

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

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‘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.’

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Grower Summary

Headline

- 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 a 14 days harvest interval.
- Application of Signum will improve disease control and increase marketable yield of roots by at least 20%.
- 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 carrot crops.

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

Germination of resting bodies

Laboratory experiments were conducted to determine the effect of temperature and soil moisture on germination of resting bodies in a sandy loam soil. Resting bodies germinated in moist soil between 10 and 25⁰C to produce fruiting bodies. The optimum temperature for germination was approximately 15⁰C. No germination occurred at 30⁰C. Few resting bodies germinated in soil at low moisture content (11% w/w), and none germinated in very dry soil.

Results showed that soil temperature and moisture are major factors affecting germination of resting bodies.

Spore (ascospore) survival

The effect of temperature and relative humidity (RH) on spore survival was monitored fortnightly for 20 weeks. High temperature (>20⁰C) and RH (>45%) were detrimental to spore survival. In general, ascospore mortality increased as temperature and RH increased.

It is unlikely that spore survival is a limiting factor to infection in carrot crops as conditions conducive to spore germination (i.e. leaf wetness and temperatures of 10-25⁰C) occur regularly within the carrot canopy once it has closed.

Development of sclerotinia disease and fruiting production in carrot crops

One field of early carrots (Monkton Cottage, *Prestwick, Ayrshire*) and four fields of main season carrots (*Aird, Delvine, Perthshire; Carriston, Glenrothes, Fife; Cults Mill, Cupar,*

Fife; Ravensby, *Barry, Angus*) with sequential sowing dates from 19 Feb until 22 May 2004, were selected for the first 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, such as oilseed rape and potatoes. All fields were sown with 'Nairobi' except Monkton Cottage, which was sown with 'Nantucket'. 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. Environmental conditions were also recorded.

The following general observations were made during crop monitoring:

- 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.
- No fruiting bodies were detected in the early sown crop (19 Feb) harvested on 7 July.
- In general, the first appearance of fruiting bodies in carrot crops (Photograph 1) occurred in July, 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.

Photograph 1.



- Mean daily soil temperatures ranged between 12-15⁰C during 2 weeks before fruiting bodies were first detected.
- Peak fruiting production at most sites occurred during late July, August and early September, after canopy enclosure.

- The primary site of infection was senescing or damaged petioles.
- At most sites, foliage disease was detected on crops 4-6 weeks after the appearance of the first fruiting bodies.
- 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 (Photograph 2). The disease then often spread by mycelium growing between diseased and healthy foliage in contact with each other.

Photograph 2.



- 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 resting bodies 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.

- Based on observations from three fungicide experiments, a large yield response to controlling low levels of disease can be expected, with less response once root disease incidence reaches approximately 10%. Further work is required to substantiate this.

Artificial inoculation of carrot crops with spores at different growth stages

Carrot foliage was artificially inoculated with spores at three different growth stages at two field sites (Aird & Ravensby). Disease development was monitored.

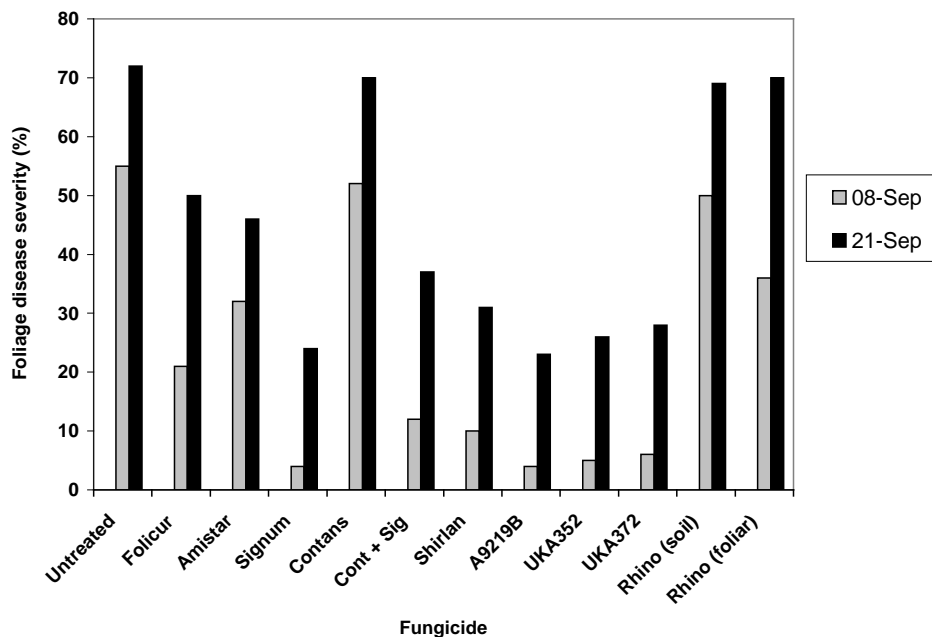
- Leaf wetness was important for infection and subsequent development of sclerotinia.
- Final foliage disease severity was greatest in plants inoculated early (canopy enclosure; main period of root growth) compared to plants inoculated late in the growing season (fresh to mature canopy, with heavy lodging and senescing leaves).
- There was no consistent relationship between crop growth stage at inoculation and marketable yield.
- Foliar sprays of Folicur (tebuconazole) prior to inoculation reduced foliage disease as well as root disease incidence and severity, and increased marketable yield of roots in some cases (no other fungicides were evaluated in this experiment).

Evaluation of current and novel fungicides

A fungicide trial was conducted within a commercially grown carrot crop (sown on 31 March, variety 'Nairobi') in Perthshire, Scotland to evaluate a range of current and experimental products against sclerotinia disease. The products were Folicur (tebuconazole, OLA) applied at 1 L/ha, Amistar (azoxystrobin, OLA) applied at 1 L/ha, and Signum (boscalid + pyraclostrobin, SOLA) applied at 0.75 kg/ha. The experimental (Exp.) fungicides were Shirlan (fluazinam, 0.5 L/ha), A9219B (1 kg/ha), UKA352 (0.75 L/ha), UKA373 (0.4 L/ha) and Rhino (flutolanil, 1.5 L/ha). Foliar sprays (3-spray programme) of each treatment were applied at approximately 21 day intervals: (T1) 28 June, 90-95% crop coverage & prior to canopy enclosure; (T2) 22 July, 100% canopy coverage and full canopy enclosure/main period of root growth; (T3) 20 August, fresh to mature canopy. Rhino was also applied (1.5 L/ha) as a soil incorporation on 6 April. A further soil treatment consisted of Contans WG, an experimental product containing a fungus, which attacks the resting bodies and kills them. This treatment was incorporated (6 kg/ha) into the soil on 6 April. Foliage disease was assessed on 8 September (heavily lodged canopy) and 2 weeks later on 21 September (heavily lodged canopy).

Untreated control plots showed 55 and 72% foliage disease severity on the 8 and 21 September, respectively

Effect of fungicide sprays applied on 28 Jun (prior to canopy closure), 22 Jul & 20 Aug on foliage disease severity on 8 and 21 Sep.



The key findings were:

- Foliar sprays of Signum (SOLA), Shirlan (Exp.), A9219B (Exp.), UKA352 (Exp.) or UKA373 (Exp.) gave good control of foliage disease.
- Foliar sprays of either Folicur (OLA) or Amistar (OLA) gave foliage disease control, but were less effective than Signum, Shirlan, A9219B, UKA352 or UKA373.
- Results suggest 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. Further trials are required to substantiate this.
- No foliage disease control was achieved with Contans WG, and this may be attributed to late soil incorporation of the product. Further trials are recommended to investigate the effect of the product on killing resting bodies and reducing soil contamination during field winter storage of crops under straw.
- Control of foliage disease by the most effective fungicides was associated with increases in yield of marketable roots (20-24% on average). Signum and Shirlan

increased marketable yields by 23.6 and 27.7 t/ha, respectively. Application of a SOLA for use of Shirlan on carrots should be considered.

Financial benefits

The results from the fungicide trials and artificial inoculation experiments illustrate the benefits, which arise from controlling sclerotinia disease in carrot crops. Findings from the first year indicate that failure to control the disease could result in crop losses of 15% on average, corresponding to losses of at least £18 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 $\geq 10\%$. Fungicide sprays have the potential to reduce these losses by at least 20-25%, an annual saving of approximately £4 million, less the cost of purchase and application.

Action points for growers

- Sclerotinia can have substantial effects on the yield of marketable roots, and action should be taken to reduce infection.
- Until further results suggest otherwise, a preventative fungicide spray programme should remain the main defence against the disease. 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. 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 control is 1 kg/ha, and two applications can be made per crop with a 14 days harvest 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 alternation 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 a QoI fungicide. To minimise the development of resistant strains of the pathogen, do not use consecutive

applications of Amistar and Signum, and select a product from a different fungicide 'group' for alternate sprays.

- 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.
- Avoid growing carrot crops close to fields of oilseed rape to minimise infection from externally generated spores. Spores can be blown at least 200 m from neighbouring oilseed rape, although this was not measured in this project.
- 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.

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

Sclerotinia sclerotiorum isolates

The isolates of *Sclerotinia sclerotiorum* used in this study were derived from sclerotia from diseased carrot plants grown in Perthshire, Scotland (KP) 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 5⁰C, and used as a stock supply for all further cultures.

Production of sclerotia

Large quantities of sclerotia (isolates KP & Ret) were produced on sterilised wheat grain (Mylchreest & Wheeler, 1987) for use in subsequent experiments. 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 121⁰ C for 15 min) in 250 ml Erlenmeyer flasks. Flasks were incubated at 18-20⁰C 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 4 or 8 weeks at 5⁰C, 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 (8 weeks at 5⁰C) 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 110⁰C for 30 min) in clear plastic tubs (250 ml volume; Bunzyl, Paisley). Tubs with lids were placed in a controlled environment cabinet at 15-18⁰C (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 5⁰C in a desiccator for no longer than 4 months.

Laboratory Experiments

Effect of temperature and relative humidity (RH) on ascospore survival

Ascospores of *S. sclerotiorum* isolate KP1 were obtained by trapping them on acetate sheets. Each sheet was rubbed with a duster to create a static charge and then held over a plastic tub containing mature apothecia. When the tub lid was removed, the resulting spore cloud impacted onto the acetate resulting in a layer of ascospores. Acetate sheets were taped to the lids of clear plastic boxes (500 ml) containing 120 ml of various saturated salt solutions in order to create specific RH levels within each box. Individual boxes were wrapped in cling film.

Five RH levels (15, 45, 79, 90 and 98%) were tested at temperatures of 5, 10, 15, 20, 25 and 30⁰C. The RH within each box was measured using a humidity probe. To test spore viability at fortnightly intervals for 20 weeks, small pieces of acetate approximately 1 cm² were cut from each temperature/humidity treatment sheet and placed face down with 0.5 ml sterile distilled water on a PDA plate. To remove the spores, the piece of acetate was then gently spread around the plate with the water to remove the spores. Germination was assessed under the microscope (x100 magnification) following incubation of plates at 20⁰C for 16 h. For each humidity/temperature combination, a 125 spores were assessed from

three random areas on each PDA plate. A spore was considered to have germinated if one or more germ-tubes were longer than half the spore length.

Effect of temperature and water potential on carpogenic germination of sclerotia

Carpogenic germination (production of apothecia) of sclerotia of *S. sclerotiorum* isolate KP was assessed at a range of temperatures and water potentials in Fife sandy-loam soil (Darvel series) in incubators with white fluorescent lighting (14-h day; 22 Wm²). The soil was passed through a 2-mm sieve and then pasteurised by autoclaving approximately 4 l for 30 min at 110⁰C. This removed fungi, which have the potential to contaminate and affect germination of *S. sclerotiorum* sclerotia. Previous research has shown that sterilizing soil by pasteurisation has no effect on carpogenic germination (Singh & Singh, 1983). After pasteurisation, the percentage moisture content of the soil was determined by weighing five samples before and after drying in an oven at 80⁰C overnight.

Soil (80 g) was dispensed into clear plastic tubs (250 ml volume; Bunzyl, Paisley) with lids. Water was added by drop with a pipette, or removed by drying in a laminar flow cabinet, and mixed thoroughly to give a range of soil water potentials. The water potentials tested were -500, -101, -33, -10, -1 kPa equivalent to 9, 11, 13, 18 and 30% moisture content (w/w). Sclerotia (20), pre-conditioned at 5⁰C for 8 weeks, were then arranged in a grid pattern on top of the soil surface and covered to a depth of 1 cm with soil. Additional water was added to this top layer of soil or removed to obtain the desired water potential. The temperatures tested were 10, 15, 18, 20, 25 and 30⁰C. There were three replicate tubs for each temperature-water potential combination. Tub were monitored fortnightly for the appearance of stipes and apothecia. A stipe was considered to have matured into an apothecium when a distinct cap was observed (≥ 3 mm in diameter).

Field work

Sites

One field of early carrots and four fields of main season carrots representative of typical commercial crops in the main growing areas of Scotland were selected for the first year of study. All the 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)	Variety	Sowing date	Straw cover (St) & harvest (H) date
Monkton Cottage <i>Prestwick, Ayrshire</i>	sandy loam	0.2	Nantucket	19 Feb 04	7 July 04 (H)
Aird <i>Delvine, Perthshire</i>	silty clay loam	2.8	Nairobi	31 Mar 04	6 Oct 04 (St) 16 Feb 05 (H)
Carriston <i>Glenrothes, Fife</i>	sandy loam	1.0	Nairobi	26 Apr 04	mid Nov (H)
Cults Mill <i>Cupar, Fife</i>	sandy loam	0.5	Nairobi	7 May 04	late Oct 04 (St) Mar 05 (H)
Ravensby <i>Barry, Angus</i>	sandy loam	1.7	Nairobi	22 May 04	mid Nov 04 (St) Apr 05 (H)

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

Five commercial fields (Monkton Cottage, Aird, Carriston, Cults Mill and Ravensby; see Table 1 for details), with sequential sowing dates from 19 February until 22 May 2004, 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 beds (each 1.8 m wide) x 120 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. At approximately 10 m intervals along each bed, the total number of apothecia and percentage of plants with sclerotinia disease (% foliage disease severity) were recorded within a 2 m² quadrat across each bed. 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
3 rd 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
Heavily 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 winter straw covering.

Effect of crop growth stage and environmental factors on the development of sclerotinia disease – artificial inoculation of carrot crops with S. sclerotiorum ascospores

Carrot foliage was artificially inoculated with *S. sclerotiorum* ascospores at three different growth stages at two field sites (Aird & Ravensby, see Table 1 for details). Disease development was monitored and environmental conditions were recorded.

The crop at each field site was grown according to common practice, but was not sprayed with fungicides. Plots were 2 m long and 4 or 5 rows wide on 1.8 m beds. Each treatment was replicated three times in a randomised block design. 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 for 2-3 min. The ascospores were dislodged with a camel's hair brush and concentrations of approximately 5.0×10^5 to 1.0×10^6 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 250 ml per plot). Immediately after inoculation, viability of the inoculum was tested by spraying 2-3 plates of PDA with the suspension.

At the Aird site, the crop was artificially inoculated on 14 July (full canopy enclosure; main period of root growth), 4 August (full canopy enclosure; first lodging of lower leaves (1) & 1-3 senescing leaves/plant) and 18 August (fresh to mature full canopy, with heavy lodging (2) and 3-4 senescing leaves/plant). At the Ravensby site, the crop was artificially inoculated on the 4 August (almost canopy enclosure; main period of root growth), 18 August (full canopy enclosure; first lodging of lower leaves (1); 1-2 senescing leaves/plant) and the 1 September (fresh to mature canopy, with lodging (1) and 3-4 senescing leaves per plant). At each inoculation time, a fungicide foliar spray [Folicur (tebuconazole); 1 l ha^{-1}] + subsequent inoculation treatment was included to investigate the effect of disease on root yield. Control plants were sprayed with water only. A fungicide control [Folicur (tebuconazole), on label approval; 1 L ha^{-1}] was also included, which consisted of a foliar spray + subsequent artificial inoculation at the first inoculation time/growth stage followed by two subsequent foliar sprays at the second and third inoculations (growth stage 2 & 3), respectively.

Foliage disease severity and growth stage were assessed at fortnightly intervals as described before until the 22 September (Aird) and the 22 October (Ravensby), prior to covering the crop with straw for winter frost protection. Environmental conditions were recorded every hour using a Delta T data logger (Aird) and Adcon Telemetry weather station (Ravensby) measuring rainfall, soil and air temperature, relative humidity, leaf wetness and soil moisture. Probes were placed within the crop canopy. All sites were monitored until October prior to winter straw covering.

Effects of inoculation on yield and sclerotinia root rot. Plots in the two artificial inoculation trials were harvested in the autumn on 22 September (Aird) and 22 October (Ravensby) by lifting a 2 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, 80 roots harvested from each plot were selected randomly and placed in a labelled polyethylene net bag. Bags were stored at 15-18⁰C 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:

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

Apothecial production by sclerotia in the field

Sclerotia of *S. sclerotiorum* isolates KP and Ret were buried in carrot crops at the Delvine (Aird) and Carriston sites every 2 weeks between April and August. 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 pre-conditioned at 5⁰C for 4 weeks in the laboratory as described before. Batches of twenty sclerotia (approximately 2-4 mm in diameter) of each isolate were placed in Terylene net bags (5 x 5 cm, mesh, 2 mm) and buried at fortnightly intervals from 6 April until 11 August (Aird),

and from 21 April until 26 August (Carriston). At each burial, five replicate bags each of 20 sclerotia were buried at a depth of 1 cm and arranged in a randomised block design. Numbers of apothecia produced from sclerotia of each replicate bag were counted at fortnightly intervals until the crop was covered in straw to protect it from frost. 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 current and novel fungicides

A trial was conducted within a commercial crop at the Aird site (see Table 1 for details) to evaluate a range of current and novel fungicides against sclerotinia disease. The Aird site was chosen, as the amount of sclerotial inoculum in the soil was particularly high. The crop (cv. 'Nairobi') was drilled on 31 March in a silty clay loam and the trial grown according to standard commercial practice, except no fungicides other than those listed in the treatment list (Table 2) were applied to the trial area. Sixty plots were marked out on 6 April. Each plot was 10 m long and 5 rows (3 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 12 treatments (see Table 2 for details) and these were arranged in a randomised block design. The trial was not artificially inoculated.

Contans WG (treatments 5 & 6) was evaluated in the trial, which is a granular formulation containing 1×10^9 spores g^{-1} of the soil fungus *Coniothyrium minitans*. The fungus attacks the sclerotia of *S. sclerotiorum* in soil and destroys them. Two applications (6 kg/ha equivalent to 12 g per plot) of the product were made on 6 April (1 week after sowing) and 17 November. For each plot, 10.8 g of product was added to 10 L of water in the spray tank, which was agitated for 10 min. The suspension (10 L) was applied evenly to each appropriate plot using a Hozelock conventional sprayer (Haddenham, Buckinghamshire) fitted with a cone nozzle, and free of fungicide and pesticide residues. Agitation was maintained continuously during spraying. A volume of 10 L was needed to provide thorough coverage of the soil. At the first application, the product was raked into the upper soil surface to a depth of approximately 3-5 cm. At the second application, the product was applied to the foliage and soil surface. The spore suspension for the first application

contained $2.2\text{-}3.4 \times 10^5$ colony forming units (CFUs) ml^{-1} and gave $1.2\text{-}1.9$ CFUs m^{-2} . The spore suspension for the second application contained $1.9\text{-}3.1 \times 10^5$ CFUs ml^{-1} and gave $1.0\text{-}1.7$ CFUs m^{-2} . Soil was taken from the top 3 cm of each *C. minitans*-treated plot at intervals, and survival of the fungus assessed by soil dilution plating on PDA containing Triton X-100 (2 ml L^{-1}) and chlortetracycline ($20 \text{ mg powder L}^{-1}$ containing 80% chlortetracycline).

Prior to the second application (24 wk after the first application) of Contans WG, approximately fifty sclerotia were collected from each untreated control and Contans WG treated plot. Sclerotia were surface-sterilised, bisected and placed on 15 mm diameter PDA discs containing chlortetracycline (20 mg l^{-1}). The viability and infection of sclerotia by *C. minitans* were assessed after 10-14 days incubation at 18°C .

Rhino (treatment 11) was also applied on 6 April and incorporated into the soil as described above.

Fungicide foliar sprays (treatments 2, 3 4, 6, 7, 8, 9, 10 & 12); 3-spray programme) were applied at approximately 21 day intervals:

T1	28 June	90-95% crop coverage, prior to canopy enclosure. Small pencil stage
T2	22 July	100% crop coverage, full canopy enclosure Main period of root growth
T3	20 August	Fresh to mature canopy Heavy lodging (2-3) and 3-4 senescing leaves/plant

Table 2. Fungicide trial 2004 (Aird) – summary of treatments.

Treatment number	Trade name	Active ingredient	Application Rate (product/ha)	Application regime	Approval status
1	Control (untreated)	-	-	-	-
2	Folicur	tebuconazole	1 L/ha	3 x foliar sprays ^a	On-label
3	Amistar	azoxystrobin	1 L/ha	3 x foliar sprays	On-label
4	Signum	boscalid + pyraclostrobin	0.75 kg/ha	3 x foliar sprays	SOLA
5	Contans WG	<i>Coniothyrium minitans</i> (biocontrol agent)	6 kg/ha	2 x soil incorp. ^b (3-5 cm depth)	Exp. ^d
6	Contans WG + Signum	<i>C. minitans</i> boscalid + pyraclostrobin	6 kg/ha 0.75 kg/ha	2 x soil incorp. 3 x foliar sprays	Exp. SOLA
7	Shirlan	fluazinam	0.5 L/ha	3 x foliar sprays	Exp.
8	A9219B	Experimental	1 kg/ha	3 x foliar sprays	Exp.
9	UKA352	Experimental	0.75 L/ha	3 x foliar sprays	Exp.
10	UKA373	Experimental	0.4 L/ha	3 x foliar sprays	Exp.
11	Rhino	flutolanil	2.5 L/ha	1 x soil incorp. ^c	Exp.
12	Rhino	flutolanil	1.5 L/ha	3 x foliar sprays	Exp.

^a A 3-spray fungicide programme was applied at approximately 21 day intervals on 28 June (prior to canopy enclosure), 22 July and 20 August.

^b Contans WG was incorporated into the soil on 6 April and 17 November.

^c Rhino (treatment 11) was incorporated into the soil on 6 April.

^d Used under Automatic Experimental Approval for the trial.

The T3 spray was delayed due to adverse weather. Fungicide sprays were applied in a minimum of 400 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 8 September [heavily lodged canopy (2) & 4-5 senescing leaves/plant] and 2 weeks later on 21 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.

Effects on yield and sclerotinia root rot. To determine the effect of fungicides on yield, plots within the fungicide trial were harvested in the autumn on 22 September 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. Roots were assessed for sclerotinia rot as described before following 14 days storage at 15-18⁰C. The trial was then protected against frost using straw and a second harvest of selected treatments taken on 14 February 2005.

Statistical analyses

Percentage data and colony counts were transformed to angular and log₁₀ values, respectively 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

Laboratory Experiments

Effect of temperature and relative humidity (RH) on ascospore survival

Ascospores survived for 20 weeks at low temperatures (5-10⁰C) with RHs below 79% (Figure 1). Germination of ascospores was >83 and 21% at 15 and 45% RH, respectively. An increase in temperature from 10 to 15⁰C was associated with a reduction in germination at all RHs and, in particular, 90 and 98% RH. Ascospore survival at 15 and 20⁰C was similar at each RH. Relative humidity in the 45-98% range was most detrimental to ascospore survival and subsequent germination when combined with temperatures at or above 20⁰C. A rapid decline in germination was observed at 30⁰C, and inhibition was reached sooner for all RHs at this temperature than at lower temperatures. The decline in ascospore survival at 90 and 98% RH was rapid at all temperatures.

Laboratory studies revealed that survival of *S. sclerotiorum* (isolate KP) ascospores was reduced markedly at high temperature (>20⁰C) and RH (>45%). Caesar & Pearson (1983) and Clarkson *et al.* (2003) have also observed this trend. Increasing the RH has been shown to decrease ascospore survival elsewhere (Abawi & Grogan, 1975). However, in our study, the ascospores survived much longer than in the previous study. For example, at 5 and 10⁰C, Caesar & Pearson (1983) reported that ascospores exposed to 98% RH did not survive more than 8 days. This compares to 6-8 weeks in our study. This difference is likely to be associated with the origin of the *S. sclerotiorum* isolates used in each study. Ascospore germination in free water and subsequent infection of plants can occur between 10 and 25⁰C (Abawi & Grogan, 1975). In carrot crops, prolonged leaf wetness and temperatures conducive to ascospore germination occurs regularly once the canopy has enclosed (see field environmental data). Consequently, it is highly unlikely that spore survival is likely to be a limiting factor to infection by the pathogen in UK carrot crops.

Figure 1. Effect of temperature and relative humidity (RH) on the survival (as measured by % germination) of ascospores of *S. sclerotiorum* (isolate KP) ejected onto acetate sheets and held over saturated salt solutions. Each line represents one RH (15, 45, 79, 90, 98%).

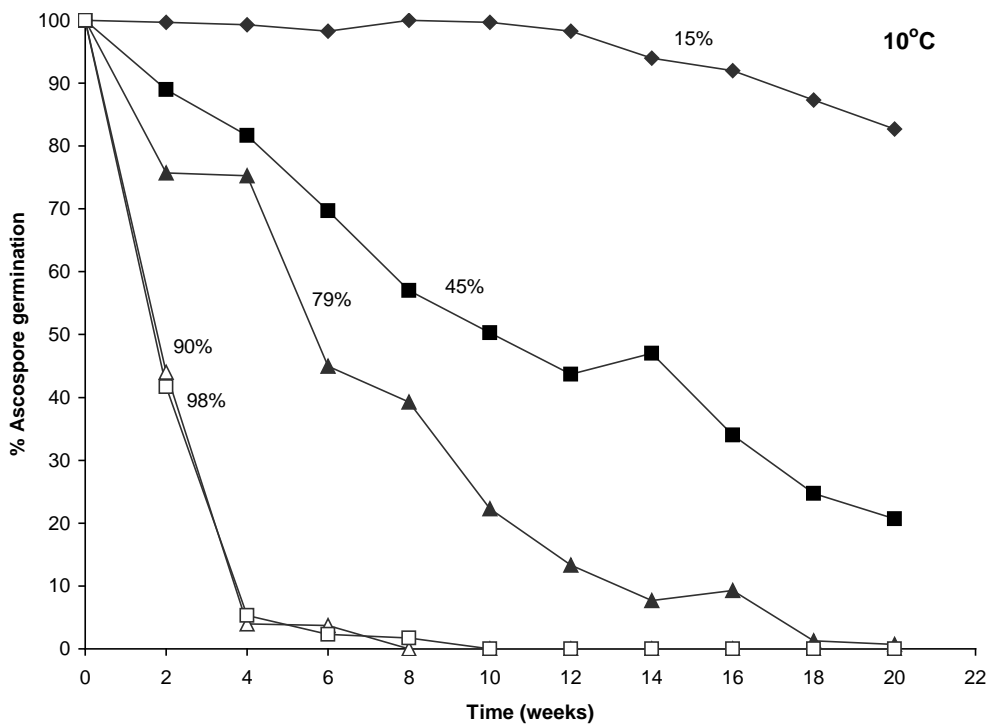
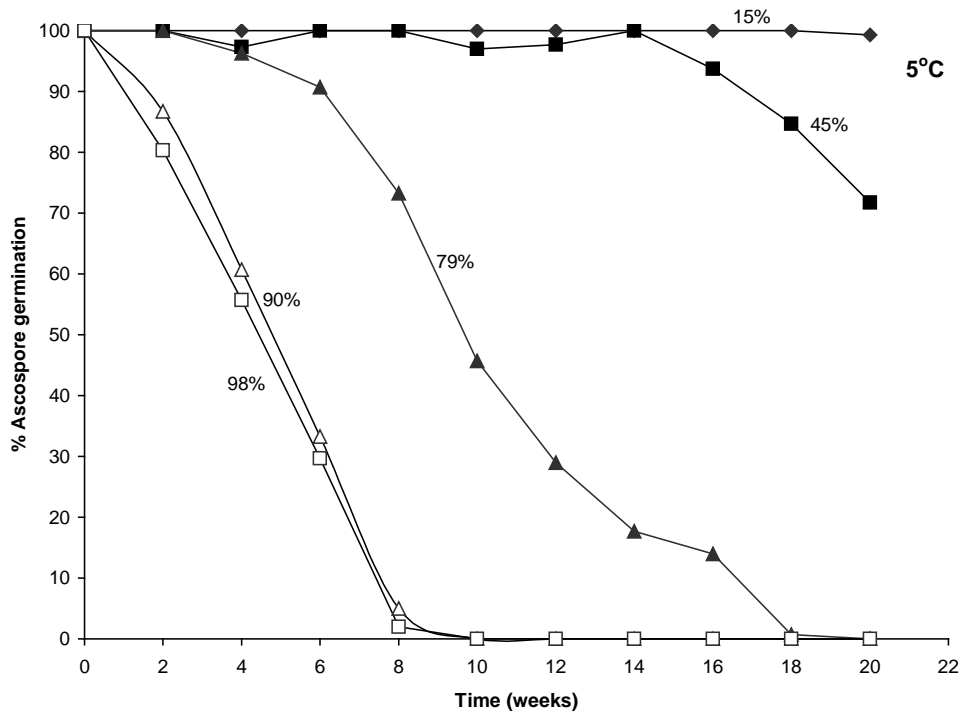


Figure 1 (continued).

Effect of temperature and relative humidity (RH) on the survival (as measured by % germination) of ascospores of *S. sclerotiorum* (isolate KP) ejected onto acetate sheets and held over saturated salt solutions. Each line represents one RH (15, 45, 79, 90, 98%).

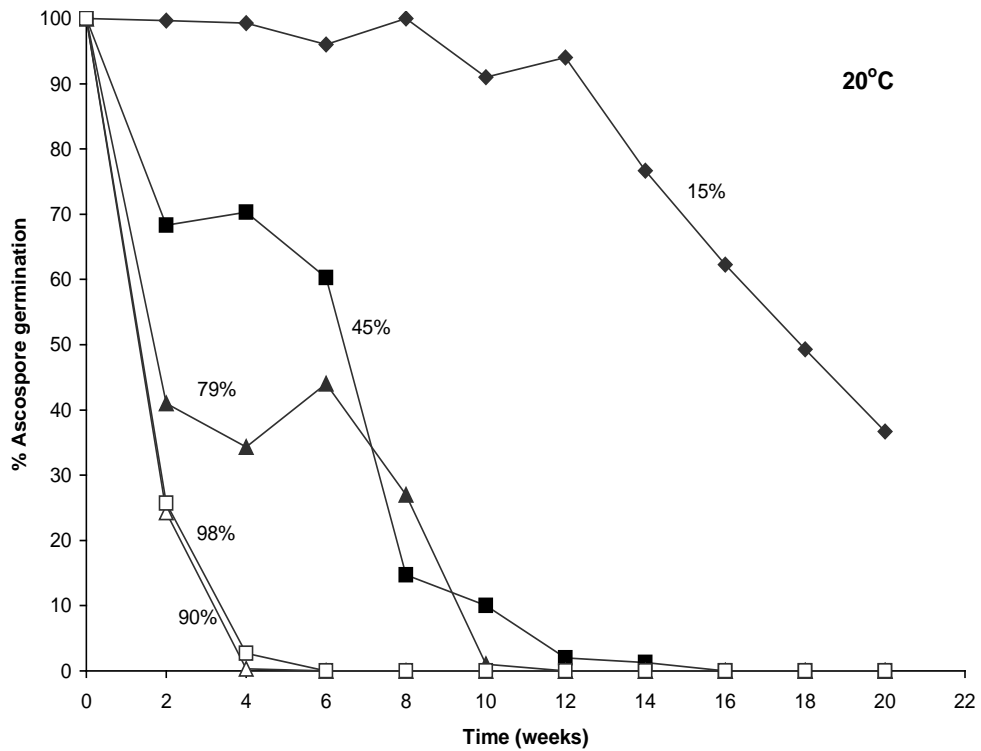
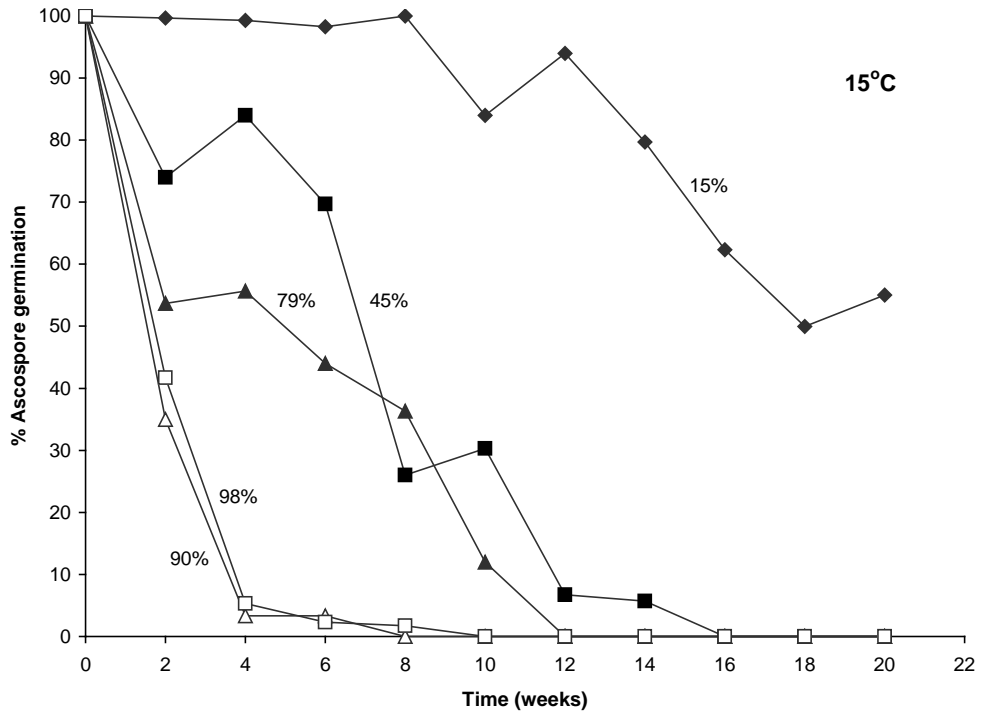
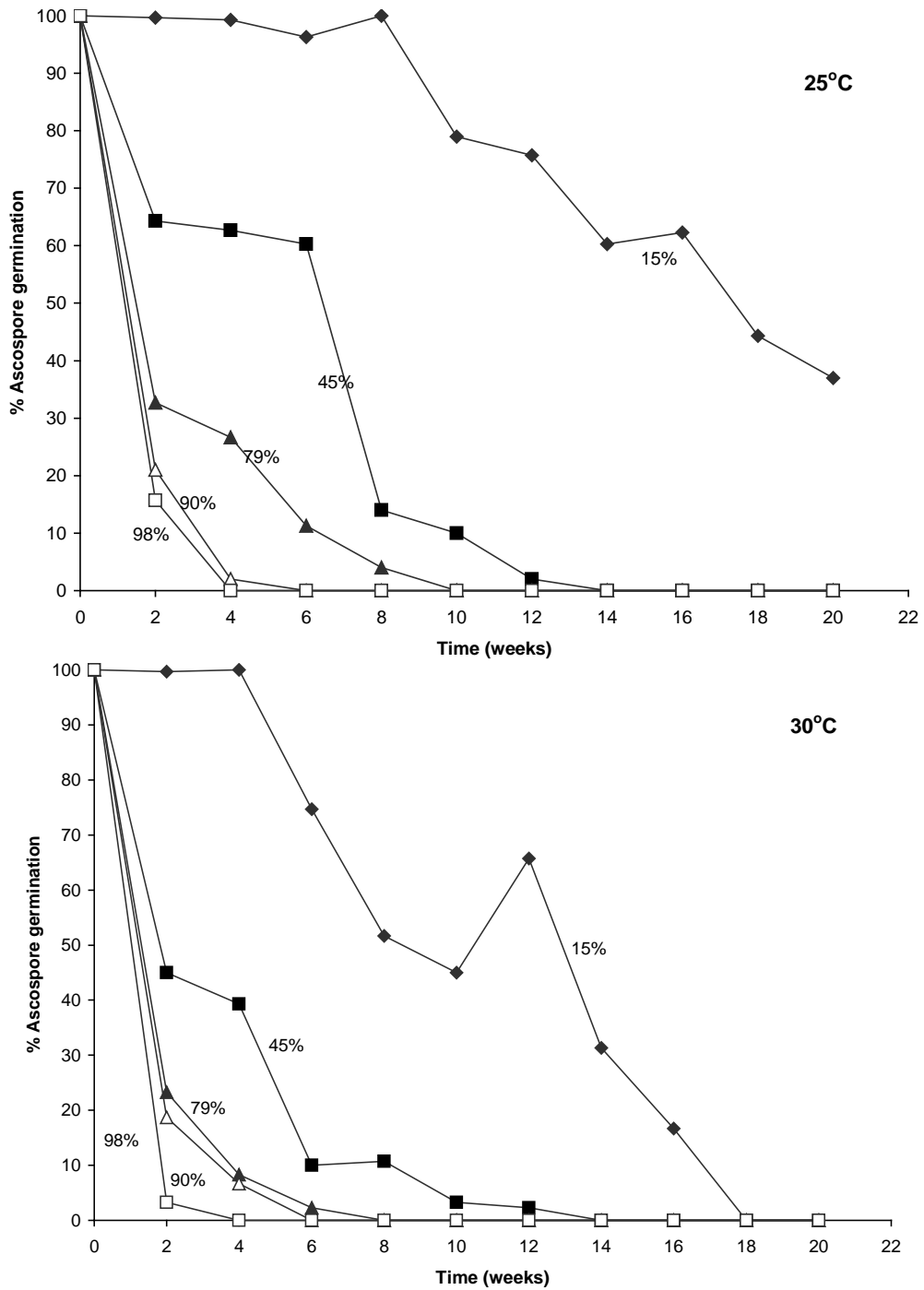


Figure 1 (continued).

Effect of temperature and relative humidity (RH) on the survival (as measured by % germination) of ascospores of *S. sclerotiorum* (isolate KP) ejected onto acetate sheets and held over saturated salt solutions. Each line represents one RH (15, 45, 79, 90, 98%).



Effect of temperature and water potential on carpogenic germination of sclerotia

Apothecia were produced by sclerotia of *S. sclerotiorum* between 10 and 25°C in the Fife sandy loam soil (Figure 2). No apothecia were produced by sclerotia at 30°C. Few apothecia were produced at -101 kPa (11% moisture content) at 10, 15, 18°C, and none at 20 or 25°C. The driest soil treatment of -500 kPa (9% moisture content) did not support any apothecial production by sclerotia. Apothecia appeared after 4-6 weeks at 15 to 20°C for those water potentials that supported germination. Results from 18°C were omitted from the graphs. Analysis of final percentage germination after 20 weeks indicated that there were significant effects of temperature ($P \leq 0.01$), water potential ($P \leq 0.01$) and interactions between temperature and water potential ($P \leq 0.01$) (Table 3). Final percentage sclerotial germination was significantly greater at 15°C compared to the other temperatures, except at a soil water potential of ≥ -101 kPa.

Table 3. Effect of water potential on final percentage of *S. sclerotiorum* (isolate KP) sclerotia producing apothecia in a Fife sandy loam (Darvel series) (-1, -10, -33, -101 kPa) at 10, 15, 18, 20, 25°C. There was no germination at 30°C at any water potential, and no germination at -500 kPa at any temperature.

Water Potential (kPa)	Temperature (°C)					Mean	LSD ^a ($P = 0.05$)
	10	15	18	20	25		
1	52.9	62.3	62.3	24.0	7.6	41.8	
10	60.0	81.4	51.8	27.7	16.2	47.4	
33	67.2	77.1	45.0	24.0	0	42.7	
101	34.2	28.9	21.3	4.31	0	17.7	2.64^c
Mean	53.5	62.4	45.1	20.0	5.96		4.20^d
LSD ($P = 0.05$)							
(within the same temperature)						5.89	
(between different temperatures)						6.34	

^aLSD is the least significant difference at a probability of 5%

^b Each value is the mean of three replicates, each of 20 sclerotia. Values were angularly transformed before ANOVA.

^c LSD between temperature means

^d LSD between water potential means

Figure 2. Effect of water potential on percentage of *S. sclerotiorum* (isolate KP) sclerotia producing apothecia in a Fife sandy loam (Darvel series) (-1, -10, -33, -101 kPa) at 10, 15, 20, 25⁰C. There was no germination at 30⁰C at any water potential, and no germination at -500 kPa at any temperature.

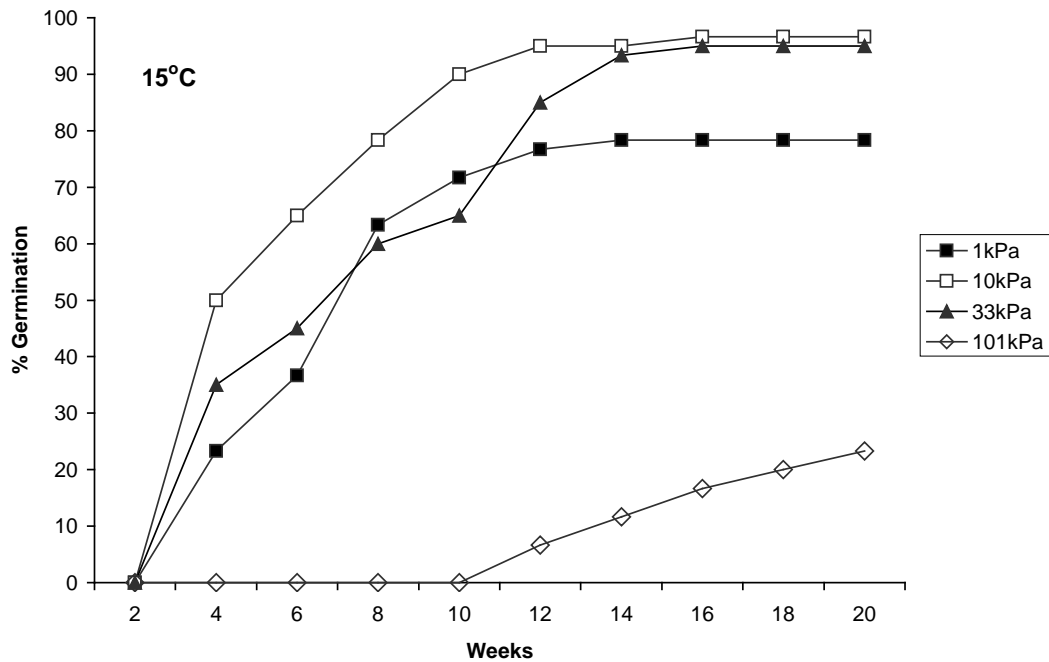
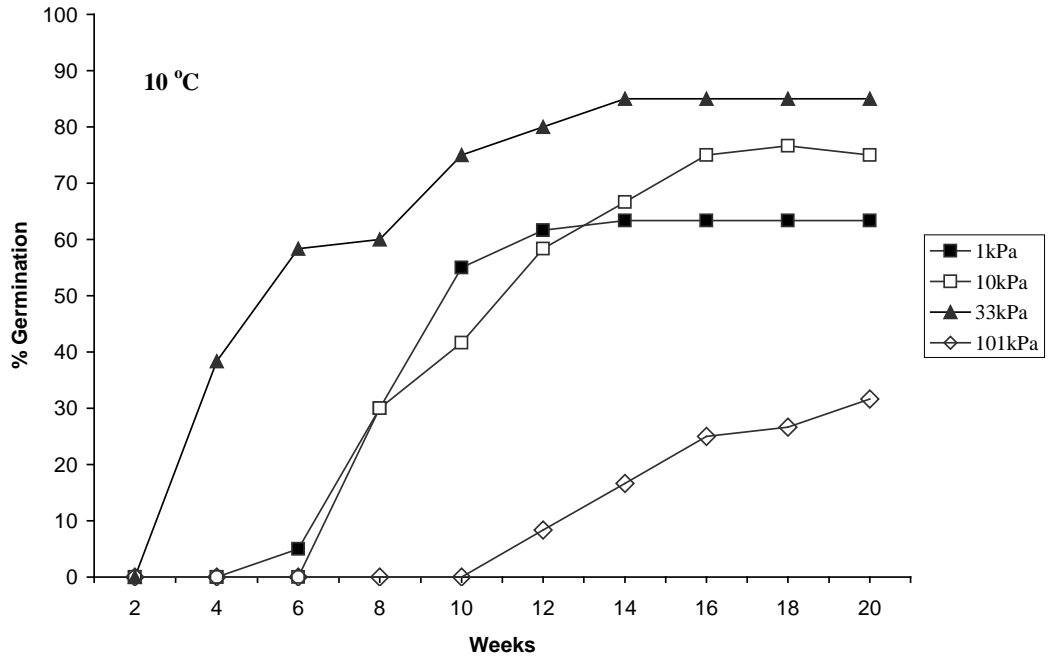
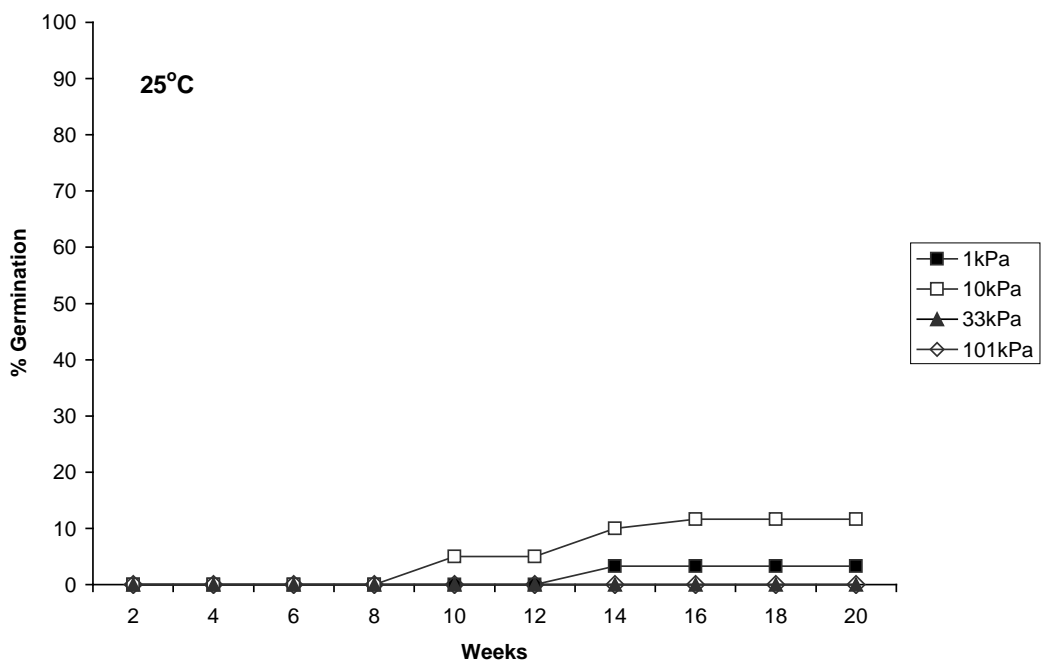
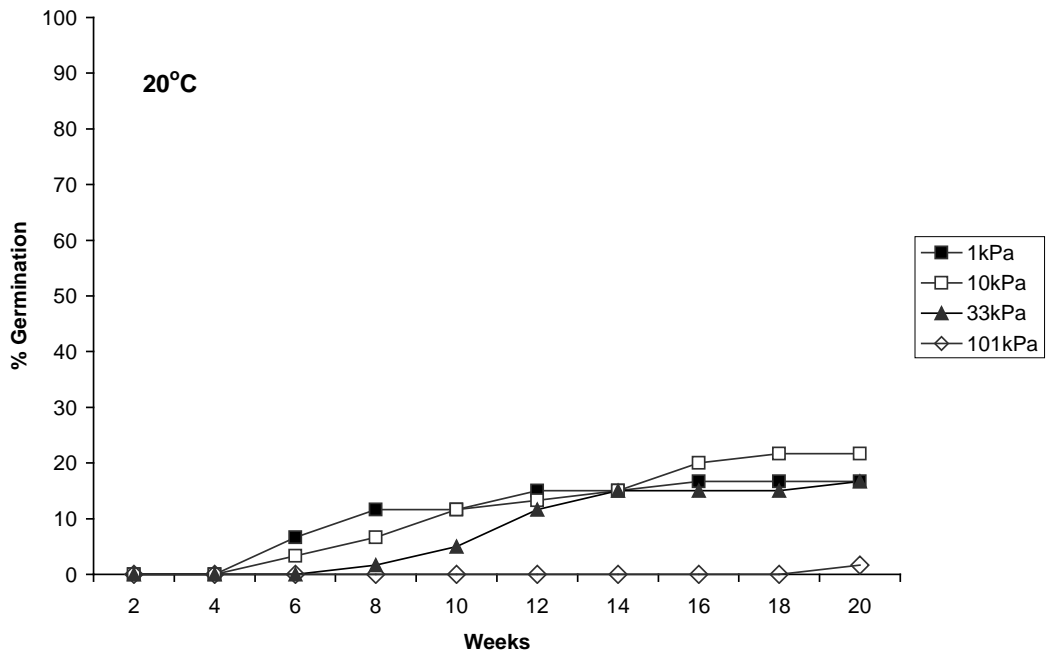


Figure 2 (continued).

Effect of water potential on percentage of *S. sclerotiorum* (isolate KP) sclerotia producing apothecia in a Fife sandy loam (Darvel series) (-1, -10, -33, -101 kPa) at 10, 15, 20, 25°C. There was no germination at 30°C at any water potential, and no germination at -500 kPa at any temperature.



Results from this study indicated that soil temperature and water potential were major factors affecting germination of *S. sclerotiorum* (isolate KP) sclerotia. There was a water potential threshold of approximately -101 kPa (11% moisture content) for the isolate in the Fife sandy loam soil. A lower water potential of -500 kPa (9% moisture content) was unable to support germination of the isolate at all temperatures tested. Some researchers have demonstrated sclerotial germination of *S. sclerotiorum* at -400 and -750 kPa (Morrall, 1977; Teo & Morrall, 1985). However, our results suggest that soil with a high moisture content is necessary for apothecial production by sclerotia, and this confirms the findings of other researchers (Grogan & Abawi, 1975; Clarkson *et al.*, 2004). Further work with more isolates would be worthwhile.

Our studies also revealed that carpogenic germination of sclerotia in soil occurred between 10 and 25°C, but not at 30°C, for water potentials of \geq -101 kPa. Germination at 5°C was not evaluated. At the optimum temperature of 15°C, sclerotia buried in soil produced apothecia within 4 weeks compared to 6-8 weeks at 10 and 20°C. Thus, temperature had a significant effect on both rate of sclerotial germination and the final number germinated.

Growth chamber and glasshouse experiments are currently in progress to determine the effect of leaf wetness duration, temperature and relative humidity on infection of carrots by ascospores of *S. sclerotiorum*.

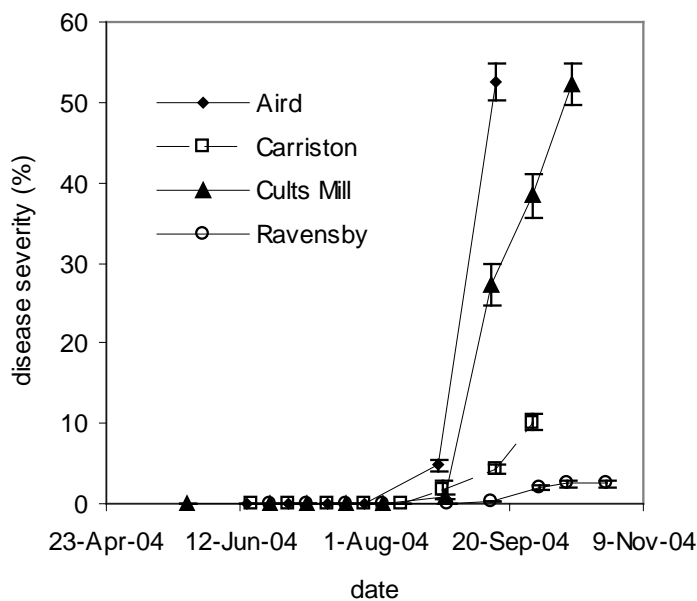
Field work

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

Field observations of five sequentially sown crops (mid Feb to late May) during the 2004 growing season revealed that apothecia first appeared within the crops between 1-14 Jul (Aird, sown 31 Mar), 29 July -11 Aug (Carriston, sown 26 Apr), and 8-21 Jul (Cults Mill, sown 7 May; Ravensby, sown 22 May) (Table 4). No apothecia were detected in the crop sown early (Monkton, 19 Feb) and harvested on 7 July. 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 12-15°C during the 2 weeks before apothecia were first detected. Peak apothecial

production at most sites occurred during late July, August and early September. Disease progress curves (DPCs) for the four main-season crops are shown in Figure 3. Figure 4 shows the DPC for each site together with the mean number of apothecia present per quadrat at each sampling bout.

Figure 3. Disease progress curves for foliar symptoms of sclerotinia at four sites in Scotland in 2004.



The results shown in Figure 4 illustrate the occurrence of a peak in apothecium production beginning in July. At Aird, Cults Mill and Ravensby the first peak in apothecium production is visible from 4-6 weeks before foliar symptoms were detected. It was noted, however, that there was no consistent relationship between the apothecium population density and the final disease severity on the foliage. This is clearly illustrated by comparing the population densities and severities for Aird and Ravensby (Fig 4(a) and (d) respectively). At Aird, the peak apothecium population density was around 0.25 apothecia per quadrat and the final mean disease severity was in the order of 50%. In contrast, the peak apothecium population density at Ravensby was about 1.75 apothecia per quadrat (i.e.

about 3 times the size of the population at Aird), but the final mean disease severity at Ravensby was only 2.5%.

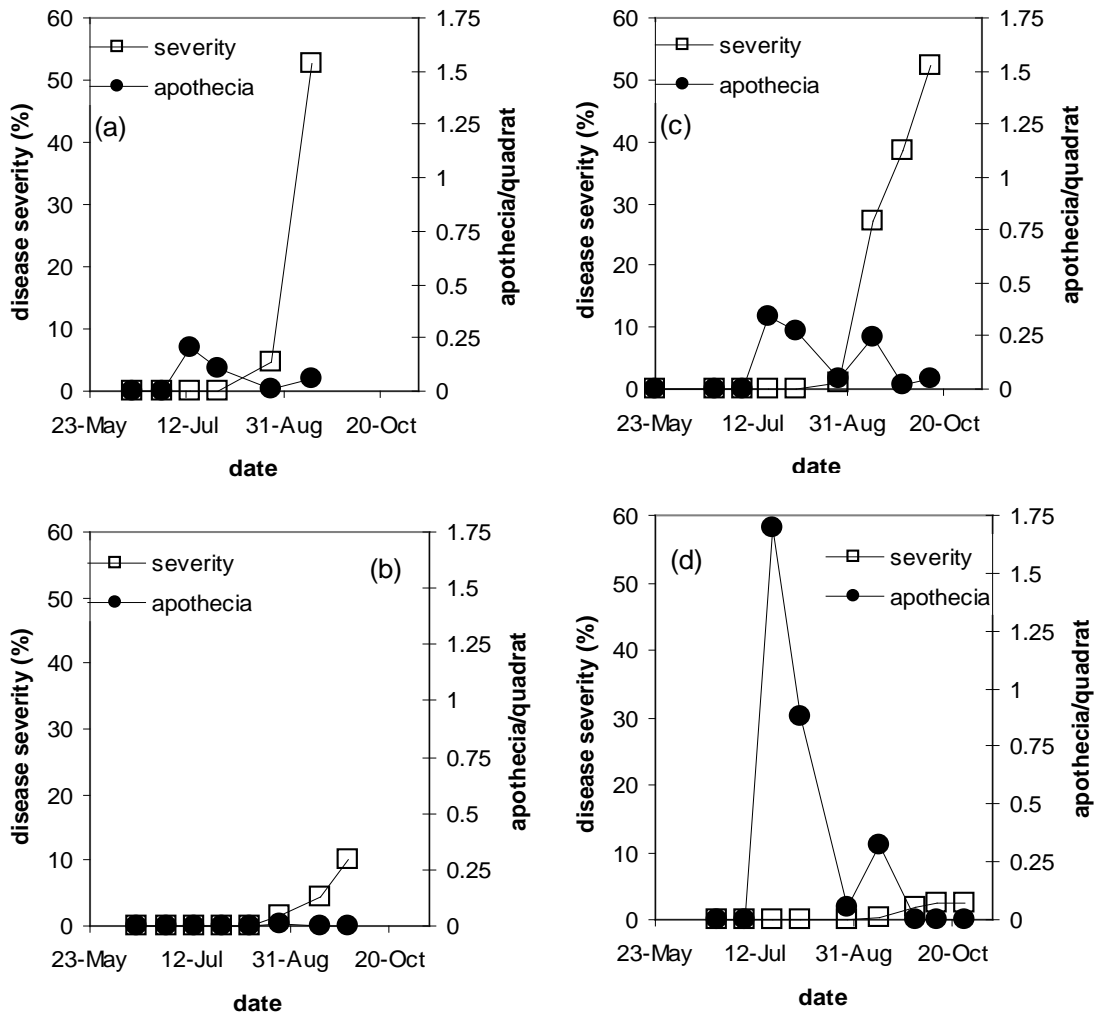


Figure 4. Mean foliar disease severity and apothecium population density for sclerotinia disease in four carrot crops in Scotland in 2004. (a) Aird; (b) Cariston; (c) Cults Mill; (d) Ravensby.

A number of factors might be involved in influencing the relationship between apothecium population size and foliar disease including; the weather conditions in the period of ascospore production, the growth stage and density of the crop, and external inoculum from neighbouring fields.

Variations in apothecium population density showed a similar pattern over the period of observation at all sites except Carriston, where apothecia were observed only at one

sampling bout in late August. At the remaining three sites, the initial flush of apothecium production in mid to late July was followed by a further flush between 54 to 63 days later, and, at Cults Mill a third peak after another 30 days (Figure 5).

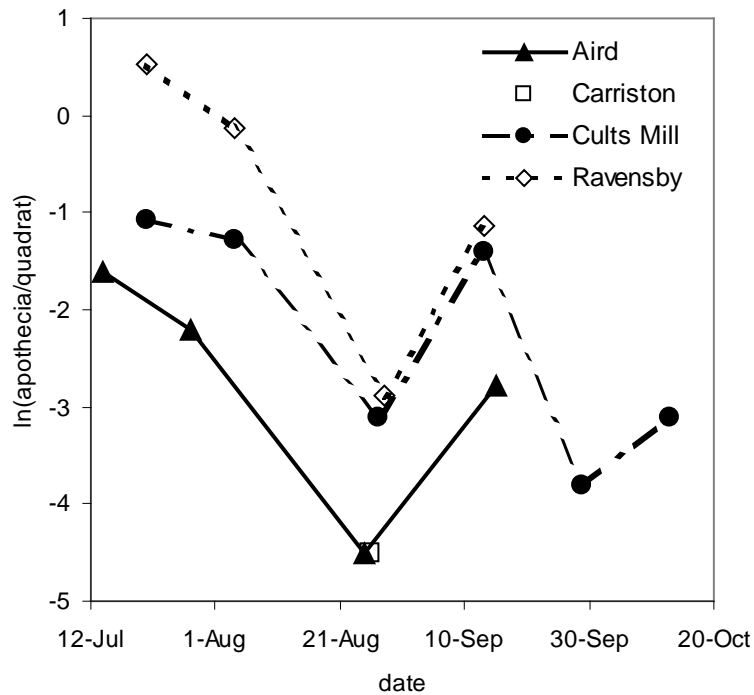


Figure 5. Temporal periodicity in apothecium population density for *S. sclerotiorum* in four carrot crops in Scotland in 2004. Mean log (base e) counts of apothecia from 90 quadrats per crop are shown.

At most sites, sclerotinia disease was detected on foliage of crops 4-6 weeks after the first appearance of apothecia, except at the Aird site where first disease was detected at the same time as the first apothecia. Senescing leaves were present in the crop at Aird when apothecia were first detected. At all sites, symptoms were first observed on petioles of lodged senescing leaves as water-soaked, dark-olive green lesions. These lesions then expanded over entire leaves, with infected tissue covered by white, cottony mycelium. In most cases, 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.

Table 4. 2004 - Production of apothecia and incidence of disease in commercially grown carrot crops [cvs. Nantucket (site 1) and Nairobi (sites 2-5)] with sequential sowing dates in relation to crop growth stage at five field sites

Site / sowing date	Soil ^a Temperature (°C)	First apothecia			First disease	
		Date ^b	Canopy enclosure	Growth stage	Date ^b	Growth stage/site of infection
1. Monkton 19 Feb	-	-	-	<i>Not detected</i>		<i>Not detected</i>
2. Aird 31 Mar	12-15 (13.6)	1- 14 Jul	Yes	Main period of root growth	1- 14 July	Main period of root growth <i>Petioles of lodged senescing leaves</i>
3. Carriston 26 Apr	13-15 (14.0)	29 Jul- 11 Aug	Yes	Main period of root growth	4- 26 Aug	First lodging <i>Petioles of lodged senescing leaves</i>
4. Cults Mill 7 May	13-15 (13.8)	8- 21 July	Almost (90% crop coverage)	Pencil + stage	4- 26 Aug	First lodging <i>Petioles of lodged senescing leaves</i>
5. Ravensby 22 May	12-15 (13.6)	8- 21 July	Almost (90% crop coverage)	Pencil + stage/main period of root growth	4- 26 Aug	Main period of root growth/early lodging <i>Senescing lower leaves</i>

^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.

Summary of General Field Observations

The following observations were made during monitoring carrot crops at different field sites throughout the 2004 growing season:

- 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.
- Peak apothecial production at most sites occurred during late July, August and early September, after canopy enclosure.
- 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 have indicated that canopy closure, the presence of apothecia within the crop and senescing leaves on the ground may be important factors in affecting disease initiation and development in the field. Further work is planned in year 2 of the project to investigate quantitative relationships between these as well as environmental factors such as soil temperature and rainfall. Studies will also be undertaken to detect whether any such

relationships between the variables could be applied to predicting the appearance of apothecia and subsequent disease.

Effect of crop growth stage and environmental factors on the development of sclerotinia disease – artificial inoculation of carrot crops with S. sclerotiorum ascospores

At both the Aird and Ravensby sites, disease symptoms developed in all plots of carrots inoculated with ascospore suspensions of *S. sclerotiorum*. Symptoms were first noticed on the 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 spread by mycelium growing between diseased and healthy plant foliage in contact with each other. No symptoms were observed prior to foliar senescence.

Table 5. Aird site 2004 - Effect of artificial inoculation at different growth stages (GS) on yield, and foliage disease severity and root rot caused by *S. sclerotiorum*. Assessments were made on 22 September.

Treatments	Marketable yield (t/ha)	Final foliage disease severity (%)	Sclerotinia infected roots (%)	Root disease severity (%)
GS 1^a (14 Jul)				
Control (water)	88.6 ^b	10.1	25.6	12.6
Folicur + inoculated	88.4	7.9	21.6	10.7
Inoculated	68.9	53.1	45.6	26.6
GS 2^a (4 Aug)				
Control (water)	95.4	4.8	29.8	14.7
Folicur + Inoculated	99.6	11.5	21.1	10.5
Inoculated	90.0	55.4	47.3	38.4
GS 3^a (18 Aug)				
Control (water)	91.8	9.3	16.3	8.2
Folicur + Inoculated	92.1	11.1	22.7	12.8
Inoculated	75.7	43.5	39.2	21.3
GS 1, 2 & 3				
Folicur + inoc. (GS 1) + Folicur (GS 2 & 3)	109.3	7.5	9.2	4.5
LSD ^c ($P=0.05$)	17.5	7.2	14.1	10.5

^a Growth stages - (1) full canopy enclosure, main period of root growth; (2) first lodging of lower leaves & 1-3 senescing leaves/plant; (3) fresh to mature full canopy, with heavy lodging & 3-4 senescing leaves/plant.

^b Values are means of three replicate plots.

^c LSD is the least significant difference at a probability of 5%.

Low levels of disease (< 10% foliage severity) developed on control plants inoculated with water. This was most likely due to infection from ascospores produced by sclerotia in the soil within the trial area.

At the Aird site, (Figure 6) the time to first symptoms was approximately 3 weeks post-inoculation for all three inoculations. Final foliage disease severity was greatest ($P = 0.05$) in plants inoculated at growth stages (GS) 1 (14 Jul) and 2 (4 Aug) compared to plants inoculated at GS 3 (18 Aug) (Table 5). However, there was no significant difference in

final foliage disease severity between plants inoculated at GS 1 and 2. At the Ravensby site (Figure 7 and Table 6) the time to first symptoms was approximately 3-4 weeks, and the disease progressed slower compared to Aird. Final foliage disease severity was greatest ($P = 0.05$) in plants inoculated at GS 1 (4 Aug) compared to plants inoculated at GS 3 (1 Sep). There was no significant difference in final foliage disease severity between plants inoculated at GS 1 and 2

At each inoculation timing/GS, final foliage disease severity, root infection and severity were significantly less ($P = 0.05$) in plants at both sites treated with a single foliar spray of Folicur (tebuconazole) prior to inoculation compared to inoculation alone. At both sites, three sequential foliar sprays of Folicur at GS 1 (prior to inoculation), GS 2 and 3 significantly ($P = 0.05$) reduced foliage disease severity, root infection and severity compared to inoculation alone at GS 1. Marketable yield was also significantly improved with this fungicide treatment. There was no consistent relationship between growth stage of carrots at inoculation and marketable yield at either site.

Table 6. Ravensby site 2004 - Effect of artificial inoculation at different growth stages (GS) on yield, and foliage disease severity and root rot caused by *S. sclerotiorum*. Assessments were made on 22 October.

Treatments	Marketable yield (t/ha)	Final foliage disease severity (%)	Sclerotinia infected roots (%)	Root disease severity (%)
GS 1^a (4 Aug)				
Control (water)	91.4 ^b	14.3	16.2	8.8
Folicur + inoculated	103.6	5.7	13.1	6.9
Inoculated	71.4	44.3	41.5	21.6
GS 2^a (18 Aug)				
Control (water)	98.6	14.3	18.6	10.5
Folicur + Inoculated	100.7	7.5	14.2	7.4
Inoculated	70.4	38.4	31.3	17.3
GS 3^a (1 Sep)				
Control (water)	98.6	12.1	16.7	9.7
Folicur + Inoculated	104.6	7.5	14.2	7.4
Inoculated	68.9	34.5	34.6	18.9
GS 1, 2 & 3				
Folicur + inoc. (GS 1) + Folicur (GS 2 & 3)	111.1	5.7	8.0	5.7
LSD ^c ($P=0.05$)	7.80	6.1	6.0	4.2

^a Growth stages - (1) almost canopy enclosure, main period of root growth; (2) full canopy enclosure, first lodging of lower leaves & 1-2 senescing leaves/plant; (3) fresh to mature full canopy, with lodging & 3-4 senescing leaves/plant.

^a Values are means of three replicate plots.

^c LSD is the least significant difference at a probability of 5%.

Rainfall was very high at both sites during August, and above average for this time of the year. The Aird site had rainfall every 2-5 days during September, but the Ravensby site was slightly drier. Air temperature in the first 12 h following inoculation ranged from 8-15^oC at Aird and 10-17^oC at Ravensby.

Results from the two artificial inoculation trials revealed that final foliage disease severity was greatest when plants were inoculated early (close to or after canopy enclosure, main period of root growth) compared to plants inoculated very late in the growth season (mature full canopy with lodging, senescing leaves).

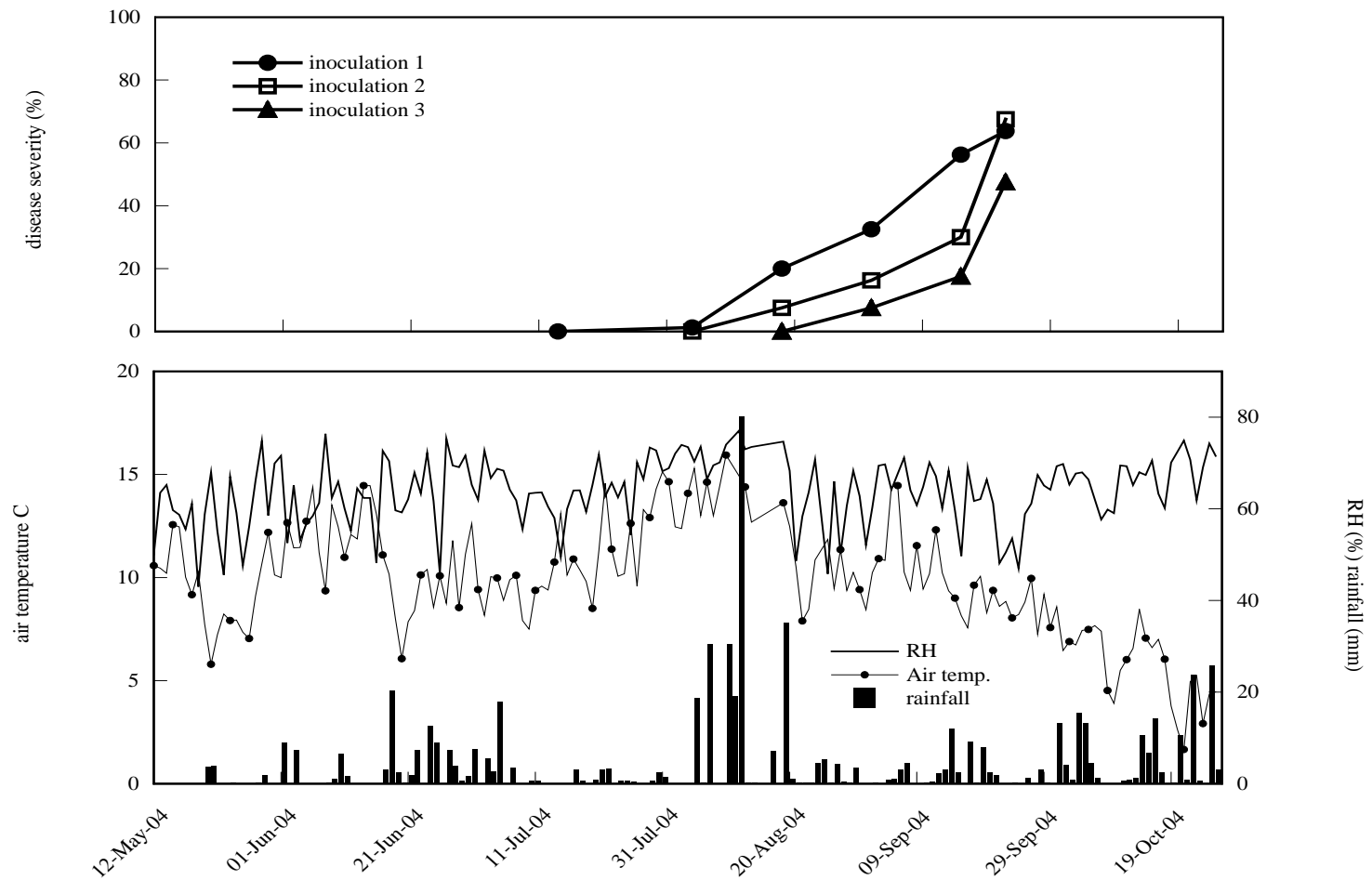


Figure 6. Disease progress (upper panel) for artificially induced epidemics of *S. sclerotiorum* and environmental data at Aird, Perth. Epidemics were initiated by foliar spray application of an ascospore suspension on 14 July, inoculation 1; 4 August, inoculation 2; 18 August, inoculation 3.

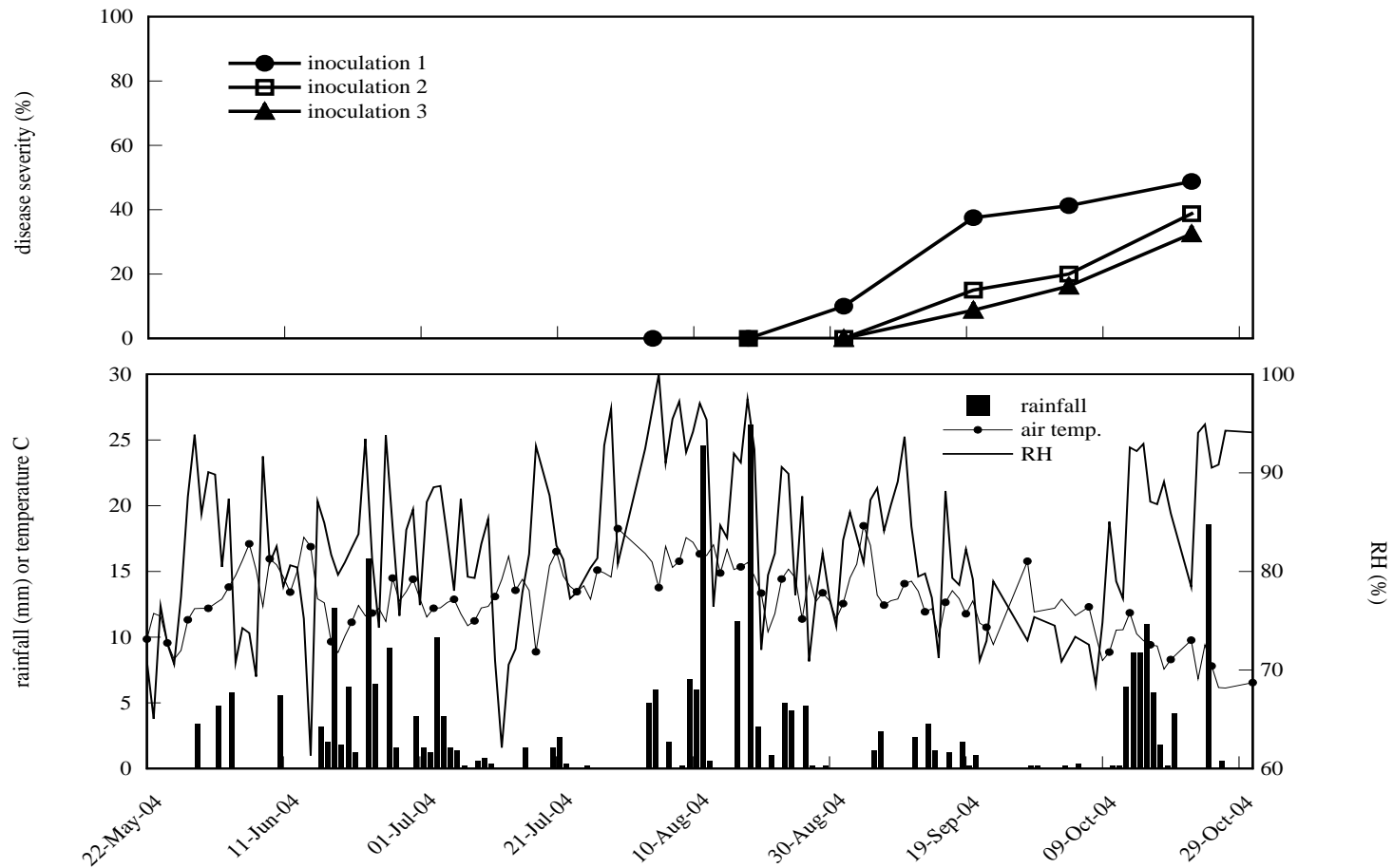


Figure 7. Disease progress (upper panel) for artificially induced epidemics of *S. sclerotiorum* and environmental data at Ravensby, Angus. Epidemics were initiated by foliar spray application of an ascospore suspension on 4 August, inoculation 1; 18 August, inoculation 2; 1 September, inoculation 3.

Considering the relationships between disease intensity and yield established by the different treatments, the correlation among disease intensity measurements (*i.e.* foliar disease severity, root disease incidence and root disease severity) and the correlation between these measurements and marketable yield differed between sites. Higher correlations among disease variables and between disease and yield loss occurred at Ravensby than at Aird. At Ravensby, the highest correlation between disease and yield was found for root disease severity ($r < -0.95$, $p < 0.001$, $n = 10$), while at Aird it was between root disease incidence ($r < -0.75$, $p < 0.006$, $n = 10$). When the data sets for both sites were pooled, correlations among disease intensity variables were all large ($r > 0.89$, $p > 0.001$, $n = 20$), while the highest correlation between disease and yield was for root disease incidence ($r < -0.82$, $p < 0.02$, $n = 20$). When these data were further pooled with those from the tests of novel fungicides (Tables 10, 11) the overall correlation between root disease

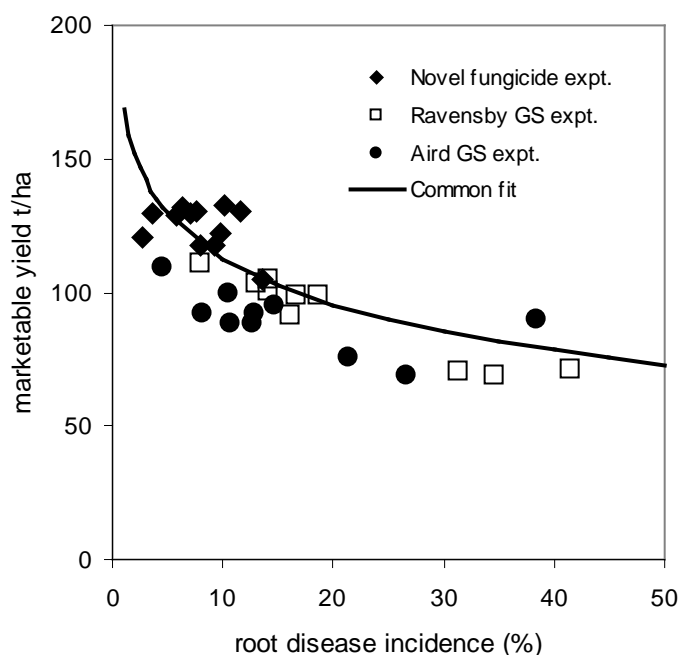


Figure 8. A yield loss relationship between marketable root yield and sclerotinia root disease incidence estimated from artificial inoculation experiments conducted at Aird, Perth and Ravensby, Angus and an experiment on novel fungicides conducted at Aird, Perth in 2004. The common regression line for the three treatments is $\text{yield (t/ha)} = 56.04 - 24.05 \ln(\text{root disease incidence})$, adjusted $R^2 = 0.77$ (30 d.f.).

incidence was significant ($r < -0.86$, $p < 0.001$, $n = 32$). A significant linear relationship between marketable yield and the logarithm of root disease incidence was present in the pooled data (Figure 8.).

Apothecial production by sclerotia in the field

Ten burials of *S. sclerotiorum* isolates KP and Ret were carried out between April and August 2004 at the Aird and Carriston field sites. No apothecia were produced by sclerotia of isolate KP buried at either Aird or Carriston. Sclerotia of both isolates were cold-conditioned at 4°C for 4 weeks prior to burial in the field. Other researchers have found that a 4 weeks cold-conditioning treatment is often insufficient to ensure adequate carpogenic of sclerotia of a number of different isolates, and recommend a minimum period of 8 weeks (Clarkson, personal communication). Lack of carpogenic germination of isolate KP in the field was most likely associated with an inadequate 4 week period of cold conditioning. In laboratory experiments, sclerotia of isolate KP produced apothecia within 4-6 weeks at 15-20°C, following cold conditioning for 8 weeks.

In the fortnightly burials of sclerotia (isolate Ret) at Aird, the time from burial to first appearance of apothecia ranged from 10 to 16 weeks (Table 8). The time from burial to first appearance of apothecia generally decreased with successive burials (16, 14, 16, 14, 12, 10, 10 for burials 1-7, respectively). Sclerotia of burials 8 (13 Jul), 9 (28 Jul) and 10 (10 Aug) did not germinate to produce apothecia before covering the crop with straw on 6 October. Apothecia produced by sclerotia buried in April (burials 1 and 2) were first seen in mid to late July and were observed throughout the growing season up to covering the crop with straw in early October. Peak apothecial production from these early burials of sclerotia was during late July and August.

At the Carriston site (Table 9), apothecial production was much lower than at the Aird site. Time to first germination of sclerotia of the early burials (1, 2 and 3 on 21 Apr, 5 May and 19 May, respectively) was 14-16 weeks, and was similar to sclerotia of the early burials at the Aird site. The time from burial to first appearance of apothecia generally decreased with successive burials, although sclerotia of burials 4 (2 Jun) and 5 (16 Jun) did not germinate (no apothecia detected before 20 Oct). Sclerotia of burials 6 (30 Jun) and 7 (14 Jul) produced apothecia after 10 weeks. However, sclerotia of the last two burials (11 and 26 Aug) did not germinate. Apothecia produced by sclerotia buried early in April and May

(burials 1, 2 and 3) were first seen in early August and were observed throughout the growing season until early October. Peak apothecial production from these early burials of sclerotia was during August and early September. At both sites, first appearance of apothecia from April and May burials occurred close to or after full canopy enclosure.

Soil moisture and rainfall data showed that the sandy clay loam at the Carriston site was consistently drier from 70-80% crop coverage onwards than the silty clay loam soil at the Aird site.

Table 8. Germination of sclerotia of *S. sclerotiorum* (isolate Ret) in 2004 buried in soil at Aird, Perthshire.

Burial No./ Date	Time to first apothecia (wk)	Number of apothecia				
		28 Jul	11 Aug	23 Aug	15 Sep	29 Sep
1. 6 Apr ^a	16	0.6 ± 0.5 ^b	9.6 ± 1.8	13.4 ± 2.2	2.2 ± 1.1	1.6 ± 0.7
2. 20 Apr	14	1.8 ± 0.8	10.0 ± 1.9	7.4 ± 3.1	3.0 ± 1.6	1.4 ± 0.8
3. 4 May	16	0.4 ± 0.2	0.4 ± 0.2	1.2 ± 0.5	5.2 ± 1.2	7.6 ± 1.5
4. 18 May	14	0	0	0.6 ± 0.2	1.8 ± 0.3	3.8 ± 0.9
5. 1 Jun	12	0	0	1.8 ± 0.8	5.0 ± 0.3	6.4 ± 1.1
6. 15 Jun	10	0	0	3.0 ± 1.5	10.6 ± 2.0	11.2 ± 1.6
7. 29 Jun	10	0	0	0	1.4 ± 0.5	1.0 ± 0.5
8. 13 Jul	ND ^c					
9. 28 Jul	ND					
10. 10 Aug	ND					

^a 1 week post sowing.

^b Each value is the mean ± SE of five replicates, with 20 sclerotia per replicate.

^c No apothecia detected before covering crop with straw on 6 Oct .

Table 9. Germination of sclerotia of *S. sclerotiorum* (isolate Ret) in 2004 buried in soil at Cariston, Fife.

Burial No./ Date	Time to first apothecia (wk)	Number of apothecia				
		11 Aug	26 Aug	8 Sep	22 Sep	6 Oct
1. 21 Apr ^a	16	1.4 ± 1.2 ^b	3.6 ± 2.4	4.6 ± 2.7	1.2 ± 0.9	0.4 ± 0.2
2. 5 May	14	0.8 ± 0.7	2.6 ± 1.1	4.0 ± 1.4	3.0 ± 1.5	2.6 ± 1.3
3. 19 May	14	0	0.8 ± 0.4	1.2 ± 0.71	1.0 ± 0.7	1.0 ± 0.5
4. 2 Jun	ND ^c					
5. 16 Jun	ND					
6. 30 June	10		0	0.4 ± 0.2	0.4 ± 0.3	0.4 ± 0.3
7. 14 July	10		0	0.2 ± 0.1	0	0
8. 28 July	ND					
9. 11 Aug	ND					
10. 26 Aug	ND					

^a 1 week post sowing.

^b Each value is the mean ± SE of five replicates, with 20 sclerotia per replicate.

^c No apothecia detected after 20 Oct .

Figure 9 shows the distribution of apothecial counts from the burial experiment at Aird in relation to accumulated thermal time (day degrees above 5 °C) from the date of burial to the day on which observations were made. Figure 10 shows both the response of time to first appearance of apothecia from the burial experiment at Aird in terms of number of elapsed days and in accumulated thermal time (day degrees above 5 °C). The results in Figure 9 illustrate the changes both in the distribution of appearance of apothecia with time and in the number of apothecia in response to delay in burial date.

Figure 10 includes an estimated value for the number of accumulated degree days (and days elapsed) for the natural field population of apothecia at Aird. Monitoring of environmental data at Aird started on 1 May 2004 at which date the mean soil temperature at 10 cm was 15.4 °C. Hence, accumulation of thermal time by the field population would have been under way by this date. In order to estimate the thermal time accumulated and the number of elapsed days prior to germination by the field population of sclerotia, it was necessary to: (a) fix a starting date for the accumulation of thermal time and elapsed days, and; (b) estimate the required values for thermal time and elapsed days. The procedure used is described below.

Scottish soils generally reach a steady temperature above 5°C in mid March. Consequently, March 15 was selected as an arbitrary estimate of the date for the start of accumulation of thermal time by the field population of sclerotia. Using the data collected by the weather station after 12 May 2005, a strong linear relationship was detected between the date and the daily rate of thermal time accumulation: the daily rate was 7.005 day degrees > 5°C per day; $R^2 > 0.95$. Based on this figure, the number of accumulated day degrees between 15 March 2004 and 12 May 2004 was calculated and used as the starting value for thermal time accumulation from 12 May 2004 onwards (when the actual data were available). A similar procedure was used to estimate the initial accumulation of thermal time for the first three burials (those on 6, 21 April and 5 May 2004) in the burial experiment. The remaining burials took place after 12 May 2004 and accumulated thermal time, in these cases, was calculated directly from the weather station soil temperature data only.

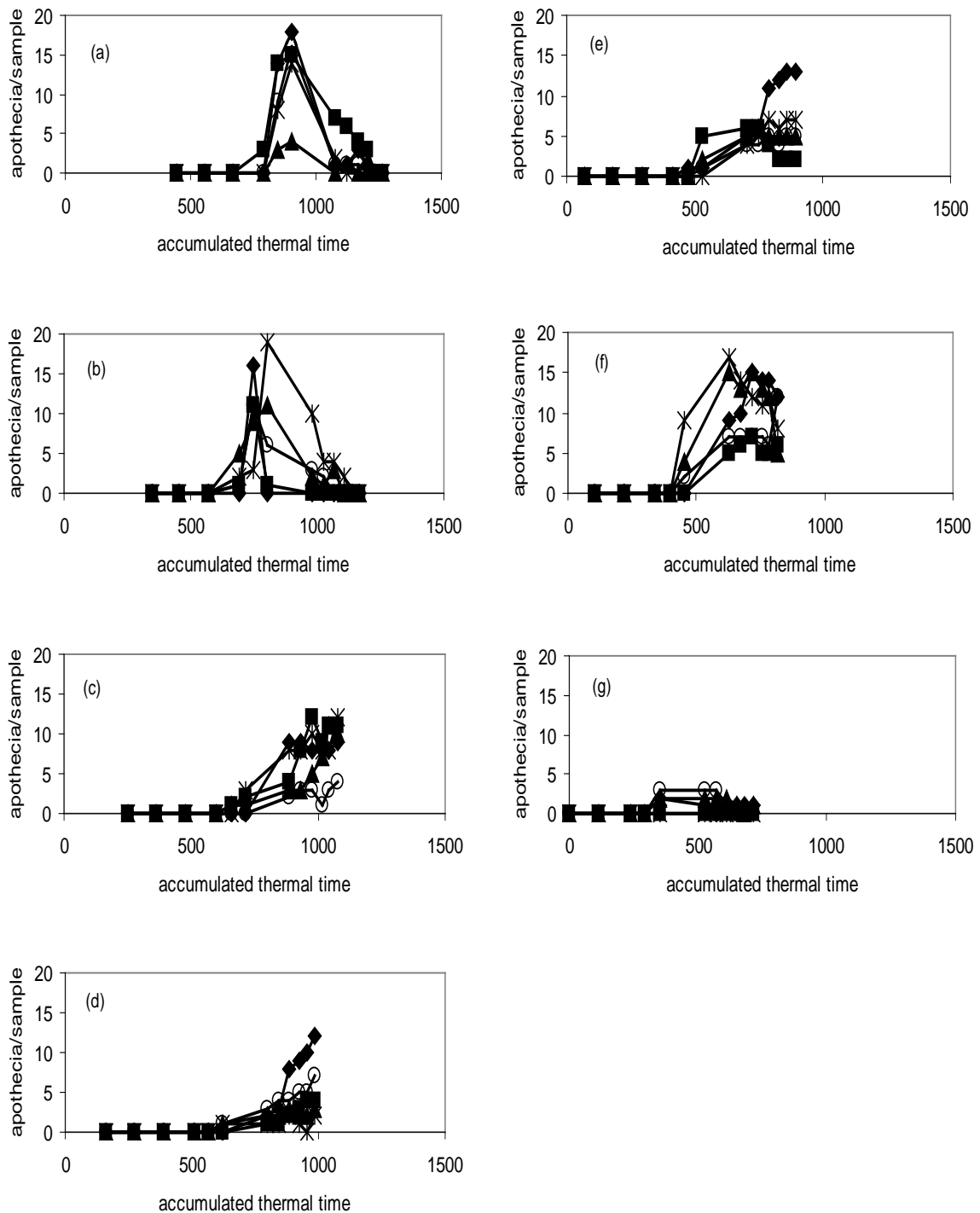


Figure 9. The distribution of apothecia with accumulated thermal time since burial (day degrees above 5⁰C) for replicate samples of sclerotia of *S. sclerotiorum* buried at Aird, Perth during 2004. Burial dates: (a) 6 April; (b) 21 April; (c) 5 May; (d) 19 May; (e) 2 June; (f) 16 June; (g) 30 June.

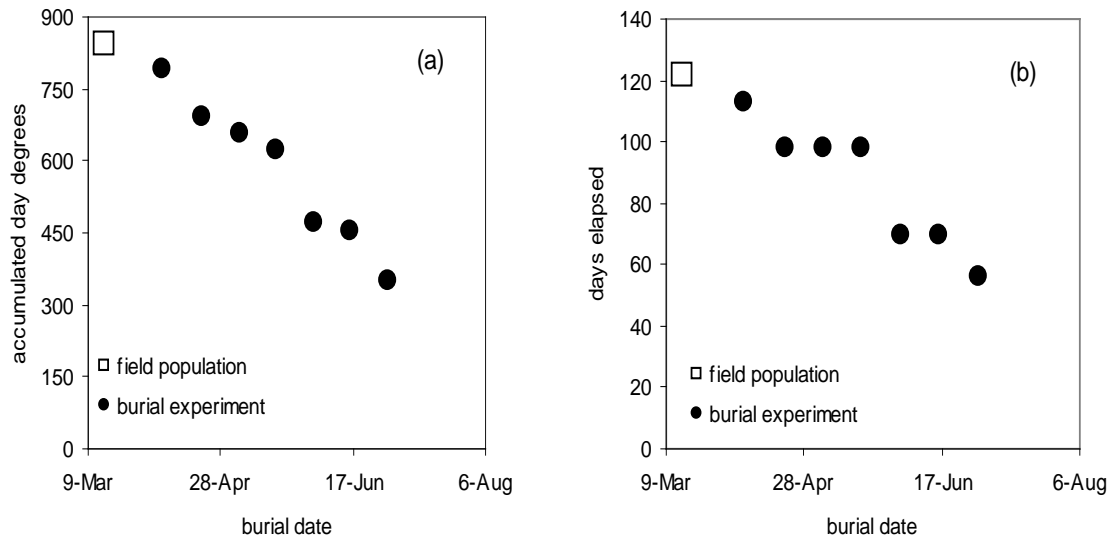


Figure 10. The effect of burial date on (a) accumulated thermal time and (b) number of days elapsed between burial (or onset of thermal time accumulation) for replicate samples of sclerotia of *Sclerotinia sclerotiorum* buried at Aird, Perth during 2004. Burial dates: (a) 6 April; (b) 21 April; (c) 5 May; (d) 19 May; (e) 2 June; (f) 16 June; (g) 30 June. Points for the natural field population are also shown for comparison. Thermal time accumulation for the field population was assumed to start on March 15.

In year 2 of the project, a preliminary model will be derived from laboratory and field data collected in year 1, and used to predict the appearance of apothecia in the field. Further data on the effect of environmental factors, especially soil temperature and rainfall, on production of apothecia in the field is required. Burials of *S. sclerotiorum* sclerotia and monitoring of apothecial production at two sites will be repeated in year 2 of the project. Sclerotia will be buried in grids laid over the soil surface to enable rate and percentage germination to be determined.

Evaluation of current and novel fungicides

Sclerotinia disease was first observed in the trial 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 10. Fungicide trial (natural infection) – Aird site, Perthshire.
Effect of fungicides on foliage disease severity caused by *S. sclerotiorum*.

Treatment ^a	Foliage disease severity (%)	
	8 September ^b	21 September ^c
Control (untreated)	54.6 (47.4) ^d	72.0 (58.2)
Folicur	21.2 (24.7)	50.0 (45.1)
Amistar	32.0 (33.2)	46.0 (41.5)
Signum	4.2 (11.5)	24.0 (28.5)
Contans WG (soil)	52.0 (45.8)	70.0 (57.6)
Contans WG (soil) + Signum	12.2 (18.9)	37.0 (36.7)
Shirlan	10.0 (17.0)	31.2 (30.7)
A9219B	4.4 (11.2)	23.0 (27.9)
UKA352	5.4 (12.3)	26.2 (28.8)
UKA373	6.2 (13.7)	28.2 (29.9)
Rhino (soil)	50.0 (44.9)	69.0 (56.5)
Rhino	36.0 (35.6)	70.0 (57.0)
LSD ^e ($P = 0.05$)	(14.70)	(13.15)

^a A 3-spray fungicide programme of each treatment was applied (except for soil incorporation of Contans WG & Rhino) at approximately 21 day intervals on 28 June (prior to canopy enclosure), 22 July and 20 August.

^b Growth stage – heavily lodged canopy (2) & 4-5 senescing leaves/plant.

^c Growth stage – heavily lodged canopy (3) & 6-7 senescing leaves/plant.

^d Values are means of five replicate plots. Figures in parenthesis are angular transformations of percentage data.

^e LSD is the least significant difference at a probability of 5%.

At the first disease assessment on 8 September (heavily lodged canopy & 4-5 senescing leaves/plant), foliar sprays of either Folicur (tebuconazole), Signum (boscalid + pyraclostrobin), Shirlan (fluazinam), A9219B, UKA352 or UKA373 significantly ($P = 0.05$) reduced foliage disease severity compared to the untreated control (Table 10). The

combined treatment of Contans WG (*Coniothyrium minitans*) soil incorporation and subsequent sprays of Signum also significantly ($P = 0.05$) reduced foliage disease severity compared to the untreated control. However, there were no significant differences between any of these effective treatments. Sprays of Amistar (azoxystrobin) or Rhino (flutolanil), or the Rhino soil incorporation were ineffective in reducing foliage disease severity compared to the untreated control.

At the second disease assessment (heavily lodged canopy & 6-7 senescing leaves/plant) on 21 September, foliage disease severity increased from 55 to 72% in the untreated control plots. All fungicide spray treatments, which were effective at the first assessment, maintained their level of control except Folicur. Foliar sprays of Amistar also significantly ($P = 0.05$) reduced foliage disease severity even though this was not observed at the first disease assessment.

Table 11. Fungicide trial (natural infection) – Aird site, Perthshire.
Effect of fungicides on yield and sclerotinia root rot. (roots were harvested on 22 September prior to covering the crop in straw).

Treatment	Total yield (t/ha)	Marketable yield (t/ha)	Sclerotinia infected roots (%)	Root disease severity (%)
Untreated	123.3 ^a	105.1	13.6 (21.5)	3.8 (11.0)
Folicur	142.4	129.7	7.0 (14.5)	1.8 (7.5)
Amistar	134.5	117.3	8.0 (14.1)	2.6 (7.7)
Signum	143.4	128.6	5.8 (13.7)	1.4 (6.7)
Contans WG (soil)	131.0	117.9	9.2 (17.5)	2.4 (8.8)
Contans WG + Signum	144.6	130.2	7.6 (13.8)	2.2 (7.4)
Shirlan	148.2	132.8	10.2 (18.1)	3.2 (9.8)
A9219B	143.1	129.4	3.6 (9.3)	1.0 (5.1)
UKA352	134.4	120.7	2.8 (7.2)	0.8 (3.9)
UKA373	144.3	131.7	6.4 (11.9)	1.8 (6.5)
Rhino (soil)	143.8	130.1	11.6 (17.5)	3.8 (9.5)
Rhino	140.4	121.8	9.8 (18.1)	2.6 (8.9)
LSD ^b ($P = 0.05$)	14.4	16.10	$P = 0.140$ NS ^c	$P = 0.256$ NS

^a Values are means of five replicate plots. Figures in parenthesis are angular transformations of percentage data.

^b LSD is the least significant difference at a probability of 5%.

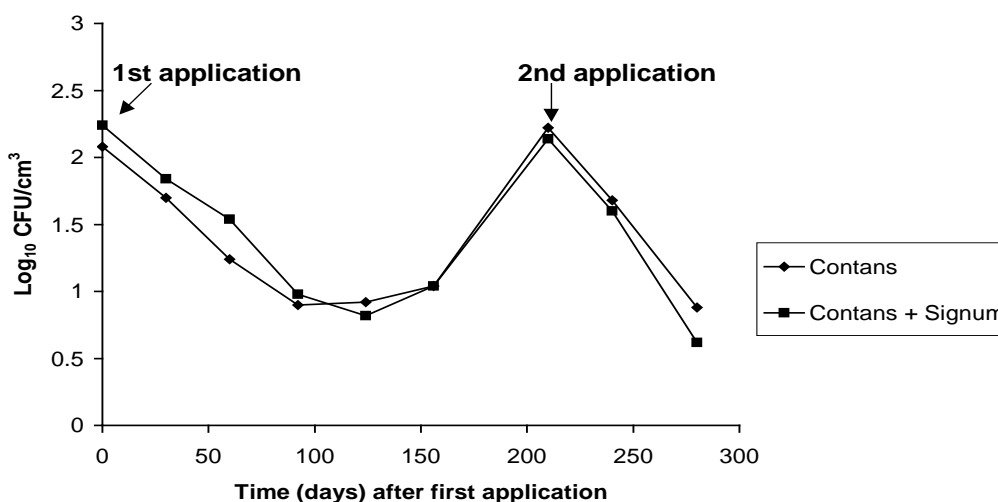
^c NS – Not significant

Effects on yield and sclerotinia root rot. Incidence of cavity spot on harvested roots was negligible. There were significant effects ($P < 0.01$) of fungicide treatments on total yield and yield of marketable roots at the first harvest (Table 11). Folicur, Signum, Contans WG + Signum, Shirlan, A9219B and UKA373 significantly ($P = 0.05$) increased both total yield and yield of marketable roots compared to the untreated control. However, there were no significant differences in yields between these fungicide treatments. The mean yield improvement of the effective treatments compared to the untreated control was 20-24%, and this was associated with a reduction in foliage disease severity. Post harvest assessments of sclerotinia root disease incidence and severity showed considerable variation, and treatment differences were not significant.

Analyses of yield and sclerotinia root rot data from the second harvest are currently in progress.

Coniothyrium minitans was detected at approximately 10^1 and 10^2 colony forming units (CFUs) cm^{-3} soil in plots treated with Contans WG throughout the trial period (6 April 2004 to 14 February 2005) (Figure 11). A second application of the product to the soil surface in mid November gave a general increase in CFUs cm^{-3} , which subsequently decreased with time.

Figure 11. Survival of *C. minitans* in field soil following application of Contans WG (6 kg/ha, soil incorporation), and Contans WG + Signum (0.75 kg /ha) 3 x foliar sprays.



After 24 weeks, Contans WG significantly decreased the percentage viability of sclerotia (65-80%) compared with the untreated control (95-100%). The percentage of sclerotia infected with *C. minitans* was significantly higher in plots treated with Contans WG (30-45%) compared with the untreated control (0-5%).

Results from the fungicide trial revealed that good control of sclerotinia foliage disease was obtained by 3-spray programmes of either Signum (boscalid + pyraclostrobin), Shirlan (fluazinam), A9219B, UKA352 or UKA373. Furthermore, these fungicides were superior to Folicur (tebuconazole) and Amistar (azoxystrobin). Control of foliage disease by most of these effective fungicides was associated with increases in yield of marketable roots. Further trials are required to assess these current and promising novel fungicides in alternating spray programmes.

In this trial, no foliage disease control was achieved with Contans WG. This may be attributed to the late application of the product at sowing, and/or long distance spread of ascospores between plots negating any localised control effects within the Contans WG-treated plots. A period of at least 8 weeks between application and sowing the crop, or a high disease risk period, is recommended to enable *C. minitans* to infect sclerotia and reduce viability. Further trials are recommended in carrot crops to investigate optimising the timing of the product in relation to sowing. Such trials would need to have plots much further apart, separated by large guard areas. Further work should also investigate 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.

C. minitans survived in soil treated with Contans WG for up to 40 weeks. Survival of the fungus for a similar period in glasshouse soil has been reported previously following soil application of the product (Jones & Whipps, 2002).

Conclusions

The following conclusions can be made based on the first year's results of the project:

Epidemiology

- High temperature and high relative humidity (RH) are detrimental to the survival of ascospores of *S. sclerotiorum*. Survival of ascospores was reduced markedly at $>20^{\circ}\text{C}$ and $\text{RH} > 45\%$.

- Soil temperature and water potential are major factors affecting carpogenic germination of sclerotia of *S. sclerotiorum*. Carpogenic germination in soil occurred between 10-25⁰C, but not at 30⁰C for water potentials of ≥ -101 kPa. Optimum temperature for germination was approximately 15⁰C.
- The appearance of lodged, senescing leaves in carrot crops usually occurs 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.
- In general, the first appearance of fruiting bodies in carrot crops occurs in July, close to or after full canopy enclosure, and is concurrent with the early onset of senescing foliage.
- Peak fruiting production in carrot crops occurs during late July, August and early September, after canopy enclosure.
- The primary site of infection by ascospores is senescing or damaged petioles. Foliage symptoms are not observed before senescence or crop damage.
- Leaf wetness is important for infection and disease development.
- At most sites, foliage disease was detected on crops 4-6 weeks after the appearance of the first fruiting bodies.
- There is no consistent relationship between apothecia population density and final foliage disease severity.
- Canopy closure, the presence of apothecia and senescing leaves on the ground are important factors in affecting disease development in the field
- Serious infection of the foliage completely defoliates the crop, leading to early infection of the crown and the production of abundant sclerotia on the soil surface.
- Disease is often very severe on damaged foliage in the outer rows of beds next to the wheelings.
- 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 have been stored under straw.
- Final foliage disease severity was greatest in plants inoculated early (canopy enclosure; main period of root growth) compared to plants inoculated late in the growing season (fresh to mature canopy, with heavy lodging and senescing leaves). There was no consistent relationship between crop growth stage at inoculation and marketable yield.

Control

- Three foliar sprays of either Signum (boscalid + pyraclostrobin), Shirlan (fluazinam), A9219B, UKA352 or UKA373 at approximately 18-21 day intervals gave the most effective foliage disease control. Amistar (azoxystrobin) and Folicur (tebuconazole) gave control but were not as effective as these fungicides. Sprays of Rhino (flutolanil), or Rhino soil incorporation were ineffective in reducing foliage disease.
- Control of foliage disease by most fungicides was associated with increases in yield of marketable roots. Further trials are required to assess these promising fungicides in alternating spray programmes.
- No disease control was obtained with Contans WG, and this may be attributed to late application of the product and infection of plants by ascospores blown into treatment plots. Further trials are required to investigate timing of application.
- Results suggest 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. Further trials are required to substantiate this.
- There is a significant relationship between root disease incidence and marketable yield. Based on observations from three fungicide experiments, a large yield response to controlling low levels of disease can be expected, with less response once root disease incidence reaches approximately 10%.

Technology Transfer

An Information Sheet outlining the basic biology and symptoms of carrot sclerotinia disease, and the objectives of the project was distributed to growers at the British Carrot Growers' Association (BGCA) Variety Day, Nottinghamshire in October 2004. A general overview of the disease and some early results were presented at the ASDA Suppliers Focus Group Meeting, Edinburgh on 11 November 2005, and to the BCGA Research & Development Committee, Perth on 18 November 2005.

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APPENDIX

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.