THE BRITISH POTATO COUNCIL

FINAL REPORT

RESISTANCE TO POTATO BLEMISH DISEASES

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1.1 Introduction

Silver scurf (*Helminthosporium solani*), black dot (*Colletotrichum coccodes*) and black scurf (*Rhizoctonia solani*) are important blemish diseases of potatoes. Symptoms caused by these diseases all result in deterioration of skin quality when tubers are kept in store. For example, both *H. solani* and *C. coccodes* cause grey, silvery lesions on the tuber surface and, whilst black dot produces small black sclerotia (Dillard, 1992), silver scurf produces dark brown spores that give lesions a sooty appearance (Brenchley and Wilcox, 1979). Infection by *R. solani* causes the formation of irregular black sclerotia on the surface of the tuber, which can range in size from 0.5 to 5 mm. Such disease symptoms significantly affect skin finish, which is an essential quality criteria in the fresh, washed, pre-packed market. BPC data suggest that growers in the UK lose £5 million annually due to contamination by silver scurf and black dot. A review of the emergence of silver scurf as an economically important disease of potato is given by Errampalli *et al.* (2001).

H. solani, R. solani and *C. coccodes* are all relatively common pathogens. A recent survey undertaken in the UK, 1989-1990 (Read *et al*., 1995) found black dot, silver scurf and black scurf in 75, 86 and 85% of potato crops surveyed, respectively. Despite these diseases being common, chemical control has had limited success and, although the fungicide Imazalil has been shown to reduce silver scurf, this treatment also resulted in increased incidence of black dot (Hide *et al*., 1994). Recent BPC projects have also illustrated the problem of fungicide resistance among these pathogens. Carnegie *et al.* (1995) showed that 60% of isolates of *H. solani* assessed displayed insensitivity to the fungicide thiabendazole. This lack of effective fungicidal control of the skin blemish pathogens highlights the future need for the production of disease resistant cultivars which can be used alone, or as part of an integrated control programme.

1.2 Project aims

1) To develop methods for assessing levels of resistance to *H. solani, C. coccodes* and *R. solani* in commercially-grown cultivars and related species of potato under optimised controlled environmental conditions and under field conditions.

2) To identify a range of resistance to the blemish diseases and to establish a crossing schedule in order to investigate the inheritance of that resistance. These findings will allow an assessment of the potential to breed cultivars more resistant to these pathogens in the future.

3) To investigate pathogen variability between isolates of each of these pathogens using differences in pathogenicity, culture morphology, fungicide sensitivity and by use of molecular techniques such as Amplified Fragment Length Polymorphisms (AFLPs). These studies will indicate whether certain pathogens will be easier to control than others. For example, some pathogens may exist as clonal populations which can be individually targeted through breeding, whilst other species will be more variable and hence more complicated to breed for resistance to.

1.3 Milestones and Target Dates

1. Obtain a wide range of isolates of *H. solani*, *C. coccodes* and *R. solani from* as many different sources as possible, and maintain them in culture (30.12.97) (Complete).
2. Review all relevant literature and write a short report (30.8.97) (Complete).
3. Grow and screen tubers of cultivars for resistance to each of the three pathogens, optimise test conditions and analyse results (30.3.98) (Complete).
4. Design a crossing schedule based on the results of Milestone 3 using parents of differing resistance (15.3.98) (Complete).
5. Plant relevant parental tubers (30.4.98) (Complete), carry out crossing according to schedule, and obtain seed from crosses (30.3.99) (Complete).
6. Examine variation for fungicide sensitivity to a range of relevant fungicides in each of the pathogens (30.6.99) (Complete).
7. Examine variation for pathogenicity in each of the three pathogens (30.9.99) (Complete).
8. Use molecular techniques to investigate variation between isolates of each the three pathogens (30.12.98) (Complete).
9. Plant a glasshouse progeny test using existing seed (30.5.98) (Complete).
10. Use tubers obtained from Milestone 9 to develop a progeny test protocol, by using a range of environmental conditions (30.2.99) (Complete).
11. Plant a glasshouse tuber progeny experiment with seed from the crossing schedule (30.5.99) (Complete).
12. Carry out progeny test on tubers obtained from Milestone 11 (30.2.00) (Complete).
13. Plant parental cultivars in the field (30.5.98) (Complete).
14. Repeat screening of field - grown cultivars and analyse results (30.2.99) (Complete).
15. Plant CPC accessions in the glasshouse (30.7.99) (Complete).
16. Screen tubers obtained from CPC accessions for resistance to each of the three pathogens and analyse results (30.2.00) (Complete).
17. Write yearly reports (30.7.98) (Complete), (30.7.99) (Complete), (30.6.00) (Complete).

2.0 Resistance to Silver scurf

2.1 Evaluating resistance to silver scurf in commercially-grown cultivars under storage and field conditions

Summary of experiments

2.1.1 Assessment of host resistance to silver scurf under optimised storage conditions

2.1.2 A comparison of severity of silver scurf on field and glasshouse-grown tubers

2.1.3 Assessment of host resistance to silver scurf under field conditions

2.1.0 Methods

Inoculation and incubation of tubers with *H.solani*

Inoculum consisted of a mix of six isolates of *H. solani*, obtained from tubers in Scotland and grown on V8 agar at 15^{o}C for 2 months. Cultures of each isolate were liquidised in 200ml of water and diluted to produce 5 litres of inoculum at a concentration of approximately 10^4 spores/ml. Mature field-grown tubers were obtained from field plots grown from seed tubers that had previously been treated with the fungicide Monceren IM (Bayer UK Ltd). At harvest, tubers were washed, then surface sterilised in 5% chloros and rinsed with water. Tubers of each cultivar or clone were then dried overnight before being inoculated by immersion in the inoculum and placed in two replicate boxes. The boxes were then placed in a controlled environment chamber at 15^{o}C with RH 95% for one month, followed by two months at 85%.

Silver scurf disease assessment

Three months after inoculation the surface area of each tuber covered in sporulating, silver scurf lesions was assessed using a 6-point scale (Table 1). The numbers of tubers falling into each scoring category was multiplied by the appropriate factor and the resultant scores were summed and divided by the total number of tubers in the box to give a final average disease score.

Table 1 Disease assessment key for potato tubers based on an estimation of surface area showing disease symptoms, together with conversion factor used for analysis of data. The total score for each box was then summed and divided by the total number of tubers in each box to give an overall disease score.

Surface area covered	0	<1/16	1/16-1/8	1/8-1/4	1/4-1/2	>1/2
Multiplying factor	0	1	3	6	12	24

Analysis of data

Differences in silver scurf disease severity between genotypes were assessed using analysis of variance, whilst similarities between tests were assessed using Spearmans rank correlation. All analysis was done using Genstat 5, Release 3.2 (Lawes Agricultural Trust, Rothamsted, UK).

2.1.1 Assessment of commercial cultivars for resistance to silver scurf under controlled environmental conditions

Field-grown tubers of 19 cultivars (Table 2) were assessed for resistance to silver scurf over three years (1997-1999). Two replicate samples of 10 tubers each were inoculated as above with a spore suspension containing a mixture of isolates of silver scurf. Tubers were then incubated under controlled environmental conditions and assessed for disease as described above.

2.1.2 A comparison of severity of silver scurf on tubers of commercial cultivars grown under glasshouse and field conditions

In 1998 and 1999 a comparison was made of the severity of silver scurf on field and glasshouse-grown tubers of 18 cultivars (Table 2). Two replicate samples of 15 daughter tubers were obtained from six-tuber field plots, and glasshouse-grown tubers obtained from five individual plants grown in pots. Tubers were inoculated with a mixture of six isolates (as above), incubated and assessed for disease severity after three months. Uninoculated controls were dipped in water.

2.1.3 Assessment of host resistance to silver scurf on tubers of commercial cultivars inoculated under field conditions

Sixteen potato cultivars (Table 2) were assessed for resistance to silver scurf under field conditions in 1999 and 2000 using the modified method of Méreda *et al.* (1994) as follows:

Inoculum of each six isolates of *H.solani* was prepared using sterilised horticultural vermiculite moistened with 2% malt extract solution. Flasks were inoculated with six plugs from the growing edge of a one-month-old isolate of *H. solani*. The inoculum was then incubated at room temperature for four weeks and shaken repeatedly to re-distribute the fungus. At planting, the inoculum of individual isolates was thoroughly mixed and seed tubers were hand-sown to a depth of 15 cm together with approximately 50g of the mixed inoculum. The number of colony forming units (CFU) of each isolate was determined using a dilution series and plating onto PDA in Petri dishes.

Seed tubers were sown by hand on April 26th 1999 and May 3rd 2000, in two-tuber plots, each with four replicates. Commercial crop management practices were used, and foliage pathogens

controlled. Tubers from each plot were harvested on September 23rd 1999 and October 16th 2000 respectively. Tubers were then washed and scored for symptoms as shown in Table 1.

2.1.4 Results and Discussion

In storage experiments, inoculation of tubers with a conidial suspension of *H. solani* (concentration of 10^4 spores ml^{-1} water) followed by incubation at a temperature of 15°C and RH 95% for one month followed by two months at RH 85% gave the best discrimination of disease severity among commercially-grown cultivars. A comparison of environmental conditions and inoculum concentrations is further discussed in Hilton *et al.* 2000. Using these conditions, significant differences ($P<0.001$) between cultivars were observed (Table 2), with the cultivars Wilja and Home Guard showing consistently more severe silver scurf disease symptoms than cvs Desiree and Romano. However, under conditions favourable for the disease, resistance among commercial cultivars is low (Hilton *et al*., 2000). This highlights the need to identify improved sources of resistance to silver scurf (Section 3). In order to identify such sources of resistance, it was necessary to develop tests that can be used on small tubers grown under glasshouse conditions. Studies showed that field and glasshouse rankings could be related, and a significant association was observed (Table 4), therefore allowing new sources of resistance to be sought amongst wild *Solanum* species.

Under field conditions, significant ($P<0.001$) differences for silver scurf disease severity between cultivars were also observed in both years. This study showed that early cultivars, such as Maris Bard and Pentland Javelin, showed more severe symptoms than maincrop cultivars, such as Romano and Kingston (Table 2). As earlier cultivars set tubers before those of the maincrop cultivars, they are in contact with soil inoculum for a longer period of time. Although there is variation in severity of silver scurf between these two groups there is also variation within the groups. This suggests that selection for an early cultivar with improved resistance to silver scurf could be obtained.

Significant associations between resistance to silver scurf in the field and in storage were observed. suggesting that the mechanisms of resistance are similar in both environments (Table 3). Although most cultivars showed similar ranking in both tests, others such as cvs Shelagh and Cara displayed severe disease symptoms in storage experiments, but lower disease severity under field conditions.

Table 2. Severity of silver scurf disease symptoms on field-grown tubers of 19 potato cultivars following inoculation with a spore suspension containing a mixture of *H. solani* isolates. Disease assessment (0-24 scale) was based on the area of individual tubers showing sporulation following storage in a controlled environment chamber at 15°C with RH 95% for one month and then RH 85%

for two months (see Table 1). This is compared to the silver scurf disease severity on 16 cultivars grown in field experiments in 1999 and 2000 following inoculation of the soil with a mixture of isolates of *H. solani*, according to the method of Méreda *et al*. (1994).

Cultivar	1997	1998	1999	Field 1999	Field 2000
Desiree (m)	4.1	1.4	4.0	3.9	2.3
Romano (m)	4.2	1.2	5.6	1.9	1.1
Kingston (m)	10.6	1.8	5.3	3.7	2.3
Shula (m)	4.9	1.1	9.1	2.1	2.9
Pentland Dell (m)	6.2	1.2	4.5	3.0	2.4
Pentland Squire	20.0	1.8	9.6	-	-
Stirling (m)	8.9	2.1	8.2	-	-
Estima (m)	12.6	3.0	14.7	6.7	6.0
Maris Piper (m)	12.0	1.9	5.9	6.1	4.0
Cara (m)	12.8	1.0	5.8	0.7	0.1
Ailsa (m)	12.2	1.8	6.7	8.6	2.8
Brodick	14.9	3.9	13.6	-	-
Shelagh (m)	9.3	1.3	8.0	2.2	0.7
Kirsty	18.9	-	10.0	-	-
Wilja (m)	18.3	3.8	14.0	9.1	7.3
Marfona (m)	-	-	-	6.2	6.4
Home Guard (e)	8.1	4.4	13.3	4.0	6.3
Arran Comet (e)	6.0		4.8	8.5	2.9
Maris Bard (e)	11.1	3.4	9.3	9.6	8.2
Pentland Javelin (e)	7.1	2.8	6.4	8.3	6.4
L.S.D ($P < 0.05$)	6.1	1.4	5.9	3.4	1.8

m = maincrop, e= early

Table 3. Relationship between resistance to silver scurf in 14 cultivars under storage conditions (1997-1999) (Section 2.1) and in the field (1999 and 2000) (Section 2.3). Associations are expressed as correlations following Spearmans rank analysis.

	1997	1998	1999	Field 1999
1998	0.36			
1999	0.51*	0.61*		
Field 1999	0.42	0.84**	0.50*	
Field 2000	0.25	0.82**	0.65**	0.84**

* $P < 0.05$; **$P < 0.001$

Table 4. A comparison of the severity of silver scurf disease symptoms on 19 field and glasshouse-grown tubers following inoculation with a mixture of isolates of *H. solani* (Section 2.2). Cultivars are listed in order of increasing disease severity of field-grown tubers with rankings in brackets.

Cultivar	Field-grown tubers	Glasshouse-grown tubers
Desiree	4.0 (1)	3.6 (1)
Pentland Dell	4.5 (2)	8.8 (9)
Arran Comet	4.8 (3)	5.4 (3)
Kingston	5.3 (4)	7.2 (6=)
Romano	5.6 (5)	4.8 (2)
Cara	5.8 (6)	6.1 (4)
Maris Piper	5.9 (7)	6.6 (5)
Pentland Javelin	6.4 (8)	9.8 (12)
Ailsa	6.7 (9)	11.6 (16)
Shelagh	8.0 (10)	7.2 (6=)
Stirling	8.2 (11)	8.1 (8)
Shula	9.1 (12)	13.8 (19)
Maris Bard	9.3 (13)	8.9 (10)
Pentland Squire	9.6 (14)	10.3 (13)
Kirsty	10.0 (15)	13.6 (18)
Pentland Guard	13.3 (16)	12.3 (17)
Brodick	13.6 (17)	9.0 (11)
Wilja	14.0 (18)	10.5 (14)
Estima	14.7 (19)	11.5 (15)
L.S.D (P<0.05) Cultivar	3.6	
Tuber type	1.2	
Cultivar x Tuber type	5.0	

2.2 Inheritance of resistance to silver scurf among commercially-grown cultivars

Summary of experiments

2.2.1 Develop a progeny test protocol using 12 progeny families (small progeny).

2.2.2 Assess a more extensive progeny to understand the inheritance of resistance to silver scurf among commercially-grown cultivars (large progeny).

2.2.1 Develop a progeny test protocol using 12 progeny families (small progeny).

A total of 12 progeny families obtained from separate crosses were used, including: (ss.12, Maris Piper x Pentland Dell; ss.15, Shula x Romano; ss.20, Cara x Shula; ss21, Desiree x Shula; ss.23, Maris Piper x Shula; ss.26, Desiree x Cara; ss.28, Wilja x Cara; ss.34, Wilja x Desiree; ss.46, Maris Piper self; ss.48, Maris Bard self; ss.19, Shula self). This limited progeny was used to identify

methods for assessing a larger number of progeny families (large progeny). For each family, 20 seedlings from each cross were sown in 4-inch pots in a glasshouse and grown to maturity. Three daughter tubers were harvested from each pot; one for inoculation in 1998 and one each for sowing in the field and glasshouse during 1999. For each progeny, two replicates of 20 tubers (each rep consisting of single tubers taken from each of the 20 individual seedlings) were inoculated, incubated and assessed for disease as described in sections 2.1. Controls consisted of glasshouse-grown tubers of parent cultivars dipped into water. The rankings of individual families were compared with results obtained in 1997 (Stewart, pers. comm.) using a Spearman rank correlation.

2.2.2 Assess a more extensive progeny to understand the inheritance of resistance to silver scurf among commercially-grown cultivars (large progeny).

In 1998, ten cultivars (Arran Comet, Cara, Desiree, Kingston, Maris Bard, Maris Piper, Pentland Dell, Shelagh, Shula and Wilja) of differing resistance to silver scurf were selected on the basis of data obtained in 1997 (Table 2). A half diallel crossing schedule without reciprocals was established and seed was obtained (1999). The following year (2000) seed was planted and the tubers from these progeny were obtained. Progeny families were then inoculated and assessed for disease as in section 2.0.

Data analysis consisted of comparing disease severity of individual families with the corresponding mid parental means (Table 6). If significant associations exist, this suggests that resistance in the parents can be passed on to its offspring. More detailed progeny analysis was performed to reveal the variation caused by genetic and environmental components (Table 7 and 8). All analysis was done using Genstat 5, Release 3.2 (Lawes Agricultural Trust, Rothamsted, UK).

2.2.3 Results and discussion

Assessment of the small progeny for resistance to silver scurf revealed that no significant differences existed between families. However, when rankings between years were compared (Stewart pers. comm.), significant ($P<0.05$) associations were found. It was concluded that repeatable progeny data

could be obtained using this inoculation and incubation method, and a more extensive progeny could therefore be assessed (Table 5).

Statistical analysis revealed significant ($P<0.001$) differences for resistance to silver scurf between families in the large progeny (Table 6). However, initial analysis found no significant associations between progeny families and mid-parent means, suggesting that there is no heritable basis to this resistance (Table 7). Indeed, more complex progeny analysis could find no significant heritable component, either GCA or SCA, that could explain the differences in severity of silver scurf between the families (Table 8). It can, therefore, be concluded that differences between progeny families were due to environmental factors.

It is suggested that breeding for resistance within this gene pool is unlikely to result in genotypes with superior resistance. This highlights the need to identify improved sources of resistance to silver scurf.

Table 5. Silver scurf disease symptom severity (0-24 scale of increasing severity) for 12 progeny families (small progeny) following inoculation with a mixture of isolates of *H. solani* during 1997 and 1999. Disease assessment was based on the area of individual tubers showing sporulation, having been stored in a controlled environment chamber set at 15ºC with RH 95% for one month and an RH 85% for two months. Disease severity was converted to a score using the scale in Table 1.

Family	1997	1999
ss.12	8.0	1.5
ss.15	9.9	1.8
ss.20	13.0	2.4
ss.21	12.4	2.0
ss.23	7.3	1.8
ss.26	15.0	2.6
ss.28	17.3	2.3
ss.34	13.1	1.7
ss.46	14.2	3.9
ss.48	16.2	2.1
ss.24	14.0	4.3
L.S.D ($P < 0.05$)	4.6	2.4

Table 6. Analysis of variance data from progeny tests (large progeny)

Source of variation	d.f.	S.S	M.S	V.R	Fpr.
rep	1	33.0	33.0	5.9	P<0.05
progeny	48	544.0	11.3	2.0	P<0.05
Residual	36	202.6	5.6		
Total	85	710.3			

Table 7. Relationship between severity of silver scurf in progeny families, related to the mid-parent means of the individual crosses carried out on field-grown tubers between 1998 and 2000 and glasshouse-grown tubers in 2000. All tubers were inoculated with a mixture of isolates of *H. solani* and then placed in a controlled environment chamber set at 15ºC with RH 95% for one month and an RH 85% for two months. Disease severity was converted to a 0–24 score. Associations are expressed as correlations coefficients.

Mid parent mean	Correlation coefficients
Field-grown tubers 1998	0.23 n.s
Field-grown tubers 1999	0.20 n.s
Field-grown tubers 2000	0.10 n.s
Glasshouse-grown tubers 2000	0.24 n.s

n.s – not significant

Table 8. Summary of progeny analysis revealing general (GCA) and specific (SCA) combining abilities (large progeny).

	d.f	s.s	m.s	v.r	FPr
Regression (GCA)	9	81.9	9.1	1.9	n.s
Residual (SCA)	39	190.1	4.9		
Total	48	272.0	5.7		

2.3 Additional potential sources of resistance to silver scurf

Summary of experiments

2.3.1 Resistance to silver scurf in clones of *Solanum phureja*

2.3.2 Resistance to silver scurf in accessions of the Commonwealth Potato Collection (CPC)

2.3.1 Resistance to silver scurf in clones of *Solanum phureja*

Seventeen clones of long-day adapted *S. phureja* (Table 9) were assessed for resistance to silver scurf under controlled environmental conditions in 1999 and 2000. The three *S. tuberosum* cultivars Shelagh, Shula and Wilja were included as controls. Five replicate boxes each containing five field-grown tubers were inoculated and assessed for disease as described in section 2.0. Control tubers consisted of uninoculated tubers of Shelagh, Shula and Wilja.

2.3.2 Resistance to silver scurf in accessions of the Commonwealth Potato Collection (CPC)

Seed of 101 accessions representing 33 species of *Solanum* was sown in the glasshouse and grown to full maturity (1999). As many species of *Solanum* are outbreeders, plants growing from seed can be considered as individuals. Therefore, ten individual tubers from each accession were grown in two replicates. At harvest, four tubers from each pot were collected, one set of 10 tubers was inoculated as in section 2.0 and the other was used as a control for each of two reps.

Silver scurf disease severity in the CPC accessions was compared to that of glasshouse-grown tubers of the three *S. tuberosum* cultivars Shelagh, Shula and Wilja. When disease assessment was made, many accessions were contaminated with soft rot and these accessions were therefore discarded and not considered in the analysis.

2.3.2 Results and discussion

Significant differences ($P < 0.001$) in silver scurf symptom severity was observed between accessions of *Solanum* (Table 10). Species that showed potential sources of resistance to silver scurf included *S. commersonii* and *S. demissum*. In contrast, *S. tuberosum* and *S. tuberosum* ssp *andigena* appeared to be susceptible. However, contamination by soft rot meant that tuber numbers for this test were reduced. To confirm findings from this work it is recommended that this test be repeated.

Significant differences ($P<0.001$) in severity of silver scurf between clones of *S. phureja* were also observed in 1999 and 2000 (Table 9). Inoculated tubers had significantly more disease than the

controls as expected. Variation in disease severity among these clones was comparable to that found in commercial cultivars used in this experiment.

These initial studies suggest that superior sources of resistance to silver scurf exist among wild species of potato. Further work is required to confirm these results and to identify the inheritance of resistance.

Table 9. Disease severity on field-grown tubers of 17 long-day adapted clones of *S. phureja* compared with the commercial cultivars Shelagh, Shula and Wilja following inoculation with a mixture of isolates of *H. solani*. Disease assessment was based on the area of individual tubers showing sporulation, having been stored in a controlled environment chamber set at 15°C with RH 95% for one month and an RH 85% for two months. Disease severity was converted to a 0-24 score using the scale in Table 1. Clones are listed in order of increasing disease severity (1999) and disease score rankings (1= most resistant) are given in brackets.

	Disease score (0-24 scale)	
Clone	1999	2000
DB378(1)	0.45 (1)	4.86
DB337(37)	1.20 (2)	9.70
DB168(11)	2.50 (3)	4.50
71T6	4.45 (4)	10.20
DB375(1)	5.00 (5)	4.55
80CP23	5.60 (6)	0.85
DB244(37)	5.65 (7)	9.15
DB333(16)	7.95 (8)	5.25
DB375(2)	10.00 (9)	5.40
DB382(1)	10.35 (10)	3.65
81S66	12.15 (11)	4.00
DB257(28)	12.45 (12)	7.30
DB358(30)	12.65 (13)	6.60
DB226(70)	13.60 (14)	9.80
71T46	13.95 (15)	9.10
DB358(23)	14.80 (16)	5.10
DB358(24)	16.60 (17)	4.05
Shelagh	8.10	13.55
Shula	1.90	2.25
Wilja	7.80	15.85
LSD	13.27	4.69

Table 10. Disease severity on glasshouse-grown tubers of 46 accessions compared with the commercial cultivars Shelagh, Shula and Wilja following inoculation with a mixture of isolates of *H. solani*. Disease assessment was based on the area of individual tubers showing sporulation, having been stored in a controlled environment chamber set at 15ºC with RH 95% for one month and an RH 85% for two months. Disease severity was converted to a score using the scale in Table 1. Accessions are listed in order of increasing disease severity.

Accession number	Disease score (range 0 - 24)
S. commersonii 5858	0.0
S. commersonii 5861	0.15
S. demissum 2245	0.15
S. demissum 2168	0.15
S. verrucosum 54	0.15
S. kurtzianum 3782	0.25
S. microdontium ssp *gigantophyllum* 7160	0.25
S. microdontium ssp *gigantophyllum* 3750	0.34
S. bukasovii 7525	0.34
S. commersonii ssp *malmaenum* 7520	0.35
S. pinnatisectum 3559	0.35
S. polytrichon 3984	0.35
S. acaule 7215	0.4
S. jamesii 3850	0.5
S. pinnatisectum 7191	0.5
S. sparsipilum 5874	0.5
S. vernii 4078	0.5
S. phureja 4364	0.55
S. sparsipilum 3572	0.6
S. phureja 4469 F	0.7
S. sparsipilum 7206	0.7
S. phureja 4598	0.75
S. vernii 4075	0.75
S. vernii 3743	0.85
S. semidemissum 7103	0.9
S. tuberosum 3302	0.9
S. verrucosum 7213	1.0
S. pinnatisectum 3863	1.0
S. commersonii ssp *malmaenum* 2727	1.25
S. spegazzinii 5926	1.25
S. phureja 4540	1.35
S. phureja 4596	1.35
S. phureja 4598	1.35
S. stoloniferum 4777	1.35
S. tuberosum ssp. *andigena* 5483	1.5
S. microdontium ssp. *gigantophyllum* 3766	1.55
S. tuberosum ssp. *andigena* 1085	1.67
S. phureja 4339	1.8
S. circanifolium ssp. *quimense* 7089	1.85
S. stoloniferum 1334	1.9
S. chacoense 5913	1.9
S. tuberosum ssp. *andigena* 1497 B	2.2
S. oplocense 7064	2.3
S. sucrense 4072	2.75
S. tuberosum 5646	3.5
S. stoloniferum 4715	4. 65
Wilja	2.1
Shula	2.15
Shelagh	3.35
L.S.D	1.77

3. 0 Resistance to black dot in commercially-grown cultivars under glasshouse and field conditions

Summary of experiments

3.1.1 Assessment of host resistance to black dot under glasshouse conditions

3.1.2 Assessment of host resistance to black dot under field conditions

3.1.1 Assessment of host resistance to black dot under glasshouse conditions

Inoculum of each of five isolates of *Colletotrichum coccodes* was prepared by inoculating flasks containing a sterilised mixture of sand-cornmeal media with six plugs from a growing edge of a one week old isolate of *C. coccodes*. The inoculum was then incubated at room temperature for 4 weeks and shaken repeatedly to redistribute the fungus. Equal amounts of inoculum of each isolate were then mixed and the concentration was determined using dilution plating.

Seed tubers of 14 (1998) and 15 cultivars (1999) (Table 11), each having 3 replicates, were sown in 6-inch pots containing SCRI compost in the glasshouse in a randomised block design. Inoculum (50g/pot) was placed on top of each tuber piece, tubers were then covered with compost, well watered and placed in a glasshouse set at 20^{o}C. Control pots were amended with 50g of autoclaved sand. Plants were then left until the end of their natural life to allow for the formation of tubers and disease development. Controls consisted of three replicates each of the cultivars Shelagh, Shula and Wilja.

Tubers were harvested, washed and the disease symptoms observed. The surface area of each tuber covered in silvering lesions characteristic of black dot was then assessed and converted to a disease score according to Table 1.

3.1.2 Assessment of host resistance to black dot under field conditions

A total of 14 (1999) and 16 (2000) cultivars were assessed for resistance to black dot under field conditions. Inoculum consisted of a mix of 5 isolates of *C. coccodes* as prepared previously (Section 3.1.1). Potato cultivars (Table 11) were sown on April 26th 1999 and May 3rd 2000, in two-tuber plots with four replicates. Tubers were hand-sown at a depth of 15 cm together with approximately 50 g of inoculum. The experiment was grown as for a commercial crop and foliage pathogens were controlled. On September 23rd 1999 and October 16th 2000 tubers were manually harvested, washed and assessed for the surface area covered in silvering lesions (Table 1).

3.1.3 Results and discussion

Reliable tests for the identification of resistance to black dot under field and glasshouse conditions were developed. Significant differences between cultivars for resistance to black dot were identified in the glasshouse in 1998 ($P<0.01$) and in the field both in 1999 ($P<0.001$) and 2000 ($P<0.05$) (Table 11). In attempting to identify new sources of resistance to black dot, and to understand the inheritance of this resistance, it was necessary to develop tests that can be used under glasshouse conditions and that reflect resistance of genotypes in the field. In this study, field and glasshouse disease rankings were related and significant associations were observed, hence such genetical studies were then performed (Table 12).

Under field conditions, early cultivars, such as Maris Bard and Pentland Javelin, showed more severe symptoms than maincrop cultivars such as Romano and Shelagh. As earlier cultivars set tubers before those of the maincrop cultivars, they are in contact with soil inoculum for a longer period of time (Table 11). However, variation in severity of black dot among both early and maincrop cultivars suggests that there is a genetic component of resistance not related to cultivar maturity. Therefore, it should be possible to select genotypes that are both early and more resistant to black dot.

The reduced severity of black dot observed in the field during 2000 can be attributed to contamination by silver scurf. Whether *H. solani* competes with *C. coccodes* for vital resources on the surface of the tuber or whether the relationship between the two pathogens is antagonistic is unclear (Table 11).

Table 11. Severity of black dot on 12 maincrop (m) and 4 early (e) cultivars between 1998 and 2000 following inoculation with isolates of *C. coccodes* in the glasshouse (Section 3.1.1) and in the field (Section 3.1.2). Cultivars are listed in order of increasing mean score over all years.

Cultivar	Glasshouse 1998	Glasshouse 1999	Field 1999	Field 2000
Romano (m)	-	0.7	-	0.1
Shelagh (m)	1.6	1.4	0.4	0.1
Cara (m)	4.2	1.4	0.6	0.1
Kingston (m)	4.5	1.8	0.5	0.2
Ailsa (m)	5.3	2.5	2.4	0.4
Pentland Dell (m)	5.5	3.7	2.4	0.1
Desiree (m)	4.7	5.7	1.3	0.1
Marfona (m)	6.3	4.1	2.2	0.5
Maris Piper (m)	7.9	3.1	2.3	0.4
Shula (m)	9.4	3.4	1.6	0.7
Wilja (m)	10.3	2.2	4.2	0.3
Estima (m)	13.3	5.8	2.8	0.6
Home Guard (e)	12.7	4.0	3.9	0.2
Arran Comet (e)	-	-	-	0.4
Maris Bard (e)	9.7	6.8	6.5	0.4
Pentland Javelin (e)	8.0	2.8	3.9	0.7
L.S.D (0.05)	7.8	n.s	2.6	0.4

Table 12. Relationship between rankings of black dot disease scores in 14 cultivars inoculated with isolates of *C. coccodes* between 1998 and 2000. Associations are expressed as correlations following Spearman rank analysis.

	1998	1999	Field 1999
1999	0.59*		
Field 1999	0.88**	0.46*	
Field 2000	0.63*	0.37	0.45*

* P<0.05, ** P<0.01

3.2. Inheritance of resistance to black dot among commercially-grown cultivars

Summary of experiments

3.2.1 Assess a progeny for resistance to black dot in order to understand the inheritance of resistance among commercially-grown cultivars.

3.2.1 Assess a progeny for resistance to black dot in order to understand the inheritance of resistance among commercially-grown cultivars.

Ten cultivars (Arran Comet, Cara, Desiree, Kingston, Maris Bard, Maris Piper, Pentland Dell, Shelagh, Shula and Wilja) of differing resistance were selected on the basis of data obtained in 1997. A half diallel crossing schedule without reciprocals was established and seed was obtained (1999). The following year (2000) seed was planted and the tubers from these progeny were obtained. Progeny families were then inoculated and grown under glasshouse conditions (Section 3.1.1). Disease was then assessed as the surface area of each tuber covered in sporulating lesions and scores were converted to a 0–24 scale (Table 1).

Data analysis consisted of comparing disease severity of individual families with the corresponding mid-parental means. If significant associations exist, this suggests that resistance in the parents can be passed on to its offspring (Table 14). More detailed progeny analysis was performed to reveal the variation caused by individual genetic and environmental components (Table 15).

3.2.2 Results and discussion

Significant ($P<0.001$) differences in severity of black dot were observed between progeny families (Table 13). Although, mid-parent means could not be related to the progeny means (Table 14) significant associations between GCA values and cultivar means were observed (data not presented) suggesting that resistance to black dot has a heritable component. More complex analysis revealed that differences between progeny families were due to significant GCA ($P<0.05$) and SCA ($P<0.01$) components (Table 15).

Assessment of GCA values for individual cultivars indicates that Cara was most effective in passing resistance onto its offspring (GCA –2.12) which was significantly greater than for the susceptible cultivars Wilja (GCA 2.96) and Maris Bard (2.01) (Table 16). It is suggested that breeding for resistance using the cv. Cara can result in genotypes with superior resistance. However, superior sources of resistance to black dot should be sought in order to develop cultivars with greatly increased resistance.

Table 13. Analysis of variance from progeny data

	d.f	s.s	m.s	v.r	FPr
Rep	1	0.002	0.00	0.98	n.s
Progeny	48	389.89	8.12	3.08	P < 0.001
Residual	48	126.64	2.64		
Total	97	516.54			

Table 14. Relationship between severity of black dot on progeny families and the mid-parent means. All tests were performed under glasshouse conditions using soil amended with inoculum containing a mixture of isolates of *C. coccodes*. Disease assessment was based on the area of individual tubers showing silvering and converted to a disease score using the scale in Table 1. Associations are expressed as correlation coefficients.

Mid parent mean	Correlation coefficients
1998	0.43***
1999	0.09 n.s
2000	0.11 n.s

*** P < 0.001

Table 15. Summary of progeny analysis revealing general (GCA) and specific (SCA) combining abilities

	d.f	s.s	m.s	v.r	FPr
Regression (GCA)	9	67.5	7.50	2.29	P<0.05
Residual (SCA)	39	127.5	3.27	1.32	P<0.01
Total	48	194.9	4.06		

Table 16. General Combining Ability (GCA) for resistance to black dot

Parent	Standard Error	GCA
Arran Comet	0.58	-0.72
Cara	0.51	-2.12
Desiree	0.51	-1.45
Kingston	0.62	0.00
Maris Bard	0.53	2.01
Maris Piper	0.51	0.24
Pentland Dell	0.53	-1.00
Shelagh	0.53	-0.10
Shula	0.53	0.65
Wilja	0.58	2.96
t (39) ($P < 0.05$)		2.02

4.0 Resistance to black scurf

4.1 Evaluating potato cultivars and wild *Solanum* species for resistance to black scurf

Summary of experiments

4.1.1 Assessment of resistance to black scurf under glasshouse conditions

4.1.2 Assessment of resistance to black scurf under field conditions

4.1.1 Assessment of resistance to black scurf under glasshouse conditions

The resistance of 14 cultivars (Table 16) of potato to black scurf were assessed during 1998 and 1999 under glasshouse conditions. Seed tubers of each cultivar were sown in 6-inch pots containing SCRI compost in the glasshouse in a randomised block design with 3 replicate blocks.

Inoculum consisted of a mixture of 5 isolates of *R. solani* (AG 3) prepared as described above for black dot. Inoculum (50 g/pot) was placed on top of each tuber piece, tubers were then covered with compost, well watered and placed in a glasshouse set at 20ºC. Control pots of Shelagh, Shula and Wilja were amended with 50 g of autoclaved sand. Plants were then left until the end of their natural life to allow for the formation of tubers and disease development.

Tubers were then harvested, washed and the disease symptoms observed. The surface area of tubers covered in black scurf sclerotia after harvest was assessed and scores were multiplied by the appropriate factor (Table 1). Tubers were also assessed using a visual 0–9 scale (Førsund, 1987) (where 0 corresponds to no symptoms and 9 are very severe symptoms). Results were then analysed using analysis of variance.

4.1.2 Assessment of resistance to black scurf under field conditions

Inoculum of five isolates of *R. solani* was prepared as above. Fourteen and sixteen cultivars of potato (Table 16) were sown on April 26th 1999 and May 3rd 2000 respectively in two tuber plots with four replicate plots. Tubers were hand-sown at a depth of 15 cm, together with approximately 50 g of inoculum. Commercial crop management practices were used, and foliage pathogens were controlled. On September 23rd 1999 and October 16th 2000 tubers were harvested, washed and then assessed for the surface area covered in sclerotia and, for the purpose of analysis, results were multiplied by the appropriate factor (Table 1). Tubers were also assessed using a visual 0–9 scale (Førsund, 1987) (where 0 corresponds to no symptoms and 9 are very severe symptoms).

4.1.3 Results and discussion

Significant differences in severity of black scurf were observed between cultivars in the field ($P<0.01$) and in the glasshouse ($P<0.05$) during 1999 using both the scoring method based on a 0-24

scale (Table 1) and a 0-9 scale (Føsund, 1987). However, a 0-9 visual scale (Føsund, 1987) was the most effective way of assessing symptoms and discriminating between cultivars. Under field conditions, early cultivars showed more severe symptoms of black dot than maincrop cultivars. As earlier cultivars set tubers before those of the maincrop cultivars, they are in contact with soil inoculum for a longer period of time.

However, tests to identify differences in resistance in commercially-grown cultivars to black scurf under field and glasshouse-grown conditions were found to be unreliable and inconsistent. Although a significant ($P<0.05$) association was found between the rankings of field and glasshouse-grown tubers in 2000 and 1999 respectively no other significant associations were observed. It is therefore concluded that other methods should be sought to identify resistance to this disease under controlled environmental and field conditions.

In contrast to silver scurf and black dot, black scurf is not characterised by infection of the periderm but by the formation of sclerotia. It is, therefore, speculated that any differences between genotypes in severity of black scurf are due to stimulation (Chemical or Physical) on the surface of the tuber.

Table 16. Severity of black scurf on 12 maincrop (m) and 4 early (e) cultivars between 1998 and 2000 following inoculation with isolates of *R. solani* in the glasshouse and in the field. Cultivars were scored using a 0-24 scale based on surface area covered in sclerotia (Table 1). Cultivars are listed in order of increasing mean score over all years.

Cultivar	Glasshouse 1998	Glasshouse 1999	Field 1999	Field 2000
Romano (m)	-	-	-	0.8
Shelagh (m)	2.6	0.4	0.2	0.7
Shula (m)	1.8	1.9	0.6	0.2
Cara (m)	2.1	1.9	0.4	0.4
Maris Piper (m)	1.7	1.2	2.0	0.3
Estima (m)	1.2	4.3	0.4	0.4
Pentland Dell (m)	2.5	2.5	1.3	0.4
Kingston (m)	1.3	5.0	0.6	0.5
Wilja (m)	2.6	3.8	1.7	0.6
Ailsa (m)	1.9	6.0	1.0	0.8
Desiree (m)	2.7	7.2	0.5	0.3
Marfona (m)	2.1	7.5	0.3	1.4
Arran Comet (e)	-	-	-	0.8
Maris Bard (e)	3.6	2.9	1.1	1.0
Pentland Javelin (e)	3.4	3.2	2.0	1.2
Home Guard (e)	2.1	7.3	2.0	0.8
L.S.D (0.05)	n.s	4.4	1.2	n.s

Table 17. Severity of black scurf on 12 maincrop (m) and 4 early (e) cultivars between 1998 and 2000 following inoculation with isolates of *R. solani* in the glasshouse and in the field. Cultivars were scored using a 0-9 scale (Føsund, 1987) based on the surface area covered in sclerotia. Cultivars are listed in order of increasing mean score over all years.

Cultivar	Glasshouse 1999	Field 1999	Field 2000
Romano (m)	4.4	-	-
Shelagh (m)	1.5	1.2	2.0
Maris Piper (m)	2.5	1.8	1.3
Shula (m)	2.8	1.6	1.3
Cara (m)	3.2	1.3	1.4
Estima (m)	4.0	1.2	1.6
Pentland Dell (m)	3.2	2.4	1.6
Kingston (m)	4.3	1.4	1.7
Desiree (m)	4.8	1.4	1.4
Wilja (m)	3.9	2.5	1.8
Ailsa (m)	5.1	1.7	1.9
Marfona (m)	5.5	1.2	2.8
Maris Bard (e)	4.0	1.6	2.1
Home Guard (e)	4.9	2.1	2.0
Pentland Javelin (e)	4.2	2.5	2.6
L.S.D (0.05)	1.5	0.8	n.s

Table 18. Relationship between rankings of black scurf in 14 cultivars inoculated with isolates of *R. solani* between 1998 and 2000. Cultivars were scored using a 0 - 24 scale based on surface area covered in sclerotia (Table 1). Associations are expressed as correlations following Spearmans rank analysis.

	1998	1999	Field 1999
1999	0.04		
Field 1999	0.14	0.40	
Field 2000	0.28	0.53*	0.09

P < 0.05, ** P < 0.01

Table 19. Relationship between rankings of black scurf in 14 cultivars inoculated with isolates of *R.solani* between 1998 and 2000. Cultivars were scored using a 0-9 scale (Førsund, 1987) based on the surface area covered in sclerotia. Associations are expressed as correlations following Spearmans rank analysis.

	1999	Field 1999
Field 1999	-0.01	
Field 2000	0.51*	0.04

* $P < 0.05$, ** $P < 0.01$

4.2 Inheritance of resistance to black scurf among commercially-grown cultivars

Summary of experiments

4.2.1 Analysis of progeny for resistance to black scurf in order to understand the inheritance of resistance to black dot among commercially-grown cultivars.

4.2.1 Analysis of progeny for resistance to black scurf in order to understand the inheritance of resistance to black dot among commercially-grown cultivars.

Ten cultivars (Arran Comet, Cara, Desiree, Kingston, Maris Bard, Maris Piper, Pentland Dell, Shelagh, Shula and Wilja) of differing resistance to blemish diseases were selected on the basis of data obtained in 1997. A half diallel crossing schedule without reciprocals was established and seed was obtained (1999). The following year (2000) seed was planted and the tubers from these progeny were obtained. Progeny families were then inoculated as previously described under glasshouse methods of inoculation. Disease was then assessed as the surface area covered in sclerotia using a 0-9 visual scale (Førsund, 1987).

Data analysis consisted of comparing disease severity of individual families with the corresponding mid-parental means. If significant associations exist, this suggests that resistance in the parents can be passed on to its offspring (Table 21). More detailed progeny analysis was performed to reveal the variation caused by individual genetic and environmental components (Table 22).

4.2.2 Results and discussion

Although significant ($P<0.001$) differences were observed between replicates for severity of black scurf, no differences were observed between progeny. The lack of significant associations between progeny families and mid-parent means of the individual crosses suggests that there is no heritable basis to this resistance

It is suggested that breeding for resistance within this gene pool is unlikely to result in genotypes with superior resistance. In order to breed cultivars with resistance to black scurf improved sources of resistance will have to be identified.

Table 20. Analysis of variance from progeny data

	d.f	s.s	m.s	v.r	FPr
Rep	1	4.67	4.67	19.76	P<0.001
Progeny	48	16.38	0.34	1.44	n.s.
Residual	48	11.35	0.24		
Total	97	32.40			

Table 21. Relationship between severity of black scurf on progeny families and the mid-parent means. All tests were performed under glasshouse conditions using soil amended with inoculum containing a mixture of isolates of *R. solani*. Disease assessment was based on the area of individual tubers showing sclerotia using a 0-9 scale (Førsund, 1987). Associations are expressed as correlation coefficients.

Mid parent mean	Correlation coefficients (0-9)[b]
1998	-0.17 ns
1999	-0.25*
2000	-0.02 n.s

* P < 0.05, ns – not significant

Table 22. Summary of progeny analysis revealing general (GCA) and specific (SCA) combining abilities

	d.f	s.s	m.s	v.r	FPr
Regression (GCA)	9	3.55	0.39	3.31	P<0.05
Residual (SCA)	39	4.64	0.12	1.01	ns
Total	48	8.19	0.17		

5.0 Characterisation, fungicide sensitivity, pathogenicity and genetic variation in isolates of *Helminthosporium solani, Colletotrichum coccodes* and *Rhizoctonia solani*.

5.1 Introduction

Knowledge of the levels of variation among isolates of *H. solani* is at present limited to studies of culture morphology (Jellis, 1972) and sensitivity to fungicides (Carnegie *et al*., 1995). Jellis (1972) observed that there were two morphologically distinct types of *H. solani* isolates when grown on agar. In one case, colonies were hairy and their colour ranged from near-white to dark grey and in the other, colonies were smaller, black, and had a smooth surface. More recently, Hide and Hall (1993) showed that when thiabendazole was applied to seed tubers, 50% of the isolates recovered were resistant to fungicide. In a survey of 4,424 isolates from the UK, over 60% were found to be insensitive on media amended with thiabendazole. No information at present has been published on variation in pathogenicity among isolates of *H. solani*.

Knowledge of the variation among isolates of *C. coccodes* is also limited and is restricted to studies on culture morphology and pathogenicity. Kendrick and Walker (1948) found that 147 isolates of *C. coccodes* from tomato fruits showed a considerable range of growth characteristics on PDA, with isolates varying in colour of substrate, in the presence of prostrate or aerial mycelia and in the size and number of sclerotia. Variation in pathogenicity and symptom development were observed in nine isolates of *C. coccodes* on foliage of the potato cultivar Russett Burbank (Barkdoll and Davis, 1992). These workers blew sand over the potato plants 1 month after planting to induce wounding, and sprayed tubers with a conidial suspension of the individual isolates.

Studying variation between isolates of *R. solani* is complicated due to the existence of several intra-specific sub groups. Although many different criteria have been used to delineate these groups, the most widely used method is based on hyphal anastomosis (fusion) (Parmeter *et al*., 1969), which has revealed 12 distinct groups (AG's) (Carling *et al*., 1994). However, although AG4, AG5 and AG9 have been recorded from potatoes, AG3 is the main group associated with black scurf on potato. Considerable differences between isolates in growth characteristics on media have been observed, including radial growth of isolates (Baird *et al*., 1996), culture characteristics (Singh and Singh, 1994), and hyphal diameter and nuclear number (Carling *et al*., 1994). Variation in pathogenicity among isolates of AG5 from a field in Maine, USA has been studied (Bandy *et al*., 1984). These workers grew potatoes in compost amended with inoculum and, 3 weeks after planting, assessed lesions on emerging sprouts. No such study has been performed on isolates of AG3.

The development of molecular biology techniques has allowed tremendous advances to be made in understanding variation within pathogenic species of fungi. Molecular characterisation of *Colletotrichum* spp. have been performed on strains from a range of hosts including: *C. acutatum, C. gloesporoides* and *C. fragariae* from strawberry plants (Buddie *et al*.,1999); and *C. graminicola* on

turfgrass (Backman *et al*.,1999). Similarly, with *R. solani*, ribosomal DNA Restriction Fragment Length Polymorphisms (RFLP's) analysis from 87 isolates obtained from different countries, a range of hosts and representing 15 AG's, have revealed genetic variation among and within intraspecific groups (Vilgalys and Gonzalez, 1990). Other studies have concentrated on variation within specific AG's; including Random Amplified Polymorphic DNA (RAPD) analysis of AG9 isolates obtained from agricultural soils in Alberta, Canada (Yang *et al*., 1996) and RFLP analysis of isolates of AG 2-1 and B1 from tobacco fields in France and Italy (Nicoletti *et al*., 1999). One study using RAPD assays on isolates of AG3 from a potato and tobacco from different geographical areas has revealed considerable variation.

However, no study on genetic variation has been performed specifically on *R. solani* (AG3), *C. coccodes* or *H. solani* on potato. Therefore, the aim of this work is to identify variation in isolates of *C. coccodes, H. solani* and *R. solani* based on culture morphology, fungicide resistance and pathogenicity on potato tubers. This data can then be related to Polymorphisms from the same isolates as identified by AFLP analysis.

5.2 General methods

Establishment of a culture collection

Isolates of *R. solani, C. coccodes* and *H. solani* were obtained from other workers in the UK, USA, New Zealand, France, Japan and Holland. Isolates have also been obtained from infected tubers and from field sites around SCRI. Cultures were maintained on Potato Dextrose Agar (PDA) at 15^{o}C, and sub-cultures were made and kept at –20^{o}C

DNA extraction

For tests requiring DNA of each of the isolates, cultures were grown on PDA (*R.solani* and *C. coccodes*) and V8 (*H. solani*) and DNA extracted using a genomic DNA extraction kit (Puregene) according to the manufacturer's instructions. DNA was rehydrated in 50 µl DNA hydration solution and stored at 4^{o}C (DNA concentration of 100 µg/ml if the total yield was 5 µg DNA).

Amplified Fragment Length Polymorphism

A protocol was adapted from Vos *et al*. (1995). A 15 µl sample of genomic DNA was digested using 10 units per µl of *Eco*RI and 4 units per per µl of *Mse*I in 4µl of 5 x RL buffer at 37^{o}C for 1 hour. To each digested sample, a 5 µl mixture containing *Mse*I (50 µM) and *Eco*RI adaptor (5 µM), 10mM ATP, 5X RL buffer and 1 unit T4 ligase was added and incubated for 3 hours at 37^{o}C. The ligated DNA was then pre-amplified using two non specific enzymes and involved adding 5 µl of sample to 15 µl mixture containing, M00 and E00 primers (50ng/µl), 5mM dNTP, 10X PCR buffer and Taq Polymerase (% Units per µl) (Amplitaq). Pre-amplified samples were diluted with 25 µl of

0.1 X TBE buffer. Selective PCR reactions were performed by labelling one of the primers in the combination (usually the E-primer) with radioactive ^{33}P. For each sample, 0.5 µl of label mix was made by adding ^{33}P ATP (ICN), unlabelled E-primer (50ng/µl), Forward reaction buffer, T4 kinase (10 units/µl) with H_2O and incubating for 1 hour at 37ºC, followed by 10 minutes at 70ºC to inactivate the kinase. This was then added to unlabelled M primer, 5mM dNTP, 10X PCR buffer, Taq polymerase (5 units/µl) and H_2O to give 15 µl per sample which was added to 5 µl of pre-amplified DNA. These samples were subjected to a PCR profile involving 12 cycles of 94ºC for 30 seconds (denaturing), 65-56ºC for 30 seconds (decrease 0.7ºC each cycle) (annealing) and then 72ºC for 60 seconds (extension), followed by 24 cycles of: 94ºC for 30 seconds, 56ºC for 30 seconds, and 72ºC for 60 seconds. Amplification products were subjected to electrophoresis on 5% denaturing polyacrylamide gel on a sequencing gel system. Gels were run for 2.5 hours, dried and exposed to X-ray film for a minimum of 2 days.

Reactions were performed on 70 isolates of *H. solani*, 33 isolates of *C. coccodes* and 67 isolates of *R. solani* with 3 primer combinations (E14/M16, E19/M14, E19M16). The *Eco*R1 (E) and *Mse 1* (M) primers used and their 3' selective bases (in brackets) are as follows: E14(+AT), M14 (+AT), M16(+CC) and E19 (+GA).

5.3 Variation in *H.solani*

Summary of experiments

5.3.1 Characterisation of isolates according to culture morphology

5.3.2 Characterisation of isolates according to sensitivity to fungicides *in-vitro*

5.3.3 Characterisation of isolates according to virulence of isolates on tubers

5.3.4 AFLP analysis of *H.solani*

5.3.1 Morphological characterisation of *H.solani*

Isolates of *H. solani* were grown on Petri dishes containing V8 agar for 3 weeks at 20ºC. Visual observations were then used to place isolates into two morphologically distinct categories; those where the colonies were hairy with colour ranging from near white to dark grey and those where the colonies were smaller, black and with a smooth surface (Jellis, 1972).

5.3.2 Sensitivity of isolates of *H.solani* to the fungicides *in-vitro*

All isolates of *H. solani* were tested for sensitivity to the fungicide thiabendazole (TBZ) in amended media. Isolates of *H. solani* were tested on V8 agar, amended with 100 mg TBZ per litre of media, and compared to an unamended control. For each isolate, two 5 mm plugs from actively growing cultures were placed in each Petri dish and two replicate plates were used per treatment. Plates were incubated at 20ºC and culture diameter measured after 3 weeks. The reaction of isolates was classified in terms of percentage inhibition of colony growth (Hide *et al*., 1988) and were classified

using the guidelines, <10% inhibition = resistant, 10-50% inhibition = intermediate and >50% inhibition = sensitive.

Isolates of *H. solani* (Table 24) were also tested for sensitivity to the fungicides fenpiclonil, fungazil and monceren IM in amended media as in above. V8 agar was amended with fenpiclonil (1 mg per litre media), fungazil (1 mg per litre media) and monceren IM (10 mg per litre media). For each isolate, a 5 mm plug from an actively growing culture was placed in each Petri dish and two replicate plates were used per treatment. Plates were incubated at 20°C and culture diameters measured after 4 weeks. Sensitivity to the fungicide was measured as a percentage reduction in colony diameter compared with the control.

5.3.3 Virulence of isolates of *H.solani*

Virulence of 12 isolates of *H. solani* was assessed in 1999 and of 30 isolates in 2000. Inoculum of each isolate (Table 25) was prepared as in section 2.1. Tubers of the cultivar Wilja were inoculated and incubated as in Section 2.1. Three months after inoculation, individual tubers were assessed for disease as described in Table 1. Two replicates consisting of 5 tubers were used for each isolate.

5.3.4 AFLP analysis of *H.solani*

AFLP was carried out on 70 isolates of *H.solani* as described in the general methods of this section (5.2).

5.3.5 Results and discussion

Considerable variation was observed among isolates of *H. solani* in terms of culture morphology, sensitivity to fungicides *in-vitro* and virulence on potato tubers. In total, only four out of 70 isolates produced black, smooth colonies whilst the remaining isolates varied considerably from almost white through to grey (Table 23). Very distinct differences among isolates were also observed in sensitivity to thiabendazole with 24 out of 65 isolates being completely insensitive and one showing an intermediate response. No relationship between the original isolation date of the isolates and sensitivity to thiabendazole was observed. Although many insensitive isolates were obtained after 1996, others such as isolate 11 were obtained pre-1990 (Table 23).

Although some differences in sensitivity of isolates of *H.solani* to fenpiclonil ($P<0.001$), fungazil ($P<0.001$) and monceren IM ($P<0.01$) were observed, these were not as distinct as those for TBZ. Therefore, no isolates were found that were completely sensitive to these fungicides and only isolate H17 was completely insensitive to fenpiclonil (Table 24).

Isolates also differed significantly ($P<0.001$) in virulence on the cv. Wilja, although data was not repeatable between years. No relationship between virulence and culture morphology or sensitivity to TBZ was observed (Table 25).

These series of experiments have revealed that considerable variation exists at a phenotypic level. However, further experiments using Amplified Fragment Length Polymorphism's (AFLPs) were carried out to attempt to reveal how much variation exists at a genetic level, and showed that little variation was evident.

Table 23. Origin, colony morphology, sensitivity to thiabendazole of isolates of *H. solani*.

Isolate name	Date of isolation	Origin	Morphology (a)	Sensitivity to thiabendazole when grown on media amended with 100 mg TBZ per litre(b)
1	1996	Scotland (SASA)	GR	S
2	1996	Scotland (SASA)	GR	R
3	1996	Scotland (SASA)	GR	S
4	1994	Scotland (SASA)	GR	S
5	1994	Scotland (SASA)	GR	R
6	1996	Scotland (SASA)	GR	R
7	1994	Scotland (SASA)	GR	R
8	1994	Lincolnshire (BPC)	GR	S
9	1994	Lincolnshire (BPC)	GR	S
10	1996	Scotland (SCRI)	GR	S
11	pre 1990	Scotland (SCRI)	GR	R
12	pre 1990	Scotland (SASA)	GR	S
13A	1993	Scotland (SCRI)	GR	S
13B	1993	Scotland (SCRI)	GR	S
14	?	Scotland (SASA)	BS	S
15	?	Scotland (SASA)	BS	S
16	?	Scotland (SASA)	GR	S
17	?	Scotland (SASA)	GR	R
18	1996	N. Ireland (DANI)	GR	S
19	1996	N. Ireland (DANI)	GR	S
20	1996	N. Ireland (DANI)	GR	R
21	1996	N. Ireland (DANI)	GR	S
Isolate	Date of isolation	Origin	Morphology	TBZ sensitivity
22	1998	Scotland (SASA)	GR	R
23	1998	Aberdeenshire	GR	S
25	1998	Forfar, Scotland	GR	R
26	1998	Scotland	GR	S
27	1998	Scotland, Fife	GR	S
28	1998	Scotland, Ross-shire	GR	S

29	1998	?	GR	R
30	1998	?	GR	R
31	1998	Scotland,	GR	S
32	1998	Scotland,	GR	S
33	1998	Scotland,	GR	S
34	1998	Scotland (SCRI)	GR	S
35	1998	Scotland (SCRI)	GR	R
36	?	USA, Minnesota	GR	R
37	?	USA, Pennsylvania	GR	nt
38	?	Canada,British Columbia	GR	I
39	?	USA, New York	GR	R
40	?	USA, North Dakota	GR	S
41	1997	Russia	GR	S
42	1996	England, Rothamsted	GR	S
43	1996	England, Rothamsted	GR	S
44	1996	England, Rothamsted	GR	R
45	1996	England, Rothamsted	GR	R
46	1996	England, Rothamsted	GR	S
47	1997	England, Rothamsted	GR	S
48	1997	England, Rothamsted	BS	R
49	1997	England, Rothamsted	GR	S
50	1997	England, Rothamsted	BS	S
51	1997	England, Rothamsted	GR	R
52	1997	England, Rothamsted	GR	S
53	1997	England, Rothamsted	GR	R
54	1997	England, Rothamsted	GR	R
55	?	England, Cambridge	GR	R
56	?	England, Cambridge	GR	S
57	?	England, Cambridge	GR	S
58	?	England, Cambridge	GR	S

Isolate	Date of isolation	Origin	Morphology	TBZ sensitivity
59	?	England, Cambridge	GR	S
60	?	England, Cambridge	GR	S
61	?	England, Cambridge	GR	R
62	?	England, Cambridge	GR	S
63	?	England, Cambridge	GR	R
64	?	England, Cambridge	GR	R

65	?	England, Cambridge	GR	R
66	?	England, Cambridge	GR	R
67	?	England, Cambridge	GR	R
68	?	England, Cambridge	GR	S
69	?	England, Cambridge	GR	S
70	?	England, Cambridge	GR	S

(a) GR, grey and hairy; BS, black and smooth, (b) S, sensitive; R, resistant; I, intermediate, nt, not tested

Table 24. a) Sensitivity of 18 isolates of *H. solani* on V8 amended with fenpiclonil (1 mg per litre media), fungazil (1 mg per litre media) and monceren IM (10 mg per litre media). Plates were incubated at 20ºC and culture diameter was measured after four weeks. Data is expressed as a percentage growth compared to an unamended control.

Isolate number	Fenpiclonil	Isolate number	Fungazil	Isolate number	Monceren IM
H1	51.4	H1	6.2	H3	42.9
H3	63.4	H3	0	H4	43.3
H4	57.5	H4	0	H5	52.8
H5	66.2	H6	0	H6	41.9
H6	71.3	H8	0	H7	40.0
H7	61.1	H9	36.7	H8	42.9
H8	62.3	H11	0	H9	51.9
H9	79.3	H12	0	H10	42.9
H10	48.8	H13a	0	H12	42.9
H12	75.0	H14	0	H13b	36.4
H14	63.4	H15	31.2	H14	42.9
H15	78.7	H18	0	H15	60.5
H16	50.3	-	-	H16	42.9
H17	105.8	-	-	H17	51.7
H18	50.4	-	-	H18	34.4
H19	45.2	-	-	H19	26.0
H20	58.5	-	-	H20	40.5
H21	53.4	-	-		
L.S.D	7.9		11.0		8.3

Table 25. Virulence of 12 isolates (1999) and 30 isolates (2000) of *H. solani* on field-grown tubers of cv. Wilja following inoculation with a spore suspension. Disease assessment was based on the area of individual tubers showing sporulation, having been stored in a controlled environment chamber set at 15ºC with RH 95% for one month and an RH 85% for two months. Tests were performed in two years and both data sets are shown. Each dataset is the mean of 2 replicates of 5 tubers.

Isolate	1999	2000
H1	2.5	12.8
H2	5.2	2.8
H3	11.6	1.9
H4	2.7	4.4
H5	8.4	2.8
H6	5.8	3.4
H7	4.5	4.3
H8	3.7	7.0
H9	1.4	2.8
H10	1.8	7.3
H11	1.9	3.3
H12	2.1	2.4
H13a	-	4.2
H13b	-	3.8
H14	-	8.9
H15	-	7.7
H16	-	2.9
H17	-	3.5
H18	-	2.8
H19	-	2.7
H20	-	1.8
H21	-	0.8
H22	-	5.5
H23	-	2.8
H25	-	3.9
H26	-	0.9
H27	-	3.2
H28	-	4.0
H29	-	4.9
H30	-	3.9
L.S.D	2.5	2.3

5.4 Variation in *Colletotrichum coccodes*.

Summary of experiments

5.4.1 Characterisation of isolates of *C.coccodes* according to culture morphology

5.4.2 Characterisation of isolates of *C.coccodes* according to sensitivity to fungicides *in-vitro*

5.4.3 Characterisation of isolates of *C.coccodes* according to virulence of isolates on tubers

5.4.4 AFLP analysis of *C.coccodes*

5.4.1 Morphological characterisation

Colony morphology of isolates of *C. coccodes* was assessed on 3-week-old cultures grown on Petri dishes containing PDA using the criteria of Chesters and Hornby (1965). Characters include size of sclerotia, area of colony covered in sclerotia, presence of aerial mycelia and colony colouration.

5.4.2 Characterisation of isolates of *C.coccodes* according to sensitivity to fungicides *in-vitro*

Isolates of *C. coccodes* (Table 27) were tested for sensitivity to the fungicides TBZ, fenpiclonil, fungazil and monceren IM. PDA was amended with TBZ (10 mg per litre media), fenpiclonil (0.1 mg per litre media), fungazil (1 mg per litre media) and monceren IM (10 mg per litre media). For each isolate, one 5 mm plug from an actively growing culture was placed in each Petri dish and two replicate plates were used per treatment. Plates were incubated at 20^{o}C and culture diameters measured after 4 weeks. Sensitivity to the fungicide was measured as a percentage reduction in colony diameter compared with the control.

5.4.3 Characterisation of isolates of *C.coccodes* according to virulence of isolates on tubers

The virulence of 10 isolates of *C.coccodes* was assessed on tubers of cv. Pentland Javelin in 1999 and 2000. Inoculum of individual isolates was prepared as in Section 3.1.1 and the colony forming units (CFU) per g was determined by dilution plating before inoculation. Six inch pots containing seed potatoes of Pentland Javelin were then inoculated as described in Section 3.1. Control pots were treated with 50 g of autoclaved sand. Pots were then laid out in randomised block design with three replicate blocks and placed in a growth cabinet with 12 h photoperiod and set at 15^{o}C during light and 10^{o}C during dark. Pots were watered so that compost remained damp and left to grow until the end of their natural life to allow for the formation and setting of tubers. Tubers were then harvested, washed and assessed for disease as in Section 3.1.

5.4.4 AFLP analysis of *C.coccodes*

AFLP was carried out on 33 isolates of *C.coccodes* as described in the general methods of this section (5.2).

5.4.5 Results and discussion

No obvious differences in culture morphology were observed between isolates of *C. coccodes* according to the criteria of Chesters and Hornby (1965). All isolates produced numerous sclerotia of approximately 1mm in diameter that covered 60-90% of the surface of the culture, and all produced white colonies, with most cultures showing no aerial mycelia.

Although isolates of *C. coccodes* showed significant ($P<0.01$) differences in sensitivity to the fungicides TBZ and Fenpiclonil *in vitro*, these differences were not large. For example, isolate C12 showed the greatest reduction (32.7% of the control), in mycelial growth on media amended with Fenpiclonil, whilst C1 showed the least reduction in growth (51.2 % of the control). Similarly, isolate C7 showed the greatest reduction in mycelial growth on media amended with TBZ compared with the control (48.3%), whilst C9 showed the least (62.6%). No significant differences in sensitivity to Fungazil and Monceren IM were observed.

Although all isolates of *C. coccodes* were pathogenic on tubers of cv. Pentland Javelin, no statistical differences between isolates were observed in terms of virulence. Results between years were comparable.

These series of experiments have revealed only limited variation at a phenotypic level among isolates of *C. coccodes.* Further experiments using Amplified Fragment Length Polymorphism (AFLPs) were conducted, and showed that there was limited genetic variation and that *C. coccodes* may exist as a clonal population.

Table 26. Origin and Morphology of isolates of *C. coccodes* on PDA according to Chesters and Hornby (1965).

Isolate	Origin	Date of Isolation	Host	Size of sclerotia (L/S)	Abundance (% sclerotial coverage)	Aerial mycelia (Y/N)	Colouration
C1	SCRI	1996	Potato	S	80	N	White
C2	WSU	1997	Potato	S	80	Y	White
C3	WSU	1997	Potato	S	80	Y	White
C4	WSU	1997	Potato	S	80	N	White
C5	SCRI	1985	Potato root	S	90	N	White
C6	SCRI	1989	Potato	S	80	Y	White
C7	SCRI	1988	Potato stem	S	80	N	White
C8	DANI	1997	Potato	S	80	N	White
C9	SASA	1997	Potato	S	80	N	White
C10	SASA	1998	Potato	S	80	N	White
C11	SASA	1998	Potato	S	80	N	White
C12	SASA	1998	Potato	S	80	N	White
C13	DANI	1998	Potato	S	80	N	White
C15	DANI	1998	Sante	S	80	N	White
C16	SCRI	-	Potato	S	80	N	White
C17	Supermarket	-	Charlotta	L	50	N	White
C18	Supermarket	-	Charlotta	S	80	N	White
C19	Rothamsted	1983	P. Crown	S	80	N	White
C20	Rothamsted	1988	Desiree	L	60	N	White
C21	Rothamsted	1988	Desiree	L	80	N	White
C22	Scotland	1991	Desiree	L	70	N	White
C23	Scotland	1991	Desiree	S	80	N	White
C24	Sutton	1997	Fianna	L	50	N	White
C25	Sutton	1997	M. Piper	S	80	N	White
C26	Sutton	1997	M. Piper	S	80	N	White
C27	Sutton	1997	Navan	S	80	N	White
C28	Sutton	1997	?	S	80	Y	White
C29	Sutton	1997	?	S	80	Y	White
C30	SCRI	1998	Estima	S	80	N	White
C31	-	1998	M. Piper	S	80	N	White
C32	-	1998	M. Piper	S	80	N	White

WSU - Washington State University

Table 27. Growth of 18 isolates of *C. coccodes* on PDA amended with fenpiclonil (0.1 mg per litre media), thiabendazole (10 mg per litre media), fungazil (1 mg per litre media) and monceren IM (10 mg per litre media). Plates were incubated at 20ºC and culture diameter was measured after 2 weeks. Data is expressed as a percentage growth compared to an unamended control.

Isolate number	Fenpiclonil	Thiabendazole	Fungazil	Monceren IM
C1	51.2	nt	49.0	67.9
C2	44.7	57.7	43.2	67.5
C3	43.0	61.7	45.4	56.2
C4	46.9	51.1	49.5	63.6
C5	36.2	48.3	47.1	56.0
C6	38.0	50.6	53.5	59.9
C7	35.4	48.3	55.2	61.2
C8	38.9	58.3	47.3	68.0
C9	33.7	62.6	45.8	64.5
C10	34.5	55.1	46.2	64.0
C11	34.4	59.5	46.2	63.1
C12	32.7	55.4	51.9	64.3
C13	40.8	56.3	53.7	69.2
C14	35.4	55.8	65.6	69.0
C15	40.2	54.2	50.5	60.5
C16	28.5	51.2	52.0	55.6
C17	41.1	58.5	50.0	68.8
C18	38.6	54.5	48.3	62.4
LSD	9.0	6.4	n.s	n.s

Table 28. Virulence of ten isolates of *C. coccodes* on tubers of cv. Pentland Javelin. Plants were inoculated by growing seed tubers in compost amended with 50 g of a sand and cornmeal mixture inoculated with mycelial plugs of *C. coccodes*. Plants were grown in a growth cabinet with 12 h photoperiod and set at 15ºC during light and 10 ºC during dark cycles. Disease assessment was based on the area of individual tubers showing silvering. Tests were performed in two years and ranking of individual isolates is shown in brackets.

Isolate	1999	2000
C1	8.2 (2)	2.2 (2)
C2	4.6 (4)	2.0 (4)
C3	0.5 (9)	1.4 (7)
C4	10.8 (1)	3.2 (1)
C5	4.0 (5)	2.0 (4)
C6	0.1 (10)	1.9 (6)
C7	0.6 (8)	2.2 (2)
C8	6.1 (3)	0.8 (9)
C9	4.0 (5)	0.4 (10)
C10	2.8 (7)	0.9 (8)
L.S.D	n.s	n.s

5.5. Variation in *R. solani.*

Summary of experiments

5.5.1 Anastomosis group typing of *R. solani*

5.5.2 Morphological characterisation of *R.solani*

5.5.3 Sensitivity of isolates of *R.solani* to fungicides

5.5.4 Virulence of isolates of R.solani

5.5.5 AFLP analysis of *R.solani*

5.5.1 Anastomosis group typing of *R. solani*

Isolates belonging to a range of *R. solani* anastomosis groups have been obtained for use as reference strains (Table 29). To identify the anastomosis group of individual isolates, a method adapted from Parmeter *et al*. (1969) was used. Mycelial disks from the edge of two actively growing isolates were paired 2–4 cm apart on Petri dishes containing 1- % water agar. A 5 mm mycelial plug of a tester isolate, of known anastomosis group, was placed at one end and the isolate to be typed was placed at the other. Plates were then incubated at 20°C for 48-72 hours until hyphae overlapped. The region on the agar plate where mycelia overlapped was removed using a scalpel and placed on a glass slide and examined under the light microscope. Anastomosis was confirmed if cell fusion and plasmalemma were observed in at least five fusion sites for each of three replicate plates.

5.5.2 Morphological characterisation of *R.solani*

Gross morphological characteristics of *R. solani* isolates in culture were observed according to criteria adapted from Singh and Singh (1994). Isolates were grown by placing a 5-mm mycelial plug on a 9 cm Petri dish containing fresh PDA and incubated in the dark at 20°C. Characters were measured after 7 and 21 days and include the presence of aerial mycelia (suppressed, abundant); substrate colour (light brown, dark brown, brown); colony area covered with sclerotia (very low 1-5%, low 6-15%, moderate 16-30%, heavy 31-50%, abundant over 51%); type of sclerotia (loose, aggregate); and sclerotia size (small >2mm, large <2mm).

5.5.3 Sensitivity of isolates of *R.solani* to fungicides

Isolates of *R. solani* were tested for sensitivity to the fungicides pencycuron (10 mg per litre of media), fenpiclonil (0.1 mg per litre media), fungazil (10 mg per litre media) and monceren IM (10 mg per litre media) in amended media, and growth was compared to that on an unamended control. For each isolate, one 5 mm plug from an actively growing culture was placed in each Petri dish and two replicate plates were used per treatment. Plates were incubated at 20°C and culture

diameters measured daily. Sensitivity to the fungicide was measured as a percentage reduction in colony diameter compared with the control. The reaction of isolates was classified in terms of percentage inhibition of colony growth (Hide *et al*., 1988) in terms of the guidelines, >10% resistant, 10-50% intermediate and <50% sensitive.

Isolates of *R. solani* (Table 30) were tested for sensitivity to the fungicides fenpiclonil, fungazil and monceren IM in amended PDA. For each isolate, one 5 mm plug from an actively growing culture was placed in an individual Petri dish, and two replicate plates were used per treatment. Plates were incubated at 20ºC and culture diameters measured after 2 days. Sensitivity to the fungicide was measured as a percentage reduction in colony diameter compared with the control.

5.5.4 Virulence of isolates of *R.solani*

Inoculum consisted of colonies of individual isolates grown in Petri dishes on PDA for one week at 20ºC. Three, 5mm mycelial plugs from the edge of individual colonies were placed on top of seed tubers of Pentland Javelin planted in 6 inch pots containing SCRI compost mix. Tubers were then covered in compost and pots were laid out in randomised block design with three replicate blocks and placed in a growth cabinet with 12 h photoperiod and set at 15ºC during light and 10ºC during dark. Control pots were treated with uninoculated plugs of PDA. Pots were watered so that compost remained damp and plants were left to grow until the end of their natural life to allow for the formation and setting of tubers. Tubers were assessed according to Føsund (1987). Four separate virulence tests were performed using different isolates but in each case the isolates R2 and R5 were used as a comparison between tests.

5.5.5 AFLP analysis of *R.solani*

AFLP was carried out on 67 isolates of *R.solani* as described in the general methods of this section (5.2)

Results and discussion

A total of 49 isolates of *R. solani* were obtained from 8 separate countries of which 34 isolates belong to Anastomosis group 3 (AG-3). Considerable variation exists between isolates of *R. solani* in terms of culture morphology both within and between individual AG's. However, no specific phenotypic character appears to be unique to AG3 (Table 29).

Significant ($P<0.001$) differences in sensitivity to Pencycuron were observed among isolates of *R. solani* with AG3 having a lower proportion of resistant isolates than other AG's. Sensitivity to Pencycuron could not be related to either culture morphology or country of origin. Significant ($P<0.001$) differences in sensitivity to Fenpiclonil, Fungazil and Monceren IM *in-vitro* were also observed. However, differences in sensitivity between these fungicides were not dramatic. For

example, R1 was most resistant isolate (72.6% growth compared to the control) and R30 the most sensitive isolate (30% growth compared to the control) when tested against fungazil (Table 30).

Testing the virulence of different isolates of *R. solani* AG3 was shown to produce repeatable results (Table 31). Data indicates that infection by *R. solani* on tubers of potato is not restricted to AG 3. Isolates of AG 1, AG 2-1, AG 2-2, AG 4, AG 6, AG 7 and AG 8 also caused symptoms of black scurf although these were less virulent than isolates of AG 3 (Table 31 and 33). Comparable results have been found in sugar beet where damping off, crown rot and foliar rot are mainly caused by AG 2-2 and 2-1. Glasshouse tests on seedlings revealed that isolates of AG 1, 3, 4 and 5 could all cause symptoms (Windels and Nabben, 1989). Significant differences ($P<0.001$) in virulence of individual AG's was also observed with isolates of AG 3 differing in their ability to produce black scurf (Table 32). However, none of the characters observed in culture could be related to virulence of individual isolates.

Considerable variation exists between isolates of *R. solani* in terms of phenotypic characters both within and between individual AG's. Unfortunately, there is a lack of suitable phytopathologic characters that can be related to specific AG groups. However, AFLP analysis has shown that isolates of AG-3 produces profiles distinct from those of other AG groups, and show a moderate degree of intra-specific variability.

Table 29. Origin, anastomosis group, colony morphology a) Aerial mycelium described as S= suppressed, M= moderate, A= abundant b) Substrate colour described as LB= light brown, B= brown, DB=dark brown c) Area covered in sclerotia described as a) very low; L = low; M= moderate; A= abundant and d) Sensitivity to pencycuron measured as a percentage reduction in colony diameter compared with the control. <10% reduction = resistant (R), 10-50% reduction = intermediate (I), >50% reduction = sensitive (S).

Isolate code	Host	Origin	AG group	Culture morphology			Sensitivity to pencycuron
				Aerial mycelia	Substrate colour	Area covered with sclerotia (c)	
R1	Potato	Scotland	3	M	B/LB	M	S
R2	Potato	Scotland	3	S	LB	H	S
R3	Potato	Scotland	3	S	LB	M	S
R4	Potato	Scotland	3	S	LB	M	S
R5	Potato	Scotland	3	S	LB	M	S
R8	Potato	Scotland	3	M	DB	L	S
R9	Potato	Scotland	3				R
R10	Potato	Scotland	3	A	LB	M	R
R12	Potato	Scotland	3	S	LB	M	S
R13	Potato	Scotland	3	S	LB	L	S
R14	Potato	N.	3	M	B/LB	L/M	S
R15	Potato	N.	3	M	B	M	R
R16	Potato	N.	3	M	LB	M	I
R17	Potato	N.	3	M/S	B/LB	M/H	S
R18	Potato	New	3	M	B/LB	L/M	S
R19	Potato	New	3	S	LB	M	S
R20	Potato	New	3	S	LB	M	S
R29			3	M	LB	M	
R31	Potato	England	3	S/M	B	H	S
R32	Potato	England	3	S	LB	L	S
R33	Potato	England	3	A	B	H	S
R34	Potato	England	3	S	LB	M	S
R36	Potato	Scotland	3	S	LB	M	S
R37	Potato	Scotland	3	S	LB	M	S
R43	Potato	France	3	S	LB	M	S

Isolate code	Host	Origin	AG group	Culture morphology			Sensitivity to pencycuron
				Aerial mycelia	Substrate colour	Area covered with sclerotia	
R44	Potato	France	3	M	LB	H	S
R45	Potato	France	3	M	LB	H	I
R46	Potato	France	3	S	B	L	S
R47	Potato	France	3	S	LB	L	S
R81	Potato	Japan	3	S	White	M	
R82	Potato		3	M	LB	H	S
R83	Potato	Japan	3	M	LB	M	
R84	Potato		3	S	LB	L	S
R85	Potato	Japan	3	S	LB	H	S
R7	Potato	Scotland	2-1	A	LB/Grey	M	S
R24			2-1	A	B	M	R
R40	Tulip	Holland	2-1	S	B	H	I
R41	Cauliflo	Holland	2-1	A	LB	L	S
R42	Cauliflo	Holland	2-1				S
R49	Potato	France	2-1				S
R38	Sugar	Holland	2-2	M	B	M	I
R39	Sugar	Holland	2-2	M	LB	H	
R50		Holland	2-2	A	LB	None	S
R51		USA	4	M	LB /	None	I
R48	Potato	France	5	S	LB	L	I
R53		Holland	5	M	yellow	H	R
R54		Holland	6	A	LB /	M	S
R55		England	8	A	LB	H	S
R56		Holland	8	S	Yellow	L	R

AG tester isolates

Isolate code	Host	Origin	AG group	Culture morphology			Sensitivity to pencycuron
				Aerial mycelia	Substrate colour	Area covered with sclerotia	
R21		England	1	A/M	yellow	M	R
R58	Rice	Japan	1-1A	M	LB	L	S
R60	Sugar beet	Japan	1-1B	S	B	M	S
R62	Sugar beet	Japan	1-1C	S	LB	M	R
R22		USA	2-1	M	B	VL	S
R23		England	2-2	A	LB	None	I
R74	Sugar beet	Japan	2-2-IV	M	LB	M	
R78	Soybean	Japan	2-3	M	LB	L	
R35		Holland	3	S	B	H	S
R25		England	4	M	yellow	M/H	R
R52		England	5	M	yellow	H	R
R26		England	6	A	LB	none	S
R27		England	7	A	LB	M	I
R28		England	8	M	LB	M	R

Table 30. Sensitivity of 21 isolates of *R. solani* on PDA amended with fenpiclonil (0.1 mg per litre media) and fungazil (10 mg per litre media). Plates were incubated at 20°C and culture diameter was measured after two days. Data is expressed as a percentage growth of an unamended control.

Isolate number	Fenpiclonil	Fungazil
R1	36.0	72.6
R2	55.2	68.6
R3	45.2	70.7
R4	47.9	67.4
R5	53.7	41.8
R7	48.3	41.9
R8	78.4	60.9
R9	38.8	43.3
R10	50.4	51.0
R12	43.1	59.8
R13	41.5	68.3
R14	45.6	67.5
R15	48.1	62.9
R16	37.5	61.5
R17	42.7	39.5
R18	41.9	57.9
R19	37.2	64.4
R20	37.7	65.4
R29	47.1	66.6
R30		30.2
LSD	9.8	8.7

Table 31. Pathogenicity of ten isolates of *R. solani* of various anastomosis groups on tubers of cv. Pentland Javelin. Plants were inoculated by placing mycelial plugs of individual isolates of *R. solani* in contact with seed tubers sown in compost. Plants were grown in a growth cabinet with 12 h photoperiod and set at 15ºC during light and 10 ºC during dark cycles. Disease assessment was based on the area of individual tubers showing sclerotia using a 1-9 scale (Føsund, 1987). Tests were performed in two years and rankings of individual isolates is shown in brackets.

Isolate	Anastomosis group	Year 1	Year 2
R2	AG 3	3.8 (2)	2.2 (3)
R4	AG 3	4.3 (1)	1.2 (6)
R5	AG 3	3.6 (3)	2.3 (1)
R11	AG 3	1.8 (8)	1.0 (7)
R14	AG 3	3.4 (4)	2.3 (1)
R21	AG 1	1.6 (9)	1.0 (7)
R22	AG 2-1	2.7 (5)	1.4 (5)
R23	AG 2-2	1.3 (10)	1.0 (7)
R25	AG 4	2.2 (7)	1.6 (4)
R26	AG 6	2.3 (6)	0.5 (10)
L.S.D Isolate		1.3 ($P < 0.05$)	
Test		0.6 ($P < 0.001$)	
Isolate x test		1.8 n.s	

Table 32. Pathogenicity of ten isolates of *R. solani AG3* on tubers of cv. Pentland Javelin. Plants were inoculated by placing mycelial plugs of individual isolates of *R. solani* in contact with seed tubers sown in compost. Plants were grown in a growth cabinet with 12 h photoperiod and set at 15ºC during light and 10ºC during dark cycles. Disease assessment was based on the area of individual tubers showing sclerotia using a 1-9 scale (Føsund, 1987).

Isolate	Anastomosis group	Disease severity (0-9)
R2	AG 3	2.6
R5	AG 3	2.8
R8	AG 3	1.5
R12	AG 3	2.2
R15	AG 3	2.6
R17	AG 3	2.3
R18	AG 3	3.3
R19	AG 3	2.5
R20	AG 3	3.2
R7	AG 2-1	2.5
L.S.D (0.05)		0.6

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