Epidemiology and Control of Black Spot in Swede and Other Plants

Project FV143

FINAL REPORT

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CONTENTS

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1 RELEVANCE TO GROWERS AND PRACTICAL APPLICATION

1.1 MAIN OBJECTIVES OF PROJECT

To identify the organisms causing swede black spot, and their source.

To investigate the effects of environmental factors on the causal pathogen.

To investigate control measures.

1.2 KEY RESULTS

: Black spot lesions varied in appearance.

: Black spot affected up to 80 % swedes.

: *Rhizoctonia solani* was the only organism to reproduce black spot-like symptoms; most *R. solani* isolates belonged to strain AG 2-1.

: *Cylindrocarpon*, *Fusarium*, *Botrytis*, *Rhizopus* and bacteria were also isolated from black spot lesions, but did not give rise to black spot when inoculated into swede.

: *R. solani* isolates from swede black spot lesions were pathogenic to seedlings of cauliflower cv. Little Rock, shepherd's purse and charlock weed; black spot *R. solani* was not pathogenic to spring barley, rye-grass or winter wheat.

: cv. Melfort was consistently the least susceptible and cvs. Airlie and Marian the most susceptible.

: Black spot incidence was higher in early sown crops.

: Soil nutritional factors, soil pH, and cropping history had no apparent effect on black spot incidence.

: Sowing depth and plant spacing had no effect on black spot incidence.

: Black spot *R. solani* isolates were sensitive *in vitro* to tolclofos methyl (Rizolex), pencycuron (Monceren) and iprodione (Rovral).

: Tolclofos methyl, pencycuron and iprodione reduced black spot incidence in the field.

1.3 PRACTICAL CONCLUSIONS FOR THE SWEDE GROWER

What is the cause of Black Spot?

1 Our data confirms that *Rhizoctonia* causes black spot symptoms, and that infection does not require initial damage by pests.

2 Pathogenic *Rhizoctonia* isolates are mostly Anastomosis Group (AG) 2-1.

3 AG 2-1 isolates prefer cool temperatures.

4 Crops sown early, in April, when soils are cooler, tend to be more susceptible to black spot than later sown crops.

Are some swede varieties more resistant than others?

5 Yes. The results were variable but varieties with a high dry matter content e.g. cv. Melfort generally had less black spot.

6 We now have started to develop a laboratory test to help identify cultivar resistance to black spot.

Can crop rotation reduce the risk of black spot?

7 No. None of the cropping patterns seemed to either reduce or increase the incidence of black spot. Although *Rhizoctonia* lives on the roots of a wide range of crops, weeds, and the debris from previous swede crops, these appear to have little influence on black spot severity.

Are some soils more prone to black spot than others?

8 No. Black spot incidence did not seem to be affected by a range of soils with different mineral nutritions and pHs.

Do fungicides control black spot?

9 No fungicide has Approval for use against black spot but pencycuron, tolclofos methyl and iprodione all provided some success in experiments resulting in 20-60 % control.

10 Having a better knowledge of the cause of black spot, and risk factors associated with crops e.g. early-sown crops and low dry matter varieties, there may be scope to assess which crops will be most at risk and so benefit from fungicide treatment.

11 Our results provide preliminary data suggesting dose rates and timings based on field and laboratory tests.

2 SUMMARY OF ACHIEVEMENTS

2.1 The lack of information on black spot (also called black crater) in the scientific literature was confirmed **(Literature survey)**.

2.2 Direct enquiry to scientists, advisers and growers at home and abroad showed that:

2.2.1 In the UK, black spot causes most damage in Scotland and Northumberland. The disease is present, but not a problem, in Yorkshire, Devon and Cornwall, and Wales, and is apparently absent from Cambridgeshire, Lincolnshire and Bedfordshire.

Black spot causes considerable damage in the Republic of Ireland. The disease is present, but not a problem, in Germany, Tasmania, and Canada, and is apparently absent from Poland and France. The disease was not known to participants at an International Conference on *Rhizoctonia* in 1995.

2.2.2 Dr J.T. Fletcher was unable to provide additional data to that already published in his original paper (per. comms. 2 February -15 November 94).

2.2.3 *Rhizoctonia solani* was considered to be the causal pathogen of black spot (pers. comm. M. McPherson, 8 December 93).

2.2.4 Experimental data on swede black spot was not available from old ADAS records (pers. comm. M. McPherson, 8 December 93) **(Objective A-2)**.

2.3 Some black lesions, found on swedes from commercial crops, corresponded to the original description of black crater disease by Fletcher & Hims (1979). Other types of lesion differed from the original description as follows: (a) some black spot-like lesions penetrated the outer vascular layer of the swede, (b) large spreading lesions of up to 12 cm diameter were never found, (c) 'pin-prick' (0.1-0.5 cm diameter) lesions were common, (d) clusters of 2-20 lesions were common, (e) some large lesions penetrated deeply to the centre of the swede.

Cabbage root fly tunnels, lined with dark, decayed host tissue, and lesions ascribed to canker and scab were common; severe club-root was seen on one occasion **(Objective A-2)**.

2.4 More than 1000 isolations, representing 500+ lesions from more than 250 swedes received from a range of commercial sources, were tested for the presence of organisms **(Objective A-2)**.

2.5 *Rhizoctonia*, *Cylindrocarpon*, *Fusarium*, *Botrytis*, *Rhizopus* and bacteria were isolated from a total of 5 %, 2 %, 11 %, 4 %, 5 % and 51 % of natural lesions; the remaining lesions failed to yield organisms (**Objective A-2**).

2.6 The isolation rate of *R. solani* from artificially induced lesions in the field was highest in the first 2-4 weeks after inoculation, and subsequently declined (**Objective A-2**).

2.7 Most *R. solani* isolates from black spot belonged to Anastomosis Group 2-1 (**Objective A-2**).

2.8 A range of *R. solani* isolates from black spot lesions were inoculated into healthy swede in the laboratory. Lesions developed from 42 % inoculations and were oval in shape, <1 cm diameter, and dark with a distinctive black margin; *R. solani* was isolated from 15 % induced lesions, and the remaining lesions either yielded bacteria or failed to yield organisms.

R. solani AG 2-1 and AG 4 (both ex Centraal Bureau Schimmelculture), together with other fungi and bacteria isolated from black spot lesions, were tested for pathogenicity. *R. solani* AG 2-1 gave rise to black spot-like lesions, whereas *R. solani* AG 4 had no effect. *Cylindrocarpon*, *Fusarium*, *Botrytis* and a range of bacterial isolates either produced lesions which differed from black spot, or had no effect (**Objective A-2**).

2.9 Black spot *R. solani* isolates, alone or in combination with *Fusarium* from black spot, gave rise to black spot-like lesions in the field; *Fusarium* alone had no effect. Re-isolation of *R. solani* from induced lesions was initially as high as 80 % but declined to zero by the end of the growing season (**Objective A-2**).

2.10 *R. solani* from black spot was pathogenic to seedlings of swede cv. Doon Major, cauliflower cv. Little Rock, potato cv. Wija, charlock and to a small percentage of spring barley cv. Alexis, but not to shepherd's purse or rye-grass cv. Bertolini, or winter wheat cv. Haven. *R. solani* was re-isolated from induced lesions except for spring barley; *R. solani* was not isolated from symptomless plants, except for shepherd's purse (**Objective A-2**).

2.11 *R. solani* was isolated from organic plant remains, chiefly shepherd's purse (**Objective A-3**).

2.12 *R. solani* from black spot was able to colonise dead cereal stubble and dead shepherd's purse weed (**Objective A-3; B-4**).

2.13 Soil from a field with black spot caused wirestem in rape seedlings and *R. solani* was isolated (**Objective A-3**).

2.14 Black spot was never seen before July; black spot incidence increased progressively during the season; in 1995, an atypically hot, dry season, there was a marked increase in black spot incidence from the end of September to mid-October **(Objective B-1)**.

2.15 Cultivars differed in their susceptibility ranking in each of three growing seasons; nevertheless cv. Melfort was consistently the least susceptible and cvs. Airlie and Marian the most susceptible **(Objective B-2)**.

2.16 Black spot incidence was not correlated with soil nutritional factors or soil pH, or to crop rotation history **(Objective B-3)**.

2.17 Black spot incidence was 90 % in a field in 1993; the following year black spot incidence was <20 % **(Objective B-3)**.

2.18 *R. solani* was isolated from lesions on the base of living shepherd's purse, present as a weed in a swede crop, and gave rise to black spot-like lesions when inoculated into healthy swede **(Objective B-4)**.

2.19 *R. solani* isolated from the stem bases of cereals, failed to give rise to black spot lesions when inoculated into swede **(Objective B-4)**.

2.20 *R. solani* from swede black spot lesions was highly sensitive to Rizolex (tolclofos methyl), Monceren (pencycuron) and Rovral (iprodione), *in vitro*. **(Objective C-1)**.

2.21 Tolclofos methyl, pencycuron and iprodione reduced black spot incidence in the field **(Objective C-1)**.

3 INTRODUCTION

The disease known as black crater of swede was found on a single farm in Northumberland in 1977, and reported as a 'new or unusual record' (Fletcher & Hims, 1979).

The key features of the report were: (a) the first siting of the disease coincided with the installation of washing equipment; therefore, the disease may have been overlooked previously; (b) the disease was present as crater-like patches on the 'root' (botanically the enlarged hypocotyl); (c) the patches were irregular in shape, often oval, with faint zone lines, and the surface of the lesions was dry and scab-like; (d) the patches were mostly at soil level; (e) black crater lesions varied in size from 1-12 cm diameter; (f) decay spread only a small distance into the 'root'; (g) in 1977-78, black crater incidence increased from 5 % to 15 % on cv. Victory (syn. cv. Magnificent) grown in the same field; other cultivars were less affected; (h) *Rhizoctonia solani* (identification by the Commonwealth Mycological Institute) was isolated in pure culture (frequency not stated); (i) symptoms similar to black crater were obtained within 3 weeks when *R. solani* from black crater lesions was inoculated into healthy swede, and the same fungus was re-isolated; (j) 18 swede cultivars differed in their 'susceptibility' to black crater, with cv. Magnificent the most susceptible and 11 cultivars, including Acme, Merrick and Ruta Otofte, the most resistant; (k) a diffuse superficial blackening, but not typical black craters, occurred on resistant cultivars; (l) losses from the disease were mainly due to the downgrading of blemish-free high quality 'shopping swedes'.

Black crater appears to be the disease now more commonly called swede black spot.

In 1992/93, the gross UK output of swede and turnip was 175,400 tons with a value of £16.2 M. Black spot affects appearance, rather than yield, therefore the losses are mainly due to the down-grading of the top quality or 'shopping swede' for supermarkets. Accurate data on the magnitude of losses from black spot are not readily available, but the indications are that about 10 % per year of the quality crop is lost.

There appear to be no other reports of symptoms corresponding to black crater or black spot as such. However, sunken putty-coloured lesions with pinkish margins (Dennis, 1941), and shiny brown, purple-rimmed shallow depressions 'dry rot' (Baker, 1972) have been reported; their relationship to black spot, if any, is not known.

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MEETINGS and CORRESPONDENCE

1993

February: Field visit with Growers in Berwickshire

June: Field visit, SAC

Invitation to Project Coordinator

October: Field visit, SAC and discussions

1994

July: Aural Examination of Sarah Clow for Interim Thesis (Dr A.R. Entwistle and Dr C. Caten, Supervisors).

September: 'Swede Black Spot': Presentation by Sarah Clow to Crop Protection Department, Scottish Agricultural College, Edinburgh.

October: Field visit, SAC and discussions

October: 'Swede Black Spot' exhibit at Scot-Grow, Edinburgh

1995

January: First Review meeting, at HRIW

June: Field visit, SAC and discussions

June: A.R. Entwistle to International *Rhizoctonia* Symposium '95, Noordwijkerhout, The Netherlands (presentation of poster, see publications above).

October: Field visit, SAC and discussions

November: 'Swede Black Spot': Presentation by Sarah Clow at HRIW.

1996

January: Second Review meeting, at HRIW

Appendix 1: Description of the work

The project is in three parts:

A. To devise research methods and determine the identity of swede black spot pathogen(s)

1a Review the scientific literature on (a) the identity of the pathogen, (b) the nature and seriousness of the disease in the UK and other areas of the world, (c) techniques available for studies of the pathogen, (d) the sources of inoculum and method of survival of the pathogen and (e) suggested control measures;

1b Devise standard methods for isolating *R. solani* and other organisms from infected plants and from soil;

2 Identify fungi isolated from black spot lesions, including *R. solani* anastomosis groups, and test pathogenicity on swede and other potential host and weed species;

3 Determine inoculum type of *R. solani* and other black spot organisms for infecting host plants; hence devise standard methods for producing inoculum, infecting host plants and producing disease symptoms;

B. Investigate factors affecting pathogenicity of black spot organisms to swede and other plants in the cropping cycle, and weeds.

1 Determine effects of temperature, moisture, pH and soil type, and nutrition on infectivity of black spot organisms to host plants;

2 Determine varietal susceptibility/tolerance to the black spot organism;

3 Determine effects of cropping history on occurrence of disease;

4 Investigate host range of black spot organisms as potential sources of the pathogen and methods of survival;

C. Control measures

1 Determine the sensitivity of black spot organisms to fungicides *in vitro* and *in vivo*;

2 Evaluate host range, crop rotation, crop hygiene as potential methods of control;

3 Evaluate potential of biological control using antagonistic organisms.

Signed: A R Entwistle

Dr A. R. Entwistle
19 December 1995

Contract between HRI & SAC (hereinafter called the "Contractors") and the Horticultural Development Council (hereinafter called the "Council") for research/development project.

1. **TITLE OF PROJECT**

Contract No: FV/143
Contract date: 20.12.93

EPIDEMIOLOGY AND CONTROL OF BLACK SPOT IN SWEDE AND OTHER PLANTS

2. **BACKGROUND AND COMMERCIAL OBJECTIVE**

Black Spot of Swede, also known as Black Crater, describes the dark sunken lesions reported to be caused by *Rhizoctonia solani*. As a result of infection high quality shopping swedes become downgraded to cattle feed or are unsaleable. There is little published information on the disease. *R. solani* is an aggregate species capable of infecting a wide range of hosts but the constituent forms, called anastomosis groups, are more restricted in their host range. The objectives of this project are to confirm the identity of the pathogen, to identify likely sources of the pathogen, to determine the method of increase and survival of the pathogen, to identify the factors governing infection, and to investigate methods of control.

3. **POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY**

The effects of swede black spot are variable in that a high incidence in one crop may be followed by a low incidence in a following crop. In severe situations the crop is rendered unsaleable as shopping swedes with a resultant loss of revenue. Information from the industry indicates that the disease becomes more prevalent with successive cropping by swede.

4. **SCIENTIFIC/TECHNICAL TARGET OF THE WORK**

1 Review the scientific literature to evaluate (a) the identity of the pathogen, (b) the nature and seriousness of the disease in the UK and other areas of the world, (c) techniques available for studies of the pathogen, (d) the sources of inoculum and method of survival of the pathogen and (e) suggested control measures;

2 Proposed laboratory and field studies

2.1 Confirm the identity of the pathogen including anastomosis groups;

2.2 Investigate likely sources of the pathogen including other plant species, and methods of survival;

2.3 Determine the role of temperature, soil moisture and other soil characteristics on activity and increase of the pathogen;

2.4 Investigate cultural and fungicidal control;

5. CLOSELY RELATED WORK - COMPLETED OR IN PROGRESS

Mr D M Mitchell and Dr S Oxley of the Scottish Agricultural College (SAC) have considerable expertise in laboratory and field investigations on swede black spot. Many producers of shopping swede in Scotland use SAC advisors for cultural advice, crop walking and trouble shooting, thus facilitating field experimentation and the practical application of research results. Research on *Allium* white rot (*Sclerotium cepivorum*), another soil-borne disease, forms the major research programme of Dr Entwistle, HRI-W, and is funded by MAFF; the white rot project provides expertise in isolation and infection techniques. Research on black root rot of bedding plants is due to start at HRI-W in summer 1992 and is to be supported financially by the HDC.

6. DESCRIPTION OF THE WORK

The project is in three parts:

A. To devise research methods and determine the identity of swede black spot pathogen(s)

1 Devise standard methods for isolating *R. solani* and other organisms from infected plants and from soil;

2 Identify fungi isolated from black spot lesions, including *R. solani* anastomosis groups, and test pathogenicity on swede and other potential host and weed species;

3 Determine inoculum type of *R. solani* and other black spot organisms for infecting host plants; hence devise standard methods for producing inoculum, infecting host plants and producing disease symptoms;

B. Investigate factors affecting pathogenicity of black spot organisms to swede and other plants in the cropping cycle, and weeds.

1 Determine effects of temperature, moisture, pH and soil type, and nutrition on infectivity of black spot organisms to host plants;

2 Determine varietal susceptibility/tolerance to the black spot organism;

3 Determine effects of cropping history on occurrence of disease;

Contract No: FV/143

TERMS AND CONDITIONS

The Council's standard terms and conditions of contract shall apply.

Signed for the Contractor(s)

Signature...

Position...

Date...

Signed for the Contractor(s)

Signature...

Position...

Date...

Signed for the Council

Signature.......[signature].......

Position.......CHIEF EXECUTIVE.......

Date.......20.12.93.......

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1 Review the scientific literature to evaluate (a) the identity of the pathogen, (b) the nature and seriousness of the disease in the UK and other areas of the world, (c) techniques available for studies of the pathogen, (d) the sources of inoculum and method of survival of the pathogen and (e) suggested control measures;

2 Proposed laboratory and field studies

2.1 Confirm the identity of the pathogen including anastomosis groups;

2.2 Investigate likely sources of the pathogen including other plant species, and methods of survival;

2.3 Determine the role of temperature, soil moisture and other soil characteristics on activity and increase of the pathogen;

4 OBJECTIVES AND RESULTS

4.1 Information Survey

Enquiries to a range of scientists, advisers and growers at home and abroad, indicated that:

: In the UK, black spot causes the most damage in Fife, Kincardineshire, Aberdeenshire, Angus, Ayrshire, Dumfriesshire and Northumberland, and the Republic of Ireland. The disease is present in Yorkshire, Devon, Cornwall, and Wales but is not a problem. The disease is apparently absent in Cambridgeshire, Lincolnshire and Bedfordshire.

: There was no additional data to that published in the original description, notably on the frequency of isolation of *Rhizoctonia*, or the production of black spot lesions following inoculation by *Rhizoctonia* originating from swede black spot lesions (pers. comm. J.T. Fletcher 15 November 94).

: Previous experimental data on swede black spot was not available (pers. comm. M. McPherson, 8 December 93).

: *Rhizoctonia* was said to be the cause of swede black spot (pers. comm. M. McPherson 8 December 93). However, there was doubt about the frequency of *Rhizoctonia* isolation.

: *R. solani* causes small brown shallow lesions on stored swede in Norway.

: Swede black spot or black crater is not known or recognised in Poland (pers. comm. W. Rondomanski, 8 February '95), France (pers. comm. F. Rouxel, 2 June '95); the disease is known in Germany but is not considered to be a problem (pers. comm. P. Mattusch, 1995). Little additional information was obtainable from Canada (pers. comms. G.M. Fortin, R. Hall, May '95). Swede black spot was not known by participants at an International Conference on *Rhizoctonia* (Entwistle, June '95).

: Losses from black spot are estimated to account for 10 % of 'shopping' swedes. The gross UK output of swede and turnip in 1992/93, including fodder crops, was 175,400 tons with a value of £16.2 M.

4.2 Objective A-1

Devise research methods for isolating R. solani and other organisms from infected plants and soil.

R. solani was isolated (a) directly by transferring parts of the leading edge of lesions to potato dextrose agar (PDA) and incubating at 15-25°C, or (b) indirectly by first placing the primary roots of cv. Doon Major swede seedlings in contact with a black spot lesion, incubating, then transferring the seedlings to PDA.

4.3 Objective A-2

Identify fungi isolated from black spot lesions, including Rhizoctonia solani anastomosis groups, and test pathogenicity on swede and other potential hosts and weeds.

4.3.1 Appearance of lesions on swedes

A total of 500+ black spots were observed from more than 250 swedes from a range of sites in Berwickshire. Some of the spots were similar in appearance to the original description of Fletcher & Hims (1979) however, others differed as follows: (a) shallow lesions of 12 cm diameter were never seen even when adjacent lesions merged (NB: extensive deeply penetrating lesions were provisionally classified as canker; see below), (b) small (0.1-0.5 cm diameter) lesions were common, (c) lesions were generally regular in shape and circular, not irregular and oval, (d) there was no evidence of 'scab-like' appearance of the surface of the lesion, (e) lesions were found on all parts of the swede below soil level, not just at the soil surface, (f) some lesions penetrated the outer vascular layer, (g) lesions were often in clusters of 2-20.

The following were also seen:

Grey-brown lesions, varying from a few centimetres in diameter to encompassing most of the swede, were common in 1993 and 1994, less so in 1995; the lesions had a wrinkled margin and penetrated deeply into the centre of the swede, sometimes causing collapse of the swede tissue at or below soil level. The lesions were attributed to canker (*Phoma lingam*).

Tunnels of the cabbage root fly (CRF) larvae on underground parts of the swede; the tunnels were lined with dark, decayed host tissue, which was somewhat similar in appearance to the tissue of black spot lesions. CRF tunnels sometimes intersected 'black spot' lesions but there was no evidence that CRF activity affected the size of black spot lesions.

Raised pustules on the top part of the swede, commonly in lines around the circumference of the swede and approximately parallel to the soil surface, were found down to 1-2 cm below soil level. The lesions were attributed to scab (*Streptomyces scabies*) and were common in all years.

4.3.2 Isolation of organisms from black spots.

Swedes from Berwickshire were washed, putative black spot lesions surface sterilised, and pieces from the edge of the lesions transferred to potato dextrose agar, and incubated at 15°C (Fletcher & Hims, 1979).

Out of more than 1000 isolations from 500+ lesions on more than 250 swedes from a range of commercial sites, *Rhizoctonia*, *Cylindrocarpon*, *Fusarium*, *Botrytis*, *Rhizopus* and bacteria were isolated a total of 5 %, 2 %, 11 %, 4 %, 5 % and 51 % times respectively; the remaining lesions did not yield organisms.

Seedlings of swede cv. Doon Major were placed in contact with black spots as a possible means of isolation. *R. solani* was isolated from 16 % of seedlings, *Cylindrocarpon*, *Phoma*, and *Rhizopus* from <1 % each.

4.3.3 Inoculation of black spot isolates into healthy swede in the laboratory.

Approximately 70 *R. solani* isolates from black spot lesions originating from a range of commercial sites gave rise to black spot-like lesions when inoculated into healthy swede; *R. solani* was re-isolated from 15 % of the induced lesions. The *R. solani* AG 2-1 tester isolate (ex Centraal Bureau Schimmelculture, CBS) gave rise to small black spot-like lesions, and *R. solani* was re-isolated, whereas *R. solani* AG 4 had no effect. *Fusarium* and *Botrytis* from black spot lesions either gave rise to lesions with little resemblance to black spot or had no effect. *Cylindrocarpon* and various bacterial isolates also had no effect on the swede.

4.3.4 Inoculation of black spot isolates into healthy swede in the field.

In 1994, four *R. solani* isolates (including three from black spot lesions in Scotland, and the AG 2-1 tester isolate), one isolate of *Fusarium* from a black spot lesion, and combinations of *Fusarium* with each of the *Rhizoctonia* isolates, were inoculated into healthy swede. Approximately 25 % of inoculations with *R. solani* alone gave rise to black spot-like lesions; combinations of *R. solani* and *Fusarium* gave similar results. Inoculation with *Fusarium* alone had no effect. *R. solani* was re-isolated from induced lesions but only at a low rate.

In 1995, all swedes inoculated in July developed symptoms, but they were not typical in appearance and became progressively difficult to detect during the growing season.

Re-isolation of *R. solani* from induced lesions was initially about 80 % but declined to zero by the end of the season. The rate of lesion initiation at later inoculation dates also declined and the success of isolation of *R. solani* from the induced lesions was progressively more variable.

4.3.5 Anastomosis Group.

Anastomosis Groups (AG) classify populations of *R. solani* which are able to fuse with each other and hence provide an opportunity for asexual genetic exchange. Thirty isolates of *R. solani* from black spot originating from a total of five sites in Berwickshire, together with 3 black spot isolates from Yorkshire, were tested against *R. solani* AG 2-1 and AG 4 tester isolates.

Twenty seven (92 %) of the Berwickshire isolates belonged to AG 2-1, one was AG-4, and two apparently belonged to neither AG. Two of the Yorkshire isolates were AG 2-1 and one did not appear to belong to either AG.

All the isolates were multinucleate and hyphae were 5-15 μm in width.

4.3.6 Host range of black spot *R. solani* isolates.

R. solani, isolated from black spot in Berwickshire, 1993 was inoculated into a range of different plant species in a glasshouse.

Lesions developed in the majority of seedlings of swede cv. Doon Major, cauliflower (*Brassica oleracea* L.) cv. Little Rock, potato cv. Wija, and charlock (*Sinapis arvensis* L.). Lesions also formed on a small percentage of spring barley (*Hordeum sativum* L.) cv. Alexis seedlings. Lesions did not develop on shepherd's purse (*Capsella bursa-pastoris* L.), rye-grass (*Lolium multiflorum* var. *italicum* L.) cv. Bertolini or winter wheat (*Triticum vulgare* L.) cv. Haven. *R. solani* was re-isolated from the induced lesions except from spring barley; *R. solani* was not isolated from symptomless plants, except from shepherd's purse.

In a second experiment, *R. solani* isolates from five different sites in Berwickshire, were pathogenic to swede and charlock, but not barley or wheat; lesions did not form on shepherd's purse, nevertheless, *R. solani* was re-isolated.

4.4 Objective A-3

Determine inoculum type of R. solani and other black spot organisms; hence devise standard methods for producing inoculum, infecting host plants and producing disease symptoms.

4.4.1 Isolation of organisms from soil.

In 1993, 40 soil samples from the site of a field experiment on black spot at Lennelhill, Berwickshire were mixed with vermiculite 10:1 by volume, and transferred to modular trays. The trays were sown with rape seed, and the seedlings examined for the presence of lesions three weeks later. Diseased seedlings were transferred to PDA, and organisms isolated and identified.

About 10 % of seedlings developed wirestem. There was no apparent relationship between black spot incidence in the field and wirestem incidence in the baiting test. *R. solani*, *Fusarium*, *Botrytis*, *Rhizopus*, *Penicillium* and bacteria were isolated from 39 %, 7 %, 2 %, 11 %, 7 % and 21 % diseased seedlings respectively; no organisms were isolated from the remainder of the diseased seedlings.

Soil was also collected from a swede trial sites at Blackburn and Drakemyre farms, Berwickshiire. Soils were sieved in running water and pieces of dead plant tissue transferred to PDA from which organisms were isolated and identified. *Rhizoctonia* was isolated from organic plant remains, chiefly the seeds of shepherd's purse.

Pieces of cereal stubble from a field, and dead shepherd's purse plants from a swede experimental site, were inoculated with *R. solani* from a black spot lesion and incubated at 15°C. *R. solani* was re-isolated from all the material.

4.4.2 Inoculation of swede and production of symptoms.

R. solani inoculum was produced on PDA or on a mixture of maize meal and sand. Inoculum was placed in contact with the skin of the swede. Inoculation of whole swedes and pieces of swede with the skin attached both resulted in infection but use of pieces was more convenient. Wounding the skin prior to inoculation had little effect.

4.5 Objective B-1

Determine effects of temperature, moisture, pH and soil type, and nutrition on infectivity of black spot organisms to host plants.

The same 30 *R. solani* isolates as used in the Anastomosis Group tests (Section 4.3.5) were grown on PDA at temperatures ranging from 5-30°C and colony diameter measured at intervals. Colony growth was also measured on PDA adjusted to different water potentials.

R. solani isolates from black spot from Berwickshire and Yorkshire had a maximum growth rate of 17-23 mm/day at 20-25°C. Black spot isolates from Berwickshire grew equally well at -0.6 and -1.2 MPa (8-14 mm/day) but growth was reduced at -1.8 MPa (6-9 mm/day). One of the Yorkshire isolates reacted similarly, but growth of the second isolate was 50 % less.

4.6 Objective B-2

Determine varietal susceptibility to the black spot organism.

Twelve cultivars of culinary swede, representing a range of different types, were compared for their susceptibility to black spot in three growing seasons.

The susceptibility ranking was not consistent over the three years; nevertheless, cv. Melfort, a green topped cultivar with a moderately high dry matter content, appeared to show resistance in all years, whilst cvs. Airlie and Marian, both of which are purple topped cultivars, were highly susceptible.

4.7 Objective B-3

Determine the effects of soil properties and cropping history on black spot incidence.

Commercial swede crops grown on a range of sites with different soil types, nutritional status, and cropping histories were monitored for black spot. In addition, replicated trials sown with swede cv. Doon Major, were carried out over the three years on contrasting soil types with different cropping histories.

There was no correlation between black spot incidence and soil pH or available nutrient levels, and black spot was found on all soil types irrespective of cropping history.

Spatial patterns of black spot development occurred in the replicated trials and in commercial crops. Unexpectedly, swedes established in the year following a crop with a high black spot incidence had a low black spot incidence.

4.8 Objective B-4 (additional)

Determine the effects of sowing date, depth of sowing and plant density on black spot incidence

The influence of sowing date was tested over three growing seasons in replicated field trials, and by monitoring commercial swede crops. The effects of sowing depth and plant density were also investigated.

Black spot incidence was higher in all early sown trials, and there was also a consistent trend of increasing black spot incidence with early sowing date in commercial crops. Sowing depth and plant spacing had no effect on black spot incidence.

4.9 Objective B-4

Investigate host range of black spot organisms as potential sources of inoculum of the pathogen and methods of survival

Shepherd's purse, growing as a weed in one of the experimental fields, was found to have lesions at soil level. Fifty lesions were tested for the presence of *R. solani*, and for their capacity to initiate black spots when transferred to swede.

R. solani was isolated from 18 % of stem base lesions, and a mixture of fungi from the remaining lesions. *R. solani* isolated from some of the diseased shepherd's purse plants also gave rise to black spot-like lesions on swede, some of which yielded *R. solani* on isolation.

4.10 Objective C-1

Determine the sensitivity of black spot organisms to fungicides in vitro and in vivo

4.10.1 Laboratory experiments.

Growth of four isolates of *R. solani* from swede black spot lesions was tested on agar containing Rizolex (tolclofos methyl), Monceren (pencycuron), or Rovral (iprodione).

All three fungicides suppressed fungal growth and the EC50 values (mg active ingredient/litre) were tolclofos methyl: 1.3; pencycuron: 0.9; iprodione: 1.8.

4.10.2 Field experiments.

The effects of high doses (tolclofos methyl: 1.25 kg a.i./ha, pencycuron: 1.50 kg a.i./ha and iprodione: 1.0 kg a.i./ha) on black spot incidence in a naturally infested trial crop in Berwickshire were tested in 1994 and 1995 in the field. The doses were much higher than in the laboratory tests because of the more challenging soil conditions in the field, and the possibility of leaching and breakdown. In addition, in 1995 the effects of pencycuron were tested on field-grown swedes which were artificially inoculated with black spot *Rhizoctonia*. Black spot incidence (%) was as follows:

Fungicide	Low infection, one spray	High infection, two sprays	Artificially inoculated (extreme), two sprays
Nil	8 %	41 %	70 %
Pencycuron	3 %	15 %	60 %
Iprodione	5 %	27 %	no data
Tolclofos methyl	1 %	23 %	no data

Under low infection conditions, symptoms were reduced, particularly by tolclofos methyl. In naturally high infection conditions, fungicides produced up to 60 % reduction in black spot incidence. In extreme conditions (inoculation), pencycuron reduced black spot incidence by only 14 %.

4.11 Objective C-2

Evaluate host range, crop rotation, and crop hygiene as potential methods of control

Evaluation of the experimental data obtained under the previous objectives suggests that crop, rotation, whether involving crucifer or cereal crops is unlikely to have potential for control. The presence of lesions on shepherd's purse and the capacity for such lesions to give rise to black spot in experimental situations suggests that control measures for this weed are advisable.

4.12 Objective C-3

Evaluate potential of biological control using antagonistic organisms.

A decision was made not to proceed with this objective.

DISCUSSION AND CONCLUSION

A range of different types of 'black spot' symptom were seen, some of them in agreement with the original description, others which were not. The black spots seen in this study were not confined to swede in the upper layers of the soil, as described by Fletcher & Hims (1979). Large shallow lesions of 12 cm diam, *sensu* Fletcher & Hims (1979), were never seen; large cavernous lesions, provisionally ascribed to canker, were common but the diagnostic pycnidia were not seen, and *Phoma* was isolated on only one occasion. This suggests that (a) black spots may have more than one cause, and (b) the distinction between small shallow lesions, possibly black spot *sensu* Fletcher & Hims (1979), and penetrating lesions, possibly canker, is unsafe without further investigation.

The role of *R. solani* in black spot requires clarification. In apparent contradiction to the Fletcher & Hims report, the isolation of *R. solani* from putative black spot lesions was generally low, as was the capacity of black spot isolates of *R. solani* to induce black spot lesions in healthy swede. Given that *R. solani* is believed to be the main organism involved in black spot, a low isolation rate suggests either a failure of *R. solani* to survive in a black spot lesion, or suppression of viable *R. solani* by other organisms which colonise the lesion. The data obtained on diminishing isolation rates of *R. solani* in ageing induced lesions supports this idea. Thus, *R. solani* appears to initiate lesions which subsequently become colonised by other organism e.g. *Phoma*, *Fusarium*, *Cylindrocarpon* and *Botrytis*, and as a result modifying the appearance of the lesion in comparison with the original description. Further study is needed to clarify the role of *Phoma* in 'black spot-like' lesions, and hence also the importance of *Phoma*-induced canker as a disease of swede.

The source of inoculum for black spot is at present unclear. Swedes are usually grown one year in five as a break crop in cereals. The progressive reduction in isolation rate of *R. solani* suggests that black spot lesions are unlikely to be a form of inoculum in the field. Cereal stem bases are often hosts to *R. solani*, but the particular strains would not be expected to be pathogenic to crucifers. The capacity of black spot *R. solani* to colonise cereal stems in soil-free systems now needs to be studied further to determine the importance, if any, of this as a means of survival and a form of inoculum. Finally, there is no evidence that the disease is seed-borne.

The reduction in black spot incidence by fungicides was additional evidence that *R. solani* is a cause of black spot, as two of the fungicides, tolclofos methyl and pencycuron, are specific to this fungus. A 50-60 % reduction in black spot incidence could potentially lead to a 50-60 % increase in marketable shopping swedes. The inoculation of swedes with *R. solani* placed against the swollen root below soil level, was an extreme situation and the poor control by pencycuron under these conditions may be expected, due to a high 'disease pressure' and possibly a lack of penetration of fungicide to the site of inoculation.

GLOSSARY

Anastomosis Group (AG): Populations of *Rhizoctonia solani* defined by reference to standard 'tester' isolates of different AGs: *Rhizoctonia* isolates which fuse with a particular AG belong to that AG, and have the opportunity for genetic exchange; conversely, lack of fusion indicates the isolates belong to a different AG in which genetic exchange is unlikely.

Black Crater spot: Name originally given by Fletcher & Hims (1979) to the disease of swede now called black spot

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