Effect of inoculum source, inoculum pressure and cultivar on development of black scurf on potatoes in South Africa

by

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Submitted in partial fulfillment of the requirements for the degree of MSc (Plant Pathology) in the Faculty of Natural and Agricultural Sciences Department of Microbiology and Plant Pathology University of Pretoria

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DECLARATION

I declare that the dissertation, which I hereby submit for the degree of MSc (Plant Pathology) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another university. Where secondary material is used, this has been carefully acknowledged and referenced in accordance with university requirements. I am aware of university policy and implications regarding plagiarism.

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Signature           Date
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CHAPTER ONE
GENERAL INTRODUCTION

The potato is the world’s most important non-grain food crop (Huang et al., 2011) and South Africa’s third most important food crop after maize and wheat (Potatoes South Africa, 2010a). In 2009, the gross value of potato production in South Africa accounted for about 53% of major vegetables, 12% of horticultural products and 3% of total agricultural production (Potatoes South Africa, 2010b). However, the potato industry in South Africa encounters numerous challenges such as severe droughts and floods, land shortages and the economy. The most frequent yield and tuber quality losses to the industry are attributed to soil and/or tuber borne pathogens (Potatoes South Africa, 2010b).

_Rhizoctonia solani_ is a soil-borne fungus consisting of morphologically similar but genetically distinct groups, referred to as Anastomosis Groups (AG’s). Research has identified 13 different AG’s of which only AG 1, 2.1, 3, 4, 5, 7 and 8 have been associated with disease in potatoes. AG 7 causes infections on stems, stolons and tubers but not on roots, while AG 8 infects potato roots (Sneh et al., 1996; Lees et al., 2002; Woodhall et al., 2007; Okubara et al., 2008). AG 5 is mostly associated with stem; root canker and black scurf (Balali et al., 1995) whilst AG 4 is only associated with stem lesions (Sneh et al., 1996; Lees et al., 2002). AG1 has been shown to cause damage to spouts (Carling & Leiner, 1990). AG 2.1 is associated with stem lesions and superficial tuber alterations (Campion et al., 2003). Two groups of AG 3 have been identified namely AG 3PT and AG 3TB, of which only AG 3PT has been reported to be the major causal agent of black scurf and stem canker (Bounou et al., 1999; Lees et al., 2002, Yanar et al., 2005). A study by Truter (2005) investigating the different AG’s associated with potato disease in South Africa showed 99.3% of isolates from tubers infected with black scurf and 82.1% of isolates from infected stems and stolons, belonged to AG 3.

The development of disease begins when _R. solani_ inoculum is present either as soil- or tuber-borne sclerotia or hyphae (Scholte, 1992; Gilligan et al., 1996; Atkinson et al., 2010). Research, however, has yet to determine which inoculum source (seed vs. soil) is more important for disease development. Stolons have a higher chance of infection by contact with soil-borne inoculum than tuber-borne inoculum (Frank & Leach, 1980).
Some studies show as little as 12-15% tuber-borne inoculum is sufficient to cause severe stem canker and black scurf symptoms (James & McKenzie, 1972; Atkinson et al., 2010). Whilst other studies prove even seed tubers not showing black scurf symptoms could contain *Rhizoctonia* hyphae, thus resulting in disease development (Du Plessis, 1999). A study by Tsror & Peretz-Alon (2005) showed that although both inoculum sources are important for disease development, it is in fact the initial inoculum load which determines intensity of disease incidence and severity.

*R. solani* causes disease at all growing stages (sprout development, vegetative growth, tuber initiation, tuber bulking and tuber maturation) of the potato plant (Banville, 1989; Simons & Gilligan, 1997; Naz et al., 2008). A study by Ahvenniemi et al. (2007) showed Rhizoctonia disease develops in four phases. The first and second phases result in infection of stolon tips prior and after emergence, respectively (Ahvenniemi et al., 2007). Infected stolons lead to death of sprouts and stems, malformed tubers and the formation of aerial tubers (James & McKenzie, 1972; Atkinson et al., 2010). The third phase in *R. solani* disease development occurs three to four weeks after emergence, resulting in scabby, sunken areas near the sprout end of young tubers (Ahvenniemi et al., 2007). The fourth phase is identifiable by the development of black scurf on progeny tubers, roots, stem bases and stolons (Ahvenniemi et al., 2007). Tubers showing black scurf symptoms are downgraded on the market and thus responsible for great economic losses to consumer markets, seed certification programs and the export industry (Platt et al., 1993; Republic of South Africa, 2002; Hamid et al., 2006).

There are many biotic and abiotic factors that influence the survival and spread of *R. solani* in soils such as temperature, soil moisture, soil pH and soil type. Physical soil characteristics such as pore size, bulk density and tortuosity influence aeration, water movement, plant root growth, water and nutrient retention, but also determine the ability of *R. solani* hyphae to invade available pore spaces and branch out (Otten et al., 2001; Harris et al., 2003; Ritz & Young, 2004). Further understanding on how physical soil conditions may be altered by factors such as tillage and irrigation, can assist in developing an integrated control strategy for *R. solani*.

Fungicides, applied as either soil and/or seed treatments, are the preferred method of control (Campion et al., 2003; Boogert & Luttikholt, 2004; Rauf et al., 2007). The efficacy
of fungicides, in vivo and in vitro, depends on the morphology, physiology, virulence and genetic composition of different R. solani sub-groups present in the field (Kataria et al., 1991). The use of some mycoparasites such as Trichoderma harzianum, T. viridae, T. hamatum, Gliocladium virens and Verticillium biguttatum have been shown to significantly reduce R. solani inoculum levels in soils (Wale, 2004; Brooks, 2007; Wilson et al., 2008). However, both chemical and biological methods are extremely complex, costly, and time consuming to implement. The use of more tolerant cultivars has been shown in past studies to be a more convenient, economical and environmentally safe method to effectively reduce inoculum levels in soils, thereby reducing the risk of disease development (Otriesko & Banville, 1992, Leach & Webb, 1993).

Most farmers rely on experience and knowledge of field factors to develop their disease management programs (Wale, 2004). To reduce the associated risk of disease, the current study focuses on determining the effect of different inoculum sources and concentrations on disease development and to investigate cultivar susceptibility of a few commonly planted cultivars in South Africa as a method of reducing soil inoculum levels for decreasing disease severity and incidence.

1.1 References:


CHAPTER TWO
LITERATURE REVIEW

2.1 Causal organism
Rhizoctonia solani Kuhn (teleomorph: Thanatephorus cucumeris) is a wide-spread, economically important soil-borne fungus, which is a species-complex consisting of morphological similar, but genetically distinct species (Beagle-Ristaino & Papavizas, 1985; Carling & Kuninaga, 1990; Lees et al., 2002; Pannecaucque et al., 2008). This pathogen is the causal agent of fruit and seed decay, damping off, foliar blight and crown rot of many crops such as soybean, maize, rice, sorghum, sugarcane, cotton and coffee (Pascual et al., 2000; Stetina et al., 2006; Brooks, 2007; Guleria et al., 2007; Thind & Aggarwal, 2008). However, on potato crops the symptoms include stem canker, stolon canker, misshapen tubers, black scurf, cracking, pitting and netting of tuber surfaces (Carling et al., 1989; Sneh et al., 1996; Stuart et al., 2008).

2.2 Taxonomy
Morphological identification of R. solani is based on a combination of hyphal traits. These include branching near the distal septum, no clamp connections, pigmentation, multinucleate and binucleate cells in vegetative hyphae and hyphal interactions (Parmeter, 1970; Sneh et al., 1996; Lees et al., 2002). Different hyphae have been shown to vary in shades of brown depending on their age. Young colonies may appear pale brown or pale yellow at times, whereas the hyphae of older colonies may vary from brown to dark brown in color (Parmeter, 1970; Hooker, 1981; Guleria et al., 2007). The brown pigmentation of hyphae cannot be used on its own for the identification of R. solani, since similar colouration can be present among other fungi (Sneh et al., 1996).

Another characteristic for the identification of R. solani is hyphal branching. In younger colonies branching is at acute angles (Fig. 1) and near the distal septum of the cells (Parmeter, 1970; Sneh et al., 1996). In older colonies the hyphae often branch at right angles (Fig. 2) and at any place along the cell. However since these characteristics are also common among most fungi, hyphal branching alone cannot be used in accurate identification (Parmeter, 1970; Sneh et al., 1996).
In addition, R. solani hyphae have multiple nuclei, usually four to eight per cell, unlike typical fungi with only two nuclei per cell. The multinucleate characteristic of R. solani has become a very reliable method in preliminary identification (Parmeter, 1970; Sneh et al., 1996; Sharon et al., 2006).

R. solani can produce actively growing mycelium or inactive sclerotia on various
substrates (Pascual et al., 2000; Harikrishnan & Yang, 2004; Ritchie et al., 2006; Brooks, 2007). Genetic variations within *R. solani* are also reflected in the type of sclerotia, which are compact masses of cells. The shape, size, shade of brown and distribution on agar plates vary between the different Anastomosis Groups (AG’s) (Parmeter, 1970; Sneh et al., 1996; Agrios, 2005; Tsror, 2010). The diversity of this species complex is also revealed by the presence of several genetically variable AG sub-groups. So far 13 different AG’s have been identified; AG-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, -12 and -13 (Carling & Kuninaga, 1990; Lees et al., 2002; Harikrishnan & Yang, 2004; Guleria et al., 2007; Woodhall et al., 2007). Past studies have shown that different AG’s are associated with different preferred hosts and disease symptoms (Parmeter, 1970; Sneh et al., 1996). Therefore, a prerequisite for a reliable disease management strategy for *R. solani* would be accurate identification of the AG’s present.

Anastomosis reactions of hyphae have been used over the years to place *R. solani* isolates into different AG’s (Cubeta & Vilgalys, 1997; Lubeck & Poulsen, 2001; Lees et al., 2002; Sharon et al., 2006; Yang et al., 2006; Lehtonen et al., 2008). There are four categories of hyphal reactions that allow grouping and differentiation of isolates, namely, “C0”, “C1”, “C2” and “C3” (Sneh et al., 1996; Cubeta & Vilgalys, 1997).

“C0” occurs between isolates that are not related and where there is no interaction, while “C1” occurs between isolates that are distantly related. Though there is contact between the hyphae, there is no evidence of wall penetration or membrane-membrane contact. In these reactions often one or both anastomosing cells and adjacent cells die (Cubeta & Vilgalys, 1997). “C2” occurs between related isolates in which there is a wall connection and the location of reaction site is visible. The anastomosing and adjacent cells always die. “C3” hyphal reactions occur between closely related isolates. The walls fuse and membranes or anastomosis points are frequently not visible. The anastomosing and adjacent cells may die but generally do not (Cubeta & Vilgalys, 1997).

The reliability of anastomosis grouping of isolates using anastomosis reactions is however jeopardized due to the occurrence of bridging isolates that can produce hyphal anastomosis reactions (C1) with isolates from more than one anastomosis group (Sneh et al., 1996). Moreover, AG’s have been described as being “genetically variable”, thus they can be considered as different species of *R. solani*. 
2.3 Molecular methods of identification

Apart from visual traits employed in *R. solani* grouping, the diversity of this pathogen is also reflected at a cellular and molecular level (Liu & Sinclair, 1993; Ceresini et al., 2002; Justesen et al., 2003; Sharon et al., 2006). Studies employing various molecular techniques such as Amplified Fragment Length Polymorphism (AFLP), Random Amplification of Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphisms (RFLP) and Polymerase Chain Reaction (PCR) have not only made it possible to identify *R. solani*, but also to differentiate among the several AG’s (Liu & Sinclair, 1993; Ceresini et al., 2002; Justesen et al., 2003; Sharma et al., 2005). These molecular techniques are normally based on the sequence variation within the internal transcribed spacer (ITS1 and ITS2) regions, which have been very useful in highlighting *R. solani* genetic diversity (Lees et al., 2002; Yang et al., 2006).

The intergenic spacer that separates the 28S and 18S rRNA subunits usually contains a 5S rRNA subunit and a 5.8S rRNA subunit. The 5.8S subunit is flanked by two internal transcribed spacer (ITS1 and ITS2) regions (Cullen et al., 2000; Lehtonen et al., 2008). Restriction analysis of the ITS1, ITS2 and 5.8S rDNA regions indicates that the AG’s of *R. solani* are genetically distinct. Comparison of rDNA-ITS nucleotide sequences has been shown to provide more genetic information than RFLP, AFLP and RAPD-PCR, and allows for the comparison of results with the GenBank databases around the world and with other studies related to *R. solani* (Kuninaga et al., 1997, 2000). *R. solani* AG-3 has since been divided into two subgroups; AG-3PT and AG-3TB, however only AG-3PT is of economic importance on potatoes as it is associated with the tuber blemish, black scurf (Kuninaga et al., 2000; Woodhall et al., 2007). Moreover, RFLP’s used by Alabouvette et al. (2003) and Liu & Sinclair (1993) showed variations within each AG and this necessitates addition of subsets to the different AG’s.

Although RFLP, AFLP and RAPD-PCR are very effective molecular techniques for differentiating among *R. solani* AG’s, conventional and real-time PCR are the preferred molecular techniques as they allow for the rapid detection of a particular *R. solani* AG present in plant tissue and in soils (Liu & Sinclair, 1993; Ceresini et al., 2002; Justesen et al., 2003). In addition, real time PCR with AG or sub-group specific primers, are highly sensitive, allowing for the detection of very low concentrations of fungal load (Lees et al., 2002).
2.4 Infection process

Disease development on a potato plant begins with the presence of the pathogen (Fig. 3). *R. solani* can assume three forms of inoculum; basidiospores, mycelium fragments or sclerotia. Basidiospores of *Thanatephorus cucumeris* (A. B. Frank) Donk, the teleomorph of *R. solani*, are normally found on soil surfaces, on stems and leaves. They are only produced under moderate temperatures, high moisture and humidity (Gutierrez *et al.*, 1997). Basidiospores are air transmitted inoculum mostly causing disease to aerial parts of the plant (Naito, 1996; Pascual *et al*., 2000). Mycelium fragments and sclerotia are present either as soil- or tuber-borne and are commonly associated with disease of below ground plant parts (Atkinson *et al*., 2010). However research over the years has not been able to determine which inoculum source plays a more important role in disease development (Atkinson *et al*., 2010; Lees *et al*., 2010).

Seed inoculum has commonly been associated with disease during the early stages of plant development, negatively affecting plant emergence (Frank & Leach, 1980). The close proximity of tuber-borne inoculum to emerging sprouts causes girdling of sprout and resultant stem cankers. Some studies (Gilligan *et al*., 1996; Naz *et al*., 2008) have shown that soil-borne inoculum can initiate stem and stolon infections as well as black scurf development. Naz *et al* (2008) showed a significant increase in black scurf on progeny tubers and stem canker with an increase in soil-borne inoculum levels. Frank & Leach (1980) suggested that as stolons move through soils, the probability of contact with soil inoculum is much greater than that with tuber-borne inoculum. However, some studies show although both inoculum sources are important for disease development, it is in fact the initial inoculum load which determines intensity of disease (Tsror & Peretz-Alon, 2005).

Optimum temperature and moisture conditions for *R. solani* can enhance disease severity and incidence by stimulating the growth and migration of *R. solani* towards the plant. When contact with the plant tissue is made, hyphal side branches or T-shaped hyphal branches give rise to infection structures. These structures, also referred to as infection cushions, comprise of swollen tips which then form infection pegs. The infection pegs penetrate the cuticle and epidermis cells and give rise to hyphae within the cells of
the plant, the end result being seedling death or stem lesions (Fig. 4) (Keijer, 1996; Weinhold & Sinclair, 1996).

*Fig. 3. Life cycle of *Rhizoctonia Solani* (Agrios, 2005)*

*Rhizoctonia solani* is capable of causing disease on tubers, stems and stolons during all five growing stages of the potato plant before harvest (sprout development, vegetative growth, tuber initiation, tuber bulking and tuber maturation). Symptoms on stem and stolons consist of dark brown, necrotic lesions (Fig. 4 A & B) (Keijer, 1996; Weinhold &
Sinclair, 1996; Simons & Gilligan, 1997; Tsror & Peretz-Alon, 2005). In severely infested fields these symptoms can result in death of sprouts and stems and girdling of stolons, resulting in malformed tubers and reduced yields due to poor stand emergence (James & McKenzie, 1972; Atkinson et al., 2010).

Infected stolons are inhibited from growing to their full length resulting in the development of tubers close to the soil surface, commonly leading to tuber greening. Tubers exposed to light change from brown to green in colour due to the production of amyloplasts from chloroplasts in the potato tuber. Green tubers also form alkaloids which pose a risk of poisoning (Zhu et al., 1984).

During the last two growth stages of the potato plant, the development of black soil-like structures on tubers, sclerotia, also referred to as black scurf is common (Woodhall et al., 2007; Thind & Aggarwal, 2008; Woodhall et al., 2008) (Fig. 5). Sclerotia are made up of loosely constructed knots of melanised hyphae which are not organized into a rind or cortex (Sneh et al., 1996; Ritchie et al., 2009). This “loose type” of sclerotia is unique to \textit{R. solani} and serves as survival structures. \textit{R. solani} can survive in the form of sclerotia in soils or on plant debris for several years (Keijer, 1996; Weinhold & Sinclair, 1996). Although these structures do not cause any mechanical damage to the tuber, they do however decrease the tubers’ market value. Therefore, black scurf is considered to be a disease of great economical importance which results in both quantitative and qualitative agricultural losses (Lees et al., 2002; Woodhall et al., 2008; Atkinson et al., 2010).

2.5 Risk factors influencing disease development

Although \textit{R. solani} is a common disease occurring throughout the world, its growth and disease development is highly influenced by various environmental factors (Simons & Gilligan, 1997; Ritchie et al., 2006, 2009; Larkin et al., 2010; Tsror, 2010). Past studies investigating the effects of temperature, pH, water potential and soil type on growth of \textit{R. solani} have concluded that all four factors are important for disease development (Ritchie et al., 2006, 2009).
Fig. 4 A & B. Stem canker on potato plant

Fig. 5. Sclerotia on tuber
A study by Richie et al. (2009) showed growth of \textit{R. solani} (AG3) mycelium to be optimal at a pH between 5 and 6. Therefore, as a cultural practice, in Scotland, soils are kept at a very low pH (Ritchie et al., 2009). This reduces the levels of \textit{R. solani} inoculum in soils, without having an effect on yield (Ritchie et al., 2009).

Disease incidence and severity are also subject to how temperature affects the host-pathogen relationship. When temperatures are optimum for the host but not the pathogen, disease development is inhibited (Agrios, 2005). Studies have shown the optimum temperature range for growth of the potato plant and for Rhizoctonia disease development are 20-25°C and 10-15°C, respectively (Beukema & Van der Zaag, 1990, Sneh et al., 1996). Research has shown low temperatures can result in late emergence of the plant leading to higher disease incidence (Beukema & Van der Zaag, 1990; Rowe et al., 1993). This poses a huge challenge to the farmer; not only is it impossible to control temperatures under field conditions, but it is also becoming increasingly difficult to predict temperatures as well as rainfall due to changing weather patterns around the world, a consequence of climate change (Norby & Luo, 2004).

2.6 Control

Chemical control of \textit{R. solani} (black scurf and stem canker) dates back to 1913 (Kataria & Gisi, 1996). It was and still is one of the most used and relied upon methods of control for both seed and soil-borne inoculum. Fungicides, either soil or seed treatments or both, or foliar applications used to control \textit{R. solani} belong to many different chemical groups viz. aromatic benzimidazoles, B-methaoxyacrylates, carboxamides, dicarboximides, hydrocarbons, morpholine, phenylpyrroles, phenylurea, triazoles and validamycin (Kataria & Gisi, 1996; Nel et al., 2003, Tomlin, 2006).

Although a wide range of fungicides are available for the control of \textit{R. solani}, the different \textit{Rhizoctonia} species and the different AG’s vary in levels of sensitivity to the active ingredients (a.i.). Research by Kataria et al. (1991), with 14 active ingredients (benodonil, benomyl, carboxin, cyproconazole, fenarimol, fenpropimorph, fusilazole, imazalil, iprodione, prochloraz, pencycuron, propiconazole, triadimenol and tolclofos methyl) and five \textit{Rhizoctonia} species (\textit{R. cerealis}, \textit{R. sacakii}, \textit{R. solani}, \textit{R. zeae} and \textit{R. oryzae}) showed varying levels of control by the different chemical groups on the five \textit{Rhizoctonia} species. Although the different levels of control could be attributed to the
varying toxicity levels of the a.i., it is in fact the distinct morphology, physiology, virulence, genetic-constitution and different teleomorphic forms between the various *Rhizoctonia* species that determine the effectiveness of the chemical employed (Kataria *et al.*, 1991).

In South Africa, chemical groups registered with the regulating body (Department of Agriculture: Act 36 of 1947) for the control of black scurf and stem canker on potato, either as seed treatments, furrow applications or both, include dichlorophen, fludioxonil, imazalil/iprodione, pencycuron, quintozene, thiaendazole, tolclofos–methyl and thiram (Nel *et al.*, 2003). Research by Truter (2005) on chemical inactivation of *R. solani* in South Africa, showed that of the twenty disinfectants tested OA5 DP was the most effective in killing sclerotia of *R. solani* and prevented progeny tubers from infection, however acute phytotoxicity towards the tubers was noted. Furthermore tolclofos-methyl was the only fungicide which gave total control of potato rhizoctoniasis (Truter, 2005).

Although registered fungicides have been proven to effectively control *R. solani*, some contain active ingredients that are highly toxic to humans and animals and result in adverse environmental consequences (Eddleston *et al.*, 2002; Tomlin, 2006). Therefore recent studies have focused on investigating integrated control management systems combining biological agents (Rhizobacteria, *Trichoderma* spp., *Gliododium* spp., *Actinomycetes* spp., non-pathogenic *Rhizoctonia* spp.), with the appropriate chemical control and cultural techniques (Strashnow *et al.*, 1985; Wale, 2004; Brooks, 2007; Wilson *et al.*, 2008).

Research by Wilson *et al.* (2008) on the combined use of biological and chemical control is one such study that highlights the importance of determining the compatibility of the different components within an integrated control strategy. In addition, this study also proved the antagonist *T. harzianum* to be compatible with the seed dressing Flutolanil, reducing the incidence of black scurf on progeny tubers by 11% to 31%. Truter (2005) reported the most inhibition of mycelial growth *in vitro* was achieved with Kresoxim-methyl followed by volatiles from roots and shoots of *B. napus, B. oleracea, var. capitata, R. sativus, S. alba* and *T. minuta*. In soils the biocontrol formulation Trykocide (*T. harzianum*) eradicated the pathogen.
Furthermore cultural techniques such as crop rotation are recommended to the potato grower as the planting of non-host crops reduces soil inoculum levels (Gilligan et al., 1996; Larkin et al., 2010). A study by Larkin et al. (2010) used three cropping systems; the Standard {SQ-2 years rotation of barley (Hordeum vulgare L.) with red clover (Trifolium pretense L.) followed by potato}, Soil–Conserving system {SC-3 year rotation with barley and forage grass timothy (Phleum pratense L.) followed by potato in the third year}, Soil-Improvement system {SI- 3 year rotation with barley/timothy- timothy- potato} and Disease–Suppressive system {DS-oriental and white mustard seed (Brassica juncea L. and Sinapis alba L.) followed by rapeseed (Brassica napus L.) in the first year. In the second year, sorgum- sudan- grass hybrid was used and winter rye (Secale cereale L.) with potato in the third year}, to investigate the effects of crop rotation on black scurf and stem canker. The results showed all rotation systems reduced stem canker by 10-50%. SQ, SC and DS systems reduced black scurf by 18-58%. Black scurf was also reduced under non-irrigated conditions in the SI system.

Other recommended cultural techniques include use of disease free propagation material, use of pathogen-free soil, soil management, irrigation, timing of harvest and haulm destruction (Beukema & Van der Zaag, 1990; Rowe et al., 1993; Wale, 2004; Hamid et al., 2006; Tsror, 2010). Black scurf development on progeny tubers has been shown to increase after haulm destruction (Beukema & Van der Zaag, 1990; Rowe et al., 1993; Tsror, 2010). Sclerotial development on progeny tubers is at a maximum 3-4 weeks after vine-killing (Gudmestad et al., 1979; Kempenaar & Struik, 2007). This is mainly due to potato tubers exuding volatiles (Beukema & Van der Zaag, 1990; Rowe et al., 1993; Tsror, 2010). Therefore, the time between haulm destruction and harvest should be as short as possible to decrease risk of disease development.

Soil characteristics such as pore size, aeration, ability to retain nutrients and water also influence the development and severity of disease. Otten et al. (2001), Harris et al. (2003) and Ritz & Young (2004) have shown growth and spread of R. solani is restricted in high density soils with tortuous and discontinuous air-filled pore space. Earlier work regarding soil physics in relation to R. solani suggest the manipulation of the physical properties of soils by tillage and irrigation can offer some control to the spread and growth of R. solani (Otten et al., 2001; Harris et al., 2003). Tillage practices such as moldboard plowing, chisel plowing and disking have been shown to successfully reduce
disease incidence and severity (Leach & Webb, 1993). Schroeder & Paulitz (2008) suggested tillage of infested soils assists in breaking or disrupting the mycelia network of *R. solani* thereby reducing the pathogen’s vigour. Some tillage methods such as the moldboard plowing technique also removes *R. solani* from the upper 10cm of soil and has the ability to bury sclerotia to depths preventing germination and infection (Leach & Webb, 1993).

Soils with high bulk densities have been shown to reduce the spread of *R. solani*. The more compact the soil, the higher its bulk density with limited pore space. The narrow pore spaces in compact soils retain more water than pore spaces in loose sandy soils (Otten et al., 1999, 2001; Harris et al., 2003 and Ritz & Young, 2004). As a result, water blocked pores restrict hyphal growth, reducing the spread of *R. solani*. Whilst some studies show *R. solani* growth is restricted by high moisture levels (>1500 bars) due to poor aeration (Ploetz & Mitchell, 1985), other studies show moisture levels have no influence on *R. solani* growth (Junior et al., 2007; Olanya et al., 2010). The varying results may be a result of moisture levels affecting the various AG’s differently. This further highlights the importance of accurate identification of AG’s present in soils before cultivation.

Although biological, chemical and cultural techniques reduce the incidence and severity of black scurf on progeny tubers, the implementation of these methods is extremely complex, costly and time consuming. Research has shown the severity and incidence of black scurf on potato tubers can also be reduced by the type of potato cultivar planted (Otrysko & Banville, 1992; Leach & Webb, 1993). Although the use of tolerant cultivars is the most practical, economical and convenient method of controlling *R. solani*, this method is not used as no resistant cultivars are available for commercial use (Harris, 1978; Otrysko & Banville, 1992; Leach & Webb, 1993)

Studies conducted by Bains et al. (2002) and Yanar et al. (2005) on AG3 tolerant cultivars showed that none of the tested cultivars were resistant. However, Bains et al. (2002) found that late-maturing cultivars showed low levels of disease as compared to early and mid-season cultivars. Yanar et al. (2005) also demonstrated that of the 22 tested cultivars, five showed low susceptibility levels. Although there may be no resistant
cultivars currently available for the farmer, cultivars which are less susceptible can be incorporated into integrated disease management systems.

In South Africa some of the commonly planted cultivars include; Astrid, Aviva, BP1, Buffelspoort, Calibra, Caren, Columbus, Darius, Devlin, Eryn, Esco, Evan, Fabula, Fianna, Hermes, Herta, Hoevelder, Lady Rosetta, Liseta, Mondial, Mnandi, Pentland Dell, Ronn, Ropedi, Sandvelder, Shepody, Up-To-Date and Vanderplank (Potatoes South Africa, 2010). A study by Du Plessis (1999) investigating susceptibility levels of 24 cultivars showed Buffelspoort had a higher tolerance to *R. solani* compared to the other cultivars tested. However, future studies should focus on determining exactly why some cultivars show more tolerance than others. Perhaps more consideration should also be given to unraveling the genetic composition of various cultivars to identify potential resistance genes.

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CHAPTER THREE
EFFECT OF RHIZOCTONIA SOLANI INOCULUM SOURCE AND SOIL TYPE ON DISEASE DEVELOPMENT IN POTATOES

ABSTRACT

*Rhizoctonia solani* inoculum can be present either as soil- or tuber-borne sclerotia or hyphae. Although both inoculum sources play a role in disease development, it is not clear which of the two is more important. Physical soil characteristics such as pore size, bulk density and tortuosity determine the ability of *R. solani* hyphae to grow and spread in soils. Two pot trials were conducted to determine the effect of tuber- and soil-borne inoculum, and stolon inoculations on *R. solani* disease development in sandy and clay loam soils. Of the two soil types, tubers harvested from inoculated sandy soils developed significantly more disease than those harvested from clay loam soils. Of the three inoculum sources, stolon inoculation and tuber-borne inoculum resulted in significantly more disease on progeny tubers than those from *R. solani* spiked soils. Although soil inoculum resulted in the lowest incidence and severity of black scurf symptoms, other symptoms, such as tuber greening and stem canker were more prominent in this treatment.

3.1 INTRODUCTION

Black scurf and stem canker on potato crops is caused by *Rhizoctonia solani* Kuhn (teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk). This pathogen causes disease on various parts of the potato plant (Ritchie *et al*., 2006; Al-Mughrabi, 2008; Lehtonen *et al*., 2009; Ritchie *et al*., 2009). Stem and stolon infections are characterized by dark brown necrotic lesions which may result in death of sprouts and stems, girdling of stolons, malformed tubers and the formation of aerial tubers (Zhu *et al*., 1984; Tsror & Peretz-Alon, 2005; Naz *et al*., 2008; Atkinson *et al*., 2010). Infected tubers develop black soil-like sclerotia on their surfaces, a cosmetic symptom referred to as black scurf (Parmeter, 1970; Sneh *et al*., 1996; Lahlali & Hijri, 2010).

Disease development begins when *R. solani* inoculum is present either as soil- or tuber-borne sclerotia or hyphae (Sneh *et al*., 1996; Agrios, 2005). Although both inoculum sources play a role in disease development, it is not clear which of the two is more important. However, research has shown that when both are present disease (black
scurf, stem canker and stolon canker) incidence and severity is intensified (Frank & Leach, 1980; Platt et al., 1993; Gilligan et al., 1996; Simons & Gilligan, 1997). Other studies have however suggested each inoculum source plays a role in disease development at different stages of the plant growth (Frank & Leach, 1980; Tsror & Peretz-Alon, 2005; Atkinson et al., 2010).

Disease development is also influenced by other factors such as temperature, pH, water potential and soil properties. Physical soil characteristics such as pore size, bulk density and tortuosity influence aeration, water movement, plant root growth, water and nutrient retention, but also determine the ability of \( R. \ solani \) hyphae to invade available pore spaces and branch out (Otten et al., 2001; Harris et al., 2003; Ritz & Young, 2004). Limited or restricted pore space has been shown to inhibit the growth of \( R. \ solani \) in soils (Otten et al., 1999; Harris et al., 2003; Ritz & Young, 2004). Therefore, further understanding on how physical soil conditions may be altered by factors such as tillage and irrigation, can assist in developing an integrated control strategy for \( R. \ solani \).

The aim of this study was to determine the effect of inoculum source on development of \( R. \ solani \) on stems, stolons and tubers of potato plants cultivated in sandy and clay loam soils.

### 3.2 MATERIALS AND METHODS

#### 3.2.1 Experimental design:

Pot trials were conducted at the University of Pretoria under greenhouse conditions. The temperature was maintained at 15°C at night and 20°C during the day. Disease-free mini-tubers (cv. BP1) were used for the trials. The trials were set out in a factorial design, consisting of three treatments (soil inoculum, seed inoculum and stolon inoculation) and two soil types (sandy and clay loam). Before planting and inoculation soil samples of each soil type were taken for soil analysis. Uninoculated soil, tubers and stolons were used as control for each soil type. Each inoculum treatment (including controls) was replicated five times for each soil type. The experiment was repeated.

#### 3.2.2 Inoculation of soil:

An \( R. \ solani \) (PPRI 9527) isolate from the Potato Pathology Program culture collection which had previously been identified as AG3 using conventional PCR, in a separate
study, was obtained. The isolate was plated on fresh PDA plates and allowed to grow for five days at 25°C. Wheat seeds were soaked overnight in sterile distilled water with 250µg/ml chloramphenicol and drained. The moist wheat seeds were autoclaved at 120°C for 1 hour. Each bag of seeds weighing 200g was inoculated with five (5mm x 5mm) colonized agar blocks cut under aseptic conditions. The wheat seeds were shaken every three days during the incubation period of 14 days at 25°C. Soil autoclaved at 120°C for 1 hour, weighing 3.4kg was potted in 4kg-capacity pots. Each pot was inoculated with 40g of *R. solani* colonized wheat seeds (inoculum). The inoculum was thoroughly mixed into the soil before planting one mini-tuber (cv. BP1) in each pot at a depth of 100mm. Soil moisture was maintained by irrigating with 200ml distilled water three times a week. After plant emergence 200ml of Culterra plant food (Multi-Kelp) was applied at a rate of 5ml in 1.5L water once a week. Uninoculated soil was used as control for each soil type.

3.2.3 Inoculation of tubers:
An *R. solani* (PPRI 9527) isolate from the Potato Pathology Program culture collection was obtained. The isolate was plated on fresh PDA plates and allowed to grow for five days at 25°C. Soil autoclaved at 120°C for 1 hour, weighing 3.4kg was potted in 4kg-capacity pots. Each mini-tuber (cv. BP1) was coated with a sludge (mud) prepared by mixing five (5mm x 5mm) colonized agar blocks with 500ml of distilled water and 700g soil (Simons & Gilligan, 1997). One coated mini-tuber was planted in each pot at a depth of 100mm. Soil moisture was maintained by irrigating with 200ml distilled water three times a week. After plant emergence 200ml of culterra plant food (Multi-Kelp) was applied at a rate of 5ml in 1.5L water once a week. Tubers planted without the inoculum coating were used as control for each soil type.

3.2.4 Inoculation of stolons:
An isolate of *R. solani* AG3 (PPRI 9527), from the Potato Pathology Program culture collection was obtained. The isolate was plated on fresh PDA plates and allowed to grow for five days at 25°C. Soil autoclaved at 120°C for 1 hour, weighing 3.4kg was potted in 4kg capacity pots. Each pot received one mini-tuber planted at a depth of 100mm. Two weeks after planting stolons below the soil surface were injected with 0.1ml hyphal suspension using a sterile hypodermic syringe. The hyphal suspension was prepared by blending five (5mm x 5mm) colonized agar blocks with 100ml of distilled water. Soil
moisture was maintained by irrigating with 200ml water three times a week. After plant emergence 200ml of Culterra plant food (Multi-Kelp) was applied at a rate of 5ml in 1.5L water once a week. Stolons not injected with the hyphal suspension were used as control for each soil type.

3.2.5 Isolation from stem lesions:
After five weeks, plants grown in *R. solani*-spiked soils showing symptoms of stem canker lesions were used for fungal isolation to confirm the causal agent. Pieces (5mm x 5mm) were cut from the stem lesions and plated on fresh PDA plates and allowed to grow for five days at 25°C. The cultures were microscopically examined to identify *R. solani*.

3.2.6 Disease assessment:
At harvest, potato tubers were placed into four disease severity categories: 0-nil (tubers with no symptoms) (Fig. 1), 1-low (<3% sclerotia on tuber surface) (Fig. 2), 2-moderate (3-25%) (Fig. 3) and 3-high (>25%) (Fig. 4). The disease severity index (s.i.) was calculated using the following formula (Tsror & Peretz-Alon, 2005):

\[ s.i. = (0 \times n) + (1 \times l) + (2 \times m) + (3 \times h) \]

Total number of tubers

At harvest, stems and stolons were evaluated using a scale of 0-5, where 0 = healthy tissue, 1 = several brown to black lesions, 2 = up to 15% of the tissue is covered with lesions, 3 = up to 30% of the tissue is covered with lesions, 4 = up to 60% of the tissue is covered with lesions and 5 = >60% of the tissue is covered with lesions (Tsror & Peretz-Alon, 2005).

3.2.7 Statistical analysis:
Data were analyzed statistically using GenStat® (Payne et al., 2011). Analysis of variance was used to test for differences between variables and means were separated by means using of Fisher’s protected F-test least significant difference.
3.3 RESULTS

Two soil types were used in this study. The laboratory analysis showed the first was a clay loam with 68% coarse sand, 10% silt and 20% clay, with a pH of 7.3. The second was a sandy soil with 84.6% coarse sand, 5% silt and 10% clay, with a pH of 6.3.

Of the two soil types, tubers harvested from inoculated sandy soils developed significantly more disease than those harvested from clay loam soils ($P \leq 0.003$) (table. 1). Of the three inoculum sources stolon inoculation and seed inoculum caused significantly more disease on progeny tubers than those from $R. solani$ spiked soils ($P \leq 0.001$) (table. 1).

No disease development was observed on plants and progeny tuber from the control pots in this trial, the mean disease severity for all the control pots was 0.0. Therefore, the statistical calculation does not include the controls.
Table. 1. Mean disease severity per inoculum treatment and soil type. 

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Mean Disease severity*</th>
<th>Soil type means (P ≤0.003)</th>
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<tr>
<td></td>
<td>Soil inoculum</td>
<td>Stolon inoculation</td>
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<tr>
<td>Clay loam</td>
<td>2.1 ^ab</td>
<td>3.0 ^b</td>
</tr>
<tr>
<td>Sandy soil</td>
<td>2.6 ^ab</td>
<td>3.4 ^c</td>
</tr>
<tr>
<td>Inoculum means</td>
<td>2.4 ^b</td>
<td>3.2 ^a</td>
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<tr>
<td>(P≤0.001)</td>
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</table>

* Values followed by the same letter do not differ significantly according to Fisher’s F-test least significant difference.

Plants grown in inoculated soils developed stem canker (Fig. 5 A & B) and green tubers (Fig. 6). *R. solani* was successfully isolated from the stem cankers. A *Fusarium* species was also isolated from the infected stem tissue.

Fig. 5 A & B. Stem lesions on plants grown in *R. solani* inoculated soils
3.4 DISCUSSION

Disease development on a potato plant begins with the presence of the pathogen as inoculum. *R. solani* can assume three forms of inoculum: basidiospores, mycelium fragments or sclerotia. Basidiospores are air transmitted inoculum mostly causing disease to aerial parts of the plant (Naito, 1996; Pascual *et al*., 2000). Mycelium fragments and sclerotia are present either as soil- or tuber-borne inoculum and are commonly associated with disease of below ground parts (Atkinson *et al*., 2010).

There is controversy surrounding the role and importance of each inoculum source (soil-borne vs. tuber-borne) in disease development. Some studies show that as little as 12-15% tuber-borne inoculum is sufficient to cause severe stem canker and black scurf symptoms (James & McKenzie, 1972; Atkinson *et al*., 2010). Other studies show significant increases in black scurf and stem canker with an increase in soil-borne inoculum (Gilligan *et al*., 1996; Naz *et al*., 2008). A comparison of disease symptoms from all three inoculum sources in the current study showed only plants grown in *R. solani* inoculated soils developed stem canker. In addition, both stolon and seed inoculum produced progeny tubers with significantly more severe black scurf symptoms than soil inoculation. These results may suggest that soil-borne inoculum plays a more important role in the development of stem canker, whilst tuber-borne inoculum and stolon infections are more important for the development of black scurf.
Although soil-borne inoculum resulted in the least black scurf symptoms, other symptoms such as tuber greening and stem canker were more prominent. Research has shown as stolons grow through soils there is a higher chance of infection by contact with soil-borne inoculum than with tuber-borne inoculum (Frank & Leach, 1980). Infected stolon tips result in stolon pruning, preventing them from growing to their full length (Struik et al., 1990). Shallow growth results in tuber development close to the soil surface and exposure to sunlight. Tubers exposed to sunlight develop poisonous alkaloids and chloroplasts from amyloplasts which turn tubers green in colour (Zhu et al., 1984).

One of the symptoms of *R. solani* disease is stem canker, described as dark brown necrotic lesions on underground stems (Keijer, 1996; Weinhold & Sinclair, 1996; Simons & Gilligan, 1997; Tsror & Peretz-Alon, 2005). *R. solani* and a *Fusarium* specie was isolated from the stem lesions observed in the current study. It is, however, not clear which of the two pathogens was responsible for this symptom or if a relationship exists between the two pathogens resulting in the development of the stem cankers. The stem lesions observed in the current study were not characteristic to stem canker, possibly due to the presence of the *Fusarium* species. A repeat of this experiment should include Koch’s postulates to determine if the *Fusarium* species isolated in this study played a role in the development of the stem cankers.

Recent research has shown how soil structure and texture can influence soil-borne fungi such as *R. solani* (Otten et al., 2001; Harris et al., 2003; Ritz & Young, 2004). Physical soil characteristics determine the ability of soils to retain water and essential nutrients. It also determines the connectivity and tortuosity of the air filled pore spaces within soils (Otten et al., 2001; Harris et al., 2003; Ritz & Young, 2004). Otten et al. (2001) and Harris et al. (2003) proved that loose sandy soils promote the spread and growth of *R. solani*, whilst compact soils inhibit hyphal spread due to the limited available pore space resulting in small, dense colonies. Results from the current study confirm those of Otten et al. (2001) and Harris et al. (2003). Black scurf symptoms were significantly more severe on tubers harvested from loose sandy soils compared to clay loam when data of the different inoculation methods were combined. These results suggest cultivating in clay loamy soils is of advantage as it reduces the disease development by limiting the
growth and spread of *R. solani*. However other studies have also shown certain soil conditions influence the pathogenicity of *R. solani* (James & McKenzie, 1972).

Another soil condition shown to influence *R. solani* mycelial growth and sclerotial development is pH. Research by Ritchie *et al.* (2009) has shown the optimum pH for mycelial and sclerotia growth is 5-6. The sandy and clay loam soil used in the current study had pH readings of 6.3 and 7.3, respectively. The near optimum pH conditions of the sandy soil used in this study may have contributed to more severe black scurf symptoms observed on harvested tubers. Future studies however should focus more on investigating the role soil pH plays on disease development and to confirm results of this study.

In summary, all three inoculum sources resulted in black scurf development, however tuber-borne and stolon inoculum proved to be of primary importance. Soil inoculum was found to be more important for stolon infection and stem canker. Furthermore, the soil structure and texture of sandy soils used in this study promoted more disease development than clay loam soils. Future work should confirm the findings of this study and focus on further investigating the possibility of clay loam soils and soil pH limiting disease development by reducing the pathogenicity of *R. solani*.

### 3.5 REFERENCES


CHAPTER FOUR
INFLUENCE OF INOCULUM PRESSURE OF RHIZOCTONIA SOLANI ON DISEASE DEVELOPMENT UNDER FIELD CONDITIONS IN SOUTH AFRICA

ABSTRACT
Successive cultivation of potato crops increases *Rhizoctonia solani* soil inoculum load resulting in an escalation in disease incidence and severity. Two field trials were conducted over two growing seasons, using three different inoculum levels and an uninoculated control treatment. Four mini plots (1.5m wide x 5m long) were used, consisting of four rows each. The first trial was planted during summer months (November 2009-February 2010) and the second trial was planted during winter months (April 2010-July 2010), where temperatures ranged between 16-29°C and 5-24°C, respectively. The cultivation of potatoes in the same soil over two growing seasons resulted in an increase in diseased (black scurf) tubers. Furthermore, results from the second trial showed black scurf was most severe on tubers from soils inoculated with the highest concentration of inoculum. There were significant disease severity differences, with initial soil inoculum levels being directly proportional to final disease severity.

4.1 INTRODUCTION
The potato is the world’s most important non-grain food crop (Huang et al., 2011). Due to its high nutritional value and ability to produce high yields under diverse environmental conditions, the potato plays an important role in the fight against hunger and poverty in many developing countries. The frequent cultivation of potatoes has led to an increased population of soil-borne pathogens resulting in higher incidence and severity of disease. One such soil-borne pathogen is *R. solani* AG3.

This soil- and tuber-borne pathogen is capable of causing disease on tubers, stems and stolons at all five growing stages of the potato plant (sprout development, vegetative growth, tuber initiation, tuber bulking and tuber maturation) (Banville, 1989; Simons & Gilligan, 1997; Naz et al., 2008). Symptoms on stems and stolons consist of dark brown, necrotic lesions (Simons & Gilligan, 1997; Tsror & Peretz-Alon, 2005). In severely infested fields these symptoms can result in death of sprouts and stems and girdling of stolons, resulting in malformed tubers and reduced yields due to poor stand emergence (James & McKenzie, 1972; Atkinson et al., 2010). During the last two growth stages
(tuber bulking and tuber maturation) of the potato plant, the development of black soil-like structures on tubers referred to as black scurf is common (Woodhall et al., 2007; Thind & Aggarwal, 2008; Woodhall et al., 2008).

The development of disease begins when *R. solani* inoculum is present either as soil- or tuber-borne sclerotia or hyphae (Scholte, 1992; Gilligan et al., 1996; Atkinson et al., 2010). Although tuber-borne inoculum has been shown in some studies to be the most important source (James & McKenzie, 1972; Gudmestad et al., 1979; Atkinson et al., 2010), it is soil-borne inoculum which proves the most difficult to control due to the wide host range of *R. solani* and its ability to survive in soils for several years, even after the cultivation of non-host crops (Gilligan et al., 1996; Sneh et al., 1996; Naz et al., 2008).

Studies have shown the successive cultivation of potato crops resulted in an increase in soil inoculum load, intensifying disease (stem canker, stolon canker and black scurf) incidence and severity (Scholte, 1992; Leach & Webb, 1993; Jager & Velvis, 1995, Mohr et al., 2011). The objective of this study is to investigate the effect of soil inoculum levels on the development, incidence and severity of stem canker, stolon canker and black scurf, over two growing seasons under field conditions.

4.2 MATERIALS AND METHODS

4.2.1 Experimental design:
Field trials were conducted over two growing seasons in the Gauteng province in South Africa. During each trial, temperature and rainfall data was recorded daily at the weather station situated on the farm. The first trial was planted during summer months (November 2009-February 2010) and the second trial was planted in the same soils during winter months (April 2010-July 2010), where temperatures ranged between 16-29°C and 5-24°C, respectively. Twenty disease free mini-tubers (cv. Mondial) were planted by hand in pre-marked rows at a depth of 100mm and 30cm apart. Approximately 105 days after planting, plant vines were physically cut off. Two weeks after haulm destruction tubers were harvested by hand.

4.2.2 Preparation of inoculum:
An *R. solani* (PPRI 9527) isolate from the Potato Pathology Program culture collection which had previously been identified as AG3 using conventional PCR, in a separate
study, was obtained. The isolate was plated onto fresh Potato Dextrose Agar (PDA) plates and allowed to grow for five days at 25°C. Wheat seeds were soaked overnight in sterile distilled water with 250µg/ml chloramphenicol and drained. The moist wheat seeds were autoclaved at 120°C for 1 hour. Each bag of seeds weighing 150g, 250g and 500g, respectively, received five (5mm x 5mm) colonized agar blocks cut up under aseptic conditions. The wheat seeds were shaken every three days during the incubation period of 14 days at 25°C.

4.2.3 Inoculation of soil:
The trials were planted in loamy-clay soil, in four mini plots, 1.5m (wide) x 5m (long), consisting of four rows each. Each mini plot consisted of three different inoculum levels (150g, 250g & 500g inoculum per row) and an uninoculated control treatment. *R. solani* inoculated wheat seeds were evenly distributed in each row after mini-tubers were placed in the furrows. Irrigation pipes were placed alongside each row in the plots. Irrigation was applied daily and adjusted to maintain soil moisture to field capacity.

4.2.4 Isolation from stem lesions:
Plants with stem lesions were used for fungal isolation to confirm the causal agent. Pieces (5mm x 5mm) were cut from the stem lesions and plated on fresh PDA plates and allowed to grow for five days at 25°C. The cultures were microscopically examined to identify *R. solani*.

4.2.5 Disease assessment:
Disease assessment of all tubers and plants was conducted for each inoculum treatment and their respective replicates, separately. Potato tubers harvested from each plant were placed into four disease severity categories: 0-healthy (tubers with no symptoms) (Fig. 1); 1-low (<3% sclerotia on tuber surface) (Fig. 2); 2-moderate (3-25%) (Fig. 3) and 3-high (>25%) (Fig. 4). The disease severity index (s.i.) was calculated using the following formula (Tsror & Peretz-Alon, 2005):

\[
\text{s.i.} = (0 \times n) + (1 \times l) + (2 \times m) + (3 \times h)
\]

Total number of tubers
At harvest, underground stems and stolons were evaluated using a scale of 0-5, where 0 = healthy tissue, 1 = several brown to black lesions, 2 = up to 15% of the tissue is covered with lesions, 3 = up to 30% of the tissue is covered with lesions, 4 = up to 60% of the tissue is covered with lesions and 5 = >60% of the tissue is covered with lesions (Tsror & Peretz-Alon, 2005).

4.2.6 Statistical analysis:
Data were analyzed statistically using GenStat® (Payne et al., 2011). Analysis of variance was used to test for differences between variables and means were separated using Fisher’s protected F-test least significant difference (P≤0.001).
4.3 RESULTS

Maximum temperatures averaged at 29°C and 24°C for the first and second trials, respectively, while temperatures dropped to a minimum of 14°C and 5°C, respectively (Fig. 5). Rainfall data collected showed the summer months received a total of 157.5mm more seasonal rain than the winter months (Fig. 6).

![Graph showing average minimum and maximum temperature per month](image)

**Fig. 5.** Average minimum and maximum temperature (°C) per month

The first field trial planted during November 2009 resulted in only 4 diseased tubers out of a total of 429 harvested tubers, results are therefore not shown. At harvest of the second field trial, 216 tubers showed black scurf symptoms. Although disease severity increased with increasing initial inoculum, there were no significant differences between the 0 and 150 inoculum levels and the 250 and 500 inoculum levels, respectively (Fig. 7).

Stem lesions not typical to *Rhizoctonia* disease were observed on above ground parts of plant stems. Isolation from these lesions did not yield *R. solani* but resulted in the positive identification of a *Fusarium* species.
**Fig. 6 A & B.** Total rainfall (mm) per month

**Fig. 7.** Mean disease severity index over all four inoculum replicates recorded during the second trial. Disease severity categories: 0-healthy (tubers with no symptoms), 1-low (<3% sclerotia on tuber surface), 2-moderate (3-25%) and 3-high (>25%) (Tsur & Peretz-Alon, 2005). Bars followed by the same letter do not differ significantly according to Fisher's F-test least significant difference (P≤0.001).
4.4 DISCUSSION

Infection of potato by *R. solani* AG3 results in black scurf, stem and stolon canker (Woodhall *et al.*, 2007; Thind & Aggarwal, 2008; Woodhall *et al.*, 2008). The incidence and severity of these symptoms is determined by many biotic and abiotic factors as well as initial inoculum load present in soils (Scholte, 1992; Gilligan *et al.*, 1996). Studies investigating the role inoculum plays in disease development have shown crop rotation to be an effective method in reducing inoculum concentrations in soils. Although rotation with non-host crops such as barley, sorghum and certain grasses has been shown to reduce soil inoculum, the correct rotation intervals are also necessary for this method to be effective. Gilligan *et al.* (1996) showed that when potato crops are cultivated consecutively a 70-80% increase in diseased tubers and plants can be expected. The cultivation of potatoes in the same soil over two growing seasons in the current study may have led to the 56% increase in diseased tubers (black scurf) observed in the second trial. Furthermore, results from the second trial showed black scurf was most severe on tubers from soil infested with the highest initial inoculum concentrations. There were significant differences between disease severity and soil inoculum levels, with inoculum levels being directly proportional to final disease severity.

The two most important abiotic factors influencing disease development are temperature and moisture (Sneh *et al.*, 1996; Agrios, 2005). Typical climatic conditions common to the Gauteng province in South Africa are hot, wet summers and cold, dry winters. Research has shown the optimum temperature range for *R. solani* disease development to be between 0-15°C (Beukema & Van Der Zaag, 1990, Sneh *et al.*, 1996). Some studies show high moisture conditions promote the development of black scurf (Rowe *et al.*, 1993; Gilligan *et al.*, 1996; Simons & Gilligan, 1997), whilst others show high moisture levels produce harvests with less extensive sclerotia (Frank & leach, 1980) The variation in results between the two field trials in the current study could be explained by the different weather patterns experienced during the two growing seasons.

The first trial was planted during the warmer months and received a total of 415.5mm of rainfall for the duration of the trial. The second field trial, which was planted during the cooler months and received a total of 258mm of rainfall, resulted in more black scurf diseased tubers than in the first trial. Rainfall during the first trial fell throughout the growing season, during the second trial rainfall was mostly received during the early
parts of the growing season. However the role the rainfall patterns played in the increase of disease severity cannot be conclusive, as the mini plots used in the current study were also irrigated daily. Although some studies have shown *R. solani* growth is restricted by high moisture levels (>1500 bars) (Ploetz & Mitchell, 1985), other studies show moisture levels have no influence on *R. solani* growth (Junior *et al.*, 2007; Olanya *et al.*, 2010). Unfortunately, in this study tensiometer readings to determine soil water content were not taken. A repeat of this experiment should include measurement of moisture and aeration of soils to investigate their role in disease development.

The development of these symptoms is influenced by temperature, soil moisture and inoculum levels (Simons & Gilligan, 1997). Stem or stolon canker symptoms were not observed during either trial, however stem lesions on above ground parts of stems were observed. These lesions did not fit the typical characteristic stem canker symptoms caused by *R. solani*, and isolation of *R. solani* from these lesions was unsuccessful. The absence of stem or stolon canker symptoms may suggest that the environmental conditions did not favour disease development. In addition, the absence of canker symptoms could also be attributed to the lack of seed inoculum, which has been associated with stolon and stem infections at early stages of the growing period (Frank & Leach, 1980).

In recent years, studies have investigated the important role soil characteristics play in soil inoculum levels and disease development (Otten *et al.*, 2001; Harris *et al.*, 2003; Ritz & Young, 2004). Soil structure and texture affects pore size, aeration, water movement, plant root growth and retention ability of nutrients and water, thereby influencing the growth and spread of soil-borne pathogens. Research has shown hyphal growth and spread is restricted in high density soils, with high water retention capacity, as pore space is limited and pores are often filled with water (Otten *et al.*, 2001; Harris *et al.*, 2003; Ritz & Young, 2004). The narrow and limited pore space available in high density soils, such as loamy-clay used in the current study may have inhibited disease development by preventing complete *R. solani* colonization of the soil (Otten *et al.*, 1999; Otten *et al.*, 2001; Harris *et al.*, 2003; Ritz & Young, 2004). Disease incidence and severity may have been higher if this experiment was conducted using loose, sandy soils. Further research investigating the role of soil type (sandy soil vs. dense soil) is discussed in chapter three.
Although the relationship between inoculum levels and disease severity has been established, our attempts at quantifying the amount of inoculum present in soils after inoculation using real time PCR in a separate study, proved unsuccessful. Knowledge of inoculum levels in soils prior to planting is essential for better disease management. Therefore, future studies should focus on using diagnostic tools such as nested PCR, microarray or real time PCR to detect and quantify \( R.\ solani \) in soils. Further research into the role of interactions between \( R.\ solani \) and the environment and other microorganisms on disease development, is necessary for better disease management decisions.

4.5 REFERENCES


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CHAPTER FIVE
SUSCEPTIBILITY OF FIVE POTATO CULTIVARS COMMONLY CULTIVATED IN SOUTH AFRICA, TO RHIZOCTONIA SOLANI

ABSTRACT
In addition to chemical and biological control, the use of tolerant cultivars can be an effective method for reducing *Rhizoctonia solani* inoculum levels in soils, thereby decreasing disease severity and incidence. Two pot trials were conducted to determine susceptibility of five cultivars, BP1, Fianna, Mondial, Up-To-Date (UTD) and Valor, to *R. solani*. The first pot trial was grown at 0-10°C at night and 15–20°C during the day; during the second trial temperatures were 21-23°C at night and 25-26°C during the day. Results showed none of the cultivars to be resistant to *R. solani*, however BP1 was less susceptible to *R. solani* at temperatures between 21-26°C. Furthermore, in comparison to the other cultivars UTD had the lowest severity index values for both trials suggesting it may be less susceptible to black scurf at a wider range of temperatures (0-26°C). A comprehensive analysis of the results from both trials showed more severe disease symptoms on all cultivars were observed under cooler temperatures.

5.1 INTRODUCTION
*Rhizoctonia solani* Kuhn (teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk) is a soil-borne fungus causing various disease symptoms on many different host crops (Brooks, 2007; Guleria *et al*., 2007; Thind & Aggarwal, 2008). Symptoms associated with the potato crop include black scurf, stolon canker and stem canker (Woodhall *et al*., 2007; Thind & Aggarwal, 2008; Woodhall *et al*., 2008). There are 13 different Anastomosis Groups (AG’s) within this species complex (Lees *et al*., 2002; Harikrishnan & Young, 2004; Guleria *et al*., 2007; Woodhall *et al*., 2007). Each AG has been shown to differ in disease symptoms, host range and control methods (Sneh *et al*., 1996; Keijer *et al*., 1997; Tewoldemedhin *et al*., 2006). However, research has commonly attributed black scurf on potato crops to AG 3 (Parmeter, 1970; Sneh *et al*., 1996; Lees *et al*., 2002; Virgen-Calleros *et al*., 2000; Back *et al*., 2006; Lehtonen *et al*., 2009; Lahlali & Hijri, 2010).

Black scurf is often referred to as a “cosmetic” disease, identifiable as black soil-like structures (sclerotia), on tuber surfaces. Potato tubers showing black scurf symptoms
are downgraded in consumer markets resulting in immense economic losses (Platt et al., 1993; Hamid et al., 2006; Al-Mughrabi, 2008). Seed markets are also susceptible to such losses as the certification of potato seed tubers in South Africa is governed by the Plant Improvement Act (ACT No. 57 of 1976) which permits the following (Republic of South Africa, 2002):

- 0% infection in G0
- 0.5-20% infection in G1-3
- 1-20% infection in G4-6
- 1-20% infection in G7-8

Reducing inoculum levels in soils and on seed tubers is therefore essential. Fungicides, applied as either soil and/or seed treatments, are the preferred method of control (Campion et al., 2003; Boogert Van der & Luttikholt, 2004; Rauf et al., 2007). However, efficacy of these fungicides is dependent on toxicity levels of the active ingredient (a.i.) and on the morphology, physiology, virulence and genetic constitution of different *Rhizoctonia* species (Kataria et al., 1991). Furthermore, the use of fungicides over time could result in development of resistance, residue build-up on harvested products and adverse effects on the environment, animal and human health (Eddleston et al., 2002; Tomlin, 2006).

Recent studies have investigated the use of biological agents for reducing disease incidence and severity. Some mycoparasites such as *Trichoderma harzianum*, *T. viridae*, *T. hamatum*, *Gliocladium virens* and *Verticillium biguttatum* have been shown to significantly reduce *R. solani* inoculum levels in soils (Wale, 2004; Brooks, 2007; Wilson et al., 2008). However, geographical variations in temperature, soil type, soil moisture and cultural practices can limit the use of biological control agents.

In addition to chemical and biological control, the use of tolerant cultivars is the most economical yet effective method for reducing inoculum levels in soils (Otrysko & Banville, 1992, Leach &Webb, 1993). Research by Du Plessis (1999) showed varying disease susceptibility levels of 16 different cultivars grown in South Africa. However there are approximately 79 different cultivars in South Africa, of which the most prominent on the consumer market is Mondial (65%), BP1 (14%) and Up-To-Date (UTD)
(5%) (Potatoes South Africa, 2010). Further research on susceptibility levels of these cultivars to *R. solani* is therefore required. This study focuses on determining susceptibility levels of five commonly planted cultivars; BP1, Mondial, Valor, Fianna and UTD, to *R. solani*.

5.2 MATERIALS AND METHODS

5.2.1 Pot trials:
Pot trials were conducted at the University of Pretoria under greenhouse conditions. Disease free mini-tubers (cv. BP1, Fianna, Mondial, UTD and Valor) were used for the trials (table 1). The pot trials were set out in a randomized complete block design and consisted of five cultivars planted in inoculated soil and uninoculated soil (control). The first pot trial started in June 2009 and was maintained at temperatures of 0-10°C at night and 15–20°C during the day. The second trial started in October 2010 and was maintained at temperatures of 21-23°C at night and 25-26°C during the day. Each treatment was replicated five times. Approximately 105 days after planting, plant vines were cut off manually. Tubers were harvested two weeks after haulm destruction.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Black scurf susceptibility/ tolerance</th>
<th>Growth period</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP1</td>
<td>Highly susceptible</td>
<td>Medium (90-110 days)</td>
</tr>
<tr>
<td>Fianna</td>
<td>Moderately susceptible</td>
<td>Medium (90-110 days)</td>
</tr>
<tr>
<td>Mondial</td>
<td>Highly tolerant</td>
<td>Medium (90-110 days)</td>
</tr>
<tr>
<td>UTD</td>
<td>Susceptible</td>
<td>Medium to long (90-120 days)</td>
</tr>
<tr>
<td>Valor</td>
<td>Moderately susceptible</td>
<td>Medium (90-110 days)</td>
</tr>
</tbody>
</table>

5.2.2 Preparation of inoculum:
An *R. solani* (PPRI 9527) isolate from the Potato Pathology Program culture collection which had previously been identified as AG3 using conventional PCR, in a separate study, was obtained. The isolate was plated on fresh Potato Dextrose Agar (PDA) plates and allowed to grow for five days at 25°C. Wheat seeds were soaked overnight in sterile distilled water with 250µg/ml chloramphenicol and drained. The moist wheat seeds were autoclaved at 120°C for 1 hour. Each bag of seeds weighing 200g was mixed with five (5mmx5mm) agar blocks of actively growing mycelium cut up under aseptic conditions.
The wheat seeds were shaken every three days during the incubation period of 14 days at 25°C (Sneh et al., 1986).

5.2.3 Inoculation of soil:
Soil autoclaved at 120°C for 1 hour, weighing 3.4kg was potted in 4kg-capacity pots. Each pot was inoculated with 40g of \textit{R. solani} colonized wheat seeds. The inoculum was thoroughly mixed into the soil before planting one seed tuber in each pot at a depth of 100mm. Soil moisture was maintained with 200ml distilled water three times a week. Plants were fertigated with 200ml of Superfeed every three weeks, applied at a rate of 1g in 1L water.

5.2.4 Disease assessment:
At harvest, potato tubers harvested from each plant were placed into four disease severity categories: 0-nil (tubers with no symptoms) (Fig. 1), 1-low (<3% sclerotia on tuber surface) (Fig. 2), 2-moderate (3-25%) (Fig. 3) and 3-high (>25%) (Fig. 4). The disease severity index (s.i.) was calculated using the following formula (Tsror & Peretz-Alon, 2005):

\[
\text{s.i.} = \left(0 \times n\right) + \left(1 \times l\right) + \left(2 \times m\right) + \left(3 \times h\right)
\]

\text{Total number of tubers}

At harvest, underground stems and stolons were evaluated using a scale of 0-5, where 0 = healthy tissue, 1 = several brown to black lesions, 2 = up to 15% of the tissue is covered with lesions, 3 = up to 30% of the tissue is covered with lesions, 4 = up to 60% of the tissue is covered with lesions and 5 = >60% of the tissue is covered with lesions (Tsror & Peretz-Alon, 2005).

5.2.5 Statistical analysis:
Data were analyzed statistically using GenStat® (Payne et al., 2011). Analysis of variance was used to test for differences between variables and means were separated using Fisher’s protected F-test least significant difference.
5.3 RESULTS

Results from the first pot trial planted during June 2009 showed varying levels of black scurf in all five cultivars. Cultivar Fianna and BP1 developed the highest level of disease symptoms, compared to the other three cultivars which showed high to moderate levels of disease symptoms. UTD showed the least amount of disease, although not significantly less than Mondial (Fig. 5). However in the second pot trial planted in October 2010 all cultivars had low (<3%) to moderate (3-25%) disease symptoms with no significant differences between the cultivars (Fig. 6). No disease development was observed on plants and progeny tuber from the control pots in this trial, the mean disease severity for all the control pots was 0.0.
**Fig. 5.** Mean disease severity per cultivar in pot trial one. Disease severity categories: 0-nil (tubers with no symptoms), 1-low (<3% sclerotia on tuber surface), 2-moderate (3-25%) and 3-high (>25%) (Tsror & Peretz-Alon, 2005). Bars followed by the same letter do not differ significantly according to Fisher’s F-test least significant difference (P≤0.001).

**Fig. 6.** Mean disease severity per cultivar in pot trial two. Disease severity categories: 0-nil (tubers with no symptoms), 1-low (<3% sclerotia on tuber surface), 2-moderate (3-25%) and 3-high (>25%) (Tsror & Peretz-Alon, 2005). Bars followed by the same letter do not differ significantly according to Fisher’s F-test least significant difference (P≤0.001).
5.4 DISCUSSION

Disease incidence and severity increases when favorable temperatures exist for the pathogen and not for the host. When temperatures are optimum for the host but not for the pathogen, disease development is inhibited (Agrios, 2005). Studies have shown that the optimum temperature range for the healthy growth of a potato plant is 20-25°C, while cooler temperatures 10-15°C results in weaker plant growth, allowing Rhizoctonia to cause more disease (Beukema & Van Der Zaag, 1990, Sneh et al., 1996). Results from the current study supports this as more severe disease symptoms were observed under cooler temperatures (0-20°C). These results also support those of Du Plessis (1999) showing higher disease incidence and severity of black scurf at temperatures between 12-15°C than at temperatures ranging between 21-28°C.

Tuber development and maturity in early cultivars occurs earlier in the growing season than that of late cultivars (Beukema & Van Der Zaag, 1990; Rowe et al., 1993). Bains et al. (2002) found that late maturing cultivars developed low levels of black scurf as compared to early and mid-season cultivars. He suggested the reason for these results were the differences in the time of tuber maturity between the cultivars. However the exact mechanism of how the time of tuber maturity affects disease development is unknown. Although none of the five cultivars used in the current study were resistant to R. solani, results showed varying levels of black scurf symptoms between the cultivars. Statistical analysis of the results showed that Mondial, a mid-season cultivar, had fewer disease symptoms when compared to the other mid-season cultivars (BP1, Fianna, and Valor). These results therefore do not agree with the findings of Bains et al. (2002). Furthermore these results are not in keeping with that of Visser (2011) who reported Mondial to be highly tolerant to black scurf (table. 1). Future research should focus more on unraveling the genetic composition of the potato tuber to identify potential resistance genes in different cultivars.

The killing of vines (haulm destruction) to induce early tuber maturation is a common practice in the potato production industry. Studies have shown the time between haulm destruction and harvest influences the development of black scurf on progeny tubers (Beukema & Van Der Zaag, 1990; Rowe et al., 1993; Kempenaar & Struik, 2007). A study by Gudmestad et al. (1979) showed that if tubers are harvested within 3-4 weeks after vine killing, disease symptoms are minimal. In the current study, tubers were
harvested two week after vine killing; however progeny tubers showed medium to high levels of black scurf symptoms. These results may suggest if initial inoculum levels are high under favorable biotic and abiotic conditions; the influence of haulm destruction on disease development may be negligible. Banville (1989) during his research on cultivar susceptibility also made mention of cultivars showing greater susceptibility levels being a result of the amount of inoculum and environmental conditions.

The role of inoculum source (seed vs soil) on disease development has been the subject of many studies (Frank & Leach, 1980; Platt et al., 1993; Gilligan et al., 1996; Tsror & Peretz-Alon, 2005; Atkinson et al., 2010; Lees et al., 2010). Research by Tsror & Peretz-Alon (2005) showed that when seed and soil inoculum is present black scurf incidence and severity increases. Furthermore, the presence of both inoculum sources under cold and moist conditions also increases the incidence and severity of stem canker and stolon canker (Frank & Leach, 1980; Platt et al., 1993; Rowe et al., 1993; Gilligan et al., 1996; Simons & Gilligan, 1997; Al-Mughrabi, 2008; Ritchie et al., 2009; Atkinson et al., 2010). In this study inoculated wheat seeds were the only source of inoculum which could explain why no stem or stolon canker was observed on any of the potato plants. It could also be that the temperature range and moisture levels in this study were not favorable for the development of stem and stolon cankers.

In summary, none of the cultivars used in this study were resistant to *R. solani* and no stem or stolon canker symptoms were observed. However, higher incidence of disease symptoms were observed on progeny tubers cultivated under cooler temperatures. Future research should focus on the possibility of cv. UTD being less susceptible to black scurf at temperatures ranging from 0-26°C; the importance of timing of lifting after haulm destruction in disease development and unraveling the genetic composition of various cultivars which could potentially provide more insight into cultivar resistance to *R. solani*.

### 5.6 REFERENCES


Rhizoctonia solani inoculum can be present either as soil- or tuber-borne sclerotia or hyphae. Although both inoculum sources play a role in disease development, it is not clear which of the two is more important. Successive cultivation of potato crops increases *R. solani* soil inoculum load resulting in an escalation in disease incidence and severity. The use of tolerant cultivars, however, can effectively reduce inoculum levels thereby decreasing disease intensity. Four pot trials were conducted; the objective of the first two pot trials was to determine the effect of tuber and soil-borne inoculum and stolon inoculations on disease development in sandy and clay loam soils. The second two pot trials were aimed at determining susceptibility levels of five cultivars. Two field trials were planted over two growing seasons in the same soils, using three inoculum levels. Results from the pot trials showed that tubers harvested from inoculated sandy soils developed significantly more disease than those harvested from clay loam soils. Of the three inoculum sources, stolon inoculation and seed-borne inoculum resulted in significantly more disease on progeny tubers than those from *R. solani* spiked soils. Although none of the cultivars proved to be tolerant to *R. solani*, BP1 was less susceptible to *R. solani* at temperatures between 21-26°C. More severe disease symptoms were observed under cooler temperatures on all cultivars. Results from the field trial showed the cultivation of potatoes in the same soil over two growing seasons resulted in an increase in diseased (black scurf) tubers. Furthermore, black scurf was most severe on tubers from soils infested with the highest concentration of inoculum. There were significant disease severity differences, with initial soil inoculum levels being directly proportional to final disease severity. Future studies in South Africa should focus on investigating the genetic composition of various cultivars; the effect of soil type and pH on the pathogenicity of *R. solani* and the use of molecular diagnostic tools to detect and quantify *R. solani* in soils.