Informing Management of Potato Diseases through Epidemiology and Diagnostics

Rhizoctonia solani Reviews

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Contents

Contents **3**
Introduction **4**
Biology **4**
Infection Process 4
Symptoms 4
Anastomosis Groups (AGs) **5**
Which Anastomosis Groups are Important on Potato? 6
Results from recent survey work 7
Effects of Individual AGs on Potato 9
Results from recent work on the effects of AG on potato disease 10
Other Differences Between AGs 12
***Rhizoctonia* Inoculum Detection** **13**
What levels of inoculum are found on seed and in soil in commercial potato production? 13
What level of inoculum can we detect in soil? 14
Inoculum detection: summary and conclusions 15
The Contribution of Hyphae and Sclerotia to *Rhizoctonia solani* Disease Development **16**
Role of Soil- and Seed-borne Inoculum in Development of *R. solani* Symptoms **17**
Crop Rotations, Potatoes and *Rhizoctonia* Diseases **23**
Control of *Rhizoctonia solani* in Potatoes **26**
References **32**
Appendix I: Examples of Symptoms caused by *R. solani* **40**

Rhizoctonia solani Reviews

Introduction

These mini reviews were produced by researchers involved in the joint levy body (Potato Council and Horticulture Australia Ltd) funded project on diagnostics and disease management. The reviews summarise the information on *Rhizoctonia solani* generated as part of the project and also make reference to other published information and previously completed levy body-funded work. They are intended to provide an overview of information on the pathogen and disease symptoms, whereas the project report (R422 "Informing Management of Potato Diseases through Epidemiology and Diagnostics") provides the details of the work carried out during the three year programme of research.

Biology

Infection Process

Infection of potato by the fungus *R. solani* occurs by the formation of infection cushions on the developing stem and penetration into the plant occurs from these cushions either with hyphae forcing their way between the epidermal cell walls or through the formation of penetration pegs (Chand *et al.,* 1985). Stomatal entry can also occur but this is considered a rare occurrence (Dodman and Flentje, 1970). Stem infection can result in the appearance of a lesion or lesions (stem canker). More severe infections result in girdling and ultimately stem death. Death of the developing sprouts forces the development of numerous secondary stems (Baker, 1970), thereby delaying, or in some cases totally preventing, emergence

Symptoms

In potato, *R. solani* infection is primarily associated with stem cankers and tuber blemishes, notably black scurf. These diseases can result in both quantitative and qualitative damage to the potato crop. Quantitative losses occur due to infection of stems, stolons and roots and observations of yield losses approaching 30% have been noted (Banville, 1989; Woodhall *et al.,* 2008). Stem and stolon canker consistently decrease tuber yields (Banville, 1989; Carling *et al.,* 1989; Hide *et al.,* 1989a) and can cause a greater number of non-target seed sizes (Simons and Gilligan, 1997). Root infection has also been observed to have an important influence on tuber yield (Woodhall *et al.,* 2008). Qualitative losses mainly occur through the production of misshapen tubers and the development of sclerotia on the tuber surface (known as black scurf). Sclerotia are irregularly shaped masses of fungal mycelium brown to black in colour adhering tightly to the surface of the tuber. Sclerotia formation does not result in physical damage to the tuber but sclerotia present on seed tubers can initiate disease in subsequent crops. Black scurf disease occurs wherever potatoes are grown, but is suspected to be more severe in temperate climates (Jeger *et al.,* 1996).

In some cases, *Rhizoctonia* potato disease is associated growth cracks (Banville *et al.,* 1996; Campion *et al.,* 2003) and tuber greening (Hide *et al.,* 1989b). Morphological alterations of the progeny tubers have been attributed to the plant's inability to process the excess photosynthate that accumulates when the flow is interrupted by stem and stolon lesions (Hartill, 1989). Netted scab symptoms, also called giraffe neck or elephant hide (russeted scabby areas on the tuber surface) have been associated with *Rhizoctonia* potato disease (Baker, 1970), although in the

UK at least, the Potato Mop Top Virus is also associated with this symptom as well as *Streptomyces* species and possibly Potato Virus Y.

In the case of some tubers symptoms (elephant hide, cracking and dry core) there may only be slight differences in the appearance of symptoms caused by *R. solani* and those caused by other factors (*Streptomyces* spp. infection, irregular soil moisture, and wireworm infection, respectively). **Appendix I provides examples of symptoms caused by *R. solani*** for reference. In some cases, *R. solani* has definitely been confirmed as the cause of the symptom (ie Koch's postulates[1] have been met). This is indicated beneath the relevant image. In other cases testing of Koch's postulates was not carried out but the researchers, based on experience, have attributed the symptoms to *R. solani* infection.

Symptoms observed above ground are likely to be secondary responses due to stem infection. Leaf rolling, stunting, rosetting and purple pigmentation in the leaves have all been observed (Banville *et al.,* 1996). Purple pigmentation is attributed to anthocyanin accumulation due to stolon infection (Baker, 1970). Late in the growing season 'white-collar' symptoms are often observed at the base of stems, particularly during moist and warm conditions (Jeger *et al.,* 1996). Basidia are typically present within the white-collar. However the significance of basidiospores in the disease cycle is currently unknown: germination of these field-grown basidiospores is difficult to induce *in vitro* and their appearance coincides with canopy closure in the potato crop which would limit wind dispersal (Jeger *et al.,* 1996).

Yield losses and symptomology are not always consistent when the causal agent is identified as *Rhizoctonia*. Factors such environment, inoculum level and disease management strategies explain this in some respects, but identification of the Anastomosis Group (AG) present (see below) can also be fundamental in understanding the pathology of the outbreak.

Anastomosis Groups (AGs)

Rhizoctonia solani is a species complex consisting of multiple anastomosis groups (AGs). Isolates are assigned to an AG on the basis of hyphal fusion. The present system for categorising hyphal fusion in *R. solani*, (originating from the work of Carling and co-workers: Carling and Leiner, 1987; Carling *et al.,* 1988) consists of four categories of reaction (C0 to C3). Perfect fusion of the hyphae is classed as C3 and usually occurs between genetically identical or near identical isolates (Cubeta and Vilgalys, 1997). These isolates are therefore considered part of the same vegetativley compatible group (VCG). 'Killing' or C2 reactions are designated when hyphal wall and membrane fusion are evident, but after a period of time cell death occurs in the fused and adjacent cells. Both C2 and C3 are usually indicative of the same AG. However, C1 reactions, designated when hyphal wall contact occurs but there is no evidence of membrane fusion (Carling and Leiner, 1987), can also occur between diverse members of an AG (e.g. between subgroups) or closely related AGs (bridging relationships). C0 is designated when 'no recognition' or 'no interaction' occurs between isolates meaning that the isolates are likely to belong to different AGs.

[1] Koch's postulates: the method for identifying the micro-organism causing a disease. It is based on four criteria that must be met before a micro-organism can be confirmed as the cause of a disease/symptom

Observation of hyphal fusion using light microscopy and determining the category of hyphal interaction occurring is the traditional method for determining AG. However, this method is laborious and can leave some ambiguity in AG determination (a C1 reaction can occur between isolates of the same AG and between different AGs). Consequently, a multitude of AG typing assays based on immunological and molecular genetic techniques have been developed to identify AG. This is reviewed in depth elsewhere (Sharon *et al.,* 2006) but techniques such as Polymerase Chain Reaction (PCR) and DNA sequencing represent powerful molecular tools to identify AGs. Real-time PCR assays have already been developed for several AGs and subgroups: AG3 (Lees *et al.,* 2002); AG8, AG2-1 (Budge *et al.,* 2009). These assays can quantify the pathogen *in planta* and can be used in conjunction with soil DNA extraction techniques. Such assays are not only a powerful tool to study the epidemiology of the disease in soil (Lees *et al.,* 2005) but also in risk prediction/decision support system for growers (OphelKeller *et al.,* 2008).

Thirteen AGs of *R. solani* are currently known to exist (Carling *et al.,* 2002). Each AG can vary in host range and often geographic locations. Table 1 lists some of the principal hosts associated with individual AGs.

Table 1. Anastomosis groups of *Rhizoctonia solani*, host, associated valid names and persons credited with discovery of the AG (adapted from Roberts, 1999)

AG	Typical hosts	Discovery credited to:	†
1	Rice, corn, bean	Parmeter *et al*. (1969)*	*T. sasakii* (AG1-IA) *T. microsclerotia* (AG1-IB)
2	Crucifers, sugar beet, carrot,	Parmeter *et al*. (1969)*	
3	Potato, tobacco	Parmeter *et al*. (1969)*	
4	Bean, cereals, root rots	Parmeter *et al*. (1969)*	*T. praticola*
5	Potato, turf grass, root rots	Ogoshi, (1976)	
6	Orchid mycorrhizal	Kuninaga *et al.* (1978)	
7	Carnation, radish, soybean, saprophyte	Homma *et al.* (1983)	
8	Cereals	Neate *et al.* (1985)	
9	Crucifers, potatoes, saprophyte	Carling *et al.* (1987)	
10	Wheat, barley	Ogoshi *et al.* (1990)	
11	Lupin, wheat	Carling *et al*. (1994)	
12	Orchid mycorrhizal	Carling *et al*. (1999a)	
13	Cotton	Carling *et al.* (1999b)	

*Presence of four anastomosis groups before Parmeter *et al.* (1969), who is credited with designation of AG1 to AG4.

†In some classifications, individual AGs have been classed as species and given specific names but this has not generally been accepted.

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Which Anastomosis Groups are Important on Potato?

In the literature there are records for all of the individual AGs occurring on potato either naturally or experimentally. However, most previous work concludes that AG3-PT (the potato subgroup) is the predominant AG in potato crops (Tsror, 2010).

AG3-PT is one of three subgroups of AG3, the other subgroups being AG3-TB, the tobacco subgroup, and a new subgroup consisting of tomato isolates (Bartz *et al.,* 2010). Isolates of AG2-1, AG2-2, AG4, AG5 and AG8 have also been consistently recovered from potato in various studies. The proportion of which varies with each study. The only exception, where AG3 was not the predominant AG, was the work of Anguiz and Martin (1989), who reported that AG4 was the predominant group in warm, lowland areas of Peru. They showed that AG4 had an optimum growth temperature of 25-28°C, compared to 20-25°C for AG3 and thus suggested that climate can influence the distribution of certain AGs. The presence of AG4 was also observed in other investigations, and again the apparent influence of climate can be seen; in the partially tropical climate of Mexico, 26.4% of infections were caused by AG4 (Virgen-Calleros *et al.,* 2000), but in the cool Alaskan climate, Carling and Leiner (1986) found no AG4 present.

Binucleate *Rhizoctonia* (BNR) and AG2-1 have been found in several surveys, but have not usually been shown to cause more than a small percentage of *Rhizoctonia* disease cases. Similarly, AG5 has been found in several investigations. Gudmestad *et al*., (1989) suggested that cropping history is an important factor in determining which AGs are present. In their study, AG4 was only present in samples grown in fields with no previous potato cropping history. In addition, they found that 56% of *R. solani* isolates recovered from wheat were AG5, thereby showing that certain groups can successfully colonise other hosts. The presence of AGs other than AG3 may have been caused by their introduction and propagation in other crops.

Several studies have recovered a high proportion of isolates that could not be successfully assigned to a particular AG. For example, Anguiz and Martin (1989) failed to assign 26% of their isolations to AGs. Similarly, Bandy *et al*., (1988) failed to assign 2.3% and Carling and Leiner (1986) 3.6% of isolates. Since most investigations of this type have used the hyphal fusion method to determine AG, such difficulties can be understood. To overcome such failures, a combination of molecular and hyphal fusion methods were used in more recent studies study to determine the relative incidence of anastomosis groups present in potato crops (Woodhall *et al.,* 2007).

Results from recent survey work

As mentioned above, AG3 occurs as different subgroups (strains) including AG3-PT which occurs on potatoes. The available PCR assays are either specific for AG3-PT or detect all subgroups of AG-3. Therefore in some parts of the review AG3-PT is mentioned and in others, such as some sections below, AG3 is discussed. This merely reflects differences in the PCR assays which were used to carry out the research..

GB

Real-time PCR was used to survey 86 batches of potato seed for AG2-1, AG3-PT, AG5 and AG8 (Table 2). It was also used to determine the presence of these AGs in soils pre-planting and post-harvest. AG3-PT was found in over half the tuber batches tested. AG2-1 was also frequently found. AG5 was only present in one batch.

In soil AG3-PT, AG2-1, AG5 AG8 were found. AG2-1 is frequently found in soil but less frequently isolated form potatoes. AG3-PT was found in more sites at, or after

harvest than at planting suggesting it was seed-borne in these cases or surviving at below detection levels in soil.

For a range of samples in both the seed and soil surveys, isolations were taken to validate and confirm the real-time PCR result. In all instances, these matched up with the PCR results except one, where a new BNR species was isolated – no PCR assay exists for this species. This suggests that the real-time PCR assays are largely correct at detecting AGs causing *Rhizoctonia* potato disease although some new uncharacterised strains may possibly remain, particularly for BNR species which is a relatively poorly studied group compared to *R. solani*.

Table 2. Summary of all data monitoring AGs in GB potato crops and all field soil samples (taken as part of project R422).

	Isolation			Real-time PCR all years		
	Stems, stolons & roots*	Tubers*	Tubers	Tubers	Soil at planting	Soil at harvest
AG2-1	4	5	2	40	40	29
AG3-PT	61	64	32	45	5	23
AG5	0	1	1	1	2	3
AG8	0	0	0	0	26	16
BNR	0	0	1	nt	nt	nt
Total number of samples	176		70	86	83	64

Australia

Potato plants were collected as volunteers from fields previously cropped to potatoes and from commercial crops (Table 3). Samples were collected from multiple sites in south-eastern South Australia (SE of SA), Kangaroo Island (KI) and Tasmania (Ta) between 2005 and 2007. Isolates of AG2-1, AG2-2 and AG3-PT were recovered. Isolates categorised as “other” were classified as binucleate *Rhizoctonia*, because of high similarity to the binucleate Rhizoctonia groups AG-A and AG-K in a search of available sequences on NCBI databases (over 98% identity).

Table 3. Number of isolates from each region classified by PCR to AGs.

	Number of isolates in each AG							
Region	**2-1**	**2-2**	**3**	**4**	**5**	**8**	**Other**	**Total**
Kangaroo Island, SA	44	44	27	0	0	0	12	127
Tasmania	3	2	28	0	0	0	0	33
Penola Region, SA	51	46	70	0	0	0	1	168
Lenswood SA	1	0	0	0	0	0	0	1
Total #	99	92	125	0	0	0	13	329
% of total	30	28	38	0	0	0	4	100

New Zealand
Isolates of *R. solani* were collected from black scurf on potato tubers of different cultivars, from 14 different potato growing regions in New Zealand. 129 *R. solani* isolates were collected and preliminary analysis revealed that 110 were AG-3PT, 18 were AG-2-1 and one was AG-5. A potentially virulent isolate of AG4 HG-I was also recovered from soil.

South Africa
Ninety-two isolates of *R. solani* were obtained from tubers displaying elephant hide and sclerotia from eleven potato growing areas of South Africa. Initial results have confirmed the presence of AG3.

Effects of Individual AGs on Potato

The influence of AG on disease of the growing plant (Stems, stolons and roots)
Often AGs of *R. solani* are limited in their ability to infect different parts of the potato plant. For example, Carling *et al*., (1998) demonstrated the infection of stems, stolons and tubers but not roots by AG7, whilst Hide and Firmager (1990) observed that AG8 was only capable of infecting potato roots and did not cause stem canker or black scurf. In a comprehensive study, Carling and Leiner (1990) compared isolates from AGs 1 to 9 in controlled environment cabinets, and found significant differences in the severity of infection on potato initiated by each of the AGs. For example, AGs other than 3, 4, 5 and 8 caused minimum damage to potato stems and roots whereas AG4 and 5 only caused significant disease at temperatures above 15°C.

Balali *et al.* (1995) found similar results to Carling and Leiner (1990) in that AGs 3, 4, 5 and 8 could all cause severe potato infections. In a glasshouse study conducted at 25°C they observed deep stem cankers, girdling and pruning with AGs 3, 4 and 5 whilst only superficial stem infections with AG8. AG4 and AG8 both caused severe root infections compared to root infections caused by AG3 or AG5. Bains and Bisht (1995) reported that AG3 caused significantly more severe stem disease compared to AGs 4 and 5. AG2-1 was reported to rarely cause stem canker and when present, the severity of symptoms was minimal (Carling and Leiner, 1986; Carling and Leiner, 1990; Chand and Logan, 1983). Lehtonen *et al.*, (2009) reported that stem disease symptoms were mild with AG2-1 compared to AG3-PT and AG5. However, Petkowksi and de Boer (2001) reported that some AG2-1 isolates could cause stem infection of a severity comparable to AG3. Woodhall *et al.,* (2007; 2008) found considerable variation in stem disease caused by AG2-1. In pathogenicity tests in glass house conditions in the UK, seven isolates of AG2-1 were tested for stem disease. A wide range of disease severity was present, with one isolate causing most severe disease compared to even 28 isolates of AG3-PT, whilst other AG 2-1 isolates caused little or no stem disease. AG3 and AG5 isolates caused considerable stem canker in these experiments, whilst an isolate of AG8 appeared to cause exclusively root disease, even under field conditions. Despite only affecting roots, this resulted in over 20% yield loss by weight. AG3 also affected roots but not to such an extent.

Temperature can also influence the severity of stem disease caused by various AGs, Carling *et al.,* (1990) observed that AG3 caused more stem damage at 10ºC compared to 15.5 and 21ºC, whilst AG5 only caused damage at 15.5 or 20ºC.

The influence of AG on disease on tubers

The severity of black scurf on potato tubers caused by different AGs also varies. Both Hide and Firmager (1990) and Balali *et al.* (1995) observed no black scurf associated with AG8 infection in controlled environment conditions. Balali *et al.,* also showed that AG4 did not cause black scurf, whereas AG5 only caused slight (1-25 sclerotia per tuber) to moderate (25-50 sclerotia) black scurf and AG3 had the ability to cause severe black scurf (50 or more sclerotia). Conversely, Campion *et al.* (2003) found that AG5 did not cause black scurf on any of the five cultivars they tested but did cause tuber deformations and superficial alterations similar to that reported by Bandy *et al.,* (1984), who found that AG5 produced sunken brown lesions on the cultivar Katahdin. Campion *et al.,* (2003) also reported that AG3 caused a 70-100% incidence of black scurf on tubers in pathogenicity tests and that whilst AG2-1 did not cause any black scurf, AG2-1 did have the ability to cause tuber deformations.

In a field experiment, Woodhall *et al*., (2008) found that AG3-PT caused considerable levels of black scurf (84% incidence). An isolate each of AG2-1 and AG5 was tested and these caused black scurf infrequently (less than 3.2%). The authors also determined that AG3 isolates have greater ability of producing sclerotia *in vitro* than isolates of AG2-1, AG4, AG5 and AG8. These results were concordant with the findings of Lehtonen *et al.,* (2009) in glass house conditions in Finland who determined that AG3 isolates had a higher ability (at least 84% incidence in all experiments) to form sclerotia on tubers than AG2-1 and AG5 isolates, with only one isolate of AG2-1 forming sclerotia relatively infrequently (13.5%) on progeny tubers in one experiment.

Results from recent work on the effects of AG on potato disease

In project R422, experiments under controlled conditions were used to determine the effects of different AGs on potato disease. The results are summarised below.

GB

A controlled environment experiment was undertaken to compare isolates of AG2-1 (four isolates), AG3 (isolates representing all three sub-groups), AG4 (isolates from two of the three HG-I, HG-II and HG-III subgroups of AG4), AG5 and the BNR isolate found in the project. The experiment was in two parts, plants were harvested after four weeks to investigate stem, stolon and root disease and also at 18 weeks to determine the effect on tubers. The experiment found that stem disease was most severe in one isolate of AG3-PT, two AG2-1 isolates, the AG5 isolate and the AG4 isolate belonging to subgroup HG-III. The BNR isolate also caused mild stem canker symptoms, similar to some AG2-1 isolates.

In tubers, elephant hide symptoms were observed with the BNR isolate, AG5 and one isolate of AG3-PT. The less aggressive stem canker isolate caused greater levels of black scurf, whilst the aggressive stem canker isolate caused more elephant hide.

The pathogenicity within AG4 was investigated in a greater range of AG4 isolates in another experiment. An isolate of AG4 HG-III caused severe stem and stolon disease whilst one AG4 HG-II isolate did not. The other isolate of AG4 HG-II caused stem death. This experiment confirms variation amongst AG4 subgroups in terms of pathogenicity to potatoes.

Australia

Plants inoculated with AG2-1, AG2-2 and AG3 in a shade-house trial all displayed stem, stolon and root necrosis and tuber sclerotia. Variation was observed within isolates from the same AG. However, one AG2-1 and one AG3 isolate caused the most severe stem necrosis and AG 3 the most severe black scurf. Yields do not reflect that produced by field plants and could not be sorted into size categories. However, plants had reduced yields compared to uninoculated plants with increased severity of symptoms of:

Root sclerotia only (AG2-1)

Stem necrosis only (AG2-1)

Stem, stolon and root necrosis and root sclerotia (AG2-2)

Stem, stolon and root necrosis and tuber and root sclerotia (AG3)

AG5 and AG8 were later tested in short term experiments and caused stem and root necrosis, respectively. One AG4 isolate was tested in a fungicide trial in which inoculated controls didn't emerge, thus statistical analysis could not be performed. However this isolate caused stem necrosis and tuber sclerotia. One BNR isolate was tested for its ability to cause disease and did not cause stem necrosis.

Table 4. Ability of individual AGs to cause disease to parts of potato in field and shade house conditions

AG	Field				Pathogenicity tests				
	stem	stolon	Root	black scurf	stem	stolon	root	black scurf	root sclerotia
AG 2-1	✓	✓	✓	✓	✓	✓	✓	✓	✓
AG 2-2	✓	✓	✓	✓	✓	✓	✓	✓	✓
AG 3	✓	✓	✓	✓	✓	✓	✓	✓	✓
AG 4	×	×	×	×	✓	✓	-	✓	-
AG 5	×	×	×	×	✓	✓	-	-	-
AG 8	×	×	×	×	×	×	✓	×	×

- Not tested

New Zealand

Experiments were conducted to determine the pathogenicity of 54 isolates to potatoes including five known AG tester isolates under two different conditions. The 49 New Zealand isolates tested in this study represented 39 isolates of AG-3PT and 11 isolates of AG-2-1, which were collected from 12 different locations in New Zealand. In pathogenicity tests, mean stolon score showed some variability ($p=0.016$), with 18 isolates having scores significantly greater than that for the uninoculated plants. These isolates consisted of all the AG-2-1 isolates and seven AG-3PT isolates collected in New Zealand. Furthermore, for tubers inoculated with the four AG-2-1 isolates there were significantly fewer numbers of stolons in comparison with the controls. Although AG-2-1 isolates were highly pathogenic on stolons, the effect was less on stems and roots. AG2-1 caused significantly less severe black scurf symptoms on tubers compared to AG3-PT. All New Zealand AG2-1 isolates were found consistently to be associated with severe tuber malformation, whereas no malformation was found for any AG-3PT isolates tested in this study. Isolates of both AG3-PT and AG2-1 were capable of yield loss by weight.

In summary, AG is an important consideration as it can affect both disease type and severity. An overview of the differences is presented in Table 5.

Table 5. Summary of disease severity and symptom caused by different AGs

	Stems & Stolons	Roots	Black Scurf	Other deformations
AG2-1	Highly variable		Mild	Yes
AG3-PT	Severe	Moderate	Severe	Yes
AG4 HG-I	Severe			
AG4 HG-II	Zero to mild			
AG4 HG-III	Severe			
AG5	Moderate		Mild	Yes
AG8	No	Severe	no	
AG9	Yes			
BNR	Mild			Yes

Other Differences Between AGs

Fungicide sensitivity

Differences in fungicide sensitivity between different AGs have been observed and several fungicides are limited in their activity against members of a particular AG. For example, Carboxin, furmecyclox, triadimenol, propiconazole and the pyrimidine fungicides fenarimol and nuarimol differ in their ability to control various AGs of *R. solani* (Kataria and Gisi, 1999). The phenylurea fungicide pencycuron has shown the highest level of selectivity between AGs, with strong activity against AG1, AG2-1, AG2-2, AG3, AG6 and AG9 and little or no activity against AG5, AG7 and AG8. Some isolates of AG4 have been reported to be highly sensitive to pencycuron whilst others are insensitive. The existence of such fungicide selectivity has led to the speculation that their continued use may force a change in the pathogen population towards insensitive AGs.

Survival

AGs also differ in their ability to survive in soil. Bell and Sumner (1987) tested soil infested with isolates of AG1 to AG4 and various BNR fungi at 86, 211 and 283 days after infestation by plating onto selective media. Isolates of AG1, 2-1, 2-2, AG4 and BNR were recovered after 283 whilst AG3 was not recovered after 83 days.

Sclerotia formation and structure also differs between AGs. Naiki and Ui (1978) found that AG3 produced less sclerotia in soil than isolates of AG1, 2-1, 2-2, 4 and 5. Internal morphology of the sclerotia differed:AG1 sclerotia had three defined layers. AG2-1 had only two well defined layers and they were found to survive poorly in soil. Isolates of AG2-2, 3, 4 and 5 had sclerotia without any defined layers and their ability to survive in soil varied in between that of AG1 and AG2-1.

The different host range of various AGs can have implications for survival throughout crop rotations. In this study AG5 has been isolated from wheat and barley. AG3 has also been isolated from barley fragments. In pathogenicity tests (Table 6), AG3 can infect wheat. AG3 has been isolated from cereals, sugar beet and a range of weed

species previously (Tsror, 2010). This could have implication for crop rotation strategies.

Table 6. Summary of the ability of alternative hosts to harbour AGs of *R. solani* in a GB UK controlled environment experiment

Host	Test	AG2-1	AG3-PT	AG5	BNR
Wheat	Symptom	Y	Y	Y	Y
	Isolation	Y	Y	Y	Y
Sugar beet	Symptom	Y	N	Y	N
	Isolation	Y	Y	Y	N
OSR	Symptom	Y	N	N	N
	Isolation	Y	N	N	N
Onion	Symptom	Y	N	Y	N
	Isolation	Y	N	Y	N

Rhizoctonia Inoculum Detection

Seed inoculum is often seen as visual black scurf symptoms. Seed tubers may be infected with *R. solani* without showing visible signs, and this can be detected using real-time PCR. The detection of seed inoculum is considered to be robust, but until recently the opportunity to quantify soil inoculum was not possible. With the development of soil sampling strategies, DNA extraction methods and real-time PCR assays for *R. solani* developed both in GB and Australia, we now have the potential to quantify soil inoculum levels and to assess the associated disease risk.

The section below is designed to pull together the relevant data collected within project R422 to answer the question; how good are the current protocols employed at detecting both seed, but in particular soil-borne inoculum? It is based mainly on the findings of work carried out in GB; 2005 – 2007 (R253) and in the current project 2009-2011 (R422). It also includes work currently ongoing, 2007- current in Australia. It refers to *R. solani* AG3 only; using the primers and probes of Lees *et al.,* (2002) in GB and in Australia the assay developed at SARDI. The assay comparability testing carried out between collaborating institutes and some additional tests carried out at JHI indicate that results generated in GB and Australia are comparable.

What levels of inoculum are found on seed and in soil in commercial potato production?

The soil sampling method employed in GB and Australia differs slightly. In GB it is based on 100 individual cores taken in a W shape from across a 4 ha area, bulked into a single sample. In Australia, where fields are often considerably larger than in GB (40ha or more), fields (paddocks) are divided into four sections and four, 20 x 20 m areas identified from which soil and tuber samples are taken. 45 cores, taken as 3 cores at 15 points along a W shape from each sampling area are bulked, resulting in four separate samples per field. Unless stated otherwise soil samples from commercial fields were sampled as above.

In GB, between 2005 and 2007, 122 commercial potato fields were sampled prior to planting. Only 17 (21 %) were found to have detectable levels of *R. solani*. A further

83 soils were sampled and tested between 2009 and 2011 and only 5 % had detectable *R. solani* AG3. A high percentage of seed stocks were contaminated with *R. solani* (real-time PCR); see Table 7.

Table 7. Naturally occurring *R. solani* AG3 soil and seed inoculum in commercial fields: GB (2005-2011)

	Number of soils tested	Percentage of soils contaminated	Number of seed stocks tested	Percentage of seed stocks contaminated (PCR)
2005	42	21	42	60
2006	44	11	43	70
2007	36	8	39	82
2008	-	-	50	60
2009	26	0	36	39
2010	30	7	-	-
2011	27	7	-	-

In Australia, a total of 232 field samples were taken in 2004 and 2005 in South Australia, Victoria and Tasmania from 10 m strips within fields. Of these, 16% had detectable levels of *R. solani* AG3 with 5% above 50 pg DNA / g soil, and 11% below 50 pg/g soil. In 2010, a further 47 fields were sampled in Australia (South Australia and Tasmania), this time taking four individual samples from each field (as described in the soil sampling paragraph above). *R. solani* AG3 was only detected in 11 of these fields, and then at very low levels < 10 pg DNA / g soil.

In contrast to soil, we are able to detect *R. solani* AG3 inoculum both frequently and in large amounts on seed stocks both in GB and Australia. For example, in GB, out of 124 seed stocks assessed both visually and with real-time PCR between 2005 and 2007, 70% had detectable levels of inoculum (PCR), and 47 % had visual symptoms. Similarly in Australia, the majority of seed stocks tested with real-time PCR had detectable levels of *R. solani* AG3 inoculum. For example, in the assessment of grower fields undertaken in 2010, 88% of planting material had detectable levels of *Rhizoctonia*.

What level of inoculum can we detect in soil?

Detection of DNA from sclerotia in soil

At Fera, *R. solani* AG3 could be detected in soil inoculated with a single sclerotia (approximately 1 mm diameter). This was equivalent to 0.2 mg sclerotia /250 g soil, or 0.0000008 g / g, or 8 x 10^{-7} g sclerotia / g soil; data not shown.

Similarly, an experiment was carried out in Australia to determine the detection of DNA of sclerotia in field soil. Varying levels of sclerotia (1-10) were added to 250 g of field soil with five replicates per treatment. Soil DNA was then extracted and *R. solani* AG-3 DNA levels determined. In field soil, the average DNA level detected was 250 pg DNA / g for 10 added sclerotia. DNA from the lowest level (1 sclerotia per 250 g field soil) was detectable but close to the detection limit of our test.

At JHI (2010), an experiment was set up to investigate the relative ability to detect inoculum in soils of different types. Detection of inoculum using real-time PCR from soil inoculated with 0.00003 g sclerotia / g soil varied between zero and 1000 pg DNA/ g soil in different soils (Figure 1). However black scurf developed on progeny tubers grown in all but one of the eleven soils inoculated at this level. The inoculum added to the soils was therefore below the threshold of detection in some soil types, but sufficient to cause disease.

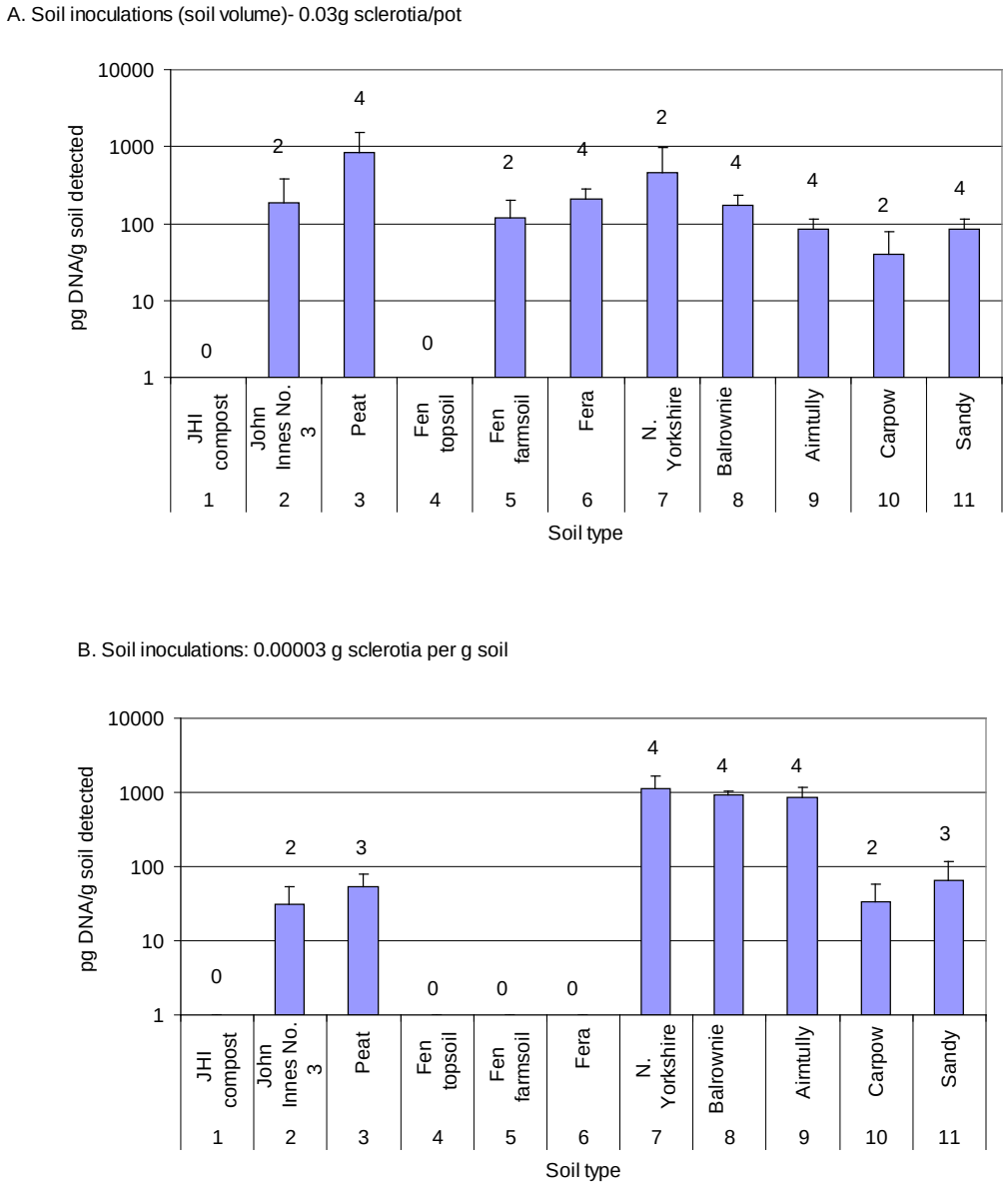

Figure 1. *R. solani* DNA (pg DNA / g soil) detected in soils inoculated with A. the equivalent of 0.03g sclerotia per pot, and with B. the equivalent of 0.00003g sclerotia per g soil; Error bars indicate standard error of mean, the number above each column indicates the number of replicates out of 4 which were positive (for more details of the experiment see the main project report)

Inoculum detection: summary and conclusions

Seed inoculum

- Potato seed in both GB and Australia is commonly infected with *R. solani* AG3 as determined by PCR and frequently displays visual black scurf symptoms.

- Seed inoculum can be detected using real-time PCR in the absence of visual symptoms.

Soil inoculum

- In both GB and Australia, *R. solani* is detected in soils (250 g) inoculated with a single sclerotia.
- A single sclerotia (1mm in diameter and weighing 0.2 mg) in 250 g soil, equates to 0.00000008 g sclerotia / g soil, or 8×10^{-7} g sclerotia / g soil.
- However, detection of low levels of inoculum may be difficult in some soil types. Detection of *R. solani* inoculum in soils inoculated with 0.00003 g sclerotia / g soil varied between zero and 1000 pg DNA/ g soil between different soil types.
- Our research in GB has shown that levels as low as 0.00003 g sclerotia / g soil can cause disease.
- We can conclude that current methods cannot reliably detect sclerotia in field soils at levels which are sufficient to cause disease, primarily due to the size of the soil sample which will not necessarily pick up low levels of unevenly distributed sclerotia, but also due to reduced detection in some soil types.

The Contribution of Hyphae and Sclerotia to *Rhizoctonia solani* Disease Development

Most work focussing on inoculum sources of *R. solani* makes a distinction between soil and tuber borne inocula. However, *R. solani* can survive in soil as both hyphae and sclerotia. The relative contribution of each to subsequent disease development is not well understood. Within this project a controlled environment experiment to understand the different effects of hyphae and sclerotia on disease development, and a field trial in which the effects of inoculum source (hyphae, sclerotia) and organic matter on disease development were carried out to establish if the type of inoculum affects disease.

The results of the controlled environment experiment showed that there were differences between inoculum types in terms of detection and the ability to produce disease. Hyphal inoculum was detected at lower levels than sclerotia. This is likely to be because sclerotia were not as uniformly distributed in soil at low inoculum levels (<0.0005g / g soil) as hyphae. Hyphal inoculum at the lowest level (0.00005g / g soil) caused disease in stems but did not produce black scurf. This could be because at low levels, inoculum did not survive long enough in compost to initiate disease on stolons or tubers. Black scurf severity and incidence was similar for all other inoculum levels

In the field trial in the low organic treatment, hyphal inoculum produced a higher incidence of stem canker than sclerotia, however this difference was not seen in the high organic treatment. At the final harvest, low organic soils inoculated with sclerotia had less disease than either, low organic soils inoculated with hyphae or high organic soils inoculated with either type of inoculum.

Overall it can be concluded that hyphal and sclerotia inoculum both have potential to cause disease. It is difficult to separate the effect of the different inoculum sources as the sclerotia will ultimately develop mycelia and although there might be a difference in the initial onset of disease, where hyphal inoculum might infect stems earlier, this is not necessarily carried through to disease incidence and severity at later assessment dates.

Role of Soil- and Seed-borne Inoculum in Development of *R. solani* Symptoms

It is widely recognized that both stem canker and black scurf may develop from both seed and soil-borne inoculum. However, the relative contribution of seed and soil borne inoculum to disease development is not clear, despite the fact that many studies have been made of the effect of source of inoculum on *Rhizoctonia* disease of potato. For example, seed-borne inoculum was found by Hide and Cayley (1982) and Adams *et al.,* (1980) to be the main cause of stem canker and shoot damage. The amount of infection which develops appears to be affected by the severity of black scurf on the tubers. James and McKenzie (1972), Gudmestad *et al.,* (1979) and Atkinson *et al.,* (2010) reported that seed tubers with a surface area coverage of more than 15-20% produced significantly more stem canker on daughter plants than seed tubers with no black scurf or with lower amounts of coverage. Simons and Gilligan (1997) also demonstrated a strong relationship between the amount of seed inoculum and the incidence of stem canker. Although Atkinson *et al.,* (2010) found that soil inoculum only occasionally played a significant role in stem and stolon disease, Scholte (1989) and Kyritsis and Wale (2002a) found that soil inoculum could also cause stem and stolon pruning, especially the latter after emergence. Black scurf development, however, was considered by Frank and Leach (1980) and Hide *et al.,* (1985) to be favoured by soil applied inoculum compared with seed-borne inoculum but Atkinson *et al.,* (2010) reported that the amount of black scurf development was similar for both sources. Gudmestad *et al.,* (1979) also found that a period of at least 4 weeks between haulm destruction and harvest was necessary to maximise the development of sclerotia on tubers although this effect was influenced by yearly environmental conditions. Tsror and Peretz-Alon (2005), found relatively low levels of seed inoculum (3% surface area covered) to be just as effective in causing disease as soil inoculum, and reported synergistic effects of soil and seed inoculum when both were present.

Within project R422, data from commercial potato fields, experimental fields, and controlled environment systems in GB and Australia have been collected to evaluate the relative importance of the two (seed vs soil) inoculum sources.
The section below is designed to pull together these data to answer the question; what evidence do we have for the relative importance of seed and soil borne *R. solani* inoculum on causing stem canker and black scurf? The data have been considered within four sub-headings as listed below:

1. What relationship is there between measurable soil and seed inoculum levels with stem canker and black scurf in commercial crops?
2. What is the relative importance of seed and soil borne inoculum in controlled environment/pot experiments?
3. What is the relative importance of seed and soil borne inoculum from field trial data?

4. Overall summary and conclusions

1. What relationship, if any, is there between detectable soil and seed inoculum levels with black scurf in commercial crops?

GB

In GB 2005-2007, to separate out the effects of seed and soil borne inoculum on disease, the incidence of black scurf on progeny tubers grown from seed which had either no visual disease symptoms or no detectable *R. solani* DNA was plotted against the level of soil inoculum. Conversely, disease on progeny tubers grown from seed which was planted into soil in which no *R. solani* was detected was plotted against seed inoculum levels.

There were 36 seed stocks in which no inoculum was detected (by real-time PCR). In 6 of the crops grown from these stocks, no inoculum was detected in the soil either, but the incidence of black scurf was greater than 1% (as indicated by the red circle in Figure 2). This indicates that in a number of cases sufficient inoculum was present on either the seed or more likely in the soil to cause disease but was not detected using the standard sampling technique and/or assay.

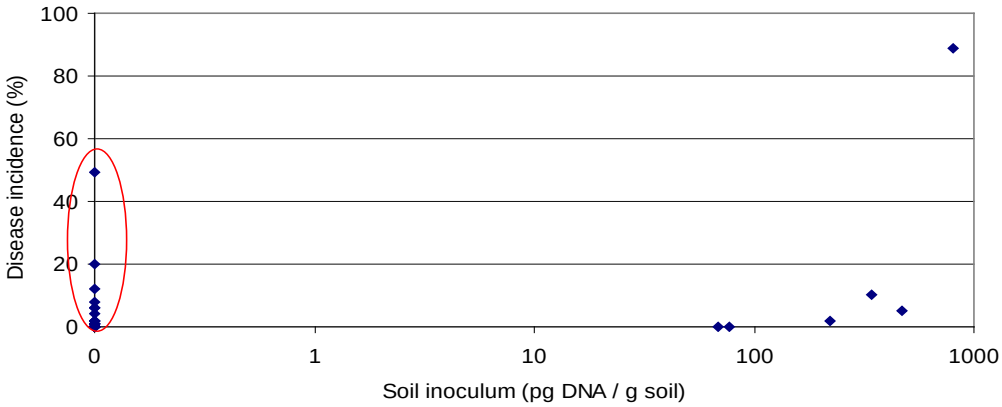

Figure 2. The relationship between soil inoculum and the incidence of black scurf on progeny tubers in crops grown from seed with no detectable inoculum (n=36).

In the 95 crops grown in soil in which no inoculum was detected, there was no relationship found between levels of seed inoculum (incidence, severity or real-time PCR results) and disease on progeny tubers. Figure 3 shows the relationship between seed inoculum based on severity and progeny tuber disease. No relationship is evident. It should be noted that the mean severity of disease on seed stocks was generally low (with only four instances where it exceeded 1%).

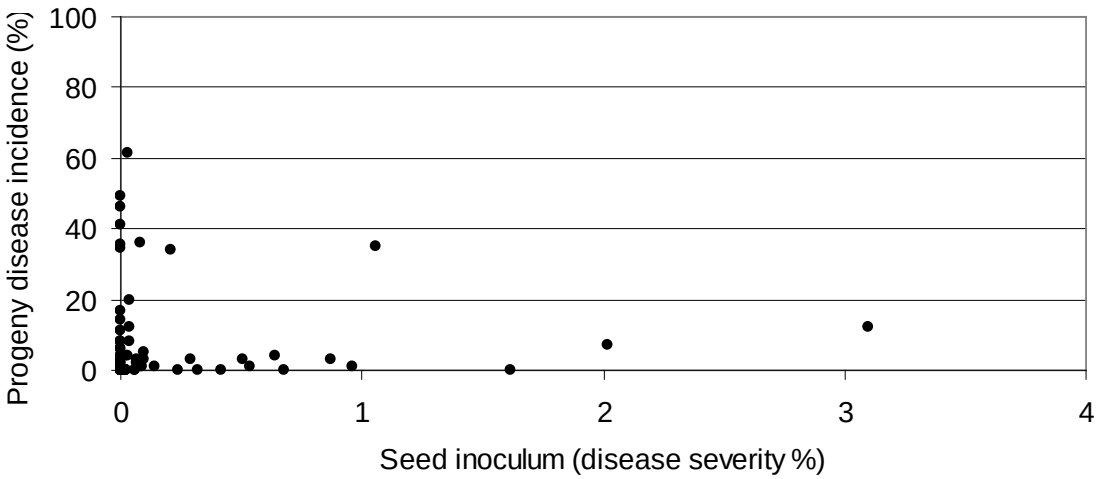

Figure 3. The relationship between seed inoculum and the incidence of black scurf on progeny tubers in crops grown in soil with no detectable inoculum (n=95).

Australia

In both South Australian fields (Figure 4) and fields in Tasmania (Figure 5), there were many soils where no inoculum of *R. solani* AG-3 was detected, but black scurf developed on progeny tubers. Where inoculum (even at a very low level) was detected, disease developed.

High levels of *R. solani* AG-3 DNA detected on seed tubers were not well-correlated with disease incidence. The additive effect of soil and seed inoculum was considered. However multiple regressions of peel DNA at planting and soil DNA pre-planting vs. black scurf severity were not significant (*F*-prob = 0.22). This indicates that disease at harvest is not directly related to the combined effect of the inoculum measured prior to planting of soil and seed.

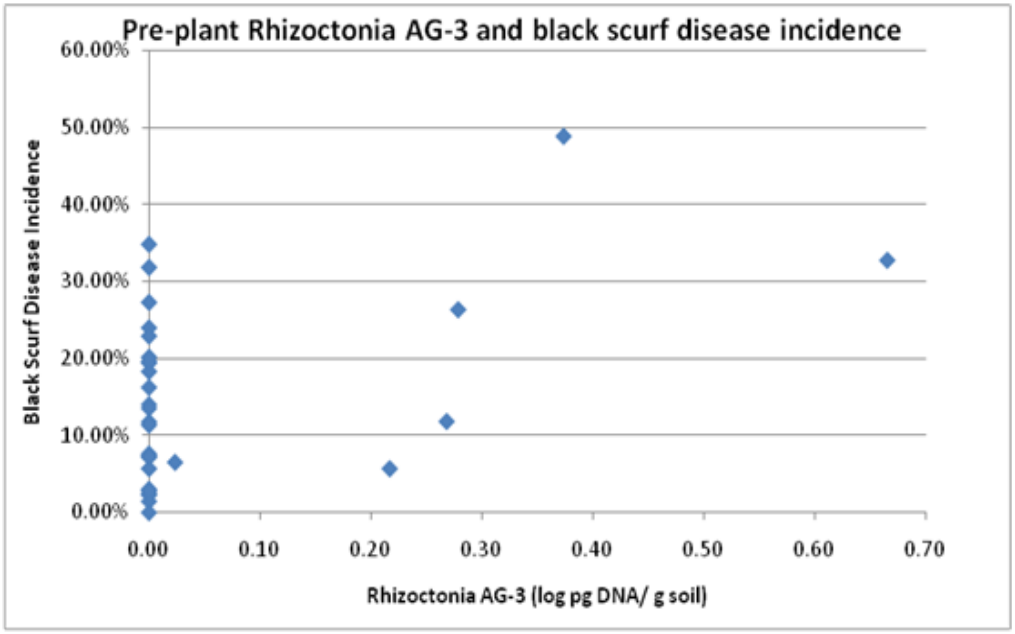

Figure 4. Relationship between pre-plant *Rhizoctonia* AG-3 DNA in soil and black scurf disease index (South Australia).

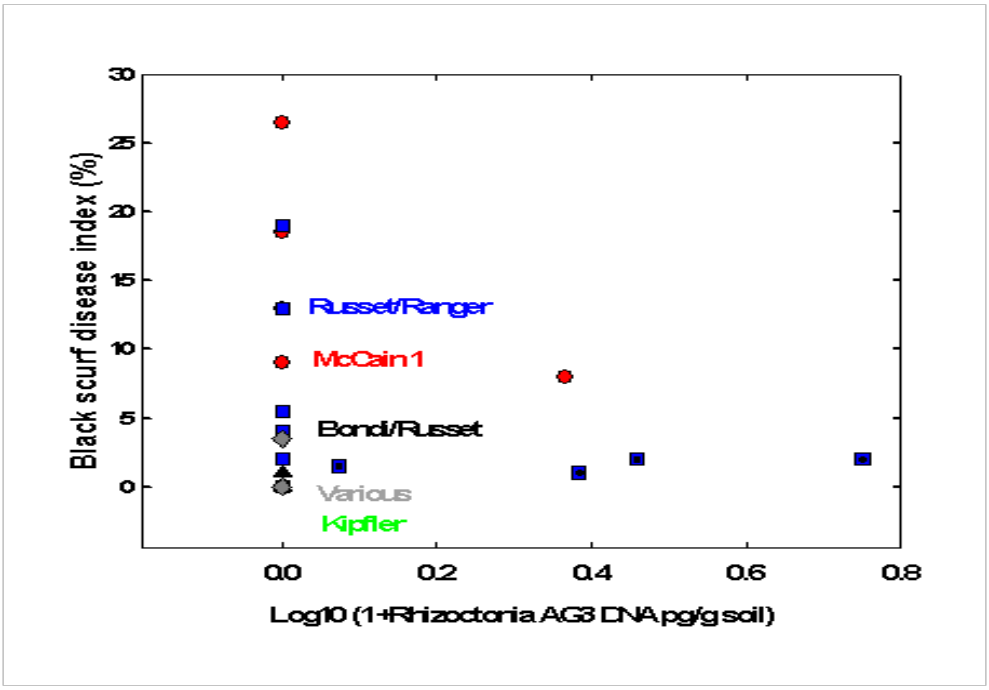

Figure 5. Relationship between pre-plant *Rhizoctonia* AG-3 and black scurf disease index (Tasmania).

2. What is the relative importance of seed and soil borne inoculum in controlled environment/pot experiments?

GB

Three experiments were carried out, at JHI (2009) and at Fera (2009 and 2010). The JHI 2009 and Fera 2010 experiments investigated the effect of seed inoculum (based on the surface area covered with black scurf) and soil inoculum (based on soil inoculated with varying amounts of inoculum). In the JHI (2009) experiment, the relative surface area of black scurf on progeny tubers was assessed. In the Fera (2010) experiment, stem canker was assessed. In the Fera (2009) experiment, soil was inoculated with sclerotia at a range of concentrations and disease on stolons and progeny tubers scored.

In the JHI (2009) experiment there was a significant seed/soil interaction ($p<0.05$), with seed inoculum having a significant effect on disease incidence and severity in the absence of soil inoculum, i.e. mini-tubers and low levels of seed inoculum (<1 and 1-10% surface area covered) resulted in less disease than the highest level of seed inoculum (> 10 % surface area covered). All soil inoculum treatments resulted in high levels of disease incidence, illustrating that the disease threshold for soil inoculum was exceeded even in the lowest inoculum treatment (estimated to be approximately 50 pg DNA/ g soil). Unfortunately, impaired inoculum detection using real-time PCR from the compost prevented an accurate quantification of inoculum in the soil treatments.

In the Fera (2009) experiment, on the addition of various amounts of sclerotia to soil, it was found that even the lowest treatment level (0.00001 g sclerotia / g soil) resulted in full disease expression (100% incidence of black scurf on progeny tubers); again indicating that the disease threshold for soil inoculum was exceeded.

In the experiment carried out at Fera (2010) no disease was present in the non-infested soil or asymptomatic seed treatments. In the absence of soil inoculum, the

high seed inoculum treatment (>15 % surface area covered) resulted in significantly higher numbers of stems killed than the low seed inoculum. In this study, soil infested with as little as 0.00005 g sclerotia / g soil caused significant levels of stem lesions and stem death, suggesting the inoculum threshold for disease to occur is somewhat lower. Disease was not assessed on progeny tubers.

Therefore, the results of the JHI 2009 and Fera 2010 experiments, show that in the absence of soil inoculum, high levels of seed inoculum (*c.* 10-15% surface area with black scurf symptoms) cause high levels of stem and tuber disease. However, the disease threshold for soil inoculum is below 0.00005 g sclerotia / g soil (or 50 pg DNA / g soil) and this level was exceeded in all soil inoculum treatments.

3. What is the relative importance of seed and soil borne inoculum from field trial data?

GB field trials 2008 (carried out as part of Potato Council-funded project R249)
The same seed stocks of six cultivars were planted at 19 field trial sites across GB. No *R. solani* inoculum was detected at any of the 19 field trial sites. Visual assessments of the six seed stocks which were planted across all field trial sites revealed that 18% of Saxon tubers had black scurf (mean 0.5% surface area coverage), and King Edward had a trace amount of black scurf.

Whilst black scurf was found on Saxon progeny at most sites (probably due to the seed-borne inoculum), at a small number of sites, black scurf was found across the majority of cultivars, being more indicative of soil-borne inoculum being present, for example, at Peakie (Figure 6).

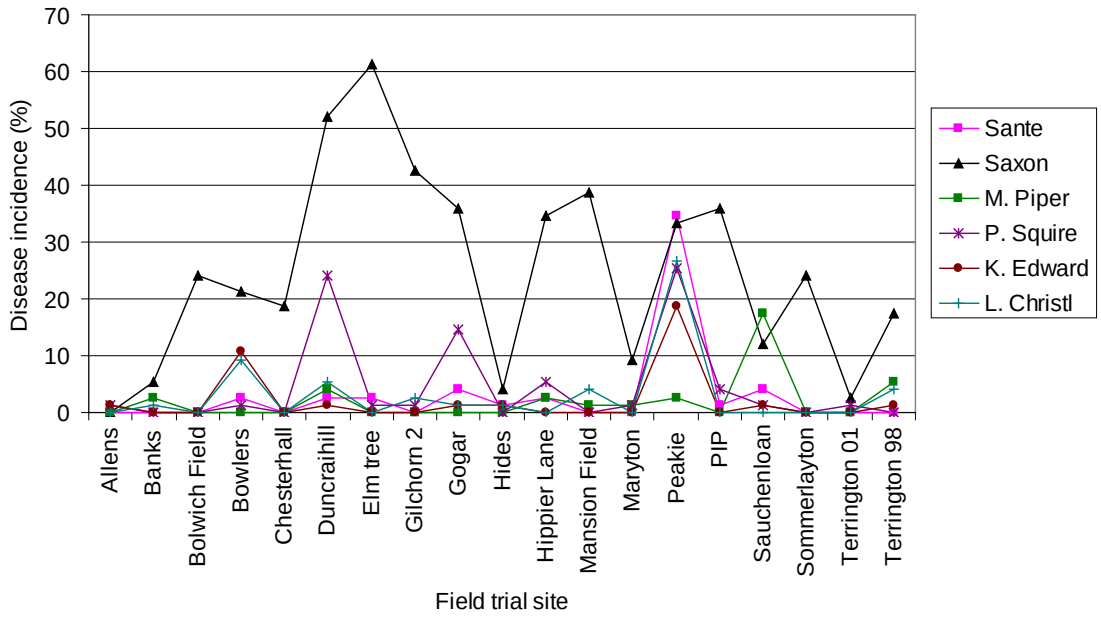

Figure 6. Average levels of black scurf incidence for six varieties grown at 19 sites across GB, all with undetectable levels of soil- borne *R. solani* inoculum.

GB field trials 2009-2011 (R422): SAC
In the five field trials, black scurf was rarely recorded on progeny tubers before haulm destruction. Overall, there were low levels of black scurf at harvest, except in Woodlands 2009 and 2011, the two trials in which large amounts of soil inoculum were detected prior to planting. Black scurf was however present in all trials, even when mini-tubers where planted at sites in which no soil inoculum was detected (Woodlands 2010 and Fingask 2010 and 2011). This illustrates that the detection of soil inoculum is not a robust method for the prediction of disease development.

GB field trial 2011 (R422): JHI
In a field trial at JHI, very little stem canker and black scurf was found in control plots (mini-tubers planted into un-inoculated field soil). In plots inoculated with very low levels of *R. solani* and planted with mini-tubers, we could not reliably detect inoculum in soil samples taken one week after inoculation, mid season or at final harvest, yet plants developed significantly more stem canker and progeny tubers more black scurf than in control plots.

5. Overall summary and conclusions

Seed inoculum

- Potato seed in both GB and Australia is commonly infected with *R. solani* AG3 as determined by PCR and frequently displays visual black scurf symptoms.
- No apparent relationship between the amount of *R. solani* AG3 inoculum detected on seed tubers and subsequent levels of black scurf on progeny tubers can be described in commercial crops either in GB or Australia. However, seed inoculum undoubtedly can cause disease (both stem canker and black scurf), this is illustrated by the set of field trials carried out in 2008, in which Saxon developed disease whilst other cultivars with clean seed did not. The results of two controlled environment experiments illustrate that in the absence of soil inoculum, seed inoculum (>10 % surface area covered in sclerotia) causes high levels of both stem canker (Fera 2010 experiment) and incidence of black scurf on progeny tubers (JHI 2009 experiment).

Soil inoculum

- In both GB and Australia *R. solani* AG3 is found infrequently in commercial potato growing fields.
- Where inoculum is detected it is generally at low levels, particularly in Australia i.e. < 100 pg DNA / g soil in Australia, compared to generally < 1000 pg DNA / g soil in GB.
- There is an indistinct relationship between soil inoculum levels and subsequent disease on progeny tubers in commercial potato crops. In both GB and Australia, black scurf was found on crops in which no soil-borne inoculum was detected, and the relative small number of fields in which inoculum is detected means that conclusions on the relationship between soil inoculum levels and disease are difficult to make.
- In a number of GB field trials; JHI 2011, SAC Woodlands 2010, Fingask 2010 and 2011, it was found that that no inoculum could be detected in the soil despite either being inoculated with *R. solani* or with a history of black scurf, but

both stem canker and black scurf developed even when mini-tubers where planted.

- From the controlled environment experiments carried out in GB, it is evident that the threshold level of soil inoculum for causing disease is very low (< 0.00003 g sclerotia/ g soil). In both the JHI and Fera experiments all soil inoculum treatments exceeded the threshold.
- Soil inoculum causes disease, but is not reliably detected in fields and therefore its effect on disease is difficult to determine in field systems.

Crop Rotations, Potatoes and *Rhizoctonia* Diseases

Introduction

The information cited in this part of the review resulted from a search of CAB Abstracts over the past 20 years using the keywords *Rhizoctonia,* potato and rotation. Of the more than 50 papers returned by this search, 28 were deemed worthy of further assessment. This assessment comprised reading only their abstracts and extracting information on one or more of the following issues: rotation length, rotation cash crops and cover or green manure crops, tillage, soil amendments, hosts including volunteer potatoes, soil DNA measures and pathogen antagonists. While many of the papers were well known to the author, a review of all papers in full is needed to properly assess the state of knowledge in this area. Other databases should also be interrogated. A number of papers emerged from particular research groups. For example, scientists from Agriculture and Agri-Food Canada and the Prince Edward Island Department of Agriculture authored 9 of the 28 papers, some of which reported on different aspects or stages of the same field experiment. A USDA-ARS group in Maine authored another 6 of the 28 papers, again with some experimental overlap in space and time. Together these groups were responsible for 9 of the 14 papers published in this area in the last 10 years. Published research on *Rhizoctonia* and rotations therefore appears to have been quite limited in geographic extent in recent times.

Rotation length

Several papers came to the already well-established conclusion that potato disease due to *Rhizoctonia* is more severe the more often potatoes are grown in the same field (Scholte 1990; Gilligan *et al.,* 1996; Honeycutt *et al.,* 1996; Carter and Sanderson 2001; Peters *et al.,* 2004; Zimny *et al,.* 2006; Larkin and Honeycutt 2006; Carter *et al.,* 2009; Larkin *et al.,* 2010), although this was not always the case (Starczewski *et al.,* 1998).

In many cases where increased potato frequency was associated with increased disease (Honeycutt *et al.,* 1996; Larkin and Honeycutt, 2006; Larkin *et al.,* 2010) the control treatment consisted of continuous potatoes and *Rhizoctonia* was less in treatments where other crops were included in the rotation. However, Carter *et al.,* (2009) and Peters *et al.,* (2003 and 2004) reported findings from a long-term trial comparing 2- and 3-year rotations each containing a single potato crop and found that *Rhizoctonia* disease was less in the 3-year rotation. Hide and Read (1991) and Gilligan *et al.,* (1996) compared 2-, 4- and 6-year rotations, and Starczewski *et al.* (1998) compared 1-, 2-, 3- and 4- year rotations and in all of these cases the longer rotations gave less disease. Further, Hide and Read (1991) planted potatoes (cv. Desiree) in all of their plots in the 7th year of their trial and found more stem canker

and black scurf with decreasing time since the last potato crop and with a greater number of previous potato crops.

There was no published evidence of rotations leading to *Rhizoctonia* suppressive soils as has been (anecdotally) reported for powdery scab.

Rotation crops

The research reported above has included a wide range of other crops in the rotation, either as cash crops or as cover crops / green manures. Those that were associated with a decreased severity of disease due to *Rhizoctonia* include Italian ryegrass, *Lolium multiflorum* (Johnston *et al.,* 1994), cereals, perennial grasses, winter rape and flax (Ivanyuk and Alexandrov 1996), lucerne (*Medicago sativa*) and red clover (*Trifolium pratense*) (Cwalina-Ambrosiak and Czajka, 2000), red clover (Carter *et al.,* 2003); canola, barley and sweet corn (Larkin and Honeycutt, 2006), broccoli (Leach *et al.,* (1993), red clover (Griffin *et al.,* 2009); and canola and rapeseed (Larkin *et al.* 2010). Larkin *et al.,* (2010) also observed that winter rye further reduced black scurf compared to canola and rapeseed on their own. In contrast, Leach *et al.,* (1993) reported that oats increased *Rhizoctonia* stem lesions in potatoes, and Larkin and Honeycutt (2006) reported that clover and soybean resulted in more disease in some years.

Peters *et al.,* (2003) isolated bacteria from the root zone of their long-term rotation trial and found the highest antibiosis activity in isolates from the endoroot tissues of rotation crops from the 3-year compared under minimum tillage. However, tillage had no effect on disease in the field (see below). In *in vitro* assays, Larkin and Griffin (2007) found that added chopped leaf matter of brassica crops and barley inhibited growth of *Rhizoctonia*, while Indian mustard almost completely inhibited its growth. Garbeva *et al.* (2008) tested the effect of adding rhizosphere extracts of maize, oat, barley and grass on Rhizoctonia and found that extracts from maize and grass rhizosphere were the most antagonistic.

van Elsas *et al.,* (2002) conducted bioassays that assessed the growth of *R. solani* AG3 hyphae through soil and found greater suppression in grassland compared to arable soils. Sturz *et al.* (1998) isolated bacterial endophytes from a red clover-potato rotation trial and found that 74% of bacteria exhibited antibiosis to *R. solani.* Celetti et al. (1990) isolated soil-borne fungi from below-ground parts of *Trifolium hybridum* and *T. pratense*, peas, soybean, Italian ryegrass, barley and wheat grown in rotation with potatoes and found that the tissue from the 2 clover species contained the most *R. solani*, followed by wheat, Italian ryegrass, soybean and barley.

Tillage

Leach *et al.,* (1993) compared chisel and mouldboard ploughing and found more *Rhizoctonia* stem lesions after mouldboard ploughing, but Carter *et al.,* (2009) in their long-term trial found no difference between conservation (tined implements) and conventional (mouldboard ploughing) tillage even though there were effects of potato rotation length on *Rhizoctonia* in the same experiment.

Soil amendments

Larkin (2008) was the only paper to report effects of soil-applied amendments on *Rhizoctonia*. They found that added aerobic compost tea and a mixture of beneficial organisms both reduced stem canker and black scurf in a 2-year rotation of barley

(undersown with ryegrass) – potato, but not in a similar rotation undersown with red clover instead of ryegrass, nor with potato followed by potato.

Hosts

On Prince Edward Island, Sturz *et al.,* (1995) survey 80 species and found 56 that harboured *R. solani*. Further inoculation trials with 61 weed species showed that 28 could be infected by the anastomosis groups AG3 and AG5. AG5 has been isolated from winter wheat (Woodhall *et al.,* 2012) and couch grass in the UK (Woodhall and Lees, 2004). *R. solani* AG-3 has also been isolated from the roots and stems of many weeds present in Spanish potato fields (*Chenopodium album, Diplotaxis eurocoides, Solanum nigrum,* and *Sorghum halepense*) (El Bakali *et al.,* 2000). Bains *et al.* (2002) found that neither *Beta vulgaris, Brassica campestris, Hordeum vulgare, Pisum sativum, Triticum aestivum* nor *Zea mays* were able to be infected by *Rhizoctonia*. However, other studies have isolated *R. solani* AG-3 from barley (Murray 1981) and sugar beet (Windels and Nabben 1989). No studies of the impact of weed potatoes (groundkeepers or volunteer potatoes) were uncovered by this literature search.

Inoculum in rotations

Gilligan *et al.,* (1996) in their experiment comparing 2-, 4- and 6-year potato rotations found that the shorter the rotation the longer it takes for inocula to fall in the intercrop period, while inoculum was replenished rapidly by growing the host crop. Jager and Velvis (1995) mapped the distribution of potato plants with black scurf (sclerotia) in a Netherlands field over 5 years and found that while the distribution was fairly homogeneous at first it became more patchy with time, which they attributed to increases in an unknown suppressive agent.

Justesen *et al.,* (2003) used DNA sequence analysis to survey genetic variability between 60 field-derived isolates of *T. cucumeris* (an anamorph of *R. solani*) and found that the population structure was not influenced by the previous crop in the rotation.

Conclusions

The more often potatoes are grown on the same ground the greater will be the risk of symptoms due to *Rhizoctonia*. While there are exceptions to this rule, rotation crops which have been associated with decreased severity of *Rhizoctonia* include cereals, brassica species and legumes especially red clover. This is still a broad group and mechanisms for disease control by these species are not clear, although pathogen antagonism by root extracts of some rotation species has been demonstrated. A further study found that soil amendments including compost tea were able to reduce the severity of disease due to *Rhizoctonia*. Studies of host species are few but indicate a wide range, which is not encouraging for control. Reports of the effect of tillage are variable probably because some work is short term and other work was extended over several years. Overall there is little to specifically recommend regarding rotation and *Rhizoctonia* except to grow potatoes as infrequently as possible, to include green manure or cover crops, especially brassicas, and achieve good weed control in intervening crops where, where feasible. Note here that in Europe and North America cover crops are usually grown over a full season ahead of potatoes, whereas in Australia they are often grown only over autumn and winter ahead of a spring potato planting. From the abstracts scanned there is no evidence of the use of DNA probes to assess *R. solani* loads in soils.

Control of *Rhizoctonia solani* in Potatoes

This overview of control considers the control of diseases caused by *R. solani* (mainly AG3) throughout the growth of the potato crop. It considers control whether the inoculum is seed- or soil-borne or both. Because different disease symptoms occur on different parts of the plant, this overview considers control of stem and stolon canker separately from tuber diseases.

Stem canker

Stem canker can arise from both seed- and soil-borne inoculum. It is generally acknowledged that infection of stems can occur from planting until the stem emerges from soil (Hide and Firmager, 1989) and further infection ceases thereafter. Mature stem tissue exhibits greater resistance to infection than immature tissues (Hide *et al*., 1985). Infection of a limited number of surface cells at an early stage of stem development results in cankers as the cells expand during stem extension and die. This early infection can result in severe cankers or even stem pruning. Later infection of stems but before the stem emerges give rise to generally smaller stem canker lesions because the cells of the stem are enlarged and the time to emergence is shorter.

Because of the proximity of seed-borne inoculum to the developing stems, this inoculum source is usually the main cause of stem canker. Whilst soil-borne inoculum can infect stems, the lack of proximity and requirement that it has to grow towards the developing stem means that under field conditions, except in exceptionally high levels of soil-borne inoculum, stem canker resulting from soil-borne inoculum is limited.

In pot experiments carried out during project R422, under ideal conditions for infection, soil infested with as little as 0.00005 g sclerotia /g soil caused significant levels of stem lesions and stem death. However, in the trials carried out in the Woodlands field where the most significant inoculum was considered to be soil-borne (healthy mini-tubers were used in the 2010 and 2011 trials), despite relatively high levels of soil-borne inoculum, stem canker severity was relatively low. This supports the view that seed-borne inoculum is the principal cause of stem canker.

Whilst stem infection is believed to stop once stems emerge, the size of the lesions formed before emergence may continue to enlarge as the infected cells of the stem expand in size. Thus, visual assessments based on severity may increase, even after emergence but no new infections are occurring. A slow, steady increase in stem canker after emergence was found in each field trial throughout project R422.

It may be anticipated that differences exist between varieties in their susceptibility to stem infection and development of stem canker. However, there are no ratings for susceptibility/resistance available. Based on pot experiments using soil-borne inoculum under typical conditions post-planting in the UK, Kyritsis (2003) found small but non-significant differences in stem canker incidence between 7 popular GB varieties. There were significant differences between varieties for stem canker severity and Sante, the variety used in field and laboratory experiments in this project in the first two years, had the lowest stem canker severity. However, all varieties showed severe stem canker and any differences in host resistance to this disease were probably small.

On the basis that seed-borne inoculum is the most important source of inoculum for stem canker, the use of healthy seed or control of seed-borne inoculum using fungicide seed treatment represent the most important control measures to reduce stem canker.

Practically, the health of seed potatoes is judged either by visual assessment or by use of eye-plug testing procedures where excised tuber eyes are incubated and the presence of pathogens determined microscopically. Visually, the presence of *R. solani* inoculum on seed tubers is determined from the existence of sclerotia (black scurf). Occasionally, dark mycelial threads may be visible to the naked eye but this is rarely the case. The absence of sclerotia, when assessed visually, does not categorically confirm a seed stock is at low risk from seed-borne inoculum as the fungus can persist as mycelial threads on the tuber surface. Hence, eye plug tests are more likely to determine the presence of mycelial threads where they exist close to the eye (it is mycelium or sclerotia close to eyes that is most likely to result in stem canker). Severe stem canker has been recorded where sclerotia were not observed (although other factors such as slow emergence may exacerbate the significance of mycelial inoculum on seed that is not visible to the naked eye).

Whilst studies have shown that increasing inoculum increases the incidence and severity of stem canker (Read *et al*., 1989; Rahmann *et al*., 1996), there are no clear guidelines on thresholds of seed-borne inoculum (assessed visually or by eye-plug tests) for judging whether *Rhizoctonia*-active seed fungicide tuber treatments are justified. It is widely accepted in the UK that seed with an incidence of 1% or more *R. solani* is an appropriate threshold for treatment (e.g. see Tesco Nurture Plant Protection Product List assessed 11 May 2011). This threshold appears to suggest that if *Rhizoctonia* is visibly present on more than 1% tubers, it is likely that it is present as mycelial threads (invisibly) on a higher percentage of tubers or that at >1% incidence control is cost effective. The consequence of the adoption of a >1% threshold is that, in practice, a large proportion of seed stocks receive a *Rhizoctonia* seed treatment.

In the field trials reported in this project, care was taken to ensure that *Rhizoctonia* seed treatment (pencycuron) was applied effectively. Coverage of as much as the tuber surface as possible, improves the chance that sclerotia or mycelial threads come into contact with the chemical. However, seed treatment manufacturers suggest that complete coverage is not essential as the fungus grows over the tuber surface and in doing so meets the fungicide even if coverage is incomplete (cf. a sporulating pathogen such as *Helminosporium solani*). Nonetheless, sufficient coverage by seed treatment is important for effective control.

The use of Amistar as a soil-applied fungicide reduced stem canker severity significantly in one out of five trials in this project. Reliance on this fungicide treatment to control stem canker may depend on the level of seed-borne inoculum. Where this is low, for example where seed tubers have limited sclerotia or mycelium, soil fungicide treatment may be sufficient to control stem canker. Where soil-borne inoculum poses the greatest threat, a soil fungicide treatment is likely to be effective in reducing stem canker. However, where seed-borne inoculum is high, it would be unrealistic to expect a soil treatment to be effective in controlling stem canker as the disease pressure adjacent to developing stems is high and the fungicide dispersed at a distance from the seed tuber. Perhaps more than with tuber fungicide tuber

treatments, careful application of soil applied fungicides is required to ensure a uniform zone of treatment.

Another factor that influences the level of stem canker is the speed of emergence. It is well known that rapid emergence reduces the level of stem canker, even where seed- or soil-borne inoculum levels are high (Harrison, 1978; Brenchley and Wilcox, 1979; Baker, 1970; Kyritsis, 2003). This is a disease escape mechanism. Thus, where a high risk for stem canker is present (based on knowledge of seed or soil inoculum), a grower can enhance the rate of emergence by chitting seed, planting into a warmer seed-bed (e.g. planting later) or by planting seed shallowly.

Unfavourable environmental conditions for the pathogen in the soil after planting can restrict infection. In practice, this usually means very wet conditions since *R. solani* has shown an ability to infect under a wide range of soil moisture conditions. Early irrigation might restrict infection but irrigation before emergence is not normal practice.

Table 8. Summary of control measure effective against stem canker and an assessment of their relative effectiveness for either seed- or soil-borne inoculum or where both are present (*=limited effectiveness, ***= effective)

Inoculum source					
Seed		**Seed + Soil**		**Soil**	
Variety resistance[1]	*	Variety resistance[1]	*	Variety resistance[1]	*
Healthy seed /seed treatment	***	Healthy seed /seed treatment	***	-	
Rapid emergence (later planting/warm seed-bed/chitting)	**	Rapid emergence (later planting/warm seed-bed/chitting)	**	Rapid emergence (later planting/warm seed-bed/chitting)	**
Soil fungicide (depends on level of black scurf)	*(*)	Soil fungicide (depends on level of black scurf)	***	Soil fungicide	***
Soil moisture (irrigation/rain)	*(*)	Soil moisture (irrigation/rain)	*(*)	Soil moisture (irrigation/rain)	*

[1] see the comments in the section above regarding differences in susceptibility to stem infection between varieties.

Stolon canker

Unlike stem canker, there is potential for *R. solani* to initiate infection of stolons and cause stolon canker throughout crop growth. There was evidence in the trials carried out in project R422 that levels of stem canker increased steadily through crop development. It is possible that stolon canker was under-estimated over the course of a trial since pruned and cankered stolons may have rotted away or been re-absorbed by the plant. Despite this, as with stem canker, it seems logical that

mature stolon tissue shows greater resistance to infection (as suggested for stems; Hide *et al*., 1985).

Infection of stolons arising from seed-borne inoculum probably occurs after mycelium has grown alongside the developing stem and stolons. Conversely, soil-borne inoculum, whilst dispersed sporadically in soil is closer to the developing stolons.

An assessment of the relative effectiveness of control measures to limit stolon canker is shown in Table 9. There are few potential control measures available. There is no evidence of differences in host resistance to stolon canker. Environmental conditions unfavourable for the pathogen may inhibit stolon infection. For example, irrigation applied from just before tuber initiation until around haulm destruction as part of good practice in a ware crop may reduce stolon canker. Wetter soil conditions, typically keeping soil moisture deficit below 15mm during tuber initiation are likely to have greater benefit than the damp conditions after the period of susceptibility to common scab when soil moisture deficits of below 40mm are sufficient to optimise yield. However, in this project an irrigation treatment in the field trials at Fingask failed to reduce stolon canker, although the two seasons when these trials were carried out were characterised by wet summers.

Control of stolon canker mainly relies on fungicide treatment where seed- and/or soil-borne inoculum is present. Where soil-borne inoculum is present, soil fungicide treatment can reduce stolon canker. This was shown in some trials reported in project R422. Control of seed-borne inoculum using a fungicide seed tuber treatment will effectively prevent inoculum growing along stem and stolon tissue. It is unlikely to have any effect on soil-borne inoculum. Soil fungicide treatment can inhibit development of soil inoculum and limit stolon infection. It may also inhibit mycelial development from seed-borne inoculum. In the trials carried out in project R422, whilst Amistar generally reduced stolon infection, reductions were generally small and not significant.

Table 9. Summary of control measure effective against stolon canker and an assessment of their relative effectiveness for either seed- or soil-borne inoculum or where both are present (*=limited effectiveness, ***= effective)

Inoculum source					
Seed		**Seed + Soil**		**Soil**	
Healthy seed /seed treatment	***	Healthy seed /seed treatment	**	-	
Soil moisture (irrigation/rain)	*(*)	Soil moisture (irrigation/rain)	*(*)	Soil moisture (irrigation/rain)	*
Soil fungicide	***	Soil fungicide	***	Soil fungicide	***

Tuber diseases

An assessment of the relative effectiveness of control measures to limit tuber diseases is shown in Table 10. The control measures listed are not applicable to

each symptom described below and reference to the text should be made for specific disease symptoms.

a. Dry core, cracking/distortion and elephant hide

The epidemiology of tuber diseases other than black scurf as a result of infection by *R. solani* is poorly understood. Dry core and cracking/distortions are all assumed to be the result of limited infection on the tuber surface affecting growth at that point. Subsequently, normal growth of the tuber at the point(s) or area(s) of infection restrict growth in those locations. Normal growth in the rest of the tuber tissue results in uneven tuber development. It seems likely that severity of symptom depends on the time tubers are infected. Early infection is likely to result in more extensive symptoms. *There was limited evidence in the project that control measures reduced the incidence of dry core. Cracking/distortions were more consistently reduced by control treatments.* Recently Keiser *et al.,* (2012) showed that dry core occurred as a result of the interaction between *R. solani* and wireworm (Keiser *et al.,* 2012).

The cause of the elephant hide symptom is even less clear and in project R422 elephant hide was unaffected by fungicide control measures, questioning whether the symptom observed in the field trials was truly caused by *R. solani*. In other studies carried out as part of project R422, elephant hide symptoms were observed in controlled environment studies with potatoes inoculated with specific isolates of AG3-PT, AG5 and a BNR species.

Dry core, cracking/distortion and elephant hide increased with time in the trials in project R422. If the symptoms in the field trials were caused by *R. solani*, this may be due in part to more obvious symptoms from earlier infection as tuber size expanded or there may have been continuous infection during tuber development. Cracking was reduced by fungicide treatment, notably Amistar, in the Woodlands trials but there was no consistent effect on dry core or elephant hide.

Infection of tubers arising from seed-borne inoculum probably occurs after mycelium has grown and developed alongside the developing stem and stolons. Conversely, soil-borne inoculum, whilst dispersed sporadically in soil is closer to the developing tubers.

There is no evidence that varietal differences in resistance exist to dry core, cracking/distortion or elephant hide. As with stem and stolon canker, unfavourable environmental conditions are likely to reduce the impact of *R. solani* inoculum. In one of the two trials at Fingask, dry core, cracking and elephant hide were significantly reduced by the irrigation treatment.

b. Black scurf

Whilst differences between varieties occur in resistance/susceptibility to development of black scurf and resistance ratings are available, in practice the rating is rarely used when considering control of *Rhizoctonia.* This is because all varieties are able to be infected to some degree. In consequence, it is not possible to carry out tests with standard varieties exhibiting the spectrum of resistances from highly susceptible to completely resistant. Therefore, with these diseases a high resistance score, whilst indicating less disease is likely, should not be taken as indicating complete resistance. In consequence, the need for other control measures such as

fungicidal control should be evaluated based on other factors such as the level of inoculum likely to be present and whether environmental conditions favour the pathogen.

Studies have shown an inconsistent relationship between the level of seed- or soil-borne inoculum or stem canker and black scurf on progeny tubers. This demonstrates that many factors other than inoculum influence black scurf development. Despite this, *control of initial inoculum on seed or in soil is an important component in limiting black scurf on progeny tubers. Thus seed tuber fungicide treatment and soil fungicide treatment targeted at seed- and soil-borne inoculum are important, and generally effective, control measures*.

As described above for other disease symptoms, environmental conditions influence the extent of black scurf development. In each of the three years that field trials were undertaken, wet conditions persisted at the end of the season. The frequently saturated soils limited black scurf development in most trials.

The development of black scurf is dependent on conditions that favour sclerotia development. In turn, these are influenced by management strategies. These include the time of haulm destruction in relation to the growth stage of the crop and the delay between haulm destruction and harvest (Spencer and Fox, 1979 a, b). Gudmestad *et al*., (1979) showed that the level of black scurf developing on tubers increased with increasing time between haulm destruction and harvest. In simple terms, delaying harvest can result in an increase in black scurf. Conversely timely harvesting limits black scurf development. Whilst this principle is generally accepted, in the trials in this project a delay of 2 weeks in harvest did not result in a large increase in black scurf in most instances. This was accounted for by the wet conditions at the end of each season of field trials.

Table 10. Summary of control measure effective against tuber diseases caused by *R. solani* and an assessment of their relative effectiveness for either seed- or soil-borne inoculum or where both are present (*=limited effectiveness, ***= effective)

Inoculum source					
Seed		**Seed + Soil**		**Soil**	
Variety resistance	*(*)	Variety resistance	*(*)	Variety resistance	*(*)
Healthy seed /seed treatment	***	Healthy seed /seed treatment	*	-	-
Soil fungicide	**(*)	Soil fungicide	***	Soil fungicide	***
Soil moisture (irrigation/rain)	*(*)	Soil moisture (irrigation/rain)	*(*)	Soil moisture (irrigation/rain)	*(*)
Early harvest	**	Early harvest	**	Early harvest	**

The control measures listed are not applicable to each symptom described below and reference to the text should be made for specific disease symptoms.

Root browning
The control of root browning as a result of infection by *R. solani* is not considered in this overview. Root infection is most likely to occur where soil-borne inoculum is present. Therefore, it is likely that the only control measure that will reduce root browning is soil fungicide treatment.

Other control measures
Crop rotations and agronomic practices
Reports in the literature confirm that some rotations can reduce soil-borne *R. solani*, although results are sometimes contradictory (see the section on rotations above) There is good evidence that in the absence of a suitable host, soil-borne *R. solani* declines annually. Crops ploughed under as green manures are generally beneficial in reducing inoculum. Deep ploughing has been shown to be more beneficial in reducing soil-borne inoculum by reducing colonisation of residues compared to shallow discing (Papavizas and Lewis, 1979). Reductions in black scurf have been achieved by separating tubers and haulm prior to harvest using methods such as haulm pulling and windrowing.

Biological control
Whilst biological control has been the subject of extensive research, there are no commercial options available. Only the mycoparasite *Verticillium biguttatum* seems to have commercial potential (Jeger *et al*., 1996). Research suggests that biological control measures should form one component of an integrated control programme. Biological control has been evaluated at several stages in potato production including seed treatment, as a treatment at green-top lifting and post-harvest. Soil suppressiveness is a concept much discussed in the literature but never clearly defined.

Host resistance
As mentioned above, breeding for resistance to *Rhizoctonia* disease in potato has only met with limited success. This is due to the presence of two phases of the disease (black scurf and stem canker), the significant effect of environmental and soil conditions on disease development (Leach and Web, 1993) and the diversity of the pathogen (Wastie, 1994). In experiments with both European (Chand and Logan, 1982; Little *et al.,* 1988 and Scholte, 1989) and North American cultivars (Leach and Web, 1993) no significant differences in susceptibility to stem canker were observed. Bains *et al.* (2002) and Kyritsis and Wale (2002b) both found differences in several cultivars for susceptibility to black scurf but none were completely resistant to the disease. However, resistance to stem canker and black scurf has been found in several wild *Solanum* species (Wastie, 1994) and crosses with these species suggest that resistance to *Rhizoctonia* is under polygenic control and recessive (Wastie, 1994). Transgenic approaches to resistance to Rhizoctonia have been investigated. One approach where potato plants (cv. Désireé) modified with a chitinase gene originating from *Trichoderma harzianum* showed complete resistance to the pathogen (Lorito *et al.,* 1998).

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Appendix I: Examples of Symptoms caused by *R. solani*

Rhizoctonia symptoms

Stem canker and stolon pruning

James Woodhall

S J Wale

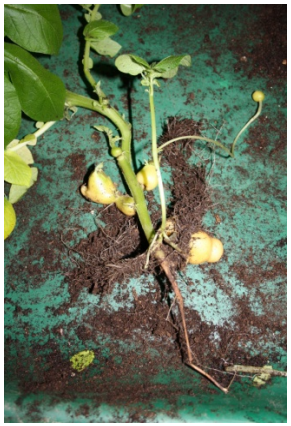
Nigel Cattlin/flpa

Top Atypical symptoms caused by BNR isolate. These are very similar to AG2-1 infection. Bottom:AG4 infection. Courtesy The Food and Environment Research Agency (Fera), Crown Copyright

Dark mycelial threads on stem Courtesy The Food and Environment Research Agency (Fera), Crown Copyright

Reddish to dark brown superficial and usually elongated, often sunken, lesions of varying size on the surface of stems below soil level. When severe the lesions may girdle stems and result in pruning. Where this occurs secondary side stems may develop. Stem canker weakens stems and can result in delayed emergence and uneven crop development. Initial and early infection of sprouts causes small brown lesions which expand as the stem grows and affected cells expand in size. Stem canker can be caused by AGs other than AG3, e.g. AG2-1, AG 4, AG5 and BNR isolates. Usually symptoms are less severe, causing brown superficial streaks. However, AG2-1 can also be more aggressive than AG3. Stem canker can originate from both seed- and soil-borne inoculum. Stem infection ceases once stems emerge above soil level but expansion of existing lesions may continue for some time. Dark mycelial threads may be present on and around lesions. Dark mycelia threads can be present even in the absence of lesions (i.e. prior to development).

Stolon canker, stolon pruning and root infection		
 Nigel Cattlin/flpa		 SRUC
Infection of stolons causes cankers and pruning similar to that found on stems. Stolon infection may result in multiple branching of stolons and greater tuber formation near the stem. Stolon infection can have a marked effect on numbers of tubers set. Stem and stolon canker result in uneven tuber size distribution within crops. Root infection results in generalised browning of the root system. Where infection of the roots is severe, black sclerotia may develop. Dark mycelial threads may be present on affected parts. Root infection can have a marked effect on yield.		

<table>
<tr><td colspan="4">Conseqences of stem canker: Delayed emergence, aerial tubers, leaf curling, leaf resetting, leaf chlorosis and white collar</td></tr>
<tr>
<td> 
Courtesy The Food and Environment Research Agency (Fera), Crown Copyright</td>
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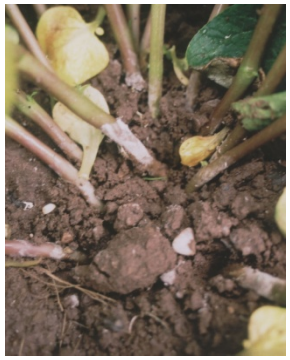
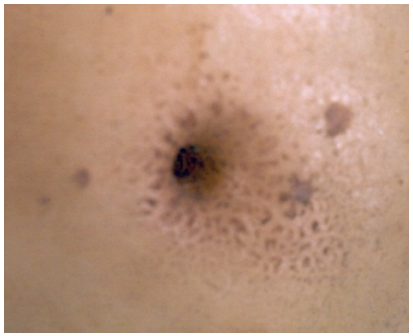
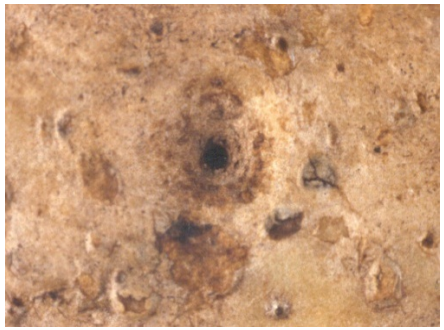
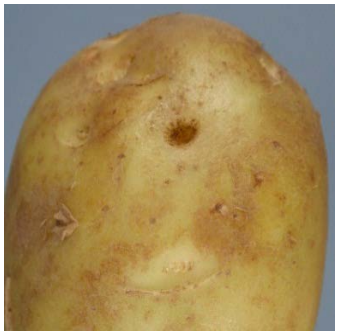
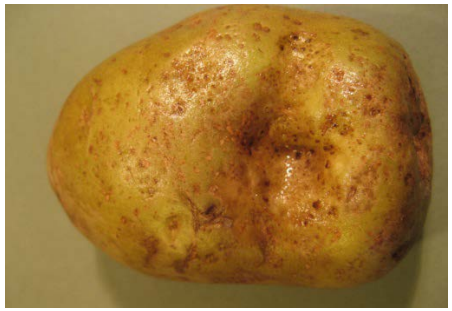
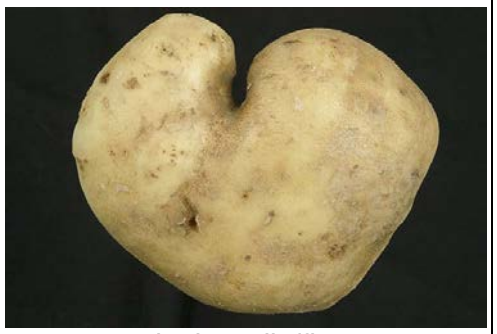
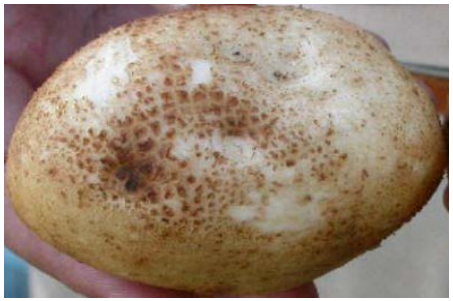
S J Wale</td>
<td> 
James Woodhall</td>
</tr>
<tr><td colspan="4">Above ground symptoms of below ground stem canker include delayed and irregular emergence (often in patches), production of green to reddish-purple aerial tubers in axils of leaves, stunting and rosetting of plant tops, in-curling and yellowing of terminal leaflets and sometimes early death of stems. Despite delays, emergence is eventually complete but crop development is uneven. White collar is the perfect stage of the pathogen. It occurs infrequently and consists of a white powdery mould on stem bases, above stem canker</td></tr>
</table>

Tuber symptoms – dry core		
 S J Wale	 S J Wale	 S J Wale
Point infection of developing tubers at an early stage, results in tuber tissue developing around the point of infection and a hole forming. The hole narrows from the outside like a vortex and at the mouth of the hole skin russetting is usually present. Koch's postulates have confirmed this symptom being caused by *R. solani*.		

Tuber symptoms – deformation and cracking			
 S J Wale	 S J Wale	 Nigel Cattlin/flpa	 Nigel Cattlin/flpa
When infection of tubers occurs along the surface of developing tubers, the tuber develops around the area of infection. This results in tuber deformation and cracking. Skin russetting is often present associated with cracking or deformation. The effect is greatest when infection occurs early in tuber development. Dark mycelial threads are rarely found associated with deformation or cracking. Koch's postulates have confirmed this symptom being caused by *R. solani*, although there may be other causes of cracking and distortion.			

Tuber symptoms – elephant hide			
 SRUC	 Courtesy The Food and Environment Research Agency (Fera), Crown Copyright	 Courtesy The Food and Environment Research Agency (Fera), Crown Copyright	 Close up of a tuber surface with a less severe form of elephant hide. A BNR isolate was isolated from the lesion. Courtesy The Food and Environment Research Agency (Fera), Crown Copyright
Elephant hide comprises a collection of small rectangular darkened patches clearly separated from each other. The area affected can vary in size. Dark mycelial threads are rarely found associated with elephant hide. The mechanism of infection is not confirmed but during the stem canker phase of the pathogen, immature tubers are infected and lesions occur. As these lesions expand they develop into the elephant hide symptom. Koch's postulates have confirmed this symptom being caused by *R. solani*. Similar elephant hide symptoms may be caused by other biotic and abiotic factors and thus exact identification may be difficult with laboratory examination.			

Black scurf		
 S J Wale	 Nigel Cattlin/flpa	 Courtesy The Food and Environment Research Agency (Fera), Crown Copyright
Black scurf describes the raised dark brown or black sclerotia or resting bodies of the fungus on the tuber surface. Black scurf can be of varying size and shape and is unsightly, thereby affecting marketability. Black scurf forms shortly before harvest and may initially be whitish in colour before rapidly darkening. Sclerotia consist of dense mats of mycelial threads. Along with black scurf individual mycelial threads may occur on the tuber surface but they are only just visible to the naked eye. On seed, these threads are as capable of initiating disease in a subsequent crop as black scurf.		