Project Title: Carrots: forecasting and integrated control of sclerotinia disease

Project number: FV 260

Project Leader Dr Mark McQuilken
Crop and Soil Research Group
Research and Development Division
Scottish Agricultural College
Ayr Campus
Auchincruive Estate
Ayr KA6 5HW

Report: Annual Report, March 2006

Key workers: Robert Redpath, Brian Pool (Laboratory and field work)
Dr Neil McRoberts (Modelling/disease forecasting)

Location: SAC, Edinburgh
Commercial crops of carrots in North East Scotland

Project co-ordinator: John Bilsland
Kettle Produce Ltd
Balmalcolm Farm
Cupar
Fife
KY15 7TJ

Date commenced: 1 March 2004

Date completed: 28 February 2007

Key words: carrots, Sclerotinia disease, *Sclerotinia sclerotiorum,* sclerotia (resting body), apothecia (fruiting body), disease forecasting, environmental factors, crop growth stage, disease control, fungicides

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

CONTENTS

## Grower Summary

## Headline

- Canopy closure, weather conditions, the presence of fruiting bodies and senescing leaves on the ground are important factors affecting initiation and development of sclerotinia disease in carrots.
- Fungicide spray programmes should start early, with the first fungicide applied just before the canopy closes, to ensure protection of senescing leaves at the base of the canopy.
- Signum (boscalid + pyraclostrobin) has been granted on-label approval (OLA) for use on carrots. The product is potentially very useful for inclusion in fungicide spray programmes against sclerotinia. The recommended rate for sclerotinia control is 1 kg/ha, and two applications can be made per crop with a14 day interval.
- Contans WG is the first product to become available that attacks the resting bodies (sclerotia) of *Sclerotinia sclerotiorum*, the causal pathogen of carrot sclerotinia disease. The product contains the naturally occurring soil fungus *Coniothyrium minitans*, which infects and kills the resting bodies of the pathogen, and thereby prevents them from producing fruiting bodies.
- Trials conducted in 2004 as part of this project revealed that 45-50% of resting bodies were infected and killed 24 weeks after soil incorporation of Contans WG.
- The approval allows Contans WG to be used as a soil incorporation pre-planting of all edible or non-edible crops. The product is not a replacement for fungicide foliar sprays, and it should be used as part of an integrated programme to long term reduction of resting bodies in the soil.

## Background and expected deliverables

Sclerotinia disease, caused by the fungus *Sclerotinia sclerotiorum*, is one of the most economically important diseases that threaten UK carrot (*Daucus carota*). It infects both the foliage and the roots, and yield losses appear to be increasing as a result of poor control. The financial losses are serious, and it has been estimated that the disease causes annual crop losses to UK growers in excess of £5 million. The fungus survives in soil as small, black resting bodies (sclerotia), which

germinate under moist soil conditions to produce tan-coloured fruiting bodies (apothecia). These fruiting bodies release millions of microscopic spores (ascospores), which spread in air currents, and are the major source of infection. Optimum timing of fungicide sprays is currently unknown and several sprays are often applied. A simple forecasting system, based on crop growth stage and environmental factors affecting fruiting body production, is needed to predict the optimum time to spray and save costs by reducing unnecessary fungicide applications.

The overall aim of this project is to develop an effective integrated control system, based on a simple predictive forecasting model and rational use of fungicides. Such an integrated control system could reduce production losses to carrot growers by 75%, an annual saving of approximately £4 million, less the cost of implementing control.

The expected deliverables from this work include:

- An understanding of the environmental conditions that promote infection of carrots in relation to crop growth stage and senescence
- The identification of periods of high sclerotinia risk
- Development of a simple predictive model, based on crop growth stage and environmental factors affecting fruiting body production and spore infection
- An evaluation of new and existing fungicides against sclerotinia
- An evaluation and validation of a developed simple forecasting system.

## Summary of the project and main conclusions

### Environmental factors affecting carrot foliage infection

A series of experiments in controlled environment cabinets and glasshouses were undertaken, using damaged carrot plants grown in pots, to determine the effect of key environmental factors on foliage infection by spores (ascopores).

- Disease was always seen first seen on either the petioles or blades of damaged leaves as water-soaked, dark olive-green lesions followed by the presence of the characteristic fluffy cotton wool like mycelium.

- Optimum conditions for foliage infection were greater than 4 days (0-8 hours minimum) of continuous leaf wetness, ≥90% (60% minimum) relative humidity (RH) and air temperatures of 10-18$^0$C (5-10$^0$C minimum).

Results clearly show that leaf wetness duration, relative humidity (RH) and air temperatures are major factors affecting carrot foliage infection by spores (ascospores) of the pathogen.

**Effect of temperature on development of sclerotinia disease**

A study was conducted in controlled environment cabinets to determine the effect of air temperature on the development of sclerotinia disease. Damaged plants grown in pots were artificially inoculated with spores, and batches placed in controlled environment rooms maintained at 8, 12, 16, 22 and 28$^0$C and 80% RH. Disease development was monitored.

- Disease appeared 6-9 days after inoculation. As before, disease was always seen first seen on either the petioles or blades of damaged leaves as water-soaked, dark olive-green lesions followed by the presence of the characteristic fluffy cotton wool like mycelium.
- Disease developed between 8-25$^0$C, with optimum temperatures between 12-18$^0$C. At 8 and 25$^0$C, disease developed more slowly.
- Air temperature is an important factor affecting Sclerotinia disease development.

**Development of sclerotinia disease and fruiting production in carrot crops**

Two fields of main season carrots (Aird, *Delvine, Perthshire*; Ravensby, *Barry*, *Angus*), with sequential sowing dates of the 14 April and 18 May 2005 were selected for the second year of study. The sites were within a sclerotinia high-risk growing region with a past history of the disease on carrots and other susceptible crops including oilseed rape and potatoes. Both fields were sown with 'Nairobi' and grown according to common practice, except that the crops were not sprayed with fungicides. Each field was monitored at fortnightly intervals

throughout the growing season for the occurrence and development of fruiting bodies and sclerotinia disease within the crop.

Field observations made during the 2005 season were very similar to those observed during the 2004 season (see Year 1 Annual Report 2004) and included the following:

- The appearance of lodged, senescing leaves in carrot crops usually occurred close to or after full canopy enclosure. The pattern of senescence in individual plants was fairly consistent, with individual leaves senescing in turn beginning with the oldest.
- The first appearance of fruiting bodies generally occurred close to or after full canopy enclosure, and was concurrent with the early onset of senescing foliage. Crops were at the pencil + stage or main period of root growth stages. Flushes of fruiting bodies appeared in crops until late September.
- Mean daily soil temperatures ranged between 13-17$^{0}$C during 2 weeks before fruiting bodies were first detected.
- The primary site of infection was senescing or damaged petioles.
- Disease was less severe in late sown crops (May).
- Symptoms (see Year 1 Annual Report for photographs) were first noticed on petioles of lodged senescing leaves as water-soaked, dark olive-green lesions. These lesions then expanded over the entire leaf, with infected tissue covered by abundant cotton wool like mycelium. The disease then often spread by mycelium growing between diseased and healthy foliage in contact with each other.
- At an advanced stage, affected tissues exhibited a bleached appearance, and entire plants collapsed and died. Sclerotia developed externally in the mycelium, or internally within the pith of the petiole.
- Serious infection of the foliage completely defoliated the crop, leading to early infection of the crown and the production of abundant sclerotia on the soil surface.
- Disease was often very severe on plants in the outer rows of beds next to the wheelings, where the crop had been damaged.
- No foliage symptoms were observed before foliar senescence or crop damage along the bed wheelings.

- Root infection resulted from infected foliage *via* the crown, but symptoms were rarely evident in the field unless the foliage had been completely defoliated, or until the roots had been stored under straw.
- There was no correlation between fruiting body population and foliage disease severity.
- Overall, at both monitoring sites, fewer apothecia were detected during the 2005 season and disease was less severe compared to the 2004 season. This observation is most likely associated with less rainfall during the 2005 season and the Scottish sites being much drier than in 2004.

**Evaluation of fungicide spray programmes & timings**

A trial was conducted within a commercially grown carrot crop (sown on 14 April, variety 'Nairobi') in Perthshire, Scotland to evaluate different fungicide spray programmes against sclerotinia disease, using fungicides (see Table 1) that performed well in 2004.

*Table 1.* Fungicide trial 2005 – details of fungicides evaluated

| Trade name | Active ingredient | Application Rate (product/ha) | Application volume (l water/ha) | Approval status |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Folicur | tebuconazole | 1 L/ha | 200-300 | On-label |
| Amistar | azoxystrobin | 1 L/ha | 200-300 | On-label |
| Signum | boscalid + pyraclostrobin | 1 kg/ha | 200-300 | On-label |
| Shirlan | | 0.5 L/ha | 200-300 | On-label |
| A9219B | fluazinam | 1 kg/ha | 200-300 | Exp.[a] |
| UKA373 | Experimental | 0.4 L/ha | 200-300 | Exp. |
| UKA374[b] | Experimental | 0.4 kg/ha | 200-300 | Exp. |
| | Experimental | | | Exp. |

[a] Used under Automatic Experimental Approval for the trial
[b] (= UKA352 evaluated in 2004 trial)

Foliar sprays of eight fungicide programmes with four timings (T1, T2, T3 & T4) at approximately 14-day intervals were evaluated (see Table 2 below for details).

*Table 2.* Fungicide trial 2005 – details of fungicide spray programme treatments and timings

| Spray programme | Timings[a] | | | |
|---|---|---|---|---|
| | T1 (5 Jul) | T2 (19 Jul) | T3 (2 Aug) | T4 (16 Aug |
| 1. | -[b] | - | - | - |
| 2. | Amistar/Folicur (tank mix) | Amistar/Folicur (tank mix) | - | Amistar/Folicur (tank mix) |
| 3. | Signum | Folicur | Signum | Folicur |
| 4. | - | Signum | Folicur | Signum |
| 5. | Signum | - | Folicur | - |
| 6. | Signum | Shirlan | Signum | Shirlan |
| 7. | UKA374 | UKA373 | UKA374 | UKA373 |
| 8. | A9219B | Folicur | A9219B | Folicur |

[a] The four fungicide timings were:

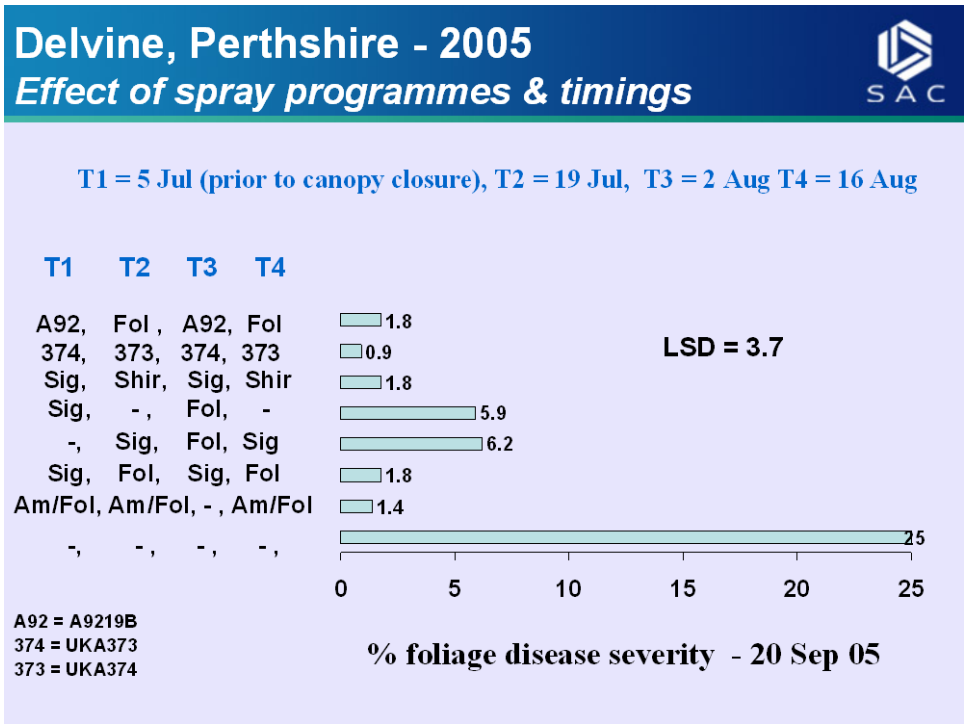
| | | |
|---|---|---|
| T1 | 5 Jul | 90-95% crop coverage, prior to canopy enclosure |
| T2 | 19 Jul | 100% crop coverage, full canopy enclosure |
| T3 | 2 Aug | Fresh full canopy, lodging & senescing leaves |
| T4 | 16 Aug | Fresh to mature canopy, heavy lodging &senescing leaves |

[b] -, No fungicide applied.

Foliage disease severity was assessed on 6 September (heavily lodged canopy) and 2 weeks later on 20 September (heavily lodged canopy). The trial was then protected against frost using straw on 6-8 October 2006. Roots were harvested subsequently in January 2006 and assessed for yield, and incidence and severity of sclerotinia on roots post harvest.

Untreated control plots showed 25% foliage disease severity on 20 September. Foliage disease severity results are summarised in Figure 1 below.

*Figure 1.*




The key findings were:

- Foliage disease was less severe (25% in untreated control plots) compared to that observed in the 2004 fungicide trial (70-80% in untreated control plots).
- Alternating 4-spray programmes at 14-day intervals of Signum (OLA) and Folicur (OLA), Signum and Shirlan (Exp.) UKA373 (Exp.) and UKA374 (Exp.) (previously tested as UKA352 in 2004), and A9219B and Folicur, with the first spray applied before canopy closure, gave good foliage disease control and were equally effective.
- A 3-spray programme of an Amistar (OLA)/Folicur tank mix with the first spray applied before canopy closure, the first two sprays applied at 14-day intervals and the final spray applied 28 days later, was as effective as the 4-spray programmes applied at 14-day intervals.
- Omission of a fungicide spray prior to canopy closure, and then applying alternating sprays of Signum and Folicur at 14-day intervals reduced foliage disease control.
- Extending the spray interval beyond 14 days and applying Signum followed by Folicur at 28-day intervals reduced foliage disease control.
- Results from this year's trial have corroborated those from last year, which indicated that it is important to apply the first fungicide early, just before the

canopy closes, to ensure protection of senescing leaves at the base of the canopy from sclerotinia.

- Results from both the 2004 and 2005 fungicide trials indicated that applications of the experimental fungicides Shirlan, UKA373, UKA374 and A9219B gave good foliage disease control. Approval for use of these fungicides on carrots should be sought.
- None of the fungicide spray programmes improved total or marketable root yield.
- Only three of the seven fungicide programmes reduced the incidence and severity of sclerotinia root rot. These included the 3-spray programme of the Amistar/Folicur tank mix, the alternating 3-spray programme of Signum and Folicur, and the alternating 4-spray programme of UKA374 and UKA373.

Despite reducing sclerotinia foliage disease severity, none of the fungicide spray programmes significantly improved marketable yield, and only three of them reduced sclerotinia root rot. This was most likely associated with the patchy low incidence of foliage disease throughout the trial. Furthermore, the incidence of foliage disease was very localised within treatment plots, and there was considerable variation in yields and sclerotinia root rot between plots and blocks. This contrasts with the 2004 fungicide trial, when control of foliage disease by the most effective fungicides (i.e. Signum, Folicur, Shirlan, UKA373, UKA374 and A9219B) was associated with increases in yield (23–28 t/ha) of marketable roots. Thus, the inability of the fungicide spray programmes to improve marketable yield and most of the programmes to reduce sclerotinia root rot in 2005 must be viewed with caution.

**Evaluation of foliar applications of Contans WG on sclerotinia disease and infection of resting bodies/Evaluation of varietal susceptibility to sclerotinia disease**

Contans WG is a granular formulation containing 1 x $10^9$ spores $g^{-1}$ of the soil fungus *Coniothyrium minitans*. The fungus attacks the resting bodies (sclerotia) of the pathogen (*S. sclerotiorium*) in soil and destroys them. It has an on-label approval (OLA), which allows it to be used as a soil incorporation pre-planting to any edible or non-edible crop. In 2004, a trial was conducted to evaluate the

effect of Contans WG soil incorporation on infection of sclerotia by *C. minitans*. Results from this trial revealed that 45% of resting bodies collected from Contans WG treated plots were infected by the mycoparasite, confirming the potential of the product to reduce the build up of resting bodies in soil.

Previous studies conducted in Canada and the Netherlands have shown that spraying *C. minitans* onto the canopies of crops susceptible to sclerotinia resulted in a high rate of infection of resting bodies (>90%), and a significant reduction in resting body viability. Consequently, a field trial was conducted in 2005 within a commercially grown carrot crop (sown on 14 April) in Perthshire, Scotland 2005 to evaluate the effect of aerial applications of Contans WG to carrot foliage on sclerotinia disease and infection of developing resting bodies. In addition, two varieties, 'Elegance' and 'Nairobi', were evaluated for susceptibility to sclerotinia disease. 'Elegance' produces light, erect foliage in contrast to 'Nairobi' which produces heavy, dense foliage that is inclined to flop over, lodge and senesce early in the growing season through shading.

Immediately after the appearance of sclerotinia foliage disease symptoms [8 Aug 2005; fresh full canopy, lodging and 1-2 senescing leaves/plant), three treatments were applied. These were: (1) control, no treatment; (2) Contans WG spore suspension [2.4 x $10^6$ spores $ml^{-1}$; recommended rate (4 kg $ha^{-1}$)]; (3) Contans WG spore suspension [4.8 x $10^6$ spores $ml^{-1}$; 2 x recommended rate (8 kg $ha^{-1}$)]. Two further applications of each treatment were made at 14-day-intervals [22 Aug 2005 (fresh to mature canopy, heavy lodging and 3-4 senescing leaves/plant)], and 5 Sep 2005 [(mature canopy, heavy lodging and 3-4 senescing leaves/plant)].

Foliage disease severity was assessed approximately 4 weeks after the third spray of Contans WG on 3 October (heavily lodged canopy & 7-8 senescing leaves/plant). Samples of resting bodies were collected from diseased foliage/crop debris and assessed for infection by *C. minitans*.

The key findings were:

*Contans WG*

- No control of foliage disease was obtained with three successive foliar sprays of Contans WG at 14-day intervals.
- Foliar sprays of Contans WG decreased the viability of resting bodies collected from diseased foliage/crop debris and 58-81% of them were infected with *C. minitans*.
- Results from this field trial reveal that Contans WG applied to carrot foliage of the growing crop has the potential to reduce the build up of resting bodies in soil long term. Contans WG has an on-label approval (OLA) in the UK, which allows it to be used as a soil incorporation before planting any edible or non edible crop. However, currently, ***it is not permitted to apply the product to the foliage of a growing crop***. In view of the promising results obtained in this study, approval to apply Contans WG to carrot foliage of a growing crop should be sought, provided that further field trials corroborate its ability to infect resting bodies developing on diseased foliage.
- Further trials are also recommended to evaluate the effect of applying the product to infected foliage, prior to covering the crop with straw for winter frost protection, on resting body production and survival.

*Varietal susceptibility*

- Foliage disease was far more severe on 'Nairobi' than 'Elegance'.
- The high susceptibility of 'Nairobi' to sclerotinia disease was confirmed in this study.
- 'Nairobi' produces heavy, dense foliage that is inclined to flop, lodge and senesce early on in the season, increasing susceptibility to infection. In contrast, 'Elegance' produces light, erect foliage which tends not to lodge and senesce early on in the growing season.
- Further trials to evaluate the susceptibility of different carrot varieties to sclerotinia disease are warranted. Cultivars may differ in susceptibility and, although none are likely to be completely resistant, some may require fewer sprays than others to minimise sclerotinia disease.

**Development of simple disease predictive model**

***Development of a Quantitative Canopy Risk Factor (Qn_CRF) for sclerotinia foliage disease in carrots***

Rather than attempt to predict the risk of disease on the basis of resting body germination of the pathogen, or the suitability of the weather for disease development, the proposed risk predictor for sclerotinia foliage disease uses a simple system for assessing canopy growth and development. This approach has been adopted for a number of reasons:

1. The difficulty in correlating disease with the apparent germination of resting bodies.
2. The almost ubiquitous suitability of the weather for disease development.
3. The well-established relationship between disease onset and canopy ground cover.
4. The evidence that disease incidence is influenced by sowing date.
5. The ease with which the relevant assessments can be included in routine crop inspections by growers.
6. The ease of implementation of the system without the need for computer technology in the field.
7. The potential for extension of the system to season-long risk assessment.

*Part 1 Managing the first protectant spray with the Quantitative Canopy Risk Factor (Qn_CRF)*

- Timing the first protectant spray for the foliage is the key to sclerotinia control within a single season.
- The simple system described here suggests that high-risk crops can be identified in advance by making simple assessments of crop growth and development during late June and early July.
- The quantitative canopy risk factor (Qn_CRF) is calculated from simple information on the status of the carrot foliage. The basic elements of the calculation are:

A. An assessment of ground cover within the bed in the form of a proportion (e.g. 70% ground cover = 0.7). Once 100% ground cover within the bed is reached

A = 1 even though further canopy growth will occur until complete canopy closure.

B. An assessment of the number of senescing, older leaves per plant.
C. An assessment of the level of lodging of the canopy which is given a simple numerical score; 0,1, 2 etc.

- Since we know that the development of the disease is correlated with the onset of canopy closure and also that the disease requires senescing or damaged tissues to infect, the Qn_CRF captures some of the most important risk factors in the development of the disease.
- To calculate the Qn_CRF, we use the following formula:

  Qn_CRF = A + [A×(B+C)], where A, B and C refer to the canopy features listed above.

The sites at which epidemics occurred (Aird 2004, 2005; Carriston, 2004; Cults Mill 2004) can be distinguished from those where no epidemic occurred (Ravensby 2004, 2005). This is by considering the value of Qn_CRF in late June to early July, and a simple system for providing an early warning of high-risk crops can be stated as follows:

***Beginning in the last week of June, crops should be examined weekly, and if the Qn_CRF calculated is 0.75 or greater in the first week of July, this indicates a crop with a high risk of severe sclerotina. The crop should be sprayed with an approved protectant fungicide prior to canopy closure.***

This guide given above will be refined during the third year of the project.

*Part 2: Managing follow-up sprays*

The work conducted to date has not revealed a simple management strategy for season-long protection against sclerotinia, and these notes are provided only for general information and guidance, pending further work.

- The importance of the pre-canopy-closure spray for sclerotinia control arises partly from the behaviour of the pathogen. In most crops, the main flush of resting body germination and first appearance of fruiting bodies of the pathogen coincides roughly with canopy closure, so protecting the foliage at that time allows the crop to escape the highest risk period for the disease.
- Fruiting bodies release spores for several weeks after their first appearance with a gradual decline as the crop progresses through its main growth stages. Our observations in 2004 suggest that the pathogen may have a second, smaller flush of resting body germination around 50-60 days after the first one, but this requires verification. Also, it is unknown how important such a flush would be to increasing the risk of infection.
- It is possible that this second phase of germination represents the second key stage for disease prevention and experiments are currently being designed for 2006 to determine whether it is possible to use a reduced number of sprays focussing on these two key stages to control the disease.

## Financial benefits

The results from the fungicide trials and field artificial inoculation experiments conducted in 2004 (see Year 1 Annual Report for details) illustrate the benefits, which arise from controlling sclerotinia disease in carrot crops. Findings from field studies indicate that failure to control the disease could result in crop losses of 15-18% on average, corresponding to losses of at least £18-20 million per annum to growers (based on 2003/04) farm gate prices). Significant losses in marketable

yield are likely to arise when root disease incidence is ≥10%. Fungicide sprays have potential to reduce these losses by at least 20-25%, an annual saving of approximately £4 million, less the cost of purchase and fungicide spray application. A simple forecasting system able to predict the optimum time to spray could reduce spray costs.

## Action points for growers

- Action should be taken to prevent foliage infection via spores, limit production of resting bodies and prevent the long-term build up of resting bodies in the soil.
- Until the simple predictive model has been evaluated and further results suggest otherwise, a preventative fungicide spray programme should remain the main defence against foliage infection. It is important to apply the first fungicide early, just before canopy closes, to ensure protection of senescing leaves at the base of the canopy. Apply further sprays at recommended intervals and rates.
- Alternate fungicides from different chemical groups, according to label instructions, in order to avoid the build up of resistance (see action points below).
- Signum (boscalid + pyraclostrobin) has been granted on-label approval (OLA) for use on carrots, and is potentially very useful for inclusion in fungicide spray programmes against sclerotinia. The recommended rate for sclerotinia is 1 kg/ha, and 2 applications can be made per crop with a 14 days interval.
- Signum contains a QoI fungicide and should be used in accordance with FRAC resistance management advice. Do not use consecutive applications of Signum and apply in alteration with fungicides from a different chemical group. Do not use more than 2 applications of Signum per crop.
- Growers should note that Amistar (azoxystrobin) also contains QoI fungicide. To minimise the development of resistant strains of the pathogen, do not use consecutive applications of Amistar and Signum on carrot crops, and select a product from a different fungicide 'group' for alternate fungicide sprays.
- Consider using 65-degree flat fan jet nozzles at 50 cm spacing on the sprayer boom to apply fungicides targeted at protecting the stem base and root crown beneath a full canopy. Recent spray application trials by Syngenta

revealed that use of such nozzles improved canopy penetration and achieved the greatest fungicide deposition on stem bases.

- Contans WG (MAPP No. 12616) has been granted on-label approval (OLA) for use as a soil incorporation before planting any edible or non edible crop. It has the potential to kill the resting bodies and to reduce the build up of the survival structures long term. The recommended pre-planting rate is 4 kg product/ha (for incorporation up to 10 cm soil depth) or 8 kg product/ha (for incorporation up to 20 cm depth). Growers are advised to seek advice on timing of soil incorporation in relation to sowing date. The product is not a replacement for fungicide foliar sprays, and it should be used as part of an integrated control programme for sclerotinia disease. Note that application of Contans WG to the growing crop *is not currently approved and permitted* in the UK.
- Consider using Perlka (calcium cyanamide) as a fertiliser. The product has been shown to kill resting bodies in soil in previous research.
- Continue to avoid short-term rotations with susceptible crops, such as oilseed rape, lettuce and potatoes. Growing cereal crops as part of the rotation may help to reduce the build up of resting bodies in the soil.
- Excessive N applications, causing extensive canopy growth, and damaging the crop increases infection. Apply correct amounts of N and avoid damaging the crop when entering fields.
- Consider reducing crop density and grow varieties, which produce light erect foliage, that tends not to lodge and senesce until late in the growing season. 'Nairobi' produces heavy dense foliage that is inclined to flop over and lodge early on in the season, increasing its susceptibility to sclerotinia disease.

## Science Section

### Introduction

Sclerotinia disease, caused by the soil-borne pathogen *Sclerotinia sclerotiorum*, is one of the most economically important diseases that threaten UK carrot (*Daucus carota*) production. The disease causes plant and root death, and renders carrots unmarketable or often causes down-grading. Although disease incidence varies greatly among years, regions and fields, yield losses are increasing as a result of poor control. The financial losses are serious, and it has been estimated that the disease causes annual crop losses to UK growers in excess of £5 million. Preliminary observations reveal that infection is more common in later growth stages once leaf senescence or crop lodging is advanced.

Periods of high Sclerotinia risk in UK carrot crops are unknown. Many growers rely on routine use of Folicur (tebuconazole; on-label), Compass (iprodione + thiophanate-methyl; SOLA) and Amistar (azoxystrobin; on-label) for sclerotinia control. However, control is often inadequate because fungicide use is based on a poor understanding of disease epidemiology, and sprays are often applied at the incorrect time. By identifying the optimum time to spray, disease control could be improved, and yield losses as well as unnecessary fungicide applications avoided.

The pathogen survives in soil as resting bodies (sclerotia), which germinate to produce fruiting bodies (apothecia). Air-borne spores (ascospores) released from fruiting bodies are the major source of infection in the majority of hosts, including carrots (Phillips, 1987). Consequently, there is an urgent need to be able to predict the appearance of fruiting bodies and identify periods of high disease risk. Such a reliable forecasting system would identify the optimum time to spray and enable rational, economic and effective use of fungicides. Previous studies have shown that soil temperature and moisture are key factors affecting fruiting body production in the field (Phillips, 1987; Hao *et al*., 2003; Clarkson *et al*., 2004). Temperature, relative humidity (RH) and leaf wetness duration also affect ascospore survival and infection of plants by the pathogen (Grogan & Abawi,

1975; Caesar & Pearson, 1983). However, none of these studies were conducted using an UK *S. sclerotiorum* isolate, originally derived from a diseased carrot.

The overall aim of this project was to develop an effective integrated control system for Sclerotinia disease in carrots, based on a simple predictive model and rational use of fungicides. The work was undertaken with the following specific objectives: (1) to identify key environmental factors affecting fruiting body production, ascospore survival and infection; (2) to develop and evaluate a simple disease predictive model, based on crop growth stage and environmental factors; (4) to evaluate current and novel fungicides; (5) to devise and evaluate an integrated control programme, using the simple disease predictive model and fungicide spray programmes.

## Materials and Methods

### *General*

Sclerotinia sclerotiorum *isolates*

The isolates of *Sclerotinia sclerotiorum* used in this study were derived from sclerotia from diseased carrot plants grown in Perthshire, Scotland (KP1) and Nottinghamshire, England (Ret). Field-collected sclerotia were surface sterilised in 50% v/v sodium hypochlorite and 70% ethanol for 5 min with agitation followed by three washes in sterile distilled water. Sclerotia were then bisected, placed on potato dextrose agar (PDA; Oxoid) and incubated for 5 weeks. Sclerotia formed in the cultures were then removed, stored at $5^0$C, and used as a stock supply for all further cultures.

*Production of sclerotia*

Large quantities of sclerotia of two isolates (KP1 & Ret) for use in subsequent experiments were produced on sterilised wheat grain (Mylchreest & Wheeler, 1987). Two agar discs (5 mm diameter) cut from the periphery of a 4-day-old *S. sclerotiorum* colony were used to inoculate sterile wheat grain (25 g wheat grain, 50 ml distilled water; autoclaved at $121^0$ C for 15 min) in 250 ml Erlenmeyer flasks. Flasks were incubated at 18-$20^0$C for 4 weeks, and shaken gently by hand at least twice a week to prevent clumping of wheat grain and mycelium, and encourage

the formation of uniform sclerotia. After 4 weeks, mature sclerotia were formed. Flasks containing sclerotia were incubated for a further 8 or 22 weeks at 5$^0$C, as a cold conditioning treatment, to ensure carpogenic germination and production of apothecia (Mylchreest & Wheeler, 1987; Sansford & Coley-Smith, 1992). After this cold conditioning treatment, sclerotia were wet sieved to recover those 2-5 mm, and the wheat grain was floated-off. Sclerotia were then dried in a laminar air-flow cabinet overnight, after which they were used immediately in experiments.

*Production of apothecia and collection of ascospores*

To produce apothecia, pre-conditioned sclerotia (approximately 25-30) were buried 1 cm deep in John Innes No. 1 compost (Arthur Bower's, William Sinclair Horticulture Ltd., Lincoln; pasteurised at 110$^o$C for 30 min) in clear plastic tubs (250 ml volume; Bunzyl, Paisley). Tubs with lids were placed in a controlled environment cabinet at 15-18$^0$C (12 light/dark), and the compost moisture was maintained at 30% (w/w) by adding appropriate amounts of water initially and maintaining the weight of each tub by further additions each week.

Mature apothecia appeared after 4-6 weeks. A tub containing apothecia was opened and ejected ascospores were trapped on a 9 cm diameter Whatman No. 1 filter paper in a Buchner funnel attached to a suction pump. Ascospores from several tubs of apothecia were collected on a single filter paper. Ascospores on filter papers were stored at 5 $^0$C in a desiccator for no longer than 4 months.

***Growth Chamber Experiments***

*Production of carrot plants and ascospore suspensions*

Individual carrot plants (cv. Nairobi) were grown in a glasshouse (12-18$^0$C) in 2-l pots containing John Innes No. 3 compost. Plants were watered from below as necessary. In all experiments, plants (6-8 weeks old, 5-7 true leaves) were damaged prior to artificial inoculation. Preliminary experiments (data not shown) revealed that damaged foliage developed disease more successfully following

artificial inoculation with ascospores. The leaf tips (approximately 30% of the length) of the oldest three leaves were cut with a pair of scissors and the clippings removed. The bases of the petioles of these leaves were also pinched and squeezed between the thumb and forefinger.

In most experiments, damaged plants were inoculated with an ascospore suspension, which was prepared immediately before use. Ascospore suspensions were prepared by soaking ascospore-laden filter papers in sterile distilled water [containing ($l^{-1}$) 62.5 mM $KH_2PO_4$ and 5.5 mM D-glucose] for 2-3 min. The ascospores were dislodged with a camel's hairbrush and concentrations of approximately 1-5 x $10^5$ ascospores $ml^{-1}$ were used. Ascospore suspensions were made immediately before use, and applied to plants to run-off using a hand-held sprayer (approximately 25-ml per plant). Viability of the inoculum was tested immediately after inoculation by spraying 2-3 plates of PDA with the suspension.

*Effect of leaf wetness on infection of carrots by ascospores*

Damaged carrot plants were inoculated with an ascopore suspension as described before, and exposed to a range of leaf wetness durations (8, 24, 48, 96,192, 288, 384 and 504 h). There were 15 plants per leaf wetness duration treatment. Continuous leaf wetness was provided within a controlled environment (CE) cabinet (15-18$^0$C, with 14-h light/10 dark) by covering the whole of each plant's foliage with a plastic bag. Plastic bags were removed from plants. Plants were then removed from the cabinet and dried with an air fan for 1 h to terminate the leaf wetness period. The control treatment was 0 h leaf wetness, where plants sprayed with ascospore suspensions were dried immediately after inoculation. Carrot plants were then returned to the CE cabinet without bags. All the plants remained in the CE cabinet until the longest wetness duration treatment had been completed, after which they were placed in a glasshouse (15-18$^0$C) and watered from below when necessary. Plants were monitored for disease symptoms at weekly intervals for 4 weeks, and the number of diseased plants was recorded at weekly. A plant was considered diseased by observing the presence of brown necrotic tissue and/or the presence of white fluffy white mycelium characteristic of *S. sclerotiorum*.

*Effect of relative humidity on infection of carrots by ascospores*

Batches of 12 damaged plants were each placed inside a CE cabinet set at an appropriate relative humidity [RH; (60, 70, 80, 90 and 98%)] and 15-18$^0$C (with 14 h light/10 h dark). For inoculation, plants were inoculated with 'dry' ascospores ejected directly from apothecia (raised in plastic tubs as described previously). Six tubs were used to inoculate 12 plants. For inoculation, tubs containing approximately 20 active apothecia were placed inside each CE cabinet in between the plants. Tub lids were removed and the cabinet doors were closed to allow ascospores ejected directly from apothecia to settle onto plants. Tubs were removed after 15 min. After a 120 h (5 day) RH exposure period, all plants were removed, transferred to a glasshouse (15-18$^0$C) and watered from below when necessary. Plants were monitored for disease symptoms, and the number of diseased plants was recorded at weekly intervals for 3 weeks as described before.

*Effect of temperature on infection of carrots by ascospores*

Damaged plants (100) were inoculated with an ascospore suspension as described before and covered with plastic bags. Batches of 20 plants were then placed inside a CE cabinet set at one of the following temperature regimes: (1) 5-10$^0$C (8 h dark at 5$^0$C, 16 h light at 10$^0$C); (2) 11-15$^0$C (8 dark at 11$^0$C, 16 h light at 15$^0$C); (3) 16-20$^0$C (8 h dark at 16$^0$C, 16 h light at 20$^0$C); (4) 21-25$^0$C (8 h dark at 21$^0$C, 16 h light at 25$^0$C). After a 120 h (5 day) exposure period, bags were removed and all plants were dried with an air fan for 1 h prior to being transferred to a glasshouse (15-18$^0$C). Plants were watered from below when necessary and monitored for disease symptoms. The number of diseased plants was recorded at weekly intervals for 3 weeks as described before.

*Effect of temperature on development of sclerotinia disease*

Damaged plants (100) were inoculated with an ascospore suspension as described before. Plants were covered in plastic bags and placed in a 'walk-in' CE growth room at 15-18$^0$C (14 light, 10 h dark) to ensure infection. After 120 h (5 days), bags were removed and batches of 20 plants were transferred to CE cabinets (80% RH), maintained at 8, 12, 16, 22 and 28$^0$C. Plants were watered from below when necessary and the number of diseased plants recorded every week for 4 weeks as described before.

***Field work***

*Sites*

Two fields of main season carrots representative of typical commercial crops in the main growing areas of Scotland were selected for the second year of study. Both sites were within a sclerotinia high-risk growing region with a past history of the disease on carrots and other susceptible crops, such as oilseed rape and potatoes. Soil samples (20-30) were collected (depth 5-8 cm) from each site using a trowel. A 'W' shaped sampling pattern, located 20 m into each field, was used to collect soil samples. Collected soil was wet-sieved to recover sclerotia (>2 mm), and the number of sclerotia $kg^{-1}$ of dry soil was determined. Details of each field site are summarised in Table 1 below.

Table 1. Details of field sites selected for the first year of study.

| Site | Soil Type | No. of sclerotia (kg $^{-1}$ soil) | Cultivar | Sowing date | Straw cover (St) & harvest (H) date |
|---|---|---|---|---|---|
| Aird<br>*Delvine, Perthshire* | Silty clay Loam | 0.42 | Nairobi & Elegance | 14 Apr 05 | 6-8 Oct 05 (St)<br>late Feb 06 (H) |
| Ravensby<br>*Barry, Angus* | Sandy loam | 0.08 | Nairobi | 18 May 05 | 25 Nov 05 (St)<br>mid Apr 06 (H) |

*Monitoring the development of naturally occurring sclerotinia disease and apothecia production in carrot crops (cv. Nairobi)*

Two commercial fields (Aird, and Ravensby; see Table 1 for details), with sequential sowing dates of the 14 April and 18 May 2005, were monitored at approximately 2 week intervals throughout the growing season for the occurrence and development of apothecia and sclerotinia disease. Within each field, an area of 10 carrot cv. 'Nairobi' beds (each 1.8 m wide) x 100 m was marked out in the centre. The crop within the monitoring area was grown according to common practice, but was not sprayed with fungicides. Within the monitoring area, 50 quadrats (1 m$^2$) were marked out at random and labelled so that the same quadrats were assessed throughout the growing season. At fortnightly intervals, the total number of apothecia and percentage of plants with sclerotinia disease (% foliage disease severity) were recorded within each 1 m$^2$ quadrat. Percentage foliage disease severity was assessed according to the key shown in Appendix 1. Observations were made to identify symptoms including water-soaked, dark olive green lesions, actively growing mycelia or sclerotia of *S. sclerotiorum* on carrot foliage. Diseased tissues were sampled regularly and plated on PDA to confirm the presence of the pathogen.

An assessment of canopy closure (defined as closed when the foliage of adjacent rows touches and soil is no longer visible) and crop growth stage was made on each monitoring date, using the following standard description provided by David Martin, Plantsystems Ltd.:

| | | |
|---|---|---|
| Pre-emergence | 0 | |
| Cotyledon stage | 1 | |
| 3rd true leaf unfolded | | 2 |
| 5 or more true leaves unfolded | 3 | |
| Small pencil stage | 4 | |
| Pencil + stage | | 5 |
| Main period of root growth | 6 | |
| First lodging of lowest leaves | | 7 |
| Fresh full canopy with over mature lodging leaves | | 8 |
| Mature full canopy with over mature lodging leaves | | 9 |
| Heaviliy lodged canopy | 10 | |
| Mature crop, no further growth required | 11 | |

Foliar senescence and canopy lodging were also assessed. Foliar senescence was rated by counting the number of senescing leaves collapsed on the soil per plant. Counts were conducted on 3-5 plants per quadrat. Canopy lodging was measured using a severity scale with four classes ranging from 0 to 3, where 0 = no lodging, with all leaves in an upright position; 1 = 1 to 3 leaves per plant slightly contacting the soil; 2 = 4 to 5 leaves per plant laying on the soil; 3 = > 5 leaves per plant laying on the soil.

Environmental conditions were recorded every hour using a data logger (Delta-T instruments, Cambridge) measuring rainfall, soil and air temperature, leaf wetness and soil moisture. Probes were placed within the crop canopy. All sites were monitored until October prior to prior to covering the crop with straw for winter frost protection.

*Apothecial production by sclerotia in the field*

Sclerotia of *S. sclerotiorum* isolates KP1 and Ret were buried in carrot crops at field sites in Delvine (Aird) and Cariston every 2 weeks between April and August 2005. This was to ensure that sclerotia were exposed to a range of environmental

conditions found during the typical carrot-growing season. Sclerotia of each isolate were produced and conditioned in the laboratory for 22 weeks as described before. A total of 200 sclerotia were buried every 2 weeks in grids laid over the soil surface (four grids of 50 sclerotia). Sclerotia were buried at a depth of approximately 1 cm in the grid cells and the grids were arranged in a randomised block design. The appearance of apothecia was recorded twice a week until the crop was covered (Aird, 6-8 Oct; Ravensby, 25 Nov) in straw to protect from frost. A sclerotium of *S. sclerotiorum* was considered to have germinated when one or more apothecia had been recorded in a grid cell. Environmental conditions were recorded every hour using a data logger (Delta-T instruments, Cambridge) measuring rainfall, soil and air temperature, leaf wetness and soil moisture. Probes were placed within the crop canopy.

*Evaluation of fungicide spray programmes and timings*

A trial was conducted within a commercial crop at the Aird site to evaluate different fungicide spray programmes against sclerotinia disease, using fungicides (see Table 2 for details) that performed well in the 2004 trial.

Table 2. Fungicide trial 2005 (Aird) – details of fungicides evaluated

| Trade name | Active Ingredient | Application Rate (product/ha) | Application volume (l water/ha) | Approval status |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Folicur | Tebuconazole | 1 L/ha | 200-300 | On-label |
| Amistar | Azoxystrobin | 1 L/ha | 200-300 | On-label |
| Signum | boscalid + pyraclostrobin | 1 kg/ha | 200-300 | On-label |
| Shirlan | | 0.5 L/ha | 200-300 | On-label |
| A9219B | fluazinam | 1 kg/ha | 200-300 | Exp.[a] |
| UKA373 | Experimental | 0.4 L/ha | 200-300 | Exp. |
| UKA374 | Experimental | 0.4 kg/ha | 200-300 | Exp. |
| (= UKA352 evaluated in 2004 trial) | Experimental | | | Exp. |

[a] Used under Automatic Experimental Approval for the trial

The Aird site was chosen, as the amount of sclerotial inoculum in the soil was particularly high. The crop (cv. 'Nairobi') was drilled on 14 April in a silty clay loam and the trial grown according to standard commercial practice, except no fungicides other than those listed (Table 2) were applied to the trial area. Forty plots were marked out in early May. Each plot was 10 m long and 5 rows (5 triple lines per row) wide on 1.8 m beds. Plots were separated by 2 m guards, and discard plots and beds were included around the perimeter of the trial. There were five replicates of eight fungicide spray programmes with four timings (T1, T2, T3 & T4) at approximately 14-day intervals (see Table 3 for details).

Table 3. Fungicide trial 2005 (Aird) – details of fungicide spray programme treatments and timings

| Spray programme | Timings[a] | | | |
|---|---|---|---|---|
| | T1 | T2 | T3 | T4 |
| 1. | -[b] | - | - | - |
| 2. | Amistar/Folicur (tank mix) | Amistar/Folicur (tank mix) | - | Amistar/Folicur (tank mix) |

| | | | | |
|---|---|---|---|---|
| 3. | Signum | Folicur | Signum | Folicur |
| 4. | - | Signum | Folicur | Signum |
| 5. | Signum | - | Folicur | - |
| 6. | Signum | Shirlan | Signum | Shirlan |
| 7. | UKA374 | UKA373 | UKA374 | UKA373 |
| 8. | A9219B | Folicur | A9219B | Folicur |

[a] The four fungicide timings were:

| | | |
|---|---|---|
| T1 | 5 Jul | 90-95% crop coverage, prior to canopy enclosure. Small pencil stage |
| T2 | 19 Jul | 100% crop coverage, full canopy enclosure Main period of root growth |
| T3 | 2 Aug | Fresh full canopy Lodging (1) and 1-2 senescing leaves/plant |
| T4 | 16 Aug | Fresh to mature canopy Heavy lodging (2-3) and 3-4 senescing leaves/plant |

[b] -, No fungicide applied

The five replicates of eight fungicide spray programmes were arranged in a randomised block design. (one replicate spray programme treatment per block), and the trial was not artificially inoculated. Fungicide sprays were sprayed in 200-300 L water $ha^{-1}$ using a Solo 425 knapsack sprayer (2-3 bar pressure), fitted with an 'even spray' flat fan nozzle.

Foliage disease severity was assessed on 6 September [heavily lodged canopy (2) & 4-5 senescing leaves/plant] and 2 weeks later on 20 September [heavily lodged canopy (3) & 6-7 senescing leaves/plant] using the sclerotinia disease key (Appendix 1) as described before. Only foliage within the central 8 m x middle three rows of each plot was assessed. The trial was then protected against frost using straw on 6-8 October 2006. Roots were harvested subsequently in January 2006 and assessed for yield, and incidence and severity of sclerotinia on roots post harvest.

*Effects on yield and sclerotinia root rot.* To determine the effects of fungicide spray programmes and timings on yield, plots within the fungicide trial were harvested in

the winter on 24 January by lifting the central 6 m section from the inner two rows of each plot. For each plot, total yield weight, root number, marketable and unmarketable roots (classified as undersized, i.e. maximum diameter < 20 mm, or < 50 g, or affected by sclerotinia disease or wet/black rot) were recorded and weight of marketable roots per hectare was calculated. To assess the incidence and severity of sclerotinia on roots post harvest, 80 roots harvested from each plot were selected randomly and placed in a labelled polyethylene net bag. Bags were stored at 15-18$^0$C for 14 days without touching, after which disease incidence was scored by counting the number of roots in the sample with at least one lesion of sclerotinia rot (cotton wool-like mycelium and sclerotia). Each root was also assigned to a severity class based on the area covered by the lesion on each diseased root: 0 = 0%; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%. Severity values were converted to a 'root disease severity index' per plot using the following equation:

$$\text{Disease severity index} = \frac{\text{Sum (severity class x number of roots in the class)}}{\text{Total number of roots assessed} \times 4} \times 100$$

*Evaluation of foliar applications of Contans WG (*Coniothyrium minitans*) on sclerotinia disease and infection of sclerotia*

Contans WG is a granular formulation containing 1 x $10^9$ spores $g^{-1}$ of the soil fungus *Coniothyrium minitans*. The fungus attacks the sclerotia of *S. sclerotiorum* in soil and destroys them. It has an on-label approval (OLA), which allows it to be used as a soil incorporation pre-planting or post harvest of any outdoor or protected edible or non-edible crop. There are two main approaches in the application of Contans WG. It can be incorporated into the soil before sowing to kill sclerotia, the source of apothecia (fruiting bodies), or applied to crop debris after harvest of an infected crop to infect mycelium and sclerotia of *S. sclerotiorum*, which reduces contamination of the soil with viable sclerotia. In 2004, a trial was conducted to evaluate the effect of Contans WG soil incorporation on

infection of sclerotia by *C. minitans*. Results from this trial revealed that 45% of resting bodies collected from Contans WG treated plots were infected by the mycoparasite, confirming the potential of the product to reduce the build up of sclerotial inoculum in soil.

In a previous study, Trutmann *et al*. (1982) found that spraying bean (*Phaseolus vulgaris* L.) plants with *C. minitans* did not reduce disease severity caused by *S. sclerotiorum*, but it significantly reduced the number of sclerotia produced on diseased plants. Gerlagh *et al*. (2003) showed that spraying *C. minitans* on the canopies of crops susceptible to *S. sclerotiorum* resulted in a high rate (>90%) of infection of sclerotia by the mycoparasite, and a significant reduction in sclerotial viability. Consequently, a field trial was conducted in 2005 to evaluate the effect of aerial applications of Contans WG to carrot foliage on sclerotinia disease and infection of developing sclerotia.

The trial was conducted within a commercial crop at the Aird site (see Table 1 for details). The crop, cvs. 'Elegance' and 'Nairobi', were drilled on 14 April in a silty clay loam and the trial grown according to standard commercial practice, except that no fungicides were applied to the trial area. The cv. 'Elegance' produces light, erect foliage in contrast to 'Nairobi' which produces heavy, dense foliage that is inclined to flop over, lodge and senesce early on in the season through shading. Sixty plots (30 plots each of 'Elegance' and 'Nairobi') were marked out in early May. Each plot was 10 m long and 5 rows (3 triple lines per row) wide on 1.8 m beds. Plots within blocks were separated by 2 m guards and between blocks by 1.8 m beds. Discard plots and beds were also included around the perimeter of the trial.

Immediately after the appearance of sclerotinia foliage disease symptoms [8 Aug 2005; fresh full canopy, lodging (1) and 1-2 senescing leaves/plant], three treatments were applied to separate plots. These were: (1) control, no treatment; (2) Contans WG spore suspension [2.4 x $10^6$ spores $ml^{-1}$; recommended rate (4 kg $ha^{-1}$)]; (3) Contans WG spore suspension [4.8 x $10^6$ spores $ml^{-1}$; 2 x recommended rate (8 kg $ha^{-1}$)]. Treatments were randomised within a two-way factorial randomised block with Contans WG spore suspension and cultivar as factors, and 10 blocks, with one replicate treatment per block. Two further applications of

each treatment were made at 14-day-intervals [22 Aug 2005 (fresh to mature canopy, heavy lodging (2-3) and 3-4 senescing leaves/plant) and 5 Sep 2005 (mature canopy, heavy lodging (3) and 3-4 senescing leaves/plant).

To apply the spore suspension of Contans WG to the foliage of each plot, 7.2 g (equivalent to 4 kg $ha^{-1}$) or 14.4 g (equivalent to 8 kg $ha^{-1}$) of product was added to 3 L of water in the spray tank and agitated for 10 min. The suspension (3 L) was applied evenly to each appropriate plot (equivalent to 1500 l $ha^{-1}$) using a Hozelock conventional sprayer (Haddenham, Bukinghamshire) fitted with a cone nozzle, and free of fungicide and pesticide residues. Agitation was maintained continuously during spraying.

*Assessment of disease and infection of sclerotia with* C. minitans. Foliage disease severity was assessed approximately 4 weeks after the third spray of *C.* minitans on 3 October [heavily lodged canopy (3) & 7-8 senescing leaves/plant] using the sclerotinia disease key (Appendix 1) as described before. Only foliage within the central 8 m x middle three rows of each plot was assessed.

Samples of 30 sclerotia per plot were collected from diseased foliage/crop debris and stored at $5^0$C prior to assessing sclerotia for infection by *C. minitans*. Recovered sclerotia were washed under a flow of tap water through a 1 mm mesh screen. Twenty sclerotia were surface sterilised by agitation in 50 ml of a 50:50 (v/v) solution of 13-15% sodium hypochlorite and absolute ethanol for 3 min. Each sclerotium was then rinsed twice individually for 1 min in 2 ml sterile distilled water (SDW) in a separate well of a 25 well Petri dish (Bibby Sterilin, Stove, UK). Washing sclerotia individually eliminated possible cross contamination of sclerotia by a heavily infected sclerotium that could occur during batch washing of sclerotia. The sclerotia were then bisected with each half being placed on a 15 mm diameter PDA disc containing chlortetracycline (20 mg $l^{-1}$) in a Petri dish. The number of sclerotia showing mycelial growth of *S. sclerotiorum* (viable) and/or infection by *C. minitans* were assessed after 10-14 days incubation at $20^0$C.

*Statistical analyses*

A standardised approach was adopted for the analyses of data from the controlled environment experiments in which Generalised Linear Models (GLMs) were fitted to the data to determine whether the factors under investigation had effects on the variable of interest. The GLMs used a binomial error distribution and a logistic function as the linear link.

Percentage data were transformed to angular values before an analysis of variance (ANOVA). Significant differences among treatments are based on the *F*-test in ANOVA. When appropriate, treatments means were compared with the least significant difference (LSD) at a probability of 5% ($P = 0.05$).

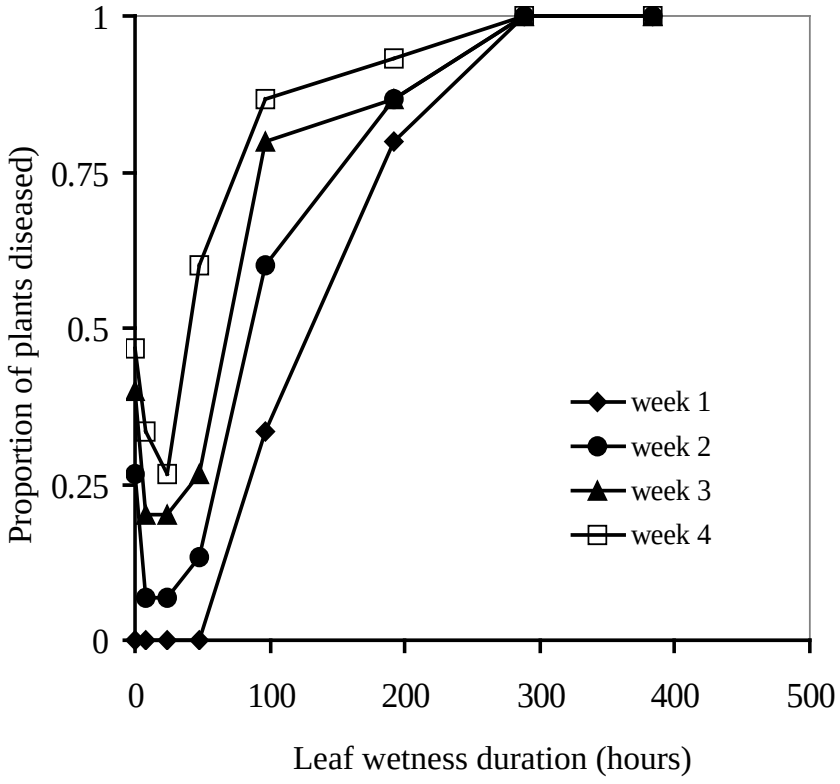
**Results and Discussion**

***Growth chamber Experiments***

In all growth chamber/glasshouse experiments, disease first appeared 5-11 days after inoculation depending on the experiment. Disease was always seen first seen on either the petioles or blades of damaged leaves as water-soaked, dark olive-green lesions followed by the presence of the characteristic fluffy cotton wool like mycelium.

*Effect of leaf wetness on infection of carrots by ascospores*

The length of leaf wetness duration had a significant effect on infection of carrot foliage by ascospores of *S. sclerotiorum* (Figure 1). When plants were exposed to long leaf wetness durations (up to 16 days) after inoculation, disease incidence reached 100% within 1 week of the end of the leaf wetness period. However, shorter leaf wetness durations resulted in some plants escaping infection. In general, a leaf wetness duration of approximately 200 h (8 days) resulted in approximately 75% of plants diseased. Shorter wetness durations than this resulted



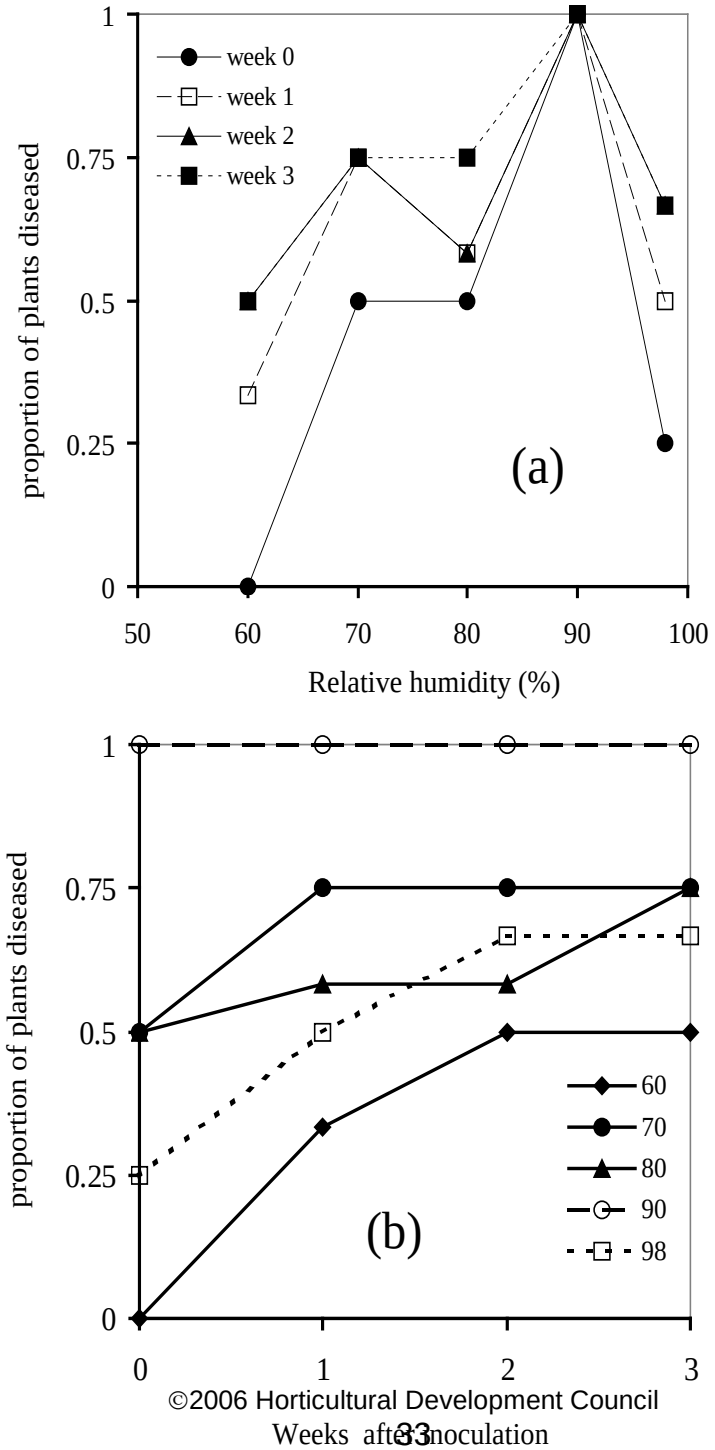

in large variability in disease incidence even after 4 weeks.

Figure 1 Effect of leaf wetness on disease incidence following infection of carrot foliage by ascospores of *S. sclerotiorum*.

*Effect of relative humidity on infection of carrots by ascospores*

The optimum RH for infection of carrot foliage by ascospores of *S. sclerotiorum* was 90% (Figure 2), at which all plants were diseased within 1 week of inoculation. The rate of disease progress was generally positively correlated with RH from 60 up to 90% RH, and declined slightly at 98% RH. The proportion of plants diseased over time for 70, 80 and 98% RH was not significantly different.

Figure 2. Effect of RH on disease incidence following infection of carrot foliage by ascospores of *S. sclerotiorum*.

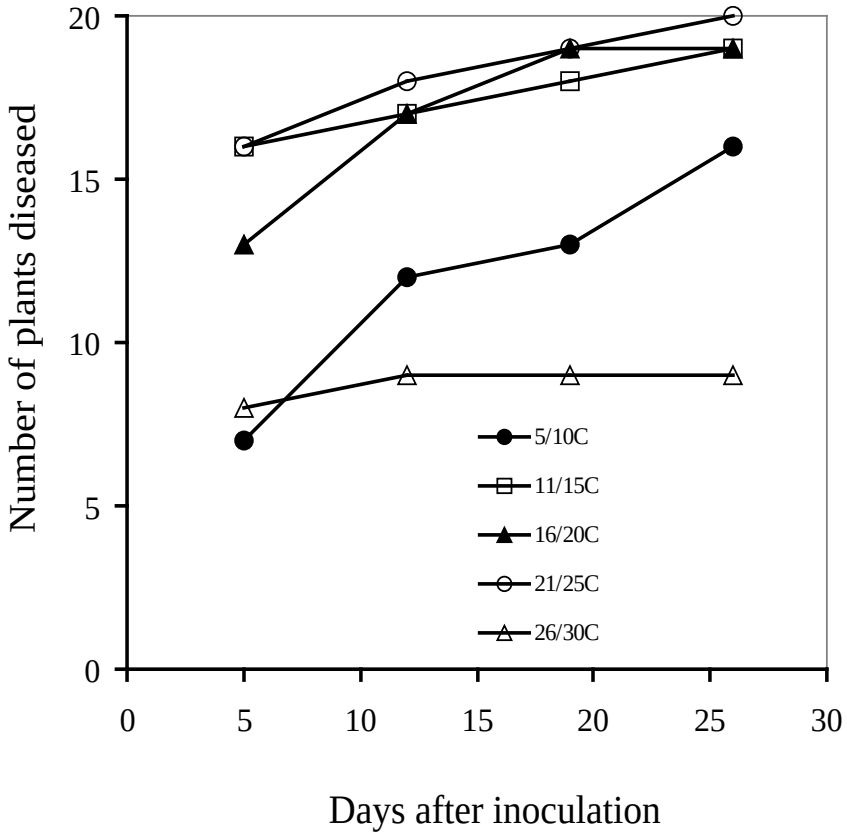


*Effect of temperature on infection of carrots by ascospores*

Infection of carrot foliage by ascospores of *S. sclerotiorum* was greatest in the range of 10 to 18$^0$C (Figure 3). The highest levels of disease incidence and most rapid rates of disease development over time were observed at this temperature range. At the highest diurnal temperature combination (26-30$^0$C), disease incidence remained below 80% over the duration of the experiment. The low temperature combination (5-10$^0$C) decreased the rate of disease development, but did not prevent a high proportion of plants eventually becoming infected.

Optimum conditions for foliage infection were greater than 4 days (0-8 hours minimum) of continuous leaf wetness, ≥90% (60% minimum) relative humidity (RH) and air temperatures of 10-18$^0$C (5-10$^0$C minimum). Results clearly show that leaf wetness duration, relative humidity (RH) and air temperatures are major factors affecting carrot foliage infection by ascospores of *S. sclerotiorum*.

Figure 3. Effect of temperature on disease incidence following infection of carrot foliage by ascospores of *S. sclerotiorum*.




*Effect of temperature on development of sclerotinia disease*

Sclerotinia disease developed most rapidly at 12 and 18$^0$C, where disease symptoms first appeared 6-9 days after inoculation (Figure 4). At 8 and 25$^0$C, disease developed more slowly. As before, disease was always seen first seen on either the petioles or blades of damaged leaves as water-soaked, dark olive-green lesions followed by the presence of the characteristic fluffy cotton wool like mycelium.

Disease developed between 8-25$^0$C, with optimum temperatures between 12-18$^0$C. The results confirm that air temperature is an important factor affecting Sclerotinia disease development.

***Field work***

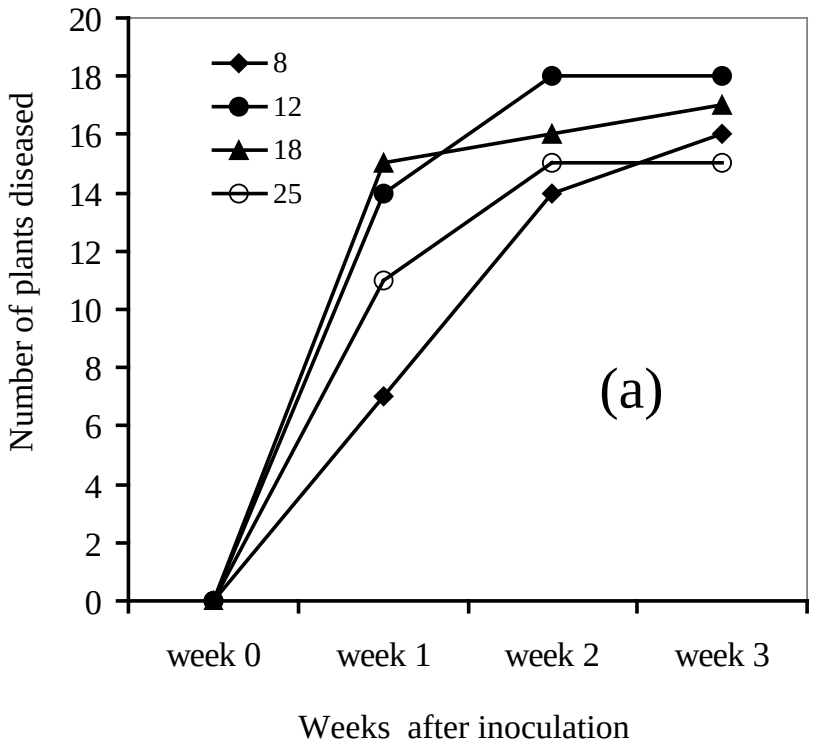
*Monitoring the development of naturally occurring sclerotinia disease and apothecia production in carrot crops*

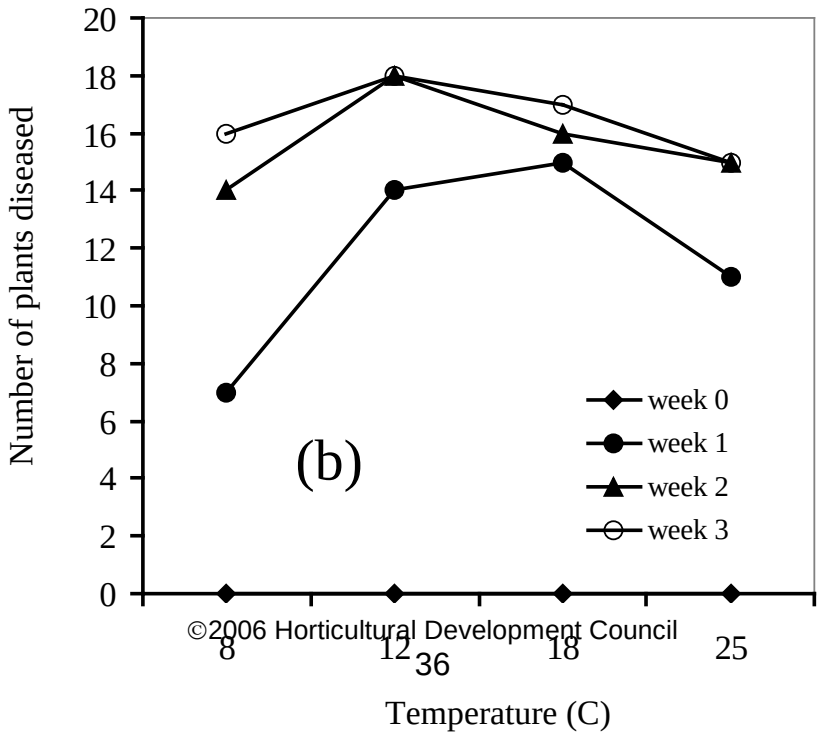
Field observations of two sequentially sown crops (mid April to mid/late May) during the 2005 growing season revealed that apothecia first appeared within the crops between 12 – 25 Aug (Aird, sown 14 Apr) and 26 Aug – 8 Sep (Ravensby, sown 24 May) (Table 4). The first appearance of apothecia generally occurred close to or after full canopy closure, when crops were at the pencil + stage or main period of root growth stages. Mean daily soil temperature ranged between 12-17$^0$C during the 2 weeks before apothecia were first detected.

At both sites, first disease was observed just before the first apothecia were detected. This is in contrast to the observations made in the 2004 season, when the first disease was detected 4-6 weeks after the appearance of the first fruiting bodies. During this season (2005), apothecial population density was much lower compared to that in the 2004 season. The differences in the timing of detecting apothecia in relation to disease incidence between the two seasons may simply be due to the difficulty in detecting apothecia at such low population densities within the crop during the 2005 season.

Senescing leaves were present in the crop at Aird when apothecia were first detected. At both sites, symptoms were first observed on petioles of lodged senescing leaves as water-

Figure 4. Effect of temperature on the development of carrot sclerotinia disease. Carrot plants were inoculated with ascospore suspensions and placed at 18-20$^0$C with continuous leaf wetness to ensure infection before exposure to different temperatures.



soaked, dark-olive green lesions. These lesions then expanded over entire leaves, with infected tissue covered by white, cottony mycelium. The disease then spread by mycelium growing between diseased and healthy foliage in contact with each other. Serious infection of the foliage completely defoliated the crop, leading to the production of abundant sclerotia on the soil surface.

Weather data recorded at the Aird and Ravensby trial sites during the 2005 growing season is summarised in Appendix 2. Average daily air temperature at both sites varied between 10 and 15$^0$C, while the RH was typically in the range of 70-90%. Both sites had regular rainfall, but this was slightly more uniform and abundant at the Aird site. During the monitoring period from late May until early October, the accumulated rainfall at Aird was 250 mm, while it was 241 mm at Ravensby. It should be noted that 53 mm of the total rainfall at Ravensby was recorded over just 2 days in October (41 mm on the 11, 12 and 13 October).

The pattern of disease recorded in early August in each of five beds at Aird is summarised in Appendix 2. Disease was first recorded in early August and increased rapidly and fairly uniform across the beds reaching a final foliage disease severity of 70-80% by early October. No disease data are shown for Ravensby because the disease was noted here only once and then only at trace levels. Despite intensive sampling at both sites, few apothecia were detected. Consequently, there was no apparent correlation between observed foliage disease severity and the apparent population of apothecia. A similar situation was observed in 2004 at two of the trial sites. At Carriston, there was a small but apparent foliage disease epidemic, but apothecia were found only once, and only after the epidemic had started, while at Ravensby, a large population of apothecia were observed, but almost no disease developed in the crop. These observations together with the results from the 2005 field monitoring are shown in Figure 5. The lines marked 'Qn_CRF' in Figure 5 show the levels of proposed canopy risk factor for sclerotinia (see later section on **'**Development of a simple predictive model'). It should be noted though that, in each case where a large epidemic of sclerotinia has been observed over the last 2 years, the risk factor is also high, while it is low in each case where disease was either absent, or occurred only at a low level.

Overall, fewer apothecia were detected during the 2005 season and disease was less severe compared to the 2004 season. This observation is most likely associated with less rainfall during the 2005 season and the sites being much drier than in 2004.

*Summary of General Field Observations*

Field observations made during the 2005 season were very similar to those observed during the 2004 season and included the following:

- The appearance of lodged, senescing leaves in carrot crops usually occurred close to or after full canopy enclosure. The pattern of senescence in individual plants was fairly consistent, with individual leaves senescing in turn beginning with the oldest.
- The first appearance of apothecia in carrot crops generally occurred close to or after full canopy enclosure, and was concurrent with the early onset of senescing foliage.
- The primary site of infection was senescing or damaged petioles. Symptoms were first noticed on petioles of lodged senescing leaves as water-soaked, dark olive-green lesions. These lesions then expanded over the entire leaf, with infected tissue covered by abundant cotton wool like mycelium. The disease then often spread by mycelium growing between diseased and healthy foliage in contact with each other. No symptoms were observed before foliar senescence or crop damage along the bed wheelings.
- At an advanced stage, affected tissues exhibited a bleached appearance, and entire plants collapsed and died. Sclerotia developed externally in the mycelium, or internally within the pith of the petiole.
- Serious infection of the foliage completely defoliated the crop, leading to the production of abundant sclerotia on the soil surface.
- Root infection resulted from infected foliage *via* the crown, but symptoms were rarely evident in the field unless the foliage had been completely defoliated, or until the roots had been stored under straw.

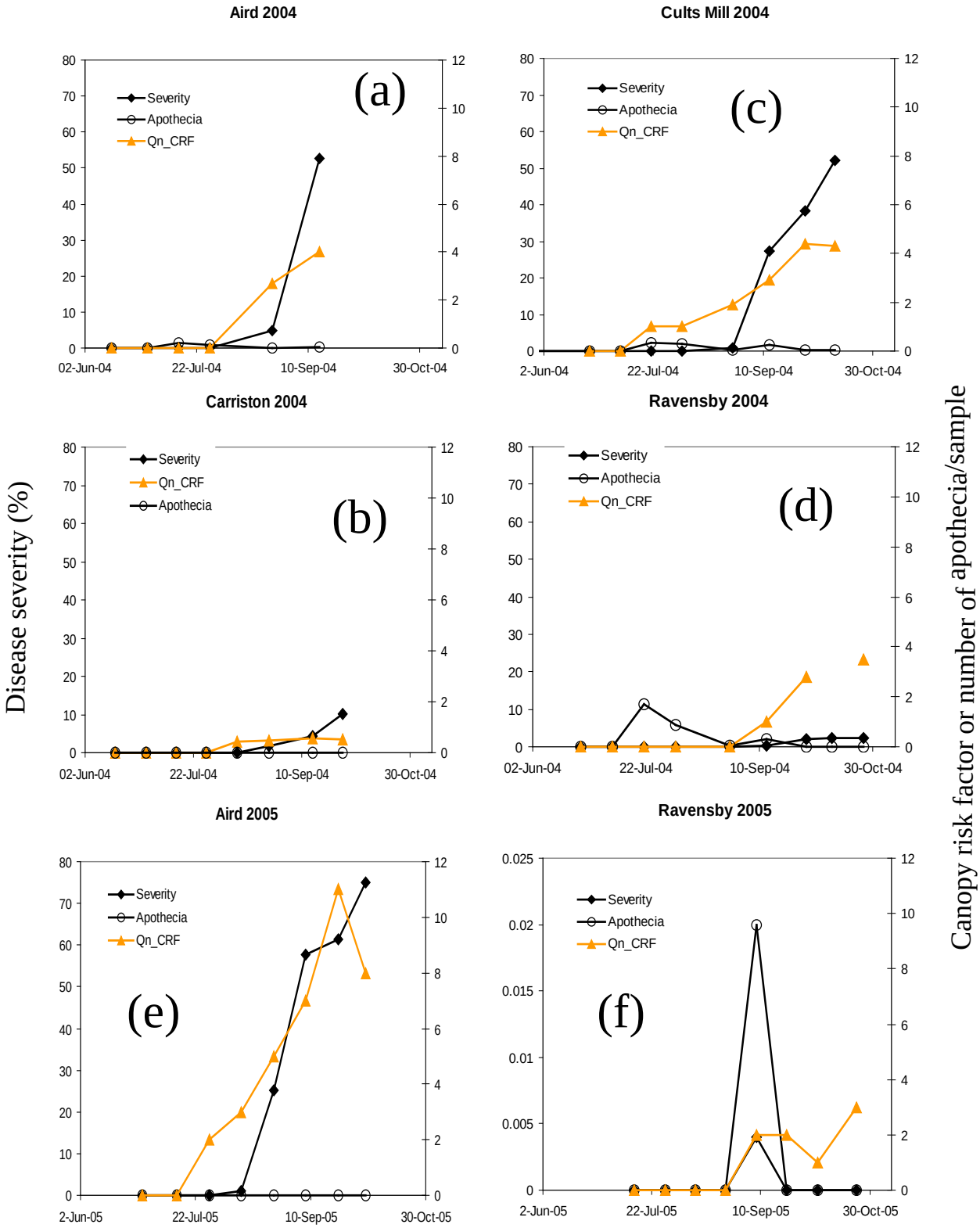
Field observations made during the 2004 and 2005 seasons have indicated that canopy closure, the presence of apothecia within the crop and senescing leaves on the ground are important factors in affecting disease initiation and development in the field.

Table 4. 2005 - Production of apothecia and incidence of disease in commercially grown carrot crops (cv. Nairobi ) with sequential sowing dates in relation to crop growth stage at two field sites.

| Site / Sowing date | Soil[a] Temperature ($^0$C) | First apothecia | | | First disease | |
|---|---|---|---|---|---|---|
| | | Date[b] | Canopy enclosure | Growth stage | Date[b] | Growth stage/*site of infection* |
| **Aird** | | | | | | |
| 31 Mar | 12-15 (13.6) | 12–**25** Aug | Yes | Main period of root growth | 15-**28** July | Main period of root growth *Petioles of lodged senescing leaves* |
| **Ravensby** | | | | | | |
| 22 May | 13-17 (14.8) | 26 Aug – **8** Sep | Almost (95% crop coverage ) | Pencil + stage/main period of root growth | 4-**26** Aug | Main period of root growth/early lodging *Senescing lower leaves* |

[a] Range and mean soil temperature during the 2 weeks preceding the 2 week period during which the first apothecia were detected.
[b] Sites were monitored fortnightly. The 1st date is the date immediately after the last assessment, and the 2nd date (in bold) is the date of the next assessment on which apothecia/disease were first detected.

Figure 5. Apothecial production, foliage disease severity and proposed quantitative risk factors (Qn_CRF) for six trial sites in 2004/05.



*Apothecial production by sclerotia buried in the field*

Fortnightly burials of *S. sclerotiorum* isolates KP and Ret were carried out within the crop between April and August 2005 at the Aird and Ravensby field sites. In the fortnightly burials of sclerotia at Aird, the time from burial to the first appearance of apothecia ranged from 70-84 to 28-42 days for isolate KP1, and from 56-70 to 14-28 days for isolate Ret (Table 5). For early burials of isolate KP1 sclerotia, made between mid April and during May (burials 1-3), apothecia appeared in 36-59 days and >35% of the sclerotia germinated. For the subsequent burials, apothecia appeared in 28-42 days, but far fewer or, in some of the cases, none of the sclerotia germinated before the crop was covered in straw in early October. A similar pattern of time to first observation of apothecia was observed for isolate Ret sclerotia, but no clear pattern of sclerotial germination was observed.

Apothecia produced by sclerotia buried early in April and May (burials 1-3) were first seen in late June to mid to late July (close to or after full canopy closure), and were observed throughout the growing season up to covering the crop with straw in early October. Peak germination and apothecial production from these early burials of sclerotia was generally during late July and August. Sclerotia (both KP1 and Ret) of burials 9 (11 Aug), and 10 (25 Aug) did not germinate to produce apothecia before covering the crop with straw on 6-8 October.

Overall, at the Aird site, the total percentage of sclerotia buried that germinated was reduced for isolate KP1 in late burials made from June to August, compared with those made between April and May.

At the Ravensby site (Table 6), apothecial production was considerably lower than at the Aird site, and no clear patterns of germination were observed. For burials of isolate KP1 sclerotia, made between early June and late July (burials 1-6), germination only reached 4%. None of the sclerotia of this isolate germinated from subsequent burials, made from August to late September, before the crop was covered in straw in late November. For burials of isolate Ret sclerotia, made in June (burials 1-3), germination only reached 2% and apothecia appeared 84-98 days after burial. For burials made in July (burials 4-6), apothecia appeared in 28-

56 days and 12-37% of sclerotia germinated. None of the sclerotia of isolate Ret germinated from subsequent burials before the crop was covered in straw in late November.

Table 5. Germination of sclerotia of *S. sclerotiorum* (isolate KP1 and Ret) in 2005 buried in soil at the Aird site, Perthshire.

| | KP1 | | Ret | |
|---|---|---|---|---|
| Burial No. /Date | Germinated (%)[a] | Time to first observation of apothecia (days) [b] | Germinated (%)[a] | Time to first observation of apothecia (days) [b] |
| 1. 21 Apr | 59 ± 4.0[c] | 70-84 | 34 ± 4.3 | 56-70 |
| 2. 5 May | 42 ± 2.9 | 42-56 | 24 ± 2.2 | 56-70 |
| 3.19 May | 36 ± 3.9 | 42-56 | 12 ± 3.3 | 42-56 |
| 4. 2 Jun | 19 ± 2.6 | 28-42 | 6 ± 1.4 | 48-56 |
| 5. 16 Jun | 14 ± 1.4 | 28-42 | 8 ± 1.9 | 48-56 |
| 6. 29 Jun | 9 ± 3.5 | 28-42 | 28 ± 5.2 | 28-42 |
| 7. 14 Jul | 6 ± 3.2 | 28-42 | 61 ± 4.5 | 28-42 |
| 8. 28 Jul | 2 ± 1.2 | 28-42 | 25 ± 5.8 | 14-28 |
| 9. 11 Aug | ND[d] | - | ND | - |
| 10. 25 Aug | ND | - | ND | - |

[a] Final percentage germination of sclerotia producing apothecia.
[b] Sites were monitored fortnightly. The 1st value is the number of days up to which no apothecia were detected; the 2nd value is the number of days after burial by which apothecia had appeared.
[c] Each value is the mean ± SE of four replicates, with 50 sclerotia per replicate.
[d] No apothecia detected before covering crop with straw on 6-8 Oct.

Table 6. Germination of sclerotia of *S. sclerotiorum* (isolates KP1 and Ret) in 2005 buried in soil at the Ravensby site, Angus.

| Burial No. /Date | KP1 | | Ret | |
|---|---|---|---|---|
| | Germinated (%)[a] | Time to first observation of apothecia (days)[b] | Germinated (%)[a] | Time to first observation of apothecia (days)[b] |
| 1. 2 Jun | 2 ± 1.5[c] | 57-70 | 1 ± 0.5 | 84-98 |
| 2. 16 Jun | ND[d] | - | 2 ± 0.8 | 84-98 |
| 3.30 Jun | ND | - | ND | - |
| 4. 2 Jul | 4 ± 1.7 | 42-53 | 20 ± 6.8 | 42-56 |
| 5. 14 Jul | 4 ± 0.9 | 28-42 | 37 ± 4.4 | 28-42 |
| 6. 28 Jul | 2 ± 1.4 | 28-42 | 22 ± 6.4 | 28-42 |
| 7. 11 Aug | ND | - | 12 ± 4.1 | 28-42 |
| 8. 25 Aug | ND | - | ND | - |
| 9. 8 Sep | ND | - | ND | - |
| 10. 22 Sep | ND | - | ND | - |

[a] Final percentage germination of sclerotia producing apothecia.
[b] Sites were monitored fortnightly. The 1st value is the number of days up to which no apothecia were detected; the 2nd value is the number of days after burial by which apothecia had appeared.
[c] Each value is the mean ± SE of four replicates, with 50 sclerotia per replicate.
[d] No apothecia detected before covering crop with straw on 25 Nov.

*Evaluation of fungicide spray programmes and timings*

Sclerotinia disease was first observed in the trial at a very low incidence in untreated plots on 28 July 2005. Typical symptoms included water-soaked, dark olive-green lesions with collapsed leaves. Infected tissue was typically covered by abundant cotton wool like, white mycelium.

Table 7. Fungicide trial (natural infection) – Aird site, Perthshire.
Effect of fungicide spray programmes and timings on foliage disease severity caused by *S. sclerotiorum*.

| Timings[a] | | | | |
|---|---|---|---|---|
| T1 (5 Jul) | T2 (19 Jul) | T3 (2 Aug) | T4 (16 Aug) | Foliage disease severity (%)[b] |
| -[c] | - | - | - | 18.8 (25.4)[d] |
| Amistar/Folicur (tank mix) | Amistar/Folicur (tank mix) | - | Amistar/Folicur (tank mix) | 0.07 (1.4) |
| Signum | Folicur | Signum | Folicur | 0.1 (1.8) |
| - | Signum | Folicur | Signum | 1.8 (6.2) |
| Signum | - | Folicur | - | 1.6 (5.9) |
| Signum | Shirlan | Signum | Shirlan | 0.1 (1.8) |
| UKA374 | UKA373 | UKA374 | UKA373 | 0.05 (0.9) |
| A9219B | Folicur | A9219B | Folicur | 0.1 (1.8) |
| *P* value | | | | <0.001 |
| LSD[e] (*P* = 0.05) | | | | (3.7) |

[a] The four fungicide timings were: T1 (prior to canopy enclosure - 5 Jul), 90-95% crop coverage & small pencil stage; T2 (19 Jul), 100% crop coverage, full canopy enclosure & main period of root growth; T3 (2 Aug), fresh full canopy, lodging & 1-2 senescing leaves/plant; T4 (16 Aug), fresh to mature canopy, heavy lodging & 3-4 senescing leaves/plant.
[b] Foliage disease severity was assessed on the 20 Sep (growth stage - heavily lodged canopy & 6-7 senescing leaves/plant). [c] -, No fungicide applied.
[d] Values are means of five replicate plots. Figures in parentheses are angular transformations of percentage data.
[e] LSD is the least significant difference at a probability of 5%.

At the first disease assessment on 6 September (heavily lodged canopy & 4-5 senescing leaves/plant), there was less than 5% foliage disease severity within the untreated plots and there were no significant differences between the fungicide spray programmes.

At the second disease assessment on 20 September, (heavily lodged canopy & 6-7 senescing leaves/plant), foliage disease severity in the untreated control plots had increased to 15-23% (Table 7). All fungicide spray programmes significantly ($P < 0.001$) reduced foliage disease severity compared to the untreated control. Alternating 4-spray programmes at 14-day intervals of Signum (boscalid + pyraclostrobin) and Folicur (tebuconazole), Signum and Shirlan (fluazinam), UKA373 and UKA374 (previously tested as UKA352 in 2004), and A9219B and Folicur, with the first spray applied before canopy closure, gave good control and were equally effective in reducing foliage disease severity compared to the untreated control (Table 7). A 3-spray programme of an Amistar (azoxystrobin)/Folicur tank mix with the first spray applied before canopy closure, the first two sprays applied at 14-day intervals and the final spray applied 28 days later, was as effective as the 4-spray programmes applied at 14-day intervals. Omission of a fungicide spray prior to canopy closure, and then applying alternating sprays of Signum and Folicur at 14-day intervals significantly increased foliage disease severity, compared to fungicide programmes in which alternating sprays were applied at 14-day intervals. Also, extending the spray interval beyond 14 days and applying Signum followed by Folicur at 28-day intervals significantly increased foliage disease severity compared to fungicide programmes in which alternating sprays were applied at 14-day intervals.

*Effects on yield and sclerotinia root rot*. Roots were harvested on 24 January 2005 after the crop was covered in straw on 6-8 October 2005. Assessments of total yield and marketable yield showed considerable variation and there were no significant ($P>0.05$) differences between the spray programme treatments and the untreated control (Table 8). None of the spray programmes improved total or marketable yield. Only three of the seven fungicide programmes significantly ($P<0.05$) reduced the incidence and severity of sclerotinia root rot. These included the 3-spray programme of the Amistar/Folicur tank mix, the alternating 3-spray

programme of Signum and Folicur, and the alternating 4-spray programme of UKA374 and UKA373.

Results from this year's fungicide trial, revealed that good control of foliage disease was obtained with alternating 4-spray programmes of Signum (boscalid + pyraclostrobin) and Folicur (tebuconazole), Signum and Shirlan (fluazinam), UKA373 and UKA374 (previously tested as UKA352 in 2004), and A9219B and Folicur applied at 14-day intervals. Good control was also obtained with a 3-spray programme of an Amistar (azoxystrobin)/Folicur tank mix, with the first two sprays applied at 14-day intervals (first spray applied before canopy) and the final spray applied 28 days later. Control was as effective as the 4-spray programmes applied at 14-day intervals. Omission of a fungicide spray prior to canopy closure gave reduced control. Extending the spray interval beyond 14 days early in the spray programme tended to reduce foliage disease control. This year's trial results have corroborated those from last year's trial, which indicated that it is important to apply the first fungicide early, just before the canopy closes, to ensure protection of senescing leaves at the base of the canopy from sclerotinia.

Results from both years' trials indicated that foliar applications of the experimental fungicides Shirlan, UKA373, UKA374 and A9219B gave good foliage disease control. Approval for use of these fungicides on carrots should be considered and sought.

Despite reducing sclerotinia foliage disease severity, none of the fungicide spray programmes significantly improved marketable yield and only three of them reduced sclerotinia root rot. This was most likely associated with the patchy low incidence of foliage disease throughout the trial. Furthermore, the incidence of foliage disease was very localised within treatment plots, and there was considerable variation in yields and sclerotinia root rot between plots and blocks. This contrasts with the 2004 fungicide trial, when control of foliage disease by the most effective fungicides (i.e. Signum, Folicur, Shirlan, UKA373, UKA374 and A9219B) was associated with increases in yield (23–28 t/ha) of marketable roots. Thus, the inability of the fungicide spray programmes to improve marketable yield

and most of the programmes to reduce sclerotinia root rot in 2005 must be viewed with caution.

An integrated control programme, using a simple disease forecasting system and fungicide programmes, will be devised and evaluated in trials conducted in 2006 (Year 3). It is anticipated that the simple forecasting will help to predict the optimum time to spray.

Table 8. Fungicide trial (natural infection) – Aird site, Perthshire.

Effect of fungicide spray programmes and timings on yield and sclerotinia root rot (roots were harvested on 24 January after the crop was covered in straw on 6-8 October 2004).

| | Timings[a] | | | | | | Percentage | |
|---|---|---|---|---|---|---|---|---|
| Trt | T1 (19 Jul) | T2 (19 Jul) | T3 (2 Aug) | T4 (16 Aug) | Total yield (t/ha) | Marketable yield (t/ha) | Sclerotinia infected roots | Root disease severity |
| 1. (Untr) | - | - | - | - | 148.2[b] | 114.7 | 29.7 (32.0)[b] | 12.4 (19.6) |
| 2. | Amistar/Folicur | Amistar/Folicur | - | Amistar/Folicur | 153.3 | 115.0 | 2.3 (8.5) | 1.0 (5.6) |
| 3. | Signum | Folicur | Signum | Folicur | 151.9 | 123.0 | 32.5 (32.5) | 12.3 (18.3) |
| 4. | - | Signum | Folicur | Signum | 139.7 | 110.5 | 16.6 (22.6) | 4.4 (11.3) |
| 5. | Signum | - | Folicur | Signum | 152.8 | 109.4 | 7.3 (12.2) | 2.8 (8.0) |
| 6 | Signum | Shirlan | Signum | Shirlan | 148.3 | 112.2 | 12.5 (20.4) | 6.3 (13.3) |
| 7. | UKA374 | UKA373 | UKA374 | UKA373 | 150.0 | 107.8 | 2.5 (7.9) | 0.6 (3.8) |
| 8. | A9219B | Folicur | A9219B | Folicur | 148.1 | 116.1 | 8.9 (15.2) | 3.1 (9.2) |
| *P* value | | | | | 0.81 (NS[c]) | 0.66 (NS) | 0.028 | 0.038 |
| LSD[d] (*P* =0.05) | | | | | - | - | (16.81) | (10.20) |

[a] See Table 7 footnote for details of fungicide timings.
[b] Values are means of five replicate plots. Figures in parentheses are angular transformations of percentage data.
[c] NS – Not significant. [d]LSD is the least significant difference at a probability of 5%

*Evaluation of foliar applications of Contans WG (*Coniothyrium minitans*) on sclerotinia disease and infection of sclerotia*

*Disease assessments*. Foliage disease severity in the untreated control was 36% on 3 October, four weeks after the final application of three successive sprays of Contans WG spore suspensions at 14-day intervals (Table 9). No significant difference ($P$ = 0.214) in foliage disease severity was obtained between the two Contans WG treatments (30 and 42% foliage disease severity, respectively) and the untreated control.

There was a significant ($P$<0.001) difference in cultivar susceptibility to sclerotinia disease between 'Nairobi' and 'Elegance' (Table 9). Foliage disease severity on the cv. 'Nairobi' was 55% compared to 17% on 'Elegance'.

*Sclerotial infection and viability*. The viability of sclerotia collected from diseased foliage/crop debris four weeks after the final application of Contans WG spore suspension is shown in Table 9. Both Contans WG spore suspension treatments applied to the foliage significantly ($P$<0.001) decreased the percentage the percentage viability of sclerotia (16 and 32%) compared to the untreated control (95%). In addition, both spore suspension treatments significantly ($P$<0.005) increased the percentage of sclerotia infected with *C. minitans* (58 and 81 %) compared to the untreated control (19%).

The results from this field trial reveal that Contans WG applied to carrot foliage of the growing crop has the potential to reduce the build up of sclerotial inoculum in soil long term. Up to 80% of the sclerotia of *S. sclerotiorum* collected from the foliage/crop debris of plots treated with spore suspensions of Contans WG were infected with *C. minitans*, and were unlikely to germinate to produce apothecia. Gerlagh *et al*. (2003) have also previously reported infection of sclerotia (90%) by *C. minitans* after spraying the canopy of oilseed rape with a spore suspension of the mycoparasite. Contans WG currently has an on-label approval (OLA) in the UK, which allows it to be used as a soil incorporation before planting or as a foliar spray to crop debris post harvest on any protected edible or non edible crop. It is

not permitted to apply the product to the foliage of a growing crop. In view of the promising results obtained in this study, approval to apply Contans WG to carrot foliage of a growing crop should be sought, provided that further field trials corroborate its ability to infect sclerotia developing on diseased foliage. Further trials are recommended in carrot crops to investigate optimising the timing of the product in relation to crop growth stage and infection by the pathogen. The effect of applying the product to infected foliage, prior to covering the crop with straw for winter frost protection, on sclerotial production and survival also merits investigation.

In this study, *C. minitans* infected sclerotia were also recovered from the control untreated plots. Water splash and mesofauna have been reported to spread *C. minitans* from the site of application to other plots in previous glasshouse trials (Williams *et al*., 1998a-c). Spread of *C. minitans* from plots sprayed with the mycoparasite to neighbouring untreated control plots in this trial could have been caused by spray drift or by secondary transmission of spores by insects.

The high susceptibility of the cultivar 'Nairobi' to sclerotinia disease was confirmed in this study. 'Nairobi' produces heavy, dense foliage that is inclined to flop, lodge and senesce early on in the season, increasing susceptibility to infection. In contrast, 'Elegance' produces light, erect foliage which tends not to lodge and senesce early on in the growing season. Further trials to evaluate the susceptibility of different carrot cultivars to sclerotinia disease are warranted. Cultivars may differ in susceptibility and, although none are likely to be completely resistant, some may require fewer sprays than others to minimise sclerotinia disease.

Table 9. 2005 Trial (Aird site, Perthshire) - Effect of three successive foliar applications of spore suspensions of Contans WG at 14 day-intervals on foliage disease severity, sclerotial viability and infection by *Coniothyrium minitans* on 3 October, 4 weeks after the final spray.

| Treatment[a]/Cultivar | Foliage disease severity (%) | Viability of sclerotia (%) | Infection of sclerotia (%) |
|---|---|---|---|
| Control (nil) | 35.7 (35.7)[b] | 95.2 (77.9)[c] | 19.3 (24.5)[c] |
| **Contans WG** | | | |
| 2.4 x $10^6$ spores $ml^{-1}$ (4 kg/ha) | 41.5 (39.1) | 16.0 (23.5) | 81.0 (65.8) |
| 4.8 x $10^6$ spores $ml^{-1}$ (8 kg/ha) | 30.0 (32.7) | 38.2 (37.9) | 58.2 (49.9) |
| *P* value | 0.214 | <0.001 | <0.005 |
| LSD (*P*=0.05) | Not significant | (9.85) | (19.0) |
| **Cultivar** | | | |
| 'Nairobi' | 54.7 (48.1) | | |
| 'Elegance' | 16.8 (23.6) | | |
| *P* value | <0.001 | | |
| LSD (*P*=0.05) | (16.1) | | |

[a] Three successive spore suspension sprays were applied at approximately 14-day intervals on 8 Aug (immediately after the appearance of sclerotinia foliage disease), 22 Aug and 5 Sep.
[b] Values are means of 10 replicate plots. Values in parentheses are angular transformed data.
[d] Values based on observation of 30 sclerotia from each of five replicate 'Nairobi' plots. Values in parentheses are angular transformed data.

**Development of a simple predictive model**

Rationale for the adopted approach

The 'disease triangle' illustrates that in order for disease to occur the following are required: (1) a susceptible host plant, (2) a pathogen, and (3) a conducive environment. Despite the inescapable logic of this simple idea, many disease prediction systems use information from only one or two "corners" of the triangle

to predict disease risk. Well known examples of this approach are the uses of 'scab' and 'blight' risk periods for apple scab and potato blight, respectively. These use only information on the weather to identify risk periods, ignoring (or taking for granted) the presence of susceptible hosts and a supply of inoculum. Our approach to predicting the risk of carrot sclerotinia mirrors this idea, but focuses on the correlation between host crop development and disease in order to predict risk.

It is assumed that infection requires the presence of senescing or lodged/damaged foliage. It is also assumed that the main flush of sclerotial germination occurs approximately at the same time when canopy closure occurs. A simple scoring system is used to record the three relevant features of the canopy development: (A) percentage ground cover within the bed (recorded as a proportion from 0 to 1); (B) number of senescing leaves per plant; (C) a simple numerical lodging index. These three numbers are combined into a quantitative canopy risk factor (Qn_CRF):

Qn_CRF = A + [A×(B+C)], where A, B and C refer to the canopy features listed above

Examples from 2004 and 2005 field data

Observations on apothecial numbers, disease severity and the Qn_CRF values for untreated carrot beds in 2004 (4 sites) and 2005 (2 sites) are summarised in Figure 5. Values of the Qn_CRF and the number of apothecia observed per sample should be read off the right hand scale; disease severity is shown on the left scale.

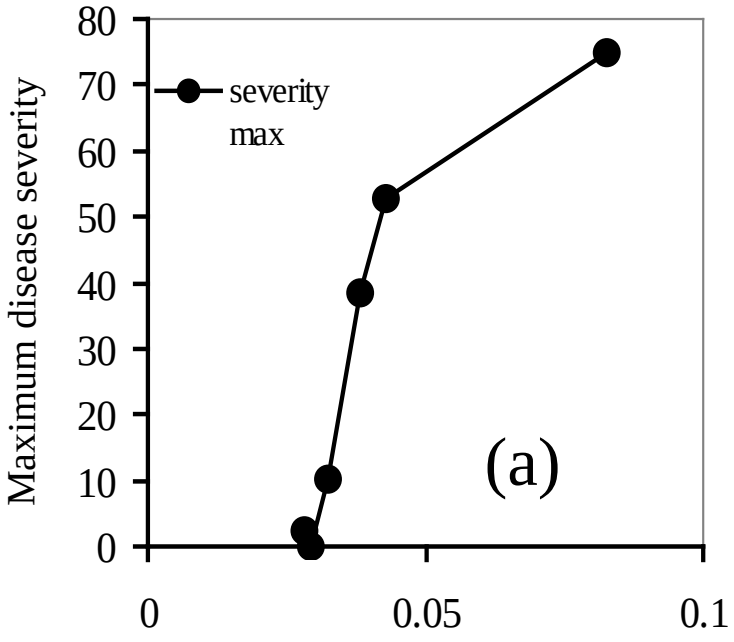
In all cases, it is noted that the Qn_CRF increases in advance of disease. Also, it is noted that in the cases where the worst epidemics occurred (Aird 2004, Cults Mill, 2004 and Aird 2005), the Qn_CRF had already started to increase before apothecia were found. At Carriston in 2004, although the Qn_CRF increased quite rapidly, the epidemic was not as bad as at Aird or Cults Mill. This might have been because fewer apothecia were observed at Carriston; certainly (as can be seen from the graphs) fewer apothecia were found at Carriston. At Ravensby in both years, there was almost no disease even though (particularly in 2004) there

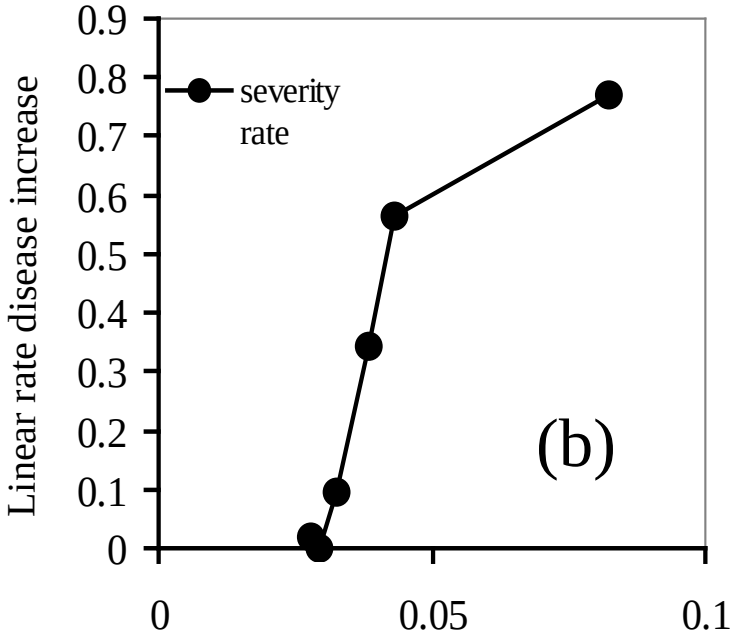
were high numbers of apothecia present. When comparing the results for Ravensby 2005 with the other 5 sites, it should be noted that the scales on the graph are much larger because the very low levels of disease and apothecia detected could not be sensibly shown on the same scale as the other 5 sites.

It is apparent from the way in which Qn_CRF is calculated that it will reach a value of 1 (or more) when canopy cover within the bed is 100%. Clearly, this value could also be reached if, for some reason, the crop suffered from early senescence or lodging during canopy development, and both of these factors would indicate an increased risk of infection as a result of the availability of damaged tissue. On the basis of the available data, we are able to suggest that, if the Qn_CRF value reaches 1 in early to mid July (indicating early crop development), there is a severe risk of sclerotinia disease. In the case of the data collected in 2004 and 2005, using a date of 15 July allowed us to differentiate with 100% accuracy those crops which had severe epidemics from those which did not (see Figure 5). This particular date is likely to be a feature of the data set we used. Consequently, in order to use the Qn_CRF in practice it would be necessary for individual growers to tailor it to their particular situations.

In practice, we suggest that the grower would note the Qn_CRF on a weekly basis during late June and early July, recording the value on a simple chart. If the value reaches 1 in early to mid July the need for a pre-canopy closure spray is indicated. Continuing to record the Qn_CRF will also allow the grower some idea of the potential risk of delaying treatment, although continuing to record the Qn_CRF beyond canopy closure will not help in timing the first spray. We have found that the rate of increase in Qn_CRF over the season is well-correlated with key epidemic parameters such as the rate of increase in disease, and the eventual final level of disease severity (Figure 6). Thus, recording Qn_CRF over the season may be informative in keeping track on the potential task facing disease control and in indicating on-going risk. These aspects of the risk prediction system will be examined in more detail in the coming season.

Figure 6. The relationship between Qn_CRF and epidemic parameters of disease at all six sites from 2 years showing how the rate of increase in Qn_CRF is correlated with the rate and final severity of the epidemic.




Linear rate of change in canopy risk factor

**Conclusions**

Based on this year's results and those from 2004, the following conclusions can be made:

*Epidemiology*

- Leaf wetness duration, relative humidity (RH) and air temperatures are major factors affecting carrot foliage infection by ascospores of the pathogen. Optimum conditions for foliage infection by ascospores were greater than 4 days (0-8 hours minimum) of continuous leaf wetness, ≥90% (60% minimum) relative humidity (RH) and air temperatures of 10-18$^0$C (5-10$^0$C minimum).
- Air temperature is an important factor affecting Sclerotinia disease development. Disease developed between 8-25$^0$C, with optimum temperatures between 12-18$^0$C. At 8 and 25$^0$C, disease developed slowly.
- The appearance of lodged, senescing leaves in carrot crops usually occurred close to or after full canopy enclosure. The pattern of senescence in individual plants is fairly consistent, with individual leaves senescing in turn beginning with the oldest.
- The first appearance of apothecia in carrot crops generally occurs close to or after full canopy enclosure, and is often concurrent with the early onset of senescing foliage.
- Apothecia may be present in carrot crops until at least late September
- The primary site of infection by ascopores is senescing or damaged petioles. Foliage symptoms are not observed before senescence or crop damage.
- Serious infection of the foliage can completely defoliate the crop, leading to the production of abundant sclerotia on the soil surface and increasing soil inoculum of *S. sclerotiorum*.
- Disease is often very severe on damaged foliage in the outer rows of beds next to the wheelings.
- The cultivar 'Nairobi', which produces dense, thick foliage that tends to lodge and senesce early in the growing season, is very susceptible to sclerotinia disease. Cultivars with light, erect foliage (e.g. 'Elegance') tend to be less susceptible to infection.

- Root infection results from infected foliage *via* the crown, but symptoms are rarely evident in the field unless the foliage has been completely defoliated, or until the roots are harvested after being stored under straw.
- Foliage disease severity tends to be most severe on crops sown in March and April compared with those sown in May.
- There is no consistent relationship between apothecia population density and final foliage disease severity.

*Control*

Alternating 4-spray programmes of Signum (boscalid + pyraclostrobin) and Folicur (tebuconazole), Signum and Shirlan (fluazinam), UKA373 and UKA374 (previously tested as UKA352 in 2004), and A9219B and Folicur applied at 14-day intervals gave good foliage disease control.

- Good control was also obtained with a 3-spray programme of an Amistar (azoxystrobin)/Folicur tank mix, with the first two sprays applied at 14-day intervals (first spray applied before canopy) and the final spray applied 28 days later. Control was as effective as the 4-spray programmes applied at 14-day intervals.
- Omission of a fungicide spray prior to canopy closure gave reduced control. Extending the spray interval beyond 14 days early in the spray programme tended to reduce foliage disease control.
- This year's trial results have substantiated those from last year's trial, which indicated that it is important to apply the first fungicide early, just before the canopy closes, to ensure protection of senescing leaves at the base of the canopy from sclerotinia.
- Results from both years' trials indicated that foliar applications of the experimental fungicides Shirlan, UKA373, UKA374 and A9219B gave good foliage disease control. Approval for use of these fungicides on carrots should be considered.
- Despite reducing sclerotinia foliage disease severity, none of the fungicide spray programmes significantly improved marketable yield and only three of them reduced sclerotinia root rot. This was most likely associated with the patchy incidence of foliage disease throughout the trial, and considerable variation in yields and sclerotinia root rot between plots and blocks.

Consequently, the inability of the fungicide spray programmes to improve marketable yield and most of the programmes to reduce sclerotinia root rot in 2005 must be viewed with caution.

- Contans WG applied to carrot foliage of the growing crop has the potential to reduce the build up of sclerotial inoculum in soil long term. Up to 80% of the sclerotia of *S. sclerotiorum* collected from infected foliage/crop debris of treated plots were infected with *C. minitans*. As these results were so promising, approval to apply Contans WG to carrot foliage of a growing crop should be considered. Further trials to evaluate the effect of applying the product to infected foliage, prior to covering the crop with straw for winter frost protection, on sclerotial production and survival are recommended.
- The very high susceptibility of the cultivar 'Nairobi' to sclerotinia disease was substantiated in this study. 'Nairobi' produces heavy, dense foliage that is inclined to flop, lodge and senesce early in the season, increasing susceptibility to infection. In contrast, 'Elegance' produces light, erect foliage which tends not to lodge and senesce early on in the growing season. Further trials are recommended to evaluate the susceptibility of different carrot cultivars to sclerotinia. Cultivars may differ in susceptibility and, although none are likely to be completely resistant, some may require fewer sprays than others to minimise sclerotinia disease.
- Cultivar susceptibility should be considered an important component of an integrated control programme.

**Technology Transfer**

An article entitled '*Beware of carrot sclerotinia disease*' and outlining the basic biology and symptoms of the disease, the objectives of the project and the key findings from the first year was published in the June/July 2004 issue of HDC News. A general overview of the disease, and key findings from the first and second years of the project were presented at the Agrimarc Focus Group Meeting, Bilsthorpe, Nottinghamshire on 10 October 2005. Dr M P McQuilken was an invited speaker at the UK Carrot Conference Exhibition 2005 (24 Nov 2005; East of England Showground, Peterborough), and delivered a paper entitled '*Sclerotinia*

– *Update of HDC Project Results'*. An abstract of the paper was published in the conference proceedings.

**References**


Abawi, G.S. and Grogan, R.G. (1975). Source of primary inoculum and effects of tempertaure and moisture on infection of beans by *Whetzelina sclerotiorum*. Phytopathology 65:300-309.

Caesar, A.J. and Pearson, R.C. (1983). Environmental factors affecting survival survival of ascospores of *Sclerotinia sclerotiorum*. Phytopathology 73:1024-1030

Clarkson, J.P., Phelps, K., Whipps, J.M., Young, C.S., Smith, J.A. and Watling, M. (2004). Forecasting sclerotinia disease on lettuce: toward developing a prediction model for carpogenic germination of sclerotia. Phytopathology 94:268-279.

Gerlagh, H. M., Goossen-van de Geijn, A.E., Hoogland, A.E. and Vereijken, P.F.G. (2003). Quantitative aspects of infection of *Sclerotinia sclerotiorum* sclerotia by *Coniothyrium minitans* – timing of application, concentration and quality of conidial suspension of the mycoparasite. European Journal of Plant Pathology 109: 489-502.

Grogan, R.G. and Abawi, G.S. (1975). Influence of water potential on growth and survival of *Whetzelina sclerotiorum*. Phytopathology 65:122-138.

Hao, J.J., Subbarao, K.V. and Duniway, J.M. (2003). Germination of *Sclerotinia minor* and *S. sclerotiorum* under various soil moisture and temperature conditions. Phytopathology 93:443-450.

Mylchreest, S.J. and Wheeler, B.E.J. (1987). A method for inducing apothecia from sclerotia of *Sclerotinia sclerotiorum*. Plant Pathology 36:16-20.

Phillips, A.J.L. (1987). Carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*: A review. Phytophylactia 19:279-283.

Sansford, C.E. and Coley-Smith, J.R. (1992). Production and germination of sclerotia of *Sclerotinia sclerotiorum*. Plant Pathology 41:154-156.

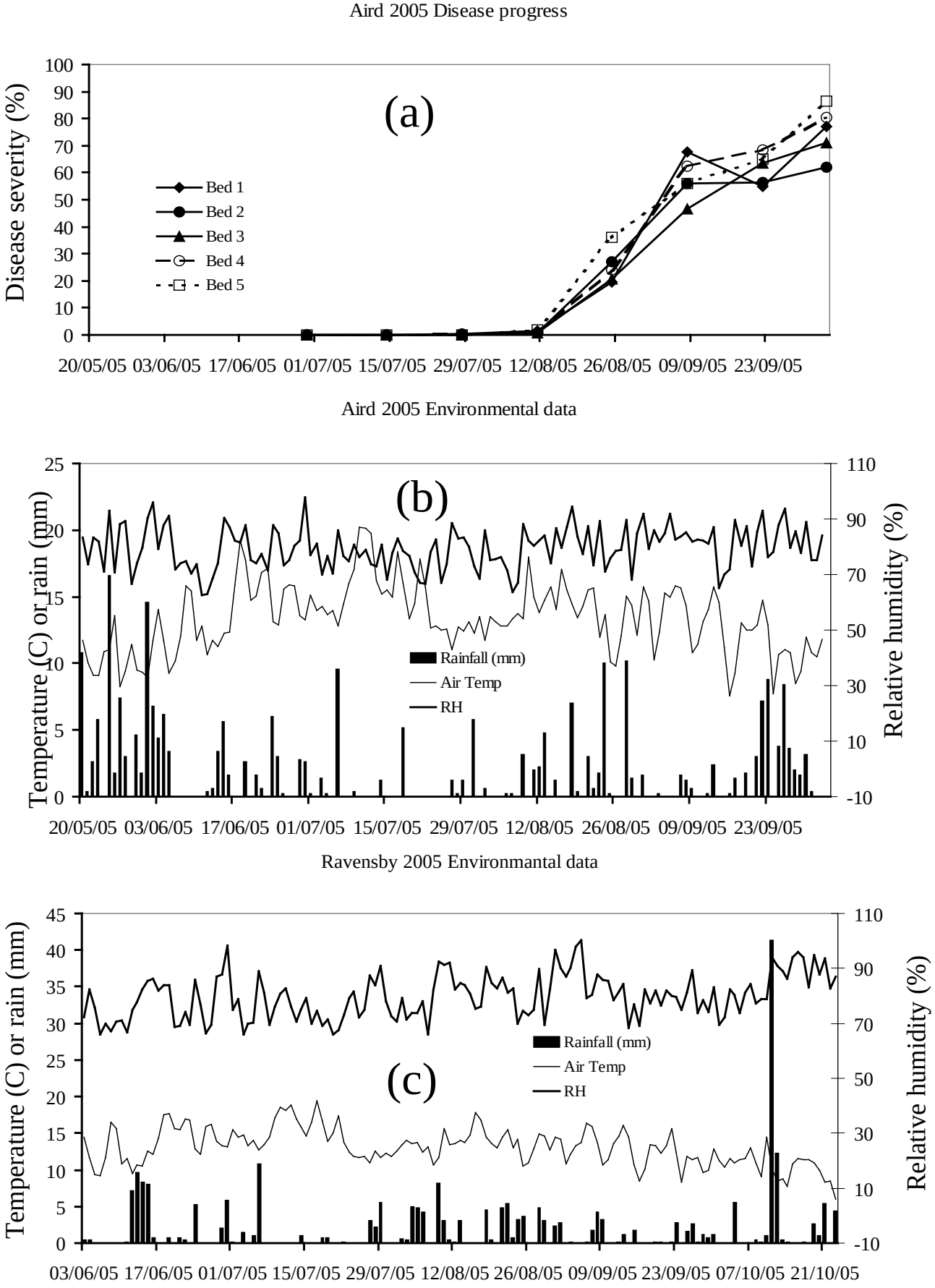
Trutmann, P., Keane, P.J. and Merriman, P.R. (1882). Biological control of *Sclerotinia sclerotiorum* on aerial parts of plants by the hyperparasite *Coniothyrium minitans*. Transactions of the British Mycological Society 78: 521-529.

**APPENDICES**

## Appendix 1

Sclerotinia disease assessment key (% foliage disease severity)

| Percentage | Description |
|---|---|
| 0 | Not seen in plots* |
| 0.1 | Trace; < 10 plants per plot with water-soaked, dark olive-green lesions present on foliage and/or petioles. |
| 10 | About one tenth of the foliage with lesions, cotton wool-like mycelia and/or sclerotia present. |
| 25 | About one quarter of the foliage with lesions, cotton wool-like mycelia and/or sclerotia present. Areas of plants may be completely defoliated/dead with sclerotia on the soil surface. |
| 50 | Half of the foliage with lesions, or bleached or dead with sclerotia present. Areas of plants may be completely defoliated/dead with sclerotia on the soil surface. |
| 75 | Approximately three quarters of the foliage bleached or dead with sclerotia present. Areas of plants may be completely defoliated/dead with sclerotia on the soil surface. |
| 100 | All of the foliage area bleached or dead with abundant sclerotia present. |




**Appendix 2.** Foliage disease severity and weather data for 2005 trial sites. (a) disease progress in 5 carrot beds (Aird); (b) weather data for Aird; (c) weather data for Ravensby.