20µl droplets containing 10^4 conidial suspension in 1 in 10 V8 and treated as in 3.	Absence
6. As 5 but inoculating damaged leaves.	Absence
7. Intact leaves were spray inoculated with 10^4 conidial suspension in water, until runoff, and treated as in 1.	Absence
8. Spray inoculated damaged leaves as in 7.	Absence
9. Intact leaves were spray inoculated with 10^4 conidial suspension in 1 in 10 V8, until runoff, and treated as in 1.	Absence
10. Spray inoculated damaged leaves as in 9.	Presence
11. Pots were preconditioned by placing plastic bags over the whole pot for 48 hours before inoculation and left after inoculation. Damaged leaves were spray inoculated with 10^6conidial suspension in neat V8 juice.	Presence
12. As in Treatment 11. Damaged leaves per spray inoculated with 10^6conidial suspensions in 1 in 10 V8 juice.	Presence
13. As in treatment 11. Damaged leaves were spray inoculated with 10^6 conidial suspensions in 1 in 10 V8 juice containing 0.05% Tween 20.	Presence
14. Preconditioned as in 11. Damaged leaves were spray inoculated with 10^5 conidial suspensions in 1: 10 V8 juice.	Presence
15. Damaged leaves were treated as in Treatment 14 .	Presence
16. As in Treatment 11. Damaged leaves were spray inoculated with 10^6 conidial suspensions in water.	Presence
17. As in Treatment 11. Damaged leaves spray inoculated with 10^5 conidial suspensions in water.	Presence
18. Preconditioned as in 11. Intact leaves spray inoculated with 10^6 conidial suspensions in 1:10 V8 juice.	Presence
19. Non-conditioned intact leaves treated as in 18.	Presence

FIGURE 3.2.1. Mean number of *Botrytis narcissicola* lesions



Results and discussion

Inoculation methods 1 – 10 (see Table 3.2.1) examined methods which previous studies had shown were successful in producing infections by *B. narcissicola* but which closely matched conditions found in the field. Results confirmed that infection will only occur if inoculations are carried out on damaged leaves. This has been previously reported by O'Neill and Mansfield (1982) who concluded that conidial inoculum typically failed to colonise healthy narcissus tissue unless inoculated tissues were damaged. Mycelial plug inoculations (method 2), on damaged leaves, developed the largest number of spreading lesions after 3 weeks, but this method and the droplet inoculation method, which produced little or no infection, do not relate closely to conditions found in the field. Subsequent inoculations were therefore carried out by applying sprays of conidial suspensions.

Leaf wetness/high humidity is an important factor determining infection by *B. narcissicola* in the field. The effect of high humidity on infection by *B. narcissicola* was investigated under glasshouse conditions by placing a plastic bag over tissues for 48 hours before inoculation. In these treatments infection was observed only if the bag was left on until onset of symptoms, with no infection if the bag was removed 24 or 48 hours after inoculation.

Conidial concentrations (10^6, 10^5, 10^4) in water and 1:10 V8 juice were sprayed on to leaves. Inoculation with conidial suspensions of 10^5 in 1:10 V8 juice gave the largest number of spreading lesions. Inoculation with an aqueous suspension of either water, or 100 % V8 juice containing 10^6 conidia ml^{-1} gave lower numbers of active lesions than equivalent treatments

with lower numbers of conidia.

Infected leaf material was taken from successful inoculation treatments and the presence of *B. narcissicola* confirmed by isolation on to PDA. Presence of *B. narcissicola* was confirmed in all cases, by characteristics such as sclerotial formation, conidial dimensions and lesion colour.

Conclusions

- Infection of narcissus leaves by *B. narcissicola* requires leaf damage.
- Optimal inoculation/incubation conditions are using spray inoculations with 10^5 conidial suspension in 1:10 V8 juice followed by humid incubation until lesion appearance.
- High humidity may be essential for successful infection**.**

Controlled environment studies on the effect of temperature and wetness duration on conidia of B. narcissicola

Introduction

Airborne fungal pathogens require the presence of environmental conditions, notably wetness, to complete critical stages in their lifecycles. The presence of bound or unbound water is a requirement for disease development by many species of *Botrytis* (Clarkson *et al*., 2000). The relationships between 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 temperature and wetness. Validated relationships derived from controlled environment experiments can be used to form the basis of a forecasting system for timing the applications of fungicides for control of *B. narcissicola*. Only robust mathematical models could be used to determine pathogen responses as conditions fluctuate in the field. Without models describing rate functions it would be impossible to assess the likely impact of environmental conditions in the field.

Materials and Methods

Production of plant material Bulbs cv. Carlton (12/14cm grade) were placed in an un-lit cold store (9˚C) in batches from 3 August to 21 September 1999. Six weeks after the start of cold storage, bulbs were planted, one bulb per FP7 pot, in a mixture of 70:30 Fisons F2 compost and sand, and were returned to the 9˚C cold store. The cold store temperature was lowered to 5˚C on 1 December 1999 and to 1˚C on 1 January 2000. This produced plants ready for use in experiments in batches from December 1999 onwards. Approximately 3 days before use plants were transferred to a glasshouse at a 16/14°C day/night temperature regime. All bulb material was grown at HRI Kirton and transported to HRI Wellesbourne 2 days prior to the experiment. Uneven, damaged or atypical plants were rejected. On the day before the plants were inoculated they were placed at high humidity prior to transfer to the controlled environment cabinets. Sixty pots (experiments 2 and 3) or one hundred pots (experiment 4 and 5) each were inoculated at 4, 8, 12, 16, 20 and 24°C.

Production of B. narcissicola inoculum and inoculation of plants Daffodil leaf extract agar (DEA) produced high conidial concentrations when cultures were grown for 7 days (see previous section). Culture plates of DEA used to produce conidia were produced as described

previously. Harvested culture plates routinely gave approximately 10^7 conidia ml^{-1}. Conidia were harvested and the concentration estimated using a haemocytometer. Final inoculation concentration of approximately 10^5 conidia ml^{-1} (experiments 2 - 4 or 5 x 10^4 conidia ml^{-1} (experiment 5 only) was produced in V8 liquid. Plants were inoculated using an atomiser. Approximately 2 ml inoculum was applied per plant in all controlled environment experiments. In Experiments 2 and 3 plants were sprayed at each temperature for 0.2 min every 9.8 min with distilled water to maintain conditions of leaf wetness after inoculation. In experiments 4 and 5 plants were maintained at a humidity of approximately 96% without leaf wetting. In experiments 2 and 3 approximately six plants were removed using a random numbering system from each temperature at 6, 12, 18, 24, 30, 36, 48, 54, 60 and 72h and air-dried. In experiments 4 and 5 ten pots were randomly removed from each cabinet at the above time periods after inoculation and air-dried. All plants were labelled before 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

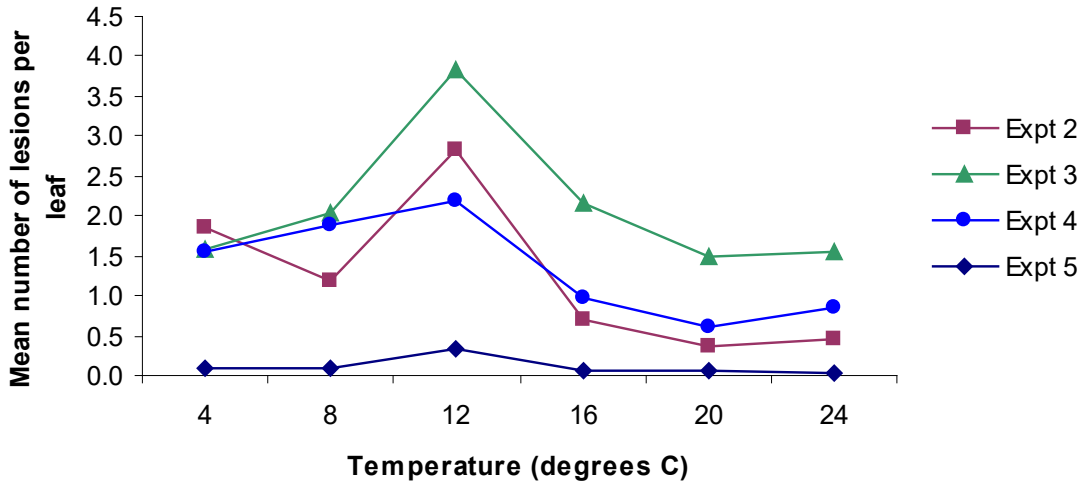
Method of simulating plant damage The plants in controlled environment experiments were damaged using two methods. In experiments 2 and 3 plants were damaged by using pins which were either drawn along the surface of the epidermis or which directly penetrated the epidermis at several pre-set points on each leaf. This produced break points on the leaf surface, which could be directly exploited by conidia of *B. narcissicola*. As this was a severe method of simulating epidermal damage, the method of producing plant damage was changed in experiments 4 and 5. In experiments 4 and 5 soft bristle brushes were used on the leaf to remove surface wax. All leaves on all plants had the brushes drawn over the upper surface of the leaf at least twice.

Results

The results of four controlled environment experiments are summarised in Figures 3.2.2 and 3.2.3. Experiment 1 was not successful, due to the failure of the wetting system in the two of the controlled environment cabinets. The results of this experiment are not presented for this reason.

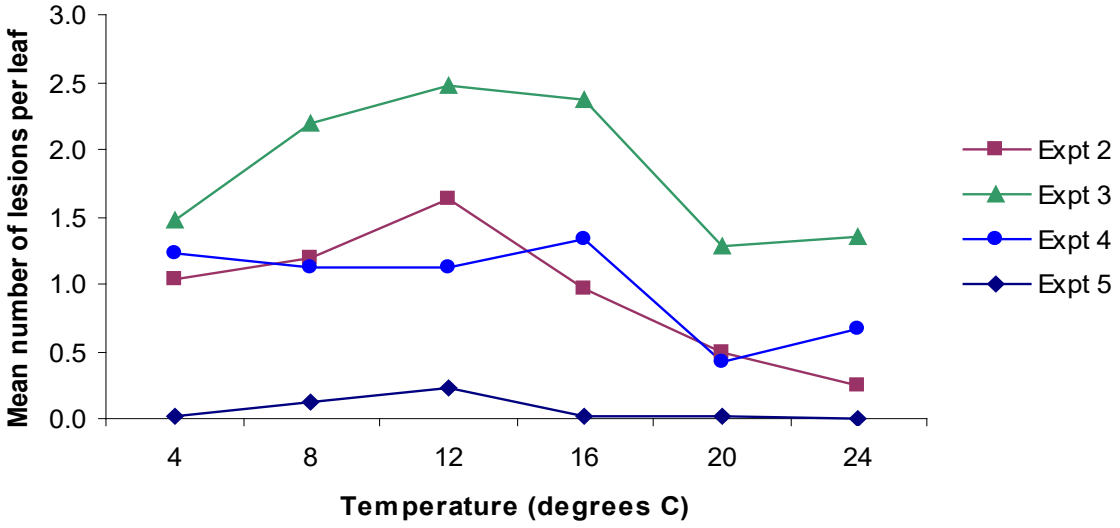
Effect of temperature and wetness duration on infection of narcissus by B. narcissicola *(6 hours wetness duration)* Short durations of wetness were sufficient for infection of narcissus by *B. narcissicola* (Figure 3.2.2). For substantial infection the presence of free water is required. However, some infection can occur at optimal temperatures under conditions of high humidity. The results show that the optimal temperature for infection was 12˚C, as there was an increase in the numbers of lesions at this temperature after 6 h wetness duration. Temperatures of 20 and 24˚C were less favourable for infection by *B. narcissicola.* There was higher infection at temperatures of 4 and 8˚C after six hours wetness.

Figure 3.2.2 Average number of lesions per leaf at 4 - 24 C (6 hours wetness duration)



Effect of temperature and wetness duration on infection of narcissus by B. narcissicola *(24 hours wetness duration)* When infection was assessed after 24 h of wetness, temperatures of 4, 8, 12 and 16˚C gave higher levels of leaf infection in comparison to temperatures of 20 and 24˚C (Figure 3.2.3). The presence of free water in comparison to relative humidities of 96% was more favourable for infection. After 24 hours wetness duration there was little difference in the level of infection on leaves at temperature of 4, 8, 12 and 16˚C.

Figure 3.2.3 Average number of lesions per leaf at 4 - 24 C (24 hours wetness duration)



Conclusions

- The presence of tissue damage was essential for infection by *B. narcissicola* on immature narcissus leaves.
- The presence of free water was required for infection of narcissus tissues by *B. narcissicola*
- Temperatures of 12˚C are optimal for infection by *B. narcissicola* at short wetness durations (approximately 6 h)
- Temperatures of 4 – 16˚C were optimal for infection *B. narcissicola* at relatively long wetness durations (approximately 24 h)
- The temperature and wetness durations favouring infection of mature narcissus tissues by *B. narcissicola* should be investigated.

Preliminary infection experiments of narcissus cv. Carlton plants with Ramularia vallisumbrosae

Introduction

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

Materials and methods

Methods of conidial production Previous studies had indicated that culture media had an important effect on which life cycle stage *R. vallisumbrosae* produced. The following culture media were used in experiments:

- CNA (Calcium Nitrate Agar)
- PDA (Potato Dextrose Agar)
- V8 juice agar
- DEA (Daffodil leaf extract agar)
- BA (Barley agar a substitute for Oatmeal agar).

All cultures were incubated at 18-20°C with a 12hour UV light/12 hour dark cycle.

The results showed that PDA, CNA, V8 juice agar and BA media resulted in production of sclerotia, DEA agar produced abundant mycelium and the fastest mycelial growth. The greatest spore production occurred on BA with some conidial production on DEA. Cultures were typically ready for spore collection after 2-3 weeks. Barley agar and daffodil leaf extract agar was used for all subsequent conidial production. Harvested conidial suspensions were then mixed prior to inoculation.

Methods of narcissus inoculations Narcissus pots containing between 6 and 10 healthy plants were greened up at 15°C and treatments started when the majority of plants had fully flowered. Plants were treated as detailed below and assessed for disease once a week, for 3 weeks or until all the leaf material had died.

Damage to the narcissus leaves was inflicted using pin-pricks. Damage in the field can be caused by weather (i.e. wind, rain damage) or by the presence of mites.

The treatments were as follows:

1 Pots were preconditioned by placing plastic bags over the whole pot for 48 hours before inoculation. Bags were replaced after inoculation. Damaged leaves were spray inoculated with 10^6 conidial suspension in a 1:10 V8 juice mixture.
2 Preconditioned as in 1. Damaged leaves were spray inoculated with 10^6 conidial suspensions in water.
3 Preconditioned as in 1. Intact leaves were spray inoculated with 10^6 conidial suspensions in 1:10 V8 juice.
4 Non-conditioned intact leaves were spray inoculated with 10^6 conidial suspensions in 1 : 10 V8 juice.

Results

Earlier experiments indicated that spray inoculation was the only reliable method of inoculation. To simulate field conditions and increase infection in the glasshouse plants were placed in plastic bags for 48 hours before inoculation. Control plants were not placed in plastic bags before inoculation. Previous studies (Gregory, 1939) had placed inoculated narcissus leaves in a moist atmosphere, either a bell jar or sealed test tubes.

Inoculation with conidial suspensions of 10^6 conidia ml $^{-1}$ in water and 1:10 V8 juice were compared. The suspension media which gave the largest number of active lesions per leaf was 1:10 V8 juice.

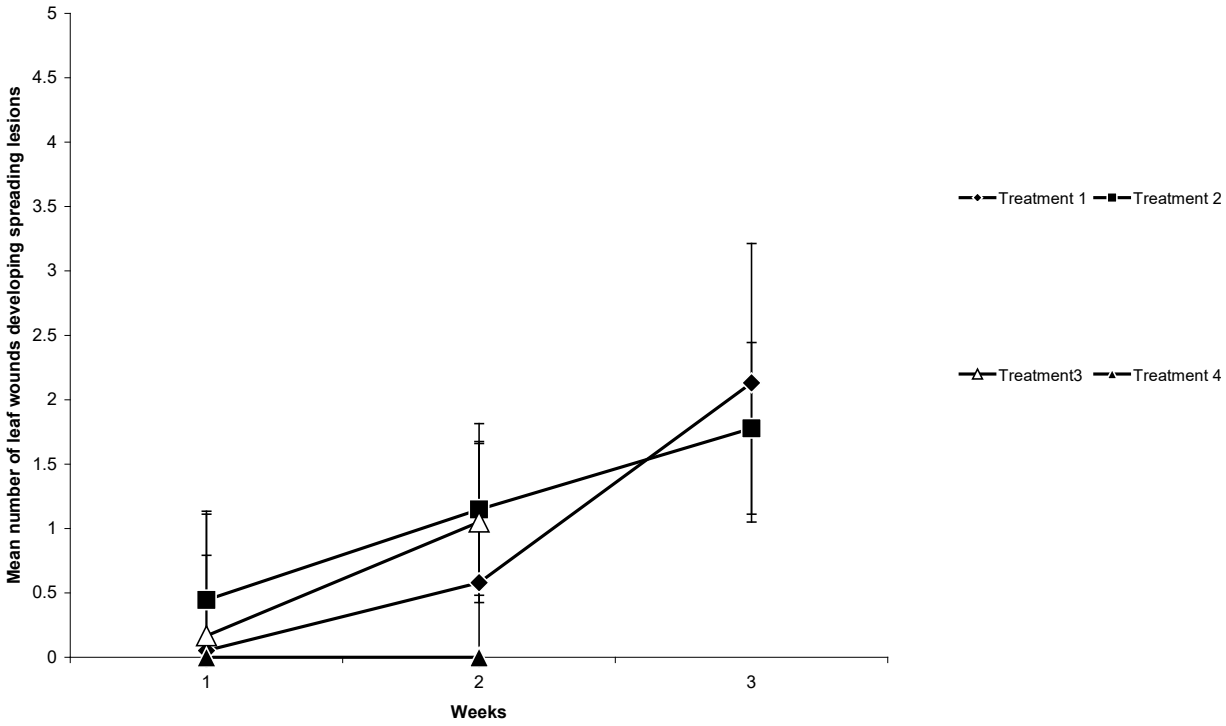
Inoculations were also carried out on intact and damaged leaves. Results (Figure 3.2.4) indicated that the pathogen could infect undamaged leaves, although a longer period of time was required for lesion spread. Some leaves remained unaffected throughout, even in a plant with infected /sporulating leaves. Plants were not assessed after 3 weeks because the majority of leaves had died. Plants without disease were still healthy after 3 weeks and symptoms of natural dieback were different from those of disease.

Discussion

- Optimal inoculation conditions seem to be preconditioned spray inoculated plants with 10^6 conidial suspension in 1:10 V8 juice on intact or damaged leaves.
- High humidity/wetness may be essential for infection to occur because:
  - Loss of free water required for conidial germination by evaporation.
  - Leaf age and tissue status, may affect infection, however placing a bag over tissues may standardise the responses of all narcissus tissue types.

Lack of narcissus material has prevented further investigations into *R. vallisumbrosae* infection.

FIGURE 3.2.4 *Ramularia vallisumbrosae* inoculation methods (mean number of lesions)



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. vallisumbrosae* and *B. narcissicola* by June 2002

No milestones due so far

Progress

This work is 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 - 4.1.3 achieved

(a) Laboratory studies

Attached leaf assays

The evaluation of novel and standard fungicides for protective and curative activity against infection by *B. narcissicola* and *R. vallisumbrosae* on narcissus leaves was completed. The methodology used was as reported for initial tests (see first Annual Report, April 1999), except that inoculated leaves were incubated in an illuminated potato store maintained at

15ºC. This was because of high temperature experienced in later glasshouse tests, which reduced the incidence of successful infection on control (untreated) leaves.

The fungicides tested were as follows:

- First set (see first Annual Report):
  - Ronilan (50% vinclozolin) at 1 ml / litre
  - Bravo 500 (500 g/litre chlorothalonil) at 3 ml / litre
  - Benlate (50% w/w benomyl) at 0.5 g / litre
  - Dithane 945 (80% w/w mancozeb) at 1.5 g / litre
  - Amistar (azoxystrobin) at 1 ml / litre
  - Stroby (50% kresoxim-methyl) at 0.625 g / litre
  - Exp H (Kif 3535) at 0.8 g / litre
  - Scala (400 g/litre pyrimethanil) at 2.0 ml / litre
  - Shirlan (50% fluazinam) at 1 ml / litre
  - Unix (50% cyprodinil) at 0.67 g / litre
  - Benlate at 0.5 g / litre in mixture with Dithane 945 at 1.5 g / litre
  - Bavistin DF (50% w/w carbendazim) at 1.1 g / litre
- Second set (not previously tested):
  - Plover (250 g/l difenoconazole) at 0.25 ml/litre
  - Folicur (250 g/l tebuconaole) at 1.0 ml/litre
  - Opus (125 g/l epoxiconazole) at 1.0 ml/litre
  - Punch C (125 g/l carbendazim + 250 g/l flusilazole) at 0.8 ml/litre
  - Compass (167 g/l iprodione + 167 g/l thiophanate-methyl) at 3.0 ml/litre
  - Bravocarb (100 g/l carbendazim + 450 g/l chlorothalonil) at 2.0 ml/litre
  - Bavistin + Dithane (50% w/w carbendazim + 80% w/w mancozeb) at 0.5+1.5 g/l

Efficacy against *Botrytis*

Fungicides in the first set which gave good control as protectant sprays, at the rates tested, were Ronilan, Bravo 500, Benlate/Dithane, Scala, Unix and Shirlan (Figure 4.1.1). Benlate, Dithane, Bavistin and Stroby appeared relatively ineffective. The result with Amistar appeared anomalous. All fungicides in the second set reduced development of *Botrytis* leaf rotting when applied as preventative sprays, with Plover, Folicur, Opus, Punch C and Compass appearing more effective than other treatments (Figure 4.1.2). The poor control of *B. narcissicola* achieved with Benlate and Bavistin may indicate resistance to the MBC group of fungicides in some isolates of the fungus.

When applied as curative sprays (2 days after inoculation), none of the treatments in either set were as effective as when used preventatively, although Ronilan, Scala, Unix, Plover and Folicur gave more than 50% control (Figures 4.1.1 and 4.1.2). The effectiveness of the new anilinopyrimidine group of fungicides (Scala, Unix and Experimental) against *Botrytis* was demonstrated.

Efficacy against *Ramularia*

In the first experiment, Benlate + Dithane, Amistar and Scala all prevented establishment of *R. vallisumbrosae* when applied as protectant sprays (Fig 4.1.3). Ronilan and Dithane were ineffective, and other treatments were intermediate.

Benlate/Dithane, Amistar and Scala were the most effective treatments when applied 2 days

after inoculation, with Bravo, Stroby, Unix, Experimental and Shirlan only slightly less effective (Figure 4.1.3). When Benlate and Dithane were used together in a mixture, the degree of control was greater than using either product alone.

A further experiment was carried out in July 1999, evaluating both protectant and curative activity of nine further fungicides (set 2). However, leaves developed secondary bacterial infection and results were largely inconclusive; there was some evidence that Bravocarb gave protectant activity.

Full results are tabulated in Appendix E.

Fig 4.1.1: Fungicide efficacy against *B. narcissicola*
Primary screen - set 1

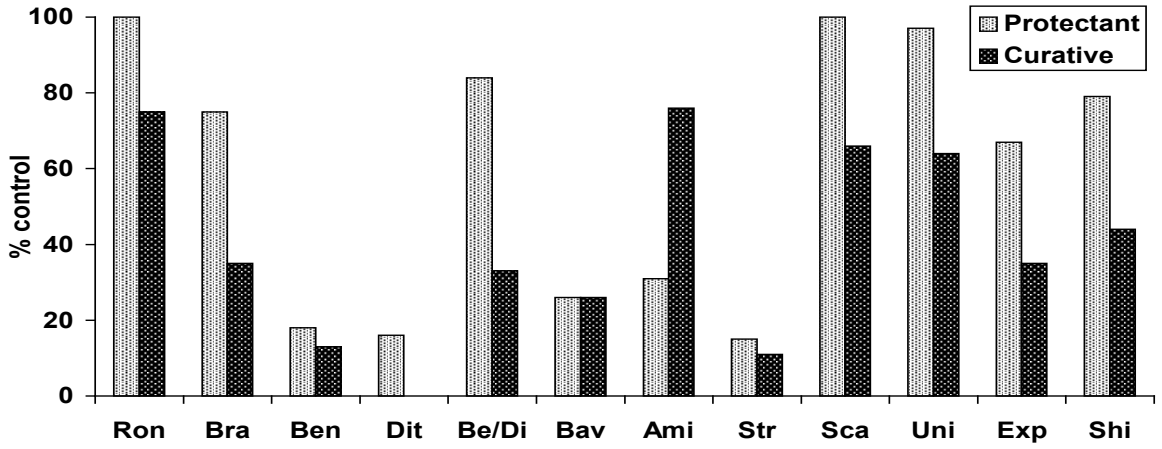


Fig 4.1.2: Fungicide efficacy against *B. narcissicola*
Primary screen - set 2

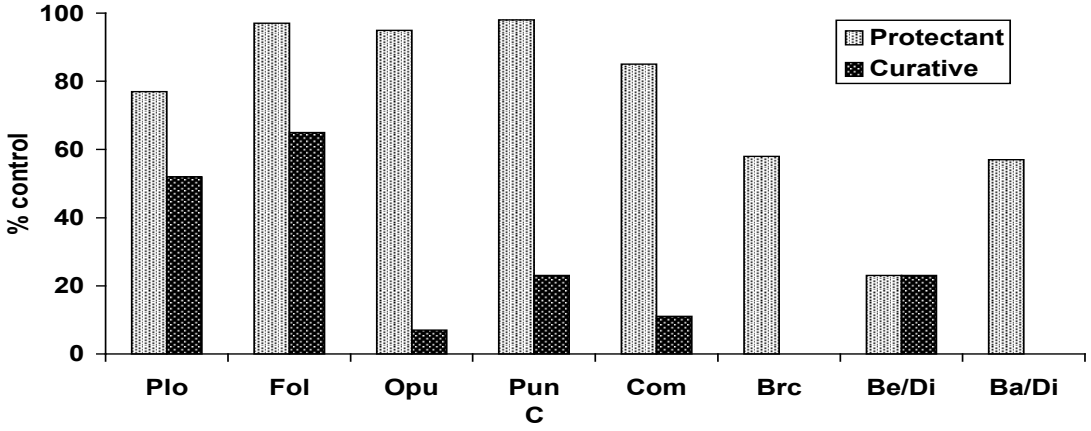
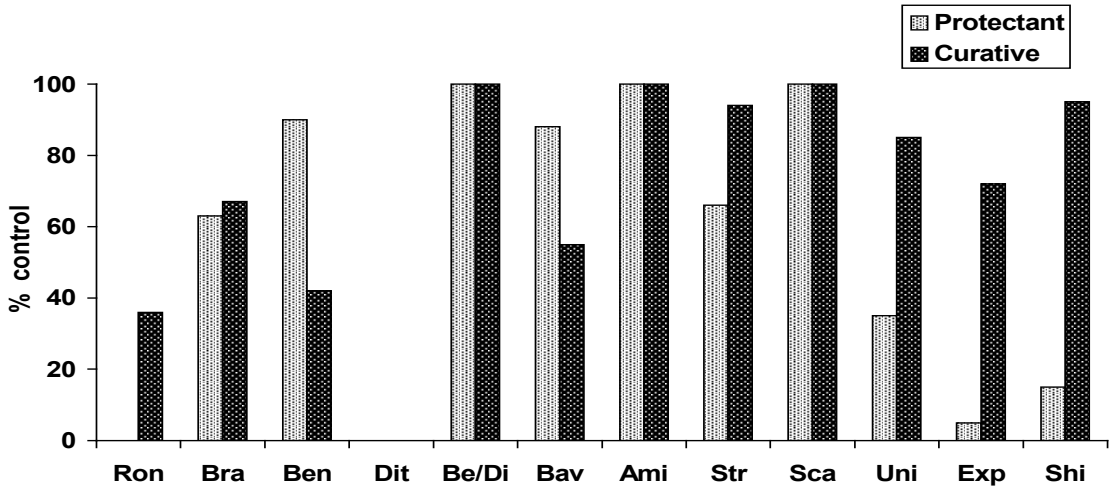


Fig 4.1.3: Fungicide efficacy against *R. vallisumbrosae*
Primary screen - set 1



(b)Field experiment – at commercial site in Cornwall

Treatment and monitoring of the field trial at Nancledra, Cornwall, was completed (see first Annual Report). The predominant disease in the crop was white mould. A final assessment was made on 7 May 1999, after flowering, when the percentage leaf area that was brown was estimated in the central two rows of each plot. There were some very clear differences

between the treatments. Three of the spray programmes resulted in very good control of white mould (Table 4.1.1), with statistically highly significant (P<0.001) differences between treatments.

Table 4.1.1 Comparison of Benlate + Dithane spray programmes for control of white mould on narcissus, cv Cheerfulness, Cornwall, 1999

No. of sprays	Timing of sprays *					Mean % leaf area dead (7 May)
	27/2	11/3	22/3	1/4	13/4	
1. Nil	-	-	-	-	-	73.7
2. Five	+	+	+	+	+	4.4
3. Four	-	+	+	+	+	5.0
4. Three	-	-	+	+	+	6.3
5. One	+	-	-	-	-	83.7
6. Two	+	+	-	-	-	56.2
Significance						<0.001
SED (15df)						4.60

* Benlate (0.5 kg/ha) + Dithane 945 (1.5 kg/ha) applied at 1,000 litres/ha

No white mould was present in the trial when the full spray programme commenced on 27 February, and a total of only 11 lesions were found in the whole trial area when the disease was first confirmed on 4 March. Interestingly, the spray programme (T4) which commenced on 22 March, 18 days after white mould was first confirmed in the trial, was as effective as the full programme (T2) which started before the disease was seen. Possible explanations for this good control from a slightly late-start programme (T4) are:

- Weather was not conducive to infection between 4 and 22 March, and white mould was still at a very low level on 22 March when T4 commenced (an average of 23.8 white mould lesions per plot (per 16 metres of ridge) was found on untreated plants on 6 April).
- The Benlate + Dithane treatment applied on 22 March was able to halt any infections which had occurred in the previous 18 days.
- Weather after 22 March, although favouring white mould development, was not highly conducive to the disease. A beneficial effect from early sprays might be expected when there is a prolonged period of weather favourable to the disease.

In due course, disease progress will be analysed in relation to weather and leaf wetness data collected at this site.

(c)Field experiments – at ADAS Arthur Rickwood and HRI Kirton

Methods

Replicated plots of cvs Carlton and Cheerfulness were planted in September 1998 at ADAS Arthur Rickwood and HRI Kirton in preparation for fungicide trials in their second year (2000). Details of the plantings are as given in the first Annual Report and in Appendix C. Further, identical planting were made in September 1999, in preparation for experimental

work in 2001.

No natural infection by white mould was observed on crops during the first year's growth in 1999. *Botrytis* occurred on many plants of both varieties after flowering. In order to increase the likelihood of disease occurrence in their second year, plots of cv. Carlton were inoculated on 13 May 1999 after flowering with *B. narcissicola* and plots of cv. Cheerfulness with *R. vallisumbrosae*. Mycelial plugs of *B. narcissicola* (98/23) were inserted into open stem ends after flower removal (ADAS Arthur Rickwood) or between the bases of leaves (HRI Kirton). Ten plants were inoculated per plot. Plots of cv. Cheerfulness were inoculated with naturally infested narcissus leaf debris bearing white mould lesions. Two mesh bags, containing debris, were pegged in position between plants in the central rows of each plot.

Plots of cv. Cheerfulness were further inoculated in spring 2000 by placing sporulating agar-plate cultures of the fungus in ridges (21 February), by addition of leaf debris bearing *R. vallisumbrosae* into guard rows (10-17 March), by inoculation of 3 plants in each guard row (17 March) and by introducing green narcissus leaves bearing white mould lesions into the trials.

Treatments were as follows:

Smoulder trial - 2000 (on cv. Carlton)
1. Untreated (double replication)
2. Benlate (0.5g/litre) + Dithane (1.5g/litre)
3. Ronilan (1ml/litre)
4. Bravo 500 (3 ml/litre)
5. Scala (2ml/litre)
6. Amistar (1ml/litre)
7. Folicur (1ml/litre)
8. Unix (0.67 g/litre)

White mould trial – 2000 (on cv. Cheerfulness)
1. Untreated (double replication)
2. Benlate (0.5g/litre) + Dithane (1.5g/litre)
3. Bavistin DF (0.5g/litre) + Dithane (1.5g/litre)
4. Bravo 500 (3 ml/litre)
5. Scala (2 ml/litre)
6. Amistar (1 ml/litre)
7. Folicur (1 ml/litre)
8. Stroby (0.625 g/litre)

Sprays were applied to each crop at 1,000 l/ha on five or six occasions at 2-3 week intervals (to date five sprays have been applied to cv. Carlton and three to cv. Cheerfulness).

All fungicide trials were irrigated with 5 mm of water, initially once and increasing to 2-3 times a week if the crop was dry. No irrigation was applied when 5 mm of rain had occurred in the previous 2-3 days, or for 48 hours after fungicide application.

Both trial areas at each site were assessed for smoulder, counting the proportion of shoots

emerging as primaries, the number of leaf lesions, the number of stem rot lesions after flower picking, and estimating the % leaf area affected by disease and % green leaf area. Samples of various leaf symptoms and stalk end rots were examined microscopically and by isolation to determine the cause.

Results

Smoulder was confirmed in all four trials. Results to date are shown in Tables 4.1.2 - 4.1.4. At the time of writing (8 May) no white mould had developed at either of the sites.

Smoulder on cv. Carlton, ADAS Arthur Rickwood, Cambs Fungicide treatment had no significant effect on the number of smoulder primaries, which ranged from 0.07 to 0.13 /100 shoots on 8 February 2000, or on the number of leaf lesions (Table 4.1.2). However, by 13 April, shortly after flower picking and following three spray applications, there were large differences between treatments (Table 4.1.2). The number of rotting stem ends was significantly reduced by Ronilan, Scala, Folicur, Unix and Amistar. Benlate + Dithane and Bravo 500 were ineffective. The cause of stem rotting was determined as *B. narcissicola*.

A large increase in the number of smoulder leaf lesions occurred in late-April/early-May. These showed as distinctive, sharply defined elongated oval lesions, grey-brown in colour, usually towards the middle of the leaf length and often on the bend of a leaf. *B. narcissicola* was consistently isolated from this symptom. With time, there was associated leaf yellowing and withering, and by 5 May the untreated plots were clearly showing more dieback caused by smoulder than all other plots. Occasionally, similar lesions also occurred on flower stems.

Smoulder on cv. Carlton, HRI Kirton, Lincs Again, fungicide treatment had not reduced the number of primary symptoms when the crop was assessed in February. By 15 April, the disease had increased considerably with over 13 lesions/100 leaves in untreated plots, and there were significant differences between treatments (Table 4.1.3). The disease was greatly reduced by Ronilan and Folicur, and moderately so by Bravo 500, Scala and Unix. Amistar had little effect, whilst Benlate plus Dithane was ineffective.

Smoulder on cv. Cheerfulness, ADAS Arthur Rickwood, Cambs. and HRI Kirton, Lincs At the Lincs site assessed on 6 April, none of the treatments had resulted in a large reduction in smoulder. At the Cambs site assessed on 4 May the incidence of smoulder on both leaves and stems appeared to be reduced by all treatments (Table 4.1.4), although differences were not statistically significant.

These trials are all continuing and further disease assessments will be made.

Discussion

These trials are continuing and after the final assessments are made the results will be examined to see what general conclusions can be drawn. Initial examination of the results indicated:

- no control of smoulder primary symptoms with any of the fungicide treatments
- good control of smoulder secondary symptoms with Ronilan, Scala, Folicur and Unix
- poor control of smoulder with Benlate + Dithane, and inconsistent control with Bravo 500
- a large increase in secondary symptoms after flower picking
- recognition of oval, grey-brown leaf lesions as a secondary symptom of smoulder.

These symptoms have been reported previously following inoculation of narcissus leaves with conidia of *B. narcissicola* (O'Neill & Mansfield, 1982), but have not described as a common field symptom.

Table 4.1.2 Comparison of fungicide sprays for control of smoulder on narcissus, cv Carlton, Cambs, 2000

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

4 sprays applied : 17 Feb, 13 Mar, 31 Mar, 19 Apr

Table 4.1.3 Comparison of fungicide sprays for control of smoulder on narcissus, cv. Carlton, Lincs, 2000

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

4 sprays applied : 17 Feb, 10 Mar, 20 Mar, 31 Mar

Table 4.1.4 Comparison of fungicide sprays for control of smoulder on narcissus, cv Cheerfulness, 2000

Treatment	Mean no. *Botrytis* lesions / 100		
	Cambs (4 May)		Lincs (6 Apr)
	Leaves	Stems	Both
1. Untreated	2.91	1.10	3.03
2. Benlate + Dithane	1.51	0.70	2.50
3. Bavistin + Dithane	1.18	0.32	3.16
4. Bravo 500	1.24	0.57	2.84
5. Scala	1.19	0.40	4.33
6. Amistar	1.58	0.27	4.16
7. Folicur	0.88	0.38	3.14
8. Stroby	1.28	0.84	2.56
Significance	NS	NS	NS
SED (25 df)			
between trts	0.806	0.569	0.694
vs control	0.698	0.493	0.601

Sprays applied: Cambs, 13 Mar, 31 Mar, 19 Apr; Lincs, 13 Mar, 31 Mar.

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

ACKNOWLEDGEMENTS

The authors would like to thank all the industrial partners for their enthusiastic support of the project, and especially where help has been given 'on site'. We thank the following for their skilful help in carrying out the work: Mr Arthur Andrews (Winchester Growers Ltd), Mrs Linda Withers and Mr Rod Asher (HRI Kirton), Mr Giles Budge (ADAS Arthur Rickwood), Miss Kathryne Byrne (HRI Wellesbourne) and Mr David Lockley (ADAS Mamhead).

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APPENDIX A
Protocols for culture of *Botrytis narcissicola* and *Ramularia vallisumbrosae*

Botrytis narcissicola

Isolates

Isolates AR 98/21, 98/23, 98/105 and 98/108 were pathogenic to wounded narcissus leaves when tested in January 1999. Isolate 98/23 was the most aggressive and should be used for inoculation experiments. This isolate also produced fleck lesions on detached, undamaged leaves.

Agar media

Mycelial growth is rapid on V8 juice agar, PDA and daffodil leaf extract agar. PDA and daffodil leaf extract agar appear slightly better that V8 for conidial production.

Conidial production

Incubate cultures in a black light unit at ambient laboratory temperature. Cultures should be supported on a shelf 320 mm below the light source. Exposure to cycling 12 hour periods of black light (wavelength 300-380 nm) and 12 hour periods dark will induce sporulation.

Sclerotial production

Sclerotial production by isolate 98/23 was abundant on V8 juice and daffodil leaf extract agar, moderate on PDA.

Ramularia vallisumbrosae

Isolates

Isolates AR/98/101, 98/102a, 98/102b and 98/120 all caused a high incidence of rotting at wound sites on narcissus leaves. Isolates 98/101 and 98/102a were slightly more aggressive than other isolates. It took around 10-14 days for lesions to appear on detached leaves, 15-21 days on attached leaves.

Agar media

R. vallisumbrosae grew very slowly (1-2 mm/day) on PDA, V8 and oat meal agar (OMA). OMA consistently resulted in the best spore production by isolates.

Conidial production

Incubate cultures on OMA at 18°C in the dark. Conidia are produced after 10-14 days.

Resting body production

Incubate cultures on OMA or V8 juice agar at 18°C in the dark.

APPENDIX B
Details of 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

APPENDIX C
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	Kirton
						2	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	Kirton
						2	Arthur Rickwood
Smoulder	Monitoring	Carlton	L W van Geest	Lincs	10-12	2	Kirton
			Lingarden	Lincs	10-12	2	Arthur Rickwood
Smoulder	Field trial (see 4.1(b))	Carlton	O A Taylor	Lincs	10-12	2	Kirton
						2	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	Kirton
						2	Arthur Rickwood
White mould	Field trial (see 4.1(b))	Cheer-fulness	Winchester Growers	Cornwall		2	Kirton
						2	Arthur Rickwood
Smoulder	Monitoring	Carlton	F Dring	Lincs		2	Kirton
			Lingarden	Lincs		2	Arthur Rickwood
Smoulder	Field trial (see 4.1(b))	Carlton	O A Taylor	Lincs		2	Kirton
						2	Arthur Rickwood

APPENDIX D

Protocol for section 3.1: Investigation of estimation of narcissus leaf wetness characteristics and crop drying time

Part 1 Tests of existing wetness sensors (leaf drying)

1. Comparisons to be made between existing Aardware design wetness sensors and wetness characteristics of narcissus leaves.
2. Three existing sensors to be placed in the crop or adjacent to 10 – 20 marked leaves in the field (one height only). Leaves are to be held in a horizontal position (may require attaching leaves to clamps or stands) *in situ*.
3. Temperature, humidity should also be logged at the same height as the wetness sensors. Rainfall amount and duration's also to be recorded outside the canopy.
4. Known amounts of water (to be ascertained according to sensor capacity) to be placed on the sensor and all leaves at the same time each day and recording made of the drying time on the sensor and on the marked leaves. Recordings should be taken on an hourly basis with details of the times of observations taken. Leaves to be confirmed as wet or dry (when no visible wetness) by use of indicator dye (details to be supplied by RK).
5. Approximately 20 different comparisons should be recorded with at least 8 – 10 observations to be taken for each event. A fresh set of leaves at approximately the same age should be used for each run.
6. Statistical tests to be made between drying times. Further variables could be added by investigating the drying times on leaves of different ages or testing sensors with different coatings (though it had been noted by RK that Aardware were not keen to pursue coating sensors). The latter may be considered if there are big differences in the estimated and observed drying times on leaves of different ages.
7. Observations would also be taken after periods of rainfall both on marked leaves and from the sensors (as described above) as a separate data set.
8. All experiments to be carried out at HRI Kirton by staff based at HRI Kirton.

Part 2 Tests of new wetness sensors designs (crop drying)

1. Aardware Design to build new sensor (based on discussions at Aardware design 23/6/99) with multiple levels of output (Sensors would be compartmentalised with possibly 4 compartments each with varying levels of rims. Aardware Design to supply details of when new sensors might be available for testing. Leaves would be marked (as in 2 above) but at two distinct heights (bottom and top of canopy). Two new sensors would be placed at each height.
2. Known amounts of water (to be ascertained according to sensor capacity) to be placed on the sensor (variable amounts per compartment) and all leaves at the same time each day and recording made of the drying time on the sensor and on the marked leaves. Hourly recordings of wetness on the leaf and sensor compartment should be taken at each height. Approximately 10 – 20 different drying events would be recorded with at least 8 – 10 observations (from leaves and sensors) taken for each event.
3. Temperature and humidity to be recorded at the same height as these wetness sensors.
4. Observations to be taken after periods of rainfall on the two sets of marked leaves and from the sensors in the same way as described above. Amount and duration of rainfall to be recorded.

5. All experiments to be carried out at HRI Kirton by staff based at HRI Kirton.

Work to provide information on:

a) Best sensor type to use in work for estimation of wetness.
b) Best position of sensor for estimation of wetness.
c) Sources of variation.

APPENDIX E
Result for section 4.1(a)

Table E.1 Evaluation of fungicides for protectant activity against *B. narcissicola* on attached leaves of narcissus cv Carlton - June 1999

Treatment	Mean lesion size (mm^2) after inoculation		% inoculation sites with lesions	
	4 days	8 days	4 days	8 days
1. Untreated	50	241	75	95
2. Plover	0	56	0	55
3. Folicur	0	8	0	10
4. Bavistin/Dithane	15	104	40	75
5. Opus	0	13	0	15
6. Punch C	0	4	0	10
7. Compass	0	35	0	35
8. Bravocarb	0	102	0	60
9. Benlate/Dithane	25	185	45	65

Tables E.2 Evaluation of fungicides for eradicant activity against *B. narcissicola* (98/23) on attached leaves of narcissus cv Carlton - June 1999

Treatment	Mean lesion size (mm^2) after inoculation		% inoculation sites with lesions	
	7 days	11 days	4 days	11 days
1. Untreated	117	205	90	95
2. Plover	66	98	65	70
3. Folicur	26	72	35	65
4. Bavistin/Dithane	89	235	85	95
5. Opus	90	190	70	75
6. Punch C	76	158	60	80
7. Compass	108	183	80	90
8. Bravocarb	114	249	85	90
9. Benlate/Dithane	71	158	70	95

Table E.3 Evaluation of fungicides for protectant activity against *R. vallisumbrosae* (98/101) on attached leaves of narcissus cv Carlton - April 1999

Treatment	Mean lesion size (mm^2) after 20 days[a]	% Inoculation sites with lesions
1. Untreated	29.7	68
2. Ronilan	52.7	67
3. Bravo	11.0	20
4. Benlate	6.0	5
5. Dithane	38.0	65
6. Amistar	0.0	0
7. Stroby	10.0	10
8. Exp (Kif 3535)	28.2	45
9. Scala	0.0	0
10.Shirlan	25.2	35
11.Unix	19.5	30
12.Benlate/Dithane	0	0
13.Bavistin	3.6	10

[a] Mean of 20 inoculation sites.

Table E.4 Evaluation of fungicides for eradicant activity against *R. vallisumbrosae* (98/33) on attached leaves of cv Carlton - May 1999

Treatment	Mean lesion size (mm^2) after 20 days	% inoculation sites with lesions
Untreated	94	95
Ronilan	68	60
Bravo	31	45
Benlate	55	20
Dithane	18	25
Amistar	0	0
Stroby	6	5
Exp	26	15
Scala	0	0
Shirlan	5	5
Unix	15	10
Benlate/Dithane	4	5
Bavistin DF	42	45

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Gordon Hanks
5 June 2000