EFFECTS OF NUTRIENT STATUS, TEMPERATURE AND PH ON MYCELIAL GROWTH, SCLEROTIAL PRODUCTION AND GERMINATION OF *RHIZOCTONIA SOLANI* FROM POTATO

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SUMMARY

The effects of nutrient status, temperature and pH on mycelial growth, sclerotial production and germination of *Rhizoctonia solani* [anastomosis groups (AGs) 2-1 and 3] from potato were studied on a range of artificial media including potato dextrose (PDA), malt yeast extract (MYA), water (WA) and soil extract (SEA) agar. Greatest sclerotium yields were obtained on MYA for AG 3 and PDA for AG 2-1. Sclerotium yields were significantly poorer for AG 2-1 isolates compared to AG 3 isolates tested. Sclerotium yields were significantly improved when mycelia grown on nutrient poor (WA) agar were transferred to nutrient rich (PDA) media after 4 d for AG 3 isolates, but not for AG 2-1 isolates. Optimum temperatures for mycelial growth of all isolates were between 20 and 25°C on all media tested. Mycelial growth of all isolates occurred between pH 4 and 9, with an optimum of pH 5.6. Sclerotium formation occurred between pH 4 and 8 and pH 5 and 6 for AG 3 and AG 2-1 isolates, respectively. Sclerotial germination for all AG 3 isolates was optimal between 20 and 30°C on all media tested, with a pH optimum between pH 5 and 6. In soil, AG 2-1 isolates grew significantly slower compared to AG-3. Optimum temperature for mycelial growth in soil was between 20 and 25°C, regardless of anastomosis group. Sclerotial germination in soil by AG 3 isolates occurred between 10°C and 30°C. The significance of these results on the pathogenicity of *R. solani* is discussed.

INTRODUCTION

Rhizoctonia solani (teleomorph *Thanatephorus cucumeris*) was initially reported as a potato pathogen nearly 150 years ago (Kühn, 1858), and has since been proven to be a widespread and destructive fungal pathogen of many plant species (Menzies, 1970). There are currently 13 anastomosis groups (AGs) of *R. solani* (Carling *et al.*, 2003), with those belonging to AG 3 predominately responsible for the infection of potato plants worldwide (Bandy and Leach, 1988; Carling and Leiner, 1990a, 1990b; Chand and Logan, 1983; Hide and Firmager, 1990; Lehtonen *et al.*, 2008). Inoculum sources of *R. solani* include seed tubers and soil, both of which can harbour mycelium and sclerotia (Tsror and Peretz-Alon, 2005). *R. solani* infects subterranean stems and stolons, and severe lesions can have a negative effect on plant growth and tuber development (Banville *et al.*, 1996).

The development of sclerotia on the surface of progeny tubers is a significant problem for growers, with severe black scurf infestations significantly reducing the quality and value of crops destined for both the ware and seed markets (Carling *et al.*, 1986; Secor and Gudmestad, 1999). Sclerotia consist of loosely constructed knots of melanised hyphae, with no cellular differentiation into a rind or medulla (Townsend and Willets, 1954; Webster, 1980). Unlike many other sclerotia-forming plant pathogens, *R. solani* sclerotia only undergo direct myceliogenic germination, whereby vegetative hyphae capable of infecting the host grow directly out of the sclerotium (Coley-Smith and Cooke, 1971). Mycelia and sclerotia can grow and develop on plant debris as well as tubers, allowing inoculum to survive in the soil as well as on seed from season to season (Dijst, 1988; Gudmestad *et al.*, 1979).

Despite the negative economic impact of sclerotia on potato, both as a tuber blemish and a source of inoculum on seed and in soil, past studies on the effects of environmental and nutritional factors on *R. solani* pathogenic to potato have tended to focus on mycelial growth (Allington, 1936; Townsend and Willets, 1954). As a result, data on factors that influence sclerotial formation or germination by *R. solani* is limited. Therefore, the objective of this study was to investigate the effect of nutrient status, temperature and pH on mycelial growth, sclerotial production and germination of *R. solani* (AGs 2-1 and 3) isolated from potato.

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MATERIALS AND METHODS

Fungal isolates and sclerotial production. Two isolates of *R. solani* AGs 2-1 and three of AG-3 from potato (Table 1) were grown on potato dextrose agar (PDA; Sigma-Aldrich, UK) at 25°C. Sclerotia were harvested aseptically by scraping from the surface of 21-day-old cultures grown at 25ºC on solid malt yeast extract media (MYA) containing (per l^{-1}) 12 g agar technical no. 3 (Oxoid, UK); 15 g malt extract (Sigma-Aldrich, UK), and 5 g yeast extract (Sigma-Aldrich, UK). Harvested sclerotia were placed in fine mesh nylon bags and sterile distilled water washed through them to remove agar pieces. Washed sclerotia were transferred onto Whatman no.1 filter papers then placed in sterile plastic Petri dishes and left to dry in a laminar flow cabinet. Following drying, sclerotia were sized (425 and 1000 µm.) by dry sieving. Only AG-3 isolates were used in sclerotial germination studies as AG-2 isolates produced very loosely constructed sclerotia, making harvesting of sufficient numbers of individual sclerotia to test germination difficult.

Mycelial growth and sclerotium yield on agar. To examine the effect of nutrient status on mycelial growth, PDA, MYA, soil extract agar (SEA; 12 g agar technical no. 3; 250 ml soil extract in 750 ml of distilled water), and distilled water agar (WA; 12 g technical no. 3 agar l^{-1} distilled water) were prepared. Soil extract was prepared by adding 100 g of a loam-sand soil (pH 6.0 Diamond Field, SAC Auchincruive, Scotland) to 1-l of distilled water. This was left at room temperature for 3 d and the suspension filtered through Whatman no. 1 filter paper to remove debris. Finally, 250 ml of the filtered extract was made up to 1-l with distilled water and the appropriate amount of agar added prior to autoclaving. All media were autoclaved at 121°C for 15 min, and 20 ml poured into 9 cm diam Petri dishes. Four replicate dishes of each medium were inoculated centrally with a 3 mm mycelial disc cut from a 3-day-old colony of *R. solani* grown on PDA and incubated at 25ºC.

The effect of nutrient status on sclerotium yield was investigated using a modification of a method previously used by Dijst (1988). Petri dishes containing PDA, MYA and WA were prepared as described previously. The medium in each Petri dish was covered with an autoclaved 90 mm cellophane disc (PT 600; British Cellophane, UK), inoculated as described previously and incubated at 25°C for 4 days, with four replicate plates per medium. After 4 days, six treatments were applied: mycelial mats transferred from nutrient-poor or nutrient-rich media or vice versa (PDA→WA: WA→PDA), mycelial mats transferred to the same medium (PDA→PDA; WA→WA) or mycelial mats not transferred (PDA or WA no transfer). Using forceps, the cellophane discs and mycelial mats were aseptically removed and transferred to fresh media. Petri dishes were resealed with Parafilm and incubated at 25°C for a further 17 days. Radial mycelial growth (mm d^{-1}) was determined 2 and 6 days after initial inoculation and sclerotium yield [total dry weight biomass (mg) per dish] after 21 days. The cellophane discs remained intact for the duration of the experiment.

To examine the effect of pH, PDA was prepared with citrate phosphate (0.05 M citric acid, 0.1 M $Na_2HPO_4 \cdot 7H_2O$) and Tris (hydroymethyl) aminoethane (0.1 M Tris, 0.1 M HCl) buffers to achieve pH ranges of 4-7 and 8-9, respectively (Gomori, 1955). Petri dishes were poured and inoculated as described previously, with radial mycelial growth determined 2 and 6 days after inoculation and sclerotium yield after 21 days.

Sclerotial germination on agar. The effect of pH and temperature on sclerotia germination was investigated by placing ten sclerotia aseptically onto 9 cm Petri dishes containing the required medium (PDA, MYA and WA for temperature and buffered PDA for pH) at least 1.5 cm apart. Petri dishes were incubated from 5 to 30°C, with sclerotial germination determined after 72 h. Germination was determined by assessing individual sclerotia for outgrowing hyphae under a stereo binocular microscope at 45X magnification. A sclerotium was considered to have germinated when outgrowing hyphae were equal to or greater than its diameter and the percentage of sclerotia germinated per plate recorded.

Table 1. Origin and anastomosis groups of *Rhizoctonia solani* isolates used.

Isolate	Anastomosis group (AG)	Potato plant part isolated from	Geographical origin	Source
x46	2-1	stolon	NW England	Harper Adams University College
x81	2-1	tuber	NE Scotland	Harper Adams University College
x72	3	tuber	NE Scotland	Harper Adams University College
UN	3	tuber	Shropshire, England	Harper Adams University College
PK	3	tuber	NE Scotland	SAC, Aberdeen

Mycelial growth and sclerotial germination in soil. A soil sandwich technique modified from Grose *et al.* (1984) was used to investigate the effect of temperature on mycelial growth in soil. A brown earth soil (Dunnington Heath series, Wellesbourne, UK) was passed through a 3 mm sieve and air-dried for 7 days. The soil was adjusted to – 0.5 MPa in accordance with a soil moisture retention curve (w/w). Water was added as a fine spray and mixed thoroughly through the soil. Soil was added to 9 cm Petri dishes and a cellulose nitrate filter (85 mm, pore size 0.45 µm; Sartorius, Germany) placed over the surface to act as a platform for mycelial growth. Two black lines were drawn across the filter at right angles through the centre using a marker pen. The centre of each filter was inoculated with a 3 mm mycelial disc of PDA and covered with a 9x9 cm square piece of nylon netting. Further soil was added to cover the nylon netting and dishes were sealed with parafilm, weighed and incubated at 5, 10, 15, 20, 25 and 30°C. Dishes were re-weighed every 3 days to maintain the desired matric potential. After 10 days, the filters were retrieved and the radial mycelial growth across the surface of the filter determined along the four axes.

To investigate sclerotial germination in soil, Petri dishes (9 cm) were half-filled with soil and covered with a 5x5 cm piece of nylon netting. Sclerotia were arranged on the netting as described previously and a larger (8x8 cm) square piece of netting was placed over the top of the sclerotia and covered in a layer of soil. All dishes were sealed with parafilm and incubated at 20°C. Dishes were re-weighed and sterile distilled water was added as required every 3 days to maintain the required matric potential. Sclerotial germination was assessed after 10 days as described previously.

Statistical analysis. Data collected on the effect of pH on mycelial growth and germination were analysed using analysis of variance (ANOVA) and treatments means compared using the least significant difference (LSD) at a probability of 5% ($P = 0.05$) using Genstat® for Windows, 7th edition. The percentages for sclerotial germination were angularly transformed before analysis. The effects of temperature and media on mycelial growth, sclerotium yield and germination on artifical media and in soil were analysed using multiple linear regression. Parameter estimates were calculated using isolate PK and MYA as the baseline reference level and pairwise t probabilities were calculated to establish significance of the results.

RESULTS

Mycelial growth and sclerotial production. Mycelial growth occurred on all media tested, with significantly ($P = 0.05$) slower mycelial growth on soil extract agar (SEA) for all isolates of AG 3 and 2-1 (Table 2). Sclerotial production was minimal on water agar (WA) and SEA, with significantly ($P = 0.05$) more sclerotia produced on potato dextrose agar (PDA) and malt yeast extract agar (MYA), particularly for AG 3 isolates. Significantly higher sclerotium yields were produced on MYA by 2 out of 3 AG 3 isolates (PK and x72) than on PDA, whereas AG 2-1 isolates produced significantly more sclerotia on PDA than MYA. Both AG 2-1 and AG 3 isolates produced fewer sclerotia when grown on WA and SEA.

No increase in sclerotium yield was observed when actively-growing *R. solani* mycelium was transferred from WA to fresh WA after 4 days or left on WA for 21 days (Table 3). When actively-growing mycelia of AG 3 isolates were transferred from WA to PDA, sclerotium yields increased significantly ($P = 0.05$), however, this did not occur with AG 2-1 isolates. All AG 3 *R. solani* isolates produced more sclerotia when initially grown

Table 2. Radial mycelial growth rate and sclerotium yield of five isolates of *R. solani* on different media at 25°C.

	Media							
	Radial mycelial growth rate				Sclerotium yield			
Isolate (AG)	WA	SEA	MYA	PDA	WA	SEA	MYA	PDA
x81 (2-1)	10.2[a]	7.4	7.3	8.3	1.0[b]	0.8	2.03	13.5
x46 (2-1)	7.8	5.1	6.9	8.4	0.0	0.9	1.7	14.7
UN (3)	10.4	7.7	9.5	9.7	0.0	0.8	46.9	46.4
PK (3)	10.7	6.4	10.0	10.2	0.0	0.5	47.2	35.8
x72 (3)	8.1	6.0	7.3	8.3	0.0	0.4	70.7	53.8
P value			0.002				<0.001	
LSD ($P = 0.05$), DF = 57[c]			2.03				7.99	

[a]Values are the mean mycelial growth rates (mm d^{-1}) of four replicates measured between 2 and 6 d.

[b]Values are the mean total dry weight biomass (mg) of sclerotia from four replicate dishes.

[c]LSD is the least significant difference at 5% ($P = 0.05$), DF = degrees of freedom in ANOVA.

Table 3. Effect of transferring mycelial mats of five isolates of *R. solani* to and from nutrient poor (WA) and nutrient rich (PDA) media on sclerotium yield.

	Original Media					
	WA			PDA		
Isolate (AG)	No transfer	Transfer to WA	Transfer to PDA	No transfer	Transfer to PDA	Transfer to WA
x81 (2-1)	3.0[a]	2.0	5.2	16.0	10.4	10.7
x46 (2-1)	0.7	0.4	9.2	14.6	7.0	7.1
UN (3)	4.4	4.6	55.6	36.4	48.4	28.0
PK (3)	8.2	9.8	32.6	38.6	56.0	29.9
x72 (3)	3.0	2.1	36.4	67.9	36.7	48.2
P value				<0.001		
LSD ($P = 0.05$), DF = 116[b]				18.41		

[a]Values are the total dry weight biomass of sclerotia (mg) from four replicates after drying for 48 h at 70°C.
[b]LSD is the least significant difference at 5% ($P = 0.05$), DF = degrees of freedom in ANOVA.

on or transferred to a nutrient rich medium (PDA and MYA), compared to growth on a nutrient poor medium (WA) alone.

Temperature had a significant effect on mycelial growth rate ($P = <0.001$), with the optimum observed for all isolates between 20 and 25°C for all media tested (Fig. 1). There were no significant differences among mycelial growth rates of any AG 3 isolates for all temperatures and media tested. Multiple linear regression showed the mycelial growth of isolate x81 (AG 2-1) on WA ($P = 0.030$) was significantly different from AG 3 isolates, having a higher mycelial growth rate at 20°C.

Multiple linear regression analysis of mycelial growth rate on PDA, MYA and WA revealed a significant effect of temperature ($P = <0.001$) on sclerotium yield, with the optimum between 20 and 25°C for all isolates (Figure 1). Sclerotium yield on WA was almost nil for all isolates at all temperatures and the data were excluded from further analyses. Sclerotia were not produced by any isolate on any media at 5°C, and there were no significant differences among AG 3 isolates for sclerotium yield. AG 2-1 isolates x81 and x46 produced significantly fewer sclerotia ($P = 0.007$ and $P = 0.002$, respectively) than AG 3 isolates.

All isolates of *R. solani* were able to grow over a pH range of 4 to 9, with the optimum for mycelial growth at pH 5.6 (Table 4). Mycelial growth was significantly restricted ($P = 0.05$) at pH 4 and 9 for all isolates compared to pH 5.6. Sclerotial formation was observed between pH 4 and 8 for AG 3 and pH 5 and 6 for isolates of AG 2-1 (Table 4).

Sclerotial germination on agar. The optimum temperature for sclerotial germination was between 20 and 30°C for all AG 3 isolates tested (Fig. 1). Sclerotial germination was restricted at 5°C, with little or no germination observed. Percentage sclerotial germination was significantly lower ($P = 0.05$) at pH 4 (57.5 to 72.5%) and 9 (42.5 to 85%) compared to pH 5 (87.5 to 100%) and pH 6 (82.5 to 100%) (Table 4).

Mycelial growth and sclerotial germination in soil. Temperature had a significant effect on mycelial growth rate through soil (P=<0.001), with the optimum observed for all isolates between 20 and 25°C (Fig. 2). Multiple linear regression showed no significant differences between the growth rates of any AG 3 isolates at any temperature. Mycelial growth of AG 2-1 isolates x81 and x46 was significantly slower (P=0.008 and 0.002, respectively) than the AG 3 isolates. Incubation temperature had a significant effect on sclerotial germination in soil (P=0.003) after 10 days, with the optimum between 15 and 25°C depending on the isolate (Fig. 2).

DISCUSSION

This study has identified differences in the effect of environmental and nutritional factors on mycelial growth and sclerotium yields of *R. solani* isolates, both between and within two AGs pathogenic to potato. It was demonstrated that the *R. solani* isolates tested produced very few or no sclerotia in nutrient-poor conditions, although such conditions were permissive for mycelial growth. There were differences in sclerotium yields between AGs in this study, with AG 3 isolates producing significantly greater sclerotium yields than AG 2-1 isolates. AG 3 and 2-1 *R. solani* cultures initially grown on, or transferred to, nutrient-rich media produced higher sclerotium yields. These were far greater for AG 3 than 2-1 isolates. A previous study on *R. solani* AG 3 pathogenic to potato showed a similar pat-

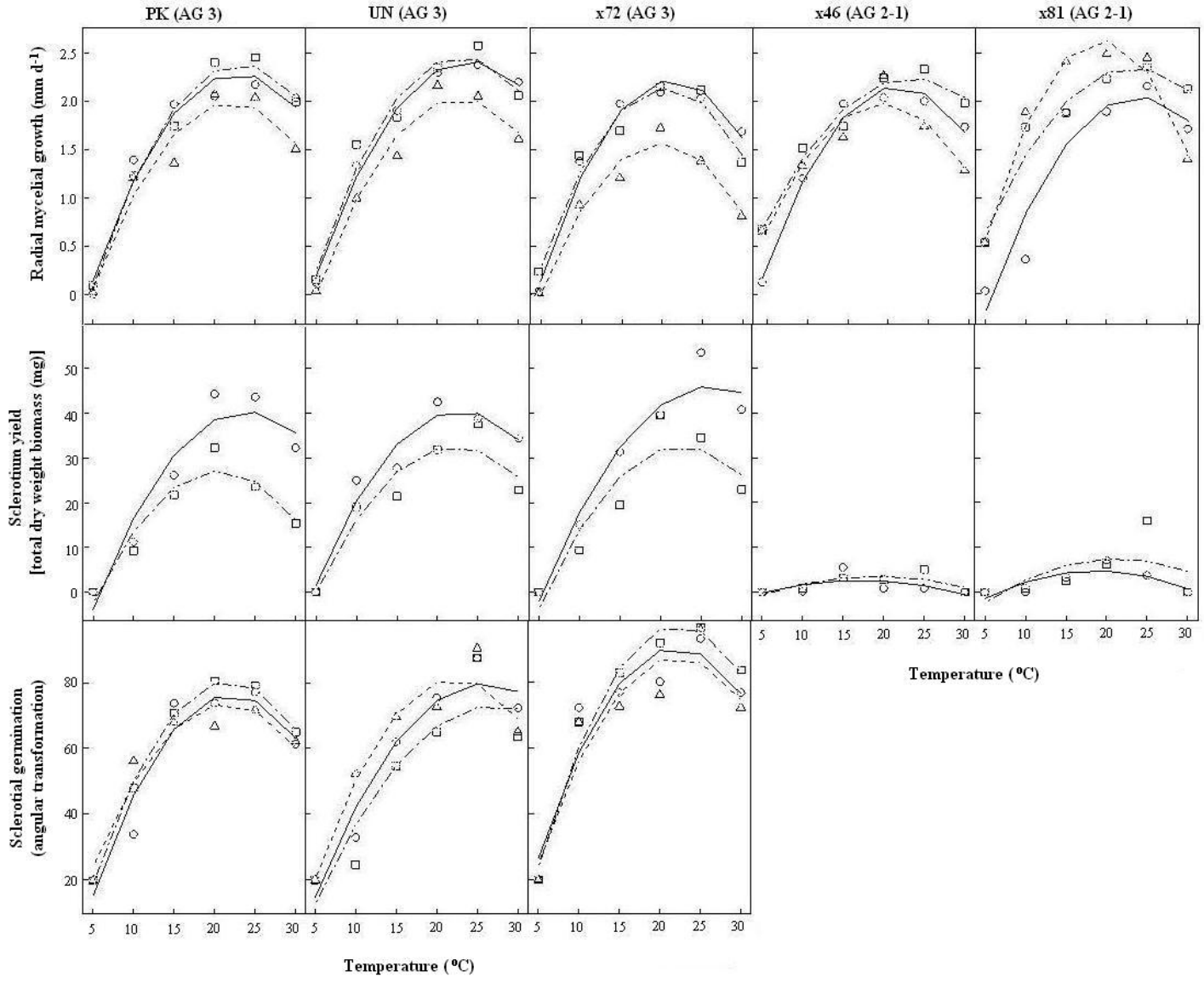

Fig. 1. Multiple linear regression analysis on the effect of temperature and media on mycelial growth rate, sclerotium yield and sclerotial germination on *R. solani*. Points represent the means of the original data values and lines show the fitted model for each medium tested (Δ --- = WA, □ – · – = PDA, ○ – = MYA). [mycelial growth rate: R^2 = 93.2, P = <0.001 (DF = 44); sclerotium yield: R^2 = 80.9, P = <0.001 (DF = 29); sclerotial germination: R^2 = 80.0, P = 0.022 (DF = 215)].

tern, in that sclerotial production was greatest when isolates were grown initially on a high-nutrient medium or were transferred from a nutrient-poor to nutrient-rich medium (Dijst, 1988).

Many fungi are able to grow over a wide pH range, with an optimum between pH 5.5 to 8 (Deacon, 1984). It has been demonstrated previously that if *R. solani* is able to initiate growth on moderately acid or alkaline media, it will usually modify the pH to one more favourable in order to grow successfully (Sherwood, 1970). In this study, *R. solani* AG 2-1 and AG 3 isolates from potato grew over a wide pH range, from pH 4 to 8 on buffered media. This corresponds well with the optimum pH range for mycelial growth of other *R. solani* isolates in previous studies. For example, a similar pH range (pH 3.5 to 7.5) was favourable for mycelial growth of *R. solani* pathogenic to poinsettia (Bateman, 1962). Another study demonstrated the optimum pH for mycelial growth of *R. solani* AG 1-1B isolates from lettuce to be between pH 5 and 6 (Grosch and Kofoet, 2003). In the current study, mycelial growth rates of *R. solani* isolates belonging to both AG 2-1 and AG 3 were optimal at pH 5.6. This is similar to a previous study where the optimum pH for mycelial growth for *R. solani* pathogenic to poinsettia was pH 5.8 (Bateman, 1962).

AG 2-1 isolates produced sclerotia over a narrower pH range (pH 5 to 6) compared with the AG 3 isolates (pH 4 to 8) in this study. Differences in sclerotial production in response to pH have been demonstrated previously between AGs, with AG 11 isolates from lupin

Table 4. Effect of pH on radial mycelial growth, sclerotium yield and germination by five isolates of *R. solani* after 21 d growth at 25°C.

	pH						
Isolate (AG)	4.0	5.0	5.6	6.0	7.0	8.0	9.0
Mycelial growth							
x81 (2-1)	1.0[a]	3.2	2.5	2.8	1.6	0.9	0.5
x46 (2-1)	2.1	4.9	6.0	3.4	3.5	1.9	2.5
UN (3)	4.9	7.7	8.1	7.9	7.3	6.1	2.9
PK (3)	3.3	4.9	9.6	7.8	4.0	3.0	2.0
x72 (3)	4.5	5.0	8.3	5.7	5.5	2.8	1.3
P value				<0.001			
LSD (*P* = 0.05), DF = 102[d]				0.90			
Sclerotium yield							
x81 (2-1)	0.0[b]	0.8	6.3	1.1	0.0	0.0	0.0
x46 (2-1)	0.0	1.2	3.7	3.4	0.0	0.0	0.0
UN (3)	5.9	8.3	27.2	18.2	12.2	0.0	0.0
PK (3)	0.0	15.1	22.0	15.0	6.5	0.0	0.0
x72 (3)	8.5	9.6	28.9	11.2	0.0	10.5	0.0
P value				<0.001			
LSD (*P* = 0.05), DF = 102				7.51			
Sclerotial germination							
UN (3)	72.5 (59.1)[c]	100 (90.0)	100 (90.0)	100 (90.0)	95.0 (80.8)	87.5 (69.5)	85.0 (67.5)
PK (3)	67.5 (55.7)	90.0 (74.1)	87.5 (69.5)	92.5 (76.2)	77.5 (62.1)	75.0 (60.3)	42.5 (40.4)
x72 (3)	57.5 (49.4)	100 (90.0)	100 (90.0)	100 (90.0)	77.5 (62.9)	65.0 (54.6)	62.5 (52.6)
P value				(0.006)			
LSD (*P* = 0.05), DF = 102				(11.01)			

[a]Values are the mean mycelial growth rates (mm d^{-1}) of four replicates measured between 2 and 6 d.
[b]Values are mean total dry weight biomass (mg) from four replicate dishes after drying for 48 h at 70°C.
[c]Values are the mean percentage of sclerotia germinated (angular transformed data) of four replicates. Analysis was carried out on transformed data.
[d]LSD is the least significant difference at 5 % (*P* =0.05), DF = degrees of freedom in ANOVA

producing sclerotia between pH 4 to 9, and AG 8 producing no sclerotia at any pH tested (Kumar *et al.,* 1999). The reasons for the effect of pH on sclerotia production by AG 2-1 and AG 3 is unclear and merits further investigation. Membrane permeability is affected by pH, therefore the ability of the fungus to take up nutrients required for mycelial growth and sclerotial production may have been affected at different media pH (Deacon, 1984). It is also possible that the buffers may have been toxic at the concentration used for efficient buffering, and this may have had an effect on both mycelial growth and sclerotium yield (Carlile *et al.,* 2001). Both germination of sclerotia and mycelial growth of AG 2-1 and AG-3 isolates in this study was found to occur over a broad pH range, from pH 4 to 8, with an optimum between pH 5 to 6, suggesting that *R. solani* isolates pathogenic to potato are likely to proliferate in most agricultural soils in which potatoes are grown. Further experiments are required to investigate the effect of soil pH on the germination of *R. solani* sclerotia.

The optimum temperatures or range for mycelial growth of the isolates of AG 2-1 and AG 3 isolates we found was between 20 and 25°C. A previous study on AG-3 and AG 2-1 isolates from potato found the optimum temperature for growth to be between 22 and 25°C (Chand and Logan, 1983). Differences in optimum temperature *in vitro* mycelial growth were found

in *R. solani* isolates from potato in Peru, with the optimum for AG 3 between 20 and 25ºC (Anguiz and Martin, 1989).

Temperature had a significant effect on the mycelial growth rate through soil of all 5 isolates of *R. solani*. The growth curves produced were similar to those observed when isolates were grown on agar, however, mycelial growth rates were much slower in soil. AG 2-1 isolates grew significantly slower than AG-3 isolates, with an optimum for mycelial growth between 20 and 25°C for all isolates. Sclerotial germination occurred over a broad temperature range (10 to 30°C).

Sclerotium yield was optimal between 20 and 25°C for both AG-3 and 2-1 isolates in this study with AG 3 producing significantly more sclerotia than AG 2-1. Differences in sclerotial production between AGs has been observed previously *in vitro*, with AG-3 isolates from potato producing numerous sclerotia at 20 to 25ºC and AG 4 isolates producing very few at 25ºC (Anguiz and Martin, 1989). Another study comparing sclerotial production by AG-3, 4 and 5 isolates from potato found only AG 3 produced sclerotia under the conditions tested (Balali *et al*., 1995).

Recent UK studies on the soil population of *R. solani* affecting potato have shown that 92% of isolates belong to AG-3, and 7 and <1% belong to AG-2 and AG-5, respectively (Woodhall, 2007). In this study, AG-3 isolates grew faster and produced significantly more sclerotia at most temperatures and pHs tested than AG 2-1 isolates. Similarly, in a previous study, AG-3 isolates grew faster and produced greater sclerotial biomass at most water potentials tested and over a wider range, than AG 2-1 isolates (Ritchie, 2007). This ability of AG-3 to grow faster and produce more abundant sclerotia under a range of environmental conditions may be a contributing factor in the dominance of this AG over others in causing disease in potato.

There appears to be no previously published studies on the effects of nutritional and environmental factors on the germination of *R. solani* sclerotia pathogenic to potato. This study has identified, for the first time, numerous factors that have an effect on germination of *R. solani* AG 3 isolates *in vitro*. Although the *in vitro* studies reported here do not directly simulate the conditions of the natural environment, the results provide an insight to the likely behaviour and growth of the pathogen in soil. Further investigation into the pathogenicity of different anastomosis groups and the survival of sclerotia under field conditions would provide a greater understanding of the biology of this plant pathogen.

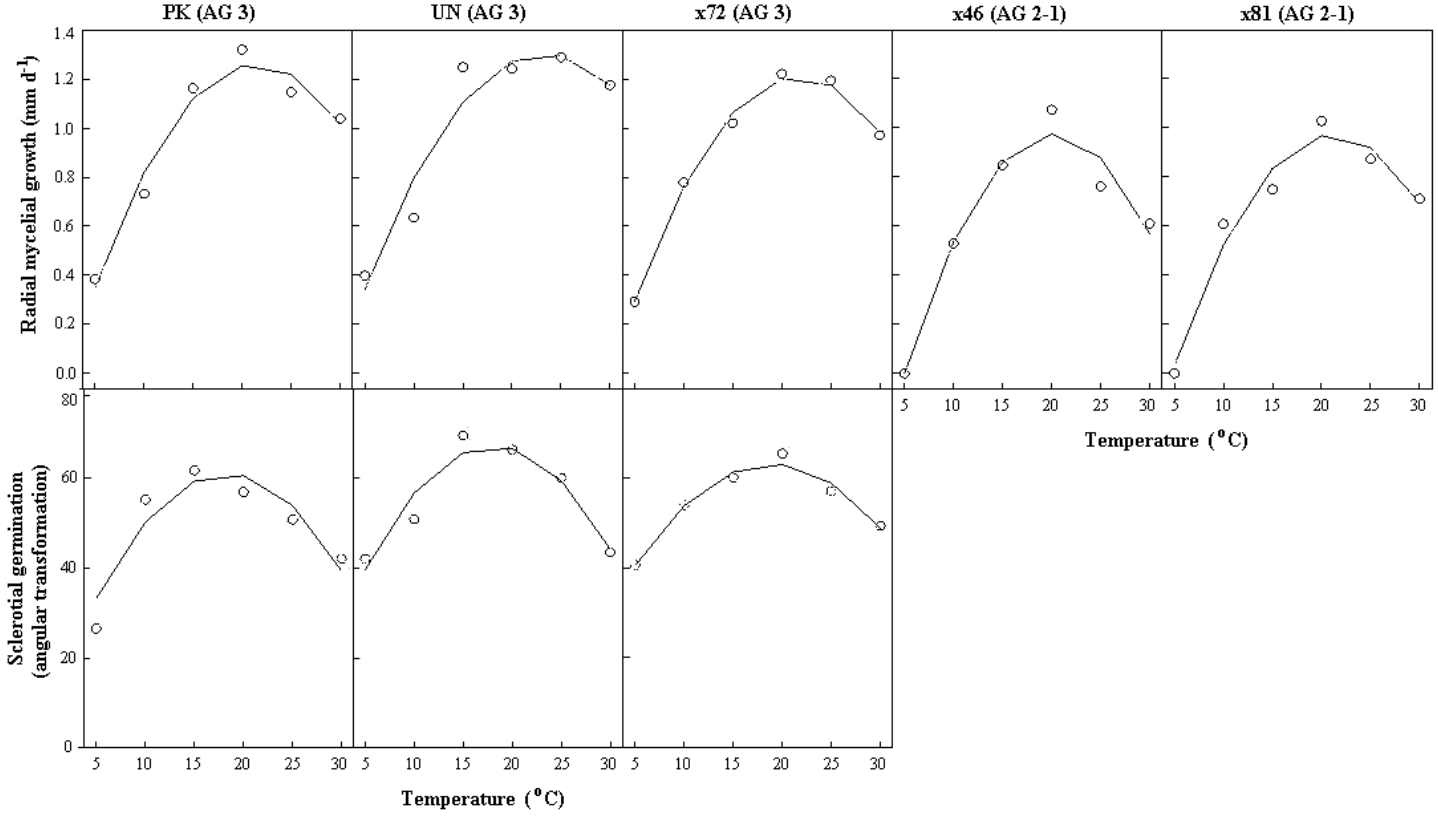

Fig. 2. Multiple linear regression analysis on the effect of temperature on mycelial growth rate and sclerotial germination in soil on *R. solani*. Points represent the means of the original data values and lines show the fitted model for each isolate tested. [mycelial growth rate: $R^2 = 70.3$, $P = 0.003$ (DF = 69); sclerotial germination: $R^2 = 85.8$, $P = <0.001$ (DF = 119)].

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