Project title:	An investigation into the prevalence and pathogenicity of *Itersonilia* spp. in commercial carrot crops and its association with leaf dieback and crown rot.
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Project leader:	Dr G M McPherson Horticulture Research International Stockbridge House Cawood Selby North Yorkshire YO8 3TZ Tel: 01757 268275 Fax: 01757 268996
Key workers:	Dr D Pattison, Miss F Pomares
Location:	HRI Stockbridge House Commercial crops in Lancashire, Morayshire and Fife
Project co-ordinator:	Mr R W Travis
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The results and conclusions in this report are based on a short term study over two years. The conditions under which the work was carried out and the results 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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Authentication

I declare that this work was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

Signature .....

Dr G M McPherson
Project Leader
Horticulture Research International
Stockbridge House

Date ..... 29/1/99 .....

Report authorised by .....

M R Bradley
Site Director
Horticulture Research International
Stockbridge House

Date ..... 29.1.99 .....

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PRACTICAL SECTION FOR GROWERS

Background and Objectives

A new disorder described as a foliar dieback and crown rot occurred for the first time in Lancashire in 1984/85. Initial investigations failed to identify known pathogens eg. *Alternaria dauci* or *Alternaria radicina*, associated with either the premature foliage dieback or the internal breakdown at the stele-cortex interface observed following over-wintering under straw (McPherson, pers comm). An *Itersonilia* sp. was recovered from both the foliage dieback in Autumn 1984 and from the roots with crown rot following over-wintering in Spring 1985. The full significance of this observation was perhaps not fully appreciated at the time, particularly as the same fungus had previously been recorded on healthy carrot foliage (Channon, 1963), and was regarded as a saprophytic leaf surface organism. Fortunately, for the UK carrot industry, the incidence of the disorder in subsequent years was much lower, though this did mean that a thorough investigation of the problem was not immediately possible.

The disorder re-appeared again in many UK production areas in 1989/90 causing significant losses and continued to do so in subsequent seasons. Random samples submitted to diagnostic laboratories again consistently failed to identify a primary pathogen, though techniques for recovery of basidiomycete fungi such as *Itersonilia* spp. were not undertaken in all cases.

However, where appropriate isolation techniques have been undertaken *Itersonilia* spp. have been recovered consistently, though the full significance of this basidiomycete fungus on carrot foliage remains undetermined. An interim report for HDC "An investigation into the Prevalence and Pathogenicity of *Itersonilia* spp. and its Association with Leaf Dieback and Crown Rot of Carrots" is available from HDC.

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Interestingly, a similar 'blight' or foliage dieback caused by *Itersonilia pastinacae* occurs on the related umbelliferous parsnip crop and this pathogen also produces a foliage 'blight' and root 'canker' not unlike the foliar dieback and crown rot symptoms observed in carrot crops. In parsnip, where canker is a recognised disease, resistance breeding has developed tolerant cultivars. In carrot, however, *Itersonilia* has not previously been recognised as a primary pathogen and it is possible that the widely grown open-pollinated cultivars are inherently resistant to this disease; hence the saprophytic occurrence of *Itersonilia* reported on carrot foliage in the 1960s. The more widespread use of F1 hybrid cultivars in recent years whilst significantly improving crop quality may have inadvertently resulted in the loss of this original cultivar tolerance. It should be noted that this remains a speculative view and currently cannot be substantiated.

Preliminary investigations with fungicides at HRI Stockbridge House demonstrated that fenpropimorph (Corbel) and carbendazim (a component of Compass) inhibited the growth of *Itersonilia* in agar plate tests. These same fungicides were subsequently found to provide a good suppression of crown rot in grower studies and in the final year of a previous HDC funded project on the disorder, conducted by ADAS (FV 34).

As a result of these early studies both Corbel and Compass were included in the HDC funded SOLA project (HDC CP 2) co-ordinated at HRI Stockbridge House on behalf of the UK carrot industry. Two new SOLAs (No's 1058/94 and 1264/94) were subsequently granted approval under emergency procedures. It is estimated that >75% of UK carrot crops now receive repeat applications of one or other of these two fungicides during the Autumn, generally in alternating programmes.

The primary objectives of this HDC funded project were to:

- determine the prevalence of *Itersonilia* spp. in commercial carrot crops, and to relate this to the incidence of leaf dieback/crown rot.

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- to monitor the same commercial crops for the presence of other potential pathogens which may be responsible for the symptoms.

- to monitor tagged plants in affected crops to determine any potential relationship between leaf tip dieback and subsequent crown rot.

- to investigate the potential of novel fungicides for preventing mycelial growth in laboratory tests.

It should be noted that a separate project, sponsored by MAFF, was conducted over the same period to further investigate the role of *Itersonilia* spp. in the crown rot disorder. The primary aim of this work was to demonstrate, where possible, whether *Itersonilia* spp. were the primary cause of the disease, by proving Koch's postulates.

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Summary of Results

A new disorder of UK grown carrots, referred to as crown rot, was investigated in this project by monitoring the incidence of *Itersonilia* spp. and other predominant organisms, in commercial carrot crops in relation to the subsequent occurrence of characteristic symptoms of leaf dieback and/or crown rot of the roots.

During the two year period of the investigation *Itersonilia* spp. were detected on carrot foliage though only at relatively low levels. Unfortunately, no symptoms of crown rot developed in the monitored crops throughout the period of study. This situation was similar to that reported throughout the UK during this period. It is possible that the widespread use of the novel approved fungicides may have reduced the inoculum in the monitored crops. This, in turn may have reduced the potential for disease in the designated monitored, untreated areas.

In laboratory evaluations of novel fungicides several products with apparent activity against the disease were identified. The most effective in suppressing mycelial growth were difenconazole (Plover), pyrimethanil (Scala) and benodanil (Calirus). Further *in-vivo* testing of these products under commercial conditions would be necessary before further progress could be made.

In separate MAFF funded work conducted during the same period as this study Koch's Postulates were partially successful. Symptoms of crown rot were successfully reproduced in carrot tissue pieces in laboratory studies. However in artificially inoculated plots of carrots at HRI Stockbridge House, established during the same period as that reported here, leaf dieback or crown rot symptoms were not reproduced successfully. Therefore Koch's Postulates were not completed satisfactorily and it is not possible to conclude that *Itersonilia* spp. are responsible for crown rot.

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Action Points for Growers

- Growers should be aware of the characteristic symptoms of the new disorder and monitor crops regularly from July onwards for the first signs of leaf dieback and discoloration of the root tissues in the crown region.

- Any suspect symptoms should be submitted to a diagnostic laboratory for detailed examination.

- Growers should ensure they are in possession of the SOLA documents (1058/94 and 1264/94) for Corbel and Compass respectively and use these products in an alternating programme during the period July-October, particularly during wet seasons, to minimise the risk of crown rot development. It should be noted that these fungicides are also likely to be effective in suppressing other foliar pathogens of carrots eg. Alternaria spp., powdery mildew.

- Growers should continue to support work, aimed at elucidating the primary cause of crown rot in UK carrot crops in the future.

- During the course of this investigation it became evident that there are several other, largely undescribed, symptoms on the roots of carrot crops. Fortunately in most cases at present, affected plants are scattered affecting less than 1% of the crop. Currently these problems are largely ignored and their primary cause remains uninvestigated. Growers and researchers alike should become more familiar with the various root problems and their primary causes on the carrot crop and it is predicted that one or more of these problems could increase significantly in the future to cause economic loss to the carrot industry.

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Practical and Financial Benefits from the Study

The study described has raised awareness of this new disorder affecting the UK carrot industry and, at the same time, drawn attention to a range of other symptoms which affect this root crop sporadically.

By identifying novel fungicides with activity against *Itersonilia* spp,, and securing their approval via the HDC sponsored SOLA project, significant benefits have been made. Since their widespread uptake by UK carrot growers the incidence of leaf dieback and crown rot has been reduced significantly, though whether it is due entirely to fungicide application or to less favourable weather conditions remains uncertain. Approval of these fungicides has also provided indirect benefits against other foliar pathogens of carrots eg. *Alternaria dauci* control with Compass (iprodione + thiophanate-methyl).

In financial terms the project has been highly successful as losses due to crown rot have become largely insignificant whereas previously severely affected crops were unmarketable due to the internal discoloration of affected roots. It is of course however, of considerable concern, that the primary incitant of crown rot remains unproven. It is recommended that further investigation should be undertaken should the disorder re-occur at appreciable levels in the future.

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EXPERIMENTAL SECTION

Introduction

The primary aim of the investigation was to determine the incidence of the basidiomycete fungus, *Itersonilia* spp. or other predominant micro-organisms, in commercial carrot crops and to relate this to the development of the leaf dieback and crown rot disorder, experienced previously by UK growers.

During a two-year period (1995/1996 and 1996/1997) a range of commercial carrot crops, selected on the basis of a potential high risk of the crown rot disorder, were monitored in detail. Sites selected for study were located largely in areas of high rainfall ie. Lancashire, Fife and Morayshire as these appeared from previous experience to have a higher risk of the disorder than other crops in Eastern England.

In Year 1 samples were submitted to HRI Stockbridge House by personnel from DMA Associates during the period from June-July 1995 to October 1995 with further root samples being collected following over-wintering in February-March 1996.

Plant samples received in the laboratory were assessed for the incidence of leaf dieback, checked for symptoms of crown rot and sub-samples taken for recovery of *Itersonilia* spp. using a selective agar technique and other micro-organisms using Potato Dextrose Agar (PDA).

In Year 2 a modified protocol was developed whereby an unsprayed area was marked out in each of four crops and individual plants tagged. By inspecting tagged plants regularly it provided an opportunity to track the development of the disorder from initial leaf tip dieback symptoms, through petiole decay, to crown rot symptoms, assuming the disorder occurred in the selected crops.

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Also, in Year 2 a selective Waxman Agar plate technique was used to monitor the air-borne spora of *Itersonilia* spp. above the selected carrot crops at each site visit. Work in Year 2 was conducted by personnel from HRI, Stockbridge House.

Finally, whilst two novel fungicides were successfully approved as Specific Off-Labels for crown rot control many other potential active ingredients had not previously been tested. Therefore a preliminary efficacy screen was conducted on fungicide amended agar using isolates of both *I. pastinacae* and *I. perplexans*.

Materials and Methods

Sites (1995/1996)

A total of 8 crops were selected for detailed monitoring during 1995/1996. Site details were as follows:

DMA 1	cv. Narman, Lancashire
DMA 2	cv. Narbonne, Lancashire
DMA 3	cv. Nerac, Lancashire
DMA 4	cv. Nairobi, Lancashire
DMA 5	cv. Nairobi, Fife, Scotland
DMA 6	cv. Narman, Fife, Scotland
DMA 7	cv. Narman, Fife, Scotland
DMA 8	cv. Narbonne, East Anglia

Sites DMA 1-7 were regarded as high risk crown rot sites, whereas DMA 8 was included as potentially a low risk 'crown rot' site.

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Sites (1996/1997)

A total of 4 crops were selected for detailed monitoring during 196/1997. Site details were as follows:

HRI, SH 1 cv. Nerac, Lancashire
HRI, SH 2 cv. Nerac, Lancashire
HRI, SH 3 cv. Narman, Fife, Scotland
HRI, SH 4 cv. Nairobi, Morayshire, Scotland

Experimental Design

Year 1 (1995/1996

At regular intervals carrot plant samples (foliage + root attached) were collected (10 lots of 10 plants collected randomly across the field) and forwarded to the Diagnostic Laboratory at HRI, Stockbridge House. On receipt samples were assessed for the presence of leaf dieback using the following scale:

0 = No dieback evident, plant healthy.
1 = Trace of leaf tip dieback on occasional leaves.
2 = Frequent dieback on most plants, affecting only individual leaflets.
3 = Severe foliage dieback, whole leaflets affected, progressing towards petiole.

From the assessment scores an index (0-100) of leaf dieback severity was calculated using the following formula:

$$\frac{1\,(1) + 2\,(2) + 3\,(3)}{\text{No. of Plants Assessed}} \times \frac{100}{3}$$

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Year 2 (1996/1997)

At each location an area, ca. 10 beds wide by 50 metres long, was marked out and identified clearly using coloured tape to delineate the area to ensure that no fungicide sprays would be applied. Within each unsprayed area 10 specific locations were identified using marker canes and at each point 10 individual carrots seedlings were tagged. Individual tagged plants could be identified by the presence of an individual numbered white label.

At regular approximately monthly intervals during the main growing season, the sites were visited by HRI personnel and individual tagged plants assessed for the presence of leaf dieback.

Dieback was scored using a 0-3 scale of severity and an Index (0-100) calculated as described previously for Year 1.

In addition, from each of the 10 locations in the untreated area, a small sample of 10 plants was collected (ie. 100 plants in total) and returned to the laboratory for further investigation.

Laboratory Evaluation (1995/1996 and 1996/1997)

At each sampling date plant samples were inspected in the laboratory and assessed in detail as described below.

Once the samples had been scored for leaf dieback according to the 0-3 scale defined above, eight tissue pieces/sample with leaf dieback (where present) were selected and attached to the lid of a Petri dish containing agar (Potato Dextrose Agar). Where dieback was not evident on the sample healthy leaf tissue pieces were selected at random instead.

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This suspended tissue test (STT) is selective for basidiomycete fungi such as *Itersonilia* spp.

A further 8 tissue pieces were plated out onto Potato Dextrose Agar (PDA) plates and incubated. All agar plates were assessed after 4-7 days for the presence of *Itersonilia* spp. or other micro-organisms.

Once the foliage samples had been taken the roots were cut longitudinally to check for crown rot symptoms. Samples were assessed using the following (0-3) scale and an Index of Disease Severity calculated:

0 = No evidence of crown rot.
1 = Slight internal discoloration of crown tissues, penetrating <1 cm into root tissues.
2 = Moderate internal discoloration of crown tissues, penetrating 1-2.5 cm into root tissues.
3 = Severe internal discoloration of crown tissues, penetrating >2.5 cm into root tissues.

Where any discoloration was noted tissue pieces were placed directly on agar (PDA) and attached to the Petri dish lid using the suspended tissue test described previously, in an attempt to recover *Itersonilia* spp.

Monitoring of Air-Borne Spora (1996/1997)

At each site visit, weather permitting, the air-borne spora of *Itersonilia* spp. was determined using a selective agar medium (Waxman egg - albumin agar). In both the unsprayed and an adjacent sprayed 'commercial' crop area 10 Waxman agar plates were exposed by laying them open on the soil surface at 5 metre intervals for a 15 minute period, between the crop rows. Following exposure the plates were collected, sealed and returned to the laboratory for incubation.

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After 48-72 hours at 20°C in the incubator plates were examined visually for the distinctive colonies of *I. pastinacae* and *I. perplexans* and colony counts of each species/plate made. Occasional colonies were checked microscopically to confirm the presence of *Itersonilia* spp. From this data it was possible to estimate the number of spores of *Itersonilia* spp. deposited over the monitored crop.

Fungicide Screening

During the period of investigation a range of novel fungicides were selected and incorporated into potato dextrose agar (PDA) at concentrations of 0, 2 and 20 ppm. Fungicide amended plates were seeded with individual isolates of both *Itersonilia perplexans* (CC16/98) and *I. pastinacae* (CC01/98) by placing a 3 mm agar plug containing the fungus onto the centre of each duplicate plate. The following fungicides were evaluated in this study.

	Active Ingredient	Product
1.	azoxystrobin	Amistar
2.	benodanil	Calirus
3.	bupirimate	Nimrod
4.	carbendazim	Bavistin DF
5.	chlorothalonil	Bravo 500
6.	cyproconazole	Alto 100
7.	difenconazole	Plover
8.	epoxiconazole	Opus
9.	fenarimol	Rubigan
10.	fenpropimorph	Corbel
11.	iprodione	Rovral WP
12.	mancozeb	Dithane 945
13.	metalaxyl + mancozeb	Fubol 58 WP
14.	myclobutanil	Systhane
15.	propamocarb-hydrochloride	Filex
16.	pyrimethanil	Scala
17.	tebuconazole	Folicur
18.	thiram	Thiram 80% WP
19.	tolclofos-methyl	Basilex
20.	triademefon	Bayleton

Plates were subsequently incubated for a period of 25 days and the mean diameter of colony growth determined.

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Discussion of Results

Year 1 (1995/1996)

Results for the laboratory evaluations, conducted in Year 1 of the project, are presented in Tables 1-4.

During the period June-July samples received exhibited no evidence of dieback (Table 1) or crown rot symptoms (Table 2) and interestingly the recovery of *Itersonilia* spp. using the Suspended Tissue Test was either nil or very low (Table 3).

During August-September the incidence and severity of leaf dieback increased and there was a corresponding increase in the recovery of *Itersonilia* sp. from leaf tissue samples. However, no evidence of crown rot was recorded at this time.

At crop maturity, prior to strawing down in October-November, the overall incidence of leaf dieback increased further, with some variation between crops. In general, however the severity was only low-moderate and there were few cases on individual plants where the dieback had progressed to affect petiole tissues. Interestingly, the lowest dieback severity was recorded in the carrot crop monitored in East Anglia (selected as a drier low risk site) and the highest in Scotland (DMA 6) and Lancashire (DMA 3) respectively, both regarded as wetter higher risk sites. By strawing down there remained no evidence of crown rot in the harvested samples. Moreover, where root samples were collected after over-wintering again crown rot symptoms were insignificant. This low incidence of the disorder concurred, in general, with other commercial crops during 1995/1996.

Overall, there was a fairly close association between the incidence and severity of leaf dieback and the subsequent recovery of *Itersonilia* spp. Unfortunately however, the lack of crown rot symptoms in any of the monitored crops during 1995/1996 prevented further interpretation of the data generated.

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Where leaf tissue samples were plated out directly onto PDA various fungi and bacteria were recovered (Table 4). The most predominant isolations were of *Cladosporium* spp., *Penicillium* spp., various unidentified bacteria and a pink yeast. *Itersonilia* spp. were only rarely recovered using this isolation technique. No micro-organisms were recovered consistently and in sufficiently high numbers to suggest they may be primarily responsible for the symptoms of leaf dieback and crown rot. However, until a primary incitant can be identified, no micro-organisms can be entirely discounted.

Year 2 (1996/1997)

Results for laboratory evaluations of samples collected from the 4 monitored sites in Lancashire (2), Fife (1) and Morayshire (1) are presented in Tables 5-6.

In contrast to 1995, symptoms of leaf dieback occurred approximately 3-4 weeks later, during early to mid September 1996 at most sites. However, where dieback occurred the severity was generally higher (Table 5). Where leaf tissue samples with dieback were taken and plated out using the Suspended Tissue Test there was a good correlation between symptoms and recovery of *Itersonilia* spp. (Table 6), and this supports earlier work.

Yet again, however, there was no evidence of crown rot development in any of the root samples taken, even after the over-wintering period (Table 6).

Where leaf tissue samples were plated out directly onto a non-selective agar medium various fungi and bacteria predominated (Table 7). Species characterisation was broadly similar to results for 1995/1996 and no organisms were recovered which would obviously account for the symptoms outlined. Again, *Itersonilia* spp. were only rarely recovered in these direct isolation plates.

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Estimate of Air-Borne Inoculum of *Itersonilia* spp.

Using a series of Petri dishes containing a selective Waxman agar (Channon, 1963) exposed at intervals in the designated 'high risk' carrot crops it was possible to quantify the inoculum of both *I. pastinacae* and *I. perplexans* in carrot crop during the period July 1996 to April 1997 (Table 8).

In July (Fife and Morayshire only), there was no evidence of *Itersonilia* spores in the air above the designated carrot crops.

During august, high rainfall levels during the site visits in Lancashire (6 August) and Scotland (21 August) prevented exposure of the Waxman plates and unfortunately data on air-borne spore inoculum was not generated. By mid September, however, a significant change was detected. At the carrot sites in Fife and Morayshire whilst a low level of spores of *I. pastinacae* were recovered, a high level of spores of *I. perplexans* was detected (ca. 1,000-1,500 spores/m^2/hr). In Lancashire a similar pattern developed, yet inoculum levels in crops, particularly for *I. perplexans* were much higher (ca. 40-440 spores/m^2/hr for *I. pastinacae* and 5,400-9,100 spores/m^2/hr for *I. perplexans*). **At the Wood End site, Lancashire this represents a total spore deposition/ha over a 24 hour period (assuming no diurnal periodicity for spore release) of 2.2 x 10^9 spores, the bulk of which would be spores of *I. perplexans*.**

By October 1996 air-borne spore inoculum, with the exception of the Morayshire site, had started to decline. By November only few spores were detected on agar plates (though most crops had been strawed down by this stage). Interestingly, where further plates were exposed in crops in spring 1997 no *Itersonilia* spp. spores were detected in the air.

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In-Vitro Evaluation of Fungicides

A range of 20 fungicides were evaluated in agar plates, amended at 2 and 20 ppm, for their effectiveness in suppressing mycelial growth of *I. pastinacae* and *I. perplexans* (Table 9).

In comparison with the mean radial growth rates on unamended agar (*I. pastinacae* - 48.1 mm, *I. perplexans* - 48.4 mm) several of the fungicides were effective in either slowing down the rate of mycelial extension or preventing it totally.

Those most effective in this test were difenconazole (Plover), pyrimethanil (Scala) and benodanil (Calirus), and these appeared equally effective against both species of *Itersonilia*. Other products which showed some activity in this test were epoxiconazole (Opus), tebuconazole (Folicur), cyproconazole (Alto 100), fenpropimorph (Corbel), carbendazim (Bavistin DF) and the dithiocarbamates mancozeb and thiram. In general terms, several of these fungicides appeared to be more effective in reducing the radial growth of *I. perplexans* than *I. pastinacae*. Interestingly, iprodione (Rovral WP), a fungicide with known activity against *Alternaria* spp. was largely ineffective against either species tested.

It should be noted that many of the fungicides listed are NOT approved for use on carrots.

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TABULATED RESULTS

Table 1: Incidence and severity of leaf dieback in a range of commercial carrot crops monitored during the 1995/1996 season.

Sample Reference	% Plants with Leaf Dieback				Severity Index (0-100) of Leaf Dieback			
	Jun/ Jul	Aug/ Sep	Oct/ Nov	Feb/ Mar	Jun/ Jul	Aug/ Sep	Oct/ Nov	Feb/ Mar
Lancashire								
DMA 1	0.0	2.0	10.0	-	0.0	0.8	4.7	-
DMA 2	0.0	11.0	28.0	†	0.0	4.3	16.4	†
DMA 3	+	5.1	25.0	-	+	3.6	18.9	-
DMA 4	0.0	1.0	18.0	-	0.0	0.6	15.1	-
Scotland								
DMA 5	0.0	4.0	*	-	0.0	1.7	*	-
DMA 6	0.0	15.0	35.0	-	0.0	7.9	21.4	-
DMA 7	0.0	+	13.0	-	0.0	+	14.7	-
East Anglia								
DMA 8	0.0	1.0	7.0	†	0.0	0.2	3.9	†

* Crop strawed down, no foliage sample available.
\- No foliage sample available.
\+ No samples received for evaluation.
† Crop harvested, no sample collected.

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Table 2: Incidence and severity of crown rot symptoms in a range of commercial carrot crops monitored during the 1995/1996 season.

Sample Reference	% Plants with Crown Rot				Severity Index (0-100) of Crown Rot			
	Jun/ Jul	Aug/ Sep	Oct/ Nov	Feb/ Mar	Jun/ Jul	Aug/ Sep	Oct/ Nov	Feb/ Mar
Lancashire								
DMA 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DMA 2	0.0	0.0	0.0	†	0.0	0.0	0.0	+
DMA 3	+	0.0	0.0	0.0	+	0.0	0.0	0.0
DMA 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Scotland								
DMA 5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DMA 6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DMA 7	0.0	+	0.0	0.0	0.0	+	0.0	0.0
East Anglia								
DMA 8	0.0	0.0	0.0	†	0.0	0.0	0.0	†

\+ No samples received for evaluation.
† Crop harvested, no sample collected.

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Table 3: **Mean % recovery of *Itersonilia* spp. from leaf and crown tissue samples in a range of commercial carrot crops monitored during the 1995/1996.**

Sample Reference	Mean % Recovery of *Itersonilia* spp.✪ from							
	Leaf Tissue Samples				Crown Tissue Samples			
	Jun/ Jul	Aug/ Sep	Oct/ Nov	Feb/ Mar	Jun/ Jul	Aug/ Sep	Oct/ Nov	Feb/ Mar
Lancashire								
DMA 1	0.0	0.0	2.0	-	0.0	0.0	0.0	0.0
DMA 2	0.0	13.0	41.5	†	0.0	0.0	0.0	†
DMA 3	+	3.5	10.0	-	+	0.0	0.0	0.0
DMA 4	0.0	0.0	12.5	-	0.0	0.0	0.0	0.0
Scotland								
DMA 5	1.0	7.5	*	-	0.0	0.0	0.0	0.0
DMA 6	0.0	14.0	39.5	-	0.0	0.0	0.0	0.0
DMA 7	0.0	+	9.5	-	0.0	+	0.0	0.0
East Anglia								
DMA 8	0.0	0.0	2.5	†	0.0	0.0	0.0	†

* Crop strawed down, no foliage sample available.
\- No foliage sample available.
† Crop harvested, no sample collected.
\+ No samples received for evaluation.
✪ Figures represent the mean % recovery of *Itersonilia* spp. from 80 tissue pieces using the Suspended Tissue Test (STT).

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Table 4: Recovery of various fungi and bacteria by Direct Isolation of leaf tissue pieces to Potato Dextrose Agar during 1995/1996.

Micro-Organism Recovered	Frequency of Recovery		
	Rare	Occasional	Common
Botrytis cinerea	✓	✓	
Alternaria spp*		✓	
Fusarium spp.			
Penicillium spp.			✓
Cladosporium spp.			✓
Itersonilia spp.	✓		
Sclerotinia sclerotiorium	✓		
Mucor spp.		✓	
Rhizopus pp.		✓	
Cylindrocarpon spp.		✓	
Pink yeast#			✓
Bacteria (various)+			✓

* Excluding *A. dauci* and *A. radicina.*

\+ Bacterial isolations generally numerous, occasionally affecting isolation of fungi due to mucoid spreading nature of colonies. No single morphologically distinct species predominating. Specific identification of species not conducted.

\# Pink yeast morphologically identical to that recovered consistently in suspended tissue tests where it was often found in association with *Itersonilia* spp.

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Table 5: Incidence and severity of leaf dieback in tagged plants *in-situ* in an untreated area of four commercial carrot crops during the 1996/1997 season.

Scotland

Date	% Plants with Leaf Dieback		Severity Index (0-100) of Leaf Dieback	
	Fife	Morayshire	Fife	Morayshire
25 July 1996	0.0	0.0	0.0	0.0
21 August 1996	0.0	5.0	0.0	1.7
18 September 1996	30.0	28.0	12.3	10.3
14 October 1996	67.0	85.0	25.6	49.3
12 November 1996	*	*	*	*

Lancashire

Date	% Plants with Leaf Dieback		Severity Index (0-100) of Leaf Dieback	
	Burscough	Wood End	Burscough	Wood End
10 July 1996	0.0	0.0	0.0	0.0
6 August 1996	0.0	0.0	0.0	0.0
4 September 1996	9.0	0.0	3.0	0.0
8 October 1996	69.0	57.0	39.0	28.0
13 November 1996	84.0	*	47.9	*

* Crop strawed down, assessment not possible.

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Table 6: **Incidence of leaf dieback and crown rot and recovery of *Itersonilia* spp. in destructive samples harvested from an untreated area of four commercial carrot crops during the 1996/1997 season.**

Fife, Scotland

Date	Leaf Dieback Index (0-100)	% Recovery of *Itersonilia* spp.*	Crown Rot Index (0-100)	% Recovery of *Itersonilia* spp.
25 July 1996	0.0	0.0 (0.0)	0.0	0.0
21 August 1996	0.0	0.0 (0.0)	0.0	0.0
18 September 1996	13.3	50.0 (37.5)	0.0	0.0
14 October 1996	30.0	32.5 (35.0)	0.0	0.0
12 November 1996	✪	✪	✪	✪
10 April 1997+	-	-	0.0	0.0

Morayshire, Scotland

Date	Leaf Dieback Index (0-100)	% Recovery of *Itersonilia* spp.*	Crown Rot Index (0-100)	% Recovery of *Itersonilia* spp.
25 July 1996	0.0	0.0 (0.0)	0.0	0.0
21 August 1996	0.0	0.0 (0.0)	0.0	0.0
18 September 1996	40.0	65.0 (65.0)	0.0	0.0
14 October 1996	33.3	18.8 (25.0)	0.0	0.0
12 November 1996	✪	✪	✪	✪
10 April 1997+	-	-	0.0	0.0

Burscough, Lancashire

Date	Leaf Dieback Index (0-100)	% Recovery of *Itersonilia* spp.*	Crown Rot Index (0-100)	% Recovery of *Itersonilia* spp.
10 July 1996	0.0	0.0 (0.0)	0.0	0.0
6 August 1996	0.0	0.0 (0.0)	0.0	0.0
4 September 1996	3.3	47.5 (80.0)	0.0	0.0
8 October 1996	33.3	41.3 (40.0)	0.0	0.0
13 November 1996	36.6	61.3 (55.0)	0.0	0.0
24 April 1997+	-	-	0.0	0.0

(cont.)

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Table 6: continued

Wood End, Lancashire

Date	Leaf Dieback Index (0-100)	% Recovery of *Itersonilia* spp.*	Crown Rot Index (0-100)	% Recovery of *Itersonilia* spp.
10 July 1996	0.0	0.0 (0.0)	0.0	0.0
6 August 1996	0.0	0.0 (0.0)	0.0	0.0
4 September 1996	3.3	60.0 (67.5)	0.0	0.0
8 October 1996	36.6	50.0 (57.5	0.0	0.0
13 November 1996	✪	✪	✪	✪
24 April 1997+	-	-	0.0	0.0

- No leaf tissues available for assessment.

* Figures represent the mean % recovery of *Itersonilia* spp. from 80 tissue pieces using the Suspended Tissue Test (STT). Figures in brackets represent the % of tissue pieces sampled with visible dieback.

\+ Crop sampled in spring after over-wintering under straw.

✪ Sample not taken, crop strawed down.

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Table 7: Recovery of various fungi and bacteria by Direct Isolation of leaf tissue pieces to Potato Dextrose Agar during 1996/1997.

Micro-Organism Recovered	Frequency of Recovery		
	Rare	Occasional	Common
Cladosporium spp.			✓
Rhizopus spp.			✓
Mucor spp.	✓		
Fusarium spp		✓	
Itersonilia spp.			
Alternaria spp*	✓		
Pink yeast#	✓		✓
Bacteria (various)+			✓
Unidentified white fungus		✓	
Trichoderma sp.		✓	
Colletotrichum sp.	✓		
Cylindrocarpon spp.		✓	
Botrytis cinerea			✓

* Excluding *A. dauci* and *A. radicina*.

\+ Bacterial isolations generally numerous, occasionally affecting isolation of fungal species, particularly where spreading mucoid colonies predominant.

\# Pink yeast morphologically identical to that recovered consistently in suspended tissue tests where it was often found in association with *Itersonilia* spp.

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Table 8: Estimate of spore concentration of *I. pastinacae* and *I. perplexans* in the air above carrot crops during the growing season in 1996/1997.

Date	Estimate of the number of *Itersonilia* spores deposited per m^2 crop/hour			
	Fife, Scotland		Morayshire, Scotland	
	I. pastinacae	*I. perplexans*	*I. pastinacae*	*I. perplexans*
25 July 1996	0.0	0.0	0.0	0.0
21 August 1996	*	*	*	*
18 September 1996	80	1240	80	1520
14 October 1996	0.0	680	0.0	2680
12 November 1996	(*)	(*)	(*)	(*)
8 April 1997	x	x	0.0	0.0

Date	Estimate of the number of *Itersonilia* spores deposited per m^2 crop/hour			
	Burscough, Lancashire		Wood End, Lancashire	
	I. pastinacae	*I. perplexans*	*I. pastinacae*	*I. perplexans*
10 July 1996	-	-	-	-
6 August 1996	*	*	*	*
4 September 1996	440	5440	40	9080
8 October 1996	0.0	1920	00	3560
13 November 1996	40	160	(*)	(*)
24 April 1997	0.0	0.0	0.0	0.0

- \- Not tested
- * Rainfall prevented exposure of Waxman agar plates
- (*) Crop strawed down, test not conducted
- x Crop harvested

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Table 9: Relative sensitivity of isolates of *I. pastinacae* and *I. perplexans* to a range of fungicide active ingredients in *in vitro* agar tests at concentrations of 2 and 20 ppm.

Fungicide	Mean Radial Growth of Fungus (mm)					
	I. pastinacae (CC01/98)			*I. perplexans* (CC16/98)		
	0 ppm	2 ppm	20 ppm	0 ppm	2 ppm	20 ppm
1. azoxystrobin (Amistar)	-	31.0	28.5	-	32.5	30.0
2. benodanil (Calirus)	-	0.0	0.0	-	0.0	0.0
3. bupirimate (Nimrod)	-	50.5	8.5	-	43.3	40.0
4. carbendazim (Bavistin DF)	-	27.5	18.3	-	27.5	0.0
5. chlorothalonil (Bravo 500)	-	36.0	30.5	-	24.0	18.5
6. cyproconazole (Alto 100)	-	26.8	13.0	-	23.5	0.0
7. difenconazole (Plover)	-	0.0	0.0	-	0.0	0.0
8. epoxiconazole (Opus)	-	17.3	0.0	-	23.8	0.0
9. fenarimol (Rubigan)	-	45.8	35.5	-	43.0	41.3
10. fenpropimorph (Corbel)	-	23.0	8.8	-	21.0	0.0
11. iprodione (Rovral WP)	-	46.5	19.5	-	40.5	33.0
12. mancozeb (Dithane 945)	-	34.8	10.5	-	22.5	0.0
13. metalaxyl + mancozeb (Fubol 58WP)	-	43.4	27.3	-	43.8	38.8
14. myclobutanil (Systhane)	-	32.8	23.5	-	24.0	9.5
15. propamocarb-HCL (Filex)	-	49.8	53.5	-	35.5	29.8
16. pyrimethanil (Scala)	-	0.0	0.0	-	20.0	0.0
17. tebuconazole (Folicur)	-	19.3	7.5	-	8.3	0.0
18. thiram (Thiram 80% WP)	-	24.8	19.5	-	51.3	0.0
19. tolclofos-methyl (Basilex)	-	✪	✪	-	✪	✪
20. triademefon (Bayleton)	-	37.3	23.5	-	42.8	27.5
Unamended agar	48.1	-	-	48.4	-	-

* It should be noted that whilst the fungicide Compass was not used specifically in this evaluation, the two primary active ingredients iprodione and an 'mbc' fungicide were tested individually.

✪ Plates contaminated, test result inconclusive.

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Conclusions

- Over a two year period (1995/1996 and 1996/1997) numerous commercial carrot crops were monitored in detail for the occurrence of leaf dieback and crown rot.

- Whilst low-moderate levels of leaf dieback symptoms occurred, particularly in the second year of investigation, no symptoms of crown rot occurred in the roots.

- A close association between the occurrence of leaf dieback and the presence of *Itersonilia* spp. on the decaying leaf tissues was found. Whether the *Itersonilia* spp. are primarily responsible for the leaf decay or merely present as a secondary opportunist colonising senescing leaf tissues remains open to speculation.

- In general terms leaf dieback and *Itersonilia* spp. occurred at higher levels in what are regarded as high 'crown rot' risk production areas (ie. Lancashire and Scotland) than in low risk areas of Eastern England (though it must be noted that data from only a single crop in Eastern counties was obtained).

- Air-borne spores of both *I. pastinacae* and *I. perplexans* were detected above all carrot crops monitored during 1996/1997. Inoculum of *I. perplexans* was significantly higher than that of *I. pastinacae*. However, the importance of the two species with respect to leaf dieback and crown rot remains unknown.

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- Air-borne spores of both species of *Itersonilia* reached a peak in early-mid September at most sites and declined thereafter, with no air-borne inoculum being detected in spring 1997 after crops had been overwintered.

  The peak air-borne inoculum detected corresponds closely with the onset of leaf dieback symptoms appearing in crops and potentially provides an effective pointer for fungicide timing, assuming *Itersonilia* spp. are subsequently proven to be primarily responsible for the disorder.

- A range of fungicides screened *in-vitro* identified several candidate products with activity against this basidiomycete fungus. Difenconazole (Plover), pyrimethanil (Scala) and benodanil (Calirus) all appeared more effective than fenpropimorph (Corbel) and carbendazim (an 'mbc' fungicide similar to thiophanate-methyl in Compass).

  It must be noted however, that currently these fungicides are only experimental and are not approved for use on the carrot crop.

- In the isolation tests conducted various other fungi were recovered sporadically from tissue samples with leaf dieback. These included *B. cinerea, Cladosporium* spp., *Alternaria* spp. (not *A. dauci* or *A. radicina*), *Penicillium* spp., *Rhizopus* spp., *Fusarium* spp., *Sclerotinia sclerotiorum, Mucor* spp., various bacteria and a pink yeast-like organism. None of these organisms were recovered as consistently as *Itersonilia* spp., though their potential role in the disorder cannot be fully discounted at this stage.

- It is recommended that the emphasis of further studies should be directed towards demonstrating Koch's Posulates with both the *Itersonilia* spp. recovered or the other fungi and bacteria detected during the investigation reported.

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