Etiology and alternative control of potato rhizoctoniasis in South Africa

by

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Potato (*Solanum tuberosum* L.) is an annual, herbaceous, dicotyledonous plant belonging to the *Solanaceae*. It is allied to tomato (*Lycopersicon esculentum* Merr.), brinjal (*Solanum melongena* L.) and capsicum (*Capsicum* spp.), as well as to potent narcotics such as tobacco (*Nicotiana tabacum* L.), henbane (*Hyoscyamus niger* L.) and belladonna (*Atropa belladonna* L.). Potato tubers contain about 18 % carbohydrates, 2.2 % protein, 0.1 % fat, 0.43 % potassium, 0.06 % phosphorus, 0.04 % chlorine, 0.03 % sulphur, 0.03 % magnesium, 0.02 % calcium, 0.005 % sodium and 0.001 % iron, as well as the vitamins ascorbic acid, niacin, retinol, riboflavin and thiamine (Graves & Taber, 1942; www.potatoes.co.za/home.asp?pid=14 30 May 2003). Most potatoes are used for human consumption, although approximately 50 % of the European stock is utilised as fodder, with as much as 25 % of the ware potatoes also being diverted to cattle feed because of defects (Hooker, 1983).

Despite being rich in various nutrients, and contradictory to the claim by Potatoes South Africa (www.potatoes.co.za/home.asp?pid=14 30 May 2003) that one serving will meet a person's daily nutrient requirements, potatoes are ill-adapted for an exclusive diet owing to the low protein content of the tubers. Indeed, some propagandists insist that potatoes should be avoided to maintain a healthy lifestyle (Van Rensburg, 2003). Potato tubers furthermore are known to contain toxic glycoalkaloids such as α-solanine and α-chaconine (Morgan & Coxon, 1987), the concentrations of which are affected by the genetic constitution of the plant (Sanford & Sinden, 1972), conditions of cultivation, and postharvest treatment of tubers (Jadhav *et al*., 1981). A study by Jelinek *et al*. (1976) has shown that extracts of healthy potatoes could be teratogenic due to the presence of solanine. Solanine levels increase when tubers are attacked by an incompatible race of the late blight pathogen, *Phytophthora infestans* (Mont.) de Bary, but not by compatible races (Kadis *et al*., 1972).

Also of considerable significance, particularly from an animal husbandry perspective, is the susceptibility of potato tubers to infection by mycotoxigenic fungi. Many of the *Fusarium* species associated with dry rot of potato tubers are capable of producing mycotoxins (Marasas *et al*., 1984). Mycotoxins that have been detected as natural contaminants in potato tubers include trichothecenes (Lafont *et al*., 1983), sambutoxin (Kim *et al*., 1995) and cytochalasin B, the latter produced by the gangrene pathogen, *Phoma exigua* Desm.

(Scott *et al*., 1975). *Fusarium oxysporum* Schltdl. em. W.C. Snyder & H.N Hansen, a major cause of dry and stem-end rot of potato tubers in South Africa (Theron, 1999), is known to produce fumonisins (Abbas *et al*., 1995; Seo *et al*., 1996), a group of related polar metabolites that have been implicated in mycotoxicoses such as porcine pulmonary edema (Osweller *et al*., 1992), equine leukoencephalomalacia (Wilson *et al*., 1990) and human oesophageal cancer (Rheeder *et al*., 1992; Marasas *et al*., 1993; Chu & Li, 1994; Yoshizawa *et al*., 1994). Fumonisins have also been reported to increase serum cholesterol levels and to induce chronic hepatotoxicity in vervet monkeys (Fincham *et al*., 1992).

Notwithstanding the above reservations there does not seem to be any aversion to potatoes in any region of the world. Many poorer communities depend on it as basic means of sustenance, whereas a diet without potatoes prepared or processed in some or other way is well-nigh unimaginable among the more affluent, not to mention the distilled brew. Consequently, potatoes not only are the most important dicotyledonous source of human food, but overall ranks fourth in world consumption after wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.) (Rowe, 1993).

In South Africa, potatoes have even a higher status, being the third most important food source after maize and wheat (Anonymous, 2002). Besides Morocco, South Africa is also the only African country that exports potatoes, albeit in limited quantities (Cilliers, 2003). Potatoes represent 2 % of the gross value of all agricultural products in the country, but are cultivated on only 0.03 % of the arable land. In 2001, 1.60 million tonnes of potatoes to a value of R2 014 million were produced on 14 101 ha dry and 39 685 ha irrigated land in the country by about 1 000 commercial and 1 100 small-scale farmers (Cilliers, 2003; Jordaan, 2003), employing almost 150 000 people at farm level alone (Van Vuuren & Le Roux, 2004). Both the area cultivated and yield declined somewhat in 2002 (47 000 ha, 1.45 million tonnes), but the value of the crop remained essentially the same (Anonymous, 2004). Disconcerting, however, is that the total production cost of potatoes in South Africa for 2002/03 is estimated at R2 381 million (R1 682 million for production under irrigation, R404 million for seed and R295 million for dry land) (Cilliers, 2003), which is R367 million more than the total value of the potato crop in 2001/02. Although the high production costs could be compensated for by higher market prices, potato growers will have to increase productivity to remain competitive.

Pests, weeds and diseases constitute major restraints to the profitable production of potatoes in all parts of the world, and their control in South Africa presently comprises

almost 11 % of the total production cost of the crop (Cilliers, 2003; Jordaan, 2003). More effective control strategies would obviously contribute to higher productivity, but to achieve this, thorough and scientifically-founded knowledge of the various disorders under local conditions is vitally important.

Of the 31 diseases caused by 30 fungal, 10 viral and three bacterial pathogens on potato in South Africa (Gorter, 1977; Denner *et al*., 1993; Theron, 1999; Crous *et al*., 2000; Millard, 2003), rhizoctoniasis induced by *Rhizoctonia solani* J.G. Kühn (teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk) is one of the oldest and most common. Infection of potato stems and stolons below the soil surface results in stem canker (Fig. 1), whereas sclerotia produced by the pathogen on tubers are referred to as black scurf (Fig. 2). Together with *Verticillium dahliae* Kleb., *Colletotrichum coccodes* (Wallr.) S. Hughes and *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven, *R. solani* is also involved in the early dying syndrome of potato (Kotcon *et al*., 1985), whereas Elarosi (1957a, b) reported a synergistic increase in the damage to potato tubers by *R. solani* and *Fusarium solani* (Mart.) Appel & Wollenw. Furthermore, since growth of potato plants infected by *R. solani* is delayed in all phases (Hide *et al*., 1985; Banville, 1989), the plants are more prone to infection by late blight and attack by aphids, which are important vectors of potato viruses (Banville *et al*., 1996).

Notwithstanding the above, yield reductions induced by *R solani* are often regarded as insignificant or not worth controlling (Hooker, 1978; Weinhold *et al*., 1982), although studies indicated that cultivar-dependent yield reductions of 7 – 64 % (average 35 %) may result if the seed source is contaminated with sclerotia (Carling & Leiner, 1986; Carling *et al*., 1989). In calculable terms, the most notable losses nevertheless occur in the seed market. Despite fairly lenient certification standards (Republic of South Africa, 1998), 0.04 % of the 4.1 million 25 kg bags of seed tubers produced in 2001/02 in South Africa were rejected and 0.6 % were downgraded due to black scurf (http://www.potatoes.co.za/uploads/105-Opbrengs%20SA%2025-07-02.gif Feb. 2003). This is considerably less than in previous years, e.g. the 1.2 % of 5.2 million bags rejected in 1998/99 (Database, Potato Seed Certification, Potatoes South Africa), but could be ascribed to more effective culling of infected seed in consignments submitted for certification, rather than a decline in disease. It is important to note that the above rejection rates do not reflect symptomless infection, i.e. the presence of viable mycelium of *R. solani* on tubers free of sclerotia, which seems to be a common source of inoculum in South Africa (Du Plessis, 1999) and elsewhere (Hide *et al*., 1973; Frank & Leach, 1980; Wicks *et al*., 1996).

Figure 1. Stem canker lesions on the underground parts of potato stems caused by *Rhizoctonia solani*.

Figure 2. Potato tubers with black scurf symptoms due to the presence of sclerotia produced by *Rhizoctonia solani*.

Information on the etiology, ecology, symptomology, pathology, epidemiology and control of potato rhizoctoniasis is voluminous. However, apart from the first recording by Doidge (1918) and subsequent referral to the prevalence of the disease (Doidge *et al*., 1953; Gorter, 1977; Crous *et al*., 2000), only Du Plessis (1999) has investigated the rhizoctoniasis complex in South Africa to some extent. Du Plessis (1999) focused mainly on chemical, cultural and varietal control, but also studied the effect of inoculum source and temperature on disease. His study assumed that *R. solani* anastomosis group (AG) 3, like in most other parts of the world (Banville *et al*., 1996), is the primary cause of rhizoctoniasis in South Africa, though subsequent screening of plants and soil occasionally yielded isolates that did not anastomose with AG-3. The present dissertation elucidates the etiology of potato rhizoctoniasis in South Africa and, cognisant of the high production costs and current emphasis on organic farming, evaluates strategies for the alternative and novel control of the diseases.

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CHAPTER 2

LITERATURE REVIEW

Introduction

Rhizoctoniasis of potato (*Solanum tuberosum* L.) is caused by the fungus *Rhizoctonia solani*, which was first described as a parasite of potato plants by Kühn (1858). Since then the species has gained the reputation of being a widespread, destructive and versatile plant pathogen. Fungi generally grouped as *R. solani* occur in all parts of the world and are capable of attacking a wide range of hosts (*ca*. 250 plant species), causing seed decay, damping-off, stem canker, root rot, fruit decay and foliage disease. This almost unlimited host range, combined with competitive saprophytic ability and lethal pathogenic potential, earn *R. solani* its status as formidable pathogen.

The current species concept stipulates that isolates of *R. solani* possess characteristics such as some shade of brown hyphal pigmentation, branching near the distal septum of cells in young vegetative hyphae, constriction of hyphae and formation of septa a short distance from the point of origin of hyphal branches, dolipore septa and multinucleate cells in young vegetative hyphae (Parmeter & Whitney, 1970). Characteristics such as monilioid cells, sclerotia, hyphae greater than 5 µm in diameter, rapid growth rate and pathogenicity are usually present, but may be lacking in some isolates. Morphological features that are never present include clamp connections, conidia, sclerotia differentiated into rind and medulla, rhizomorphs and pigments other than brown. However, no single feature, except the teleomorph, *Thanatephorus cucumeris* (A.B. Frank) Donk, serves to distinguish *R. solani* from related fungi. The holomorph can be described as follows: Basidiome effused, pellicular; subicular hyphae brownish, multinucleate, often constricted near branching point, relatively wide (some >10 µm), without clamp connections; brown sclerotia present; cystidia and other sterile hymenial elements lacking; basidia homobasidious, hyaline, thin-walled, barrel-shaped to sub-cylindrical, (10-)12-20(-23) x 8-12(-13) µm, with (2-)4(-5) sterigmata; protosterigmata (epibasidia) not swollen; basidiospores ovoid to ellipsoid, (7-)8-13.5 x 4-5(-7) µm (Hawksworth *et al*., 1995; Stalpers & Andersen, 1996).

Anastomosis groups of *Rhizoctonia solani*

R. solani is a species complex composed of morphologically similar fungi that are quite

variable in karyotype (Keijer *et al*., 1996), cultural appearance, growth characteristics and pathogenicity (Butler & Bracker, 1970). The various biotypes are divided into anastomosis groups (AGs) based on the ability of their hyphae to fuse *in vitro*. Presently, 13 AGs (designated AG-1 through AG-13) and 21 subgroups (designated AGs 1-IA, 1-IB, 1-IC, 1-ID, 2-1, 2-2-IIIB, 2-2-IV, 2-2-LD, 2-3, 2-4, 2-BI, 3-IIA, 3-IIB, 3-IIC, 3-TB, 4-HG-I, 4-HG-II, 6-GV, 6-HG-I, 9-TX, 9-TP) are recognised (Ogoshi, 1987; Naito & Kanematsu, 1994; Carling, 1996; Hyakumachi *et al*., 1998; Carling *et al.,* 1999, 2002a, b; Kuninaga *et al*., 2000; Priyatmojo *et al*., 2001). Subgroups within AGs are partially based on differences in one or more biochemical, genetic, or pathogenic characteristic (Ogoshi, 1987). Some isolates of certain groups will anastomose with members of some other AGs, e.g. AG-2, AG-3, AG-6 and AG-8, but most isolates, particularly those of AG-1, AG-4, AG-5, AG-7 and AG-9 anastomose only with members of their own group (Carling, 1996).

AGs and subgroups have been compared by means of DNA/DNA hybridisation, ribosomal DNA restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA PCR, isozyme electrophoresis, pectic zymograms and DNA restriction mapping in the internal transcribed spacer 5.8 S rDNA region, which indicated that different AGs and subgroups are discrete evolutionary units (Vilgalys, 1988; Cruickshank, 1990; Vilgalys & Gonzalez, 1990; Laroche *et al*., 1992; Liu *et al*., 1993; MacNish *et al*., 1993; Balali *et al*., 1996; Kuninaga *et al*., 1997; Carling *et al*., 2000b). RFLP in the nuclear encoded ribosomal DNA repeat of *R. solani* revealed considerable molecular variation among and within subgroups that have been recognised previously on the basis of anastomosis, morphology and pathogenicity (Vilgalys & Gonzalez, 1990).

AG-3 is the principal cause of potato rhizoctoniasis (Carling & Leiner, 1986, 1990b; Bandy *et al*., 1988; Bains & Bisht, 1995), hence its classification as Fusion gruppe F “kartoffel” by Richter & Schneider (1953). Reports also refer to AG-1 (Carling & Leiner, 1990a), AG-2 (subgroups -1 and -2) (Chand & Logan, 1983; Carling & Leiner, 1986, 1990a), AG-4 (Anguiz & Martin, 1989; Carling & Leiner, 1990a; Bains & Bisht, 1995; Balali *et al*., 1995), AG-5 (Bandy *et al*., 1984; Carling & Leiner, 1990a; Bains & Bisht, 1995; Balali *et al*., 1995), AG-7 (Carling *et al*., 1998), AG-8 (Carling & Leiner, 1990a; Hide & Firmager, 1990; Balali *et al*., 1995; Banville *et al*., 1996) and AG-9 (Carling *et al*., 1987; Carling & Leiner, 1990a) being pathogenic to potato.

AG-1 and AG-2 cause only minor damage to sprouts (Carling & Leiner, 1990a). Isolates of AG-2 were collected from sclerotia on potato tubers and from hymenia and lesions on stems (Chand & Logan, 1983; Carling & Leiner, 1986).

AG-3 is by far the most aggressive AG on potato, and indiscriminately attacks roots, stolons and subterranean portions of the main stem (Carling & Leiner, 1990a; Bains & Bisht, 1995). Although AG-3 is virulent across a broad range of temperatures (5 to 25 °C), it is particularly aggressive at 10 to 15 °C, where other AGs generally become less damaging (Carling & Leiner, 1990a). On average, isolates from hymenia were significantly more virulent than isolates from lesions, but neither differed significantly in virulence from isolates obtained from sclerotia or soil (Carling & Leiner, 1986, 1990b; Hill & Anderson, 1989).

AG-4 causes damage to sprouts, roots and underground stems of the potato plant (Anquiz & Martin, 1989; Carling & Leiner, 1990a; Bains & Bisht, 1995; Balali *et al*., 1995). AG-4 isolates have been shown to cause damping-off of potato seedlings, resulting in a seedling mortality of 70 % (Anquiz & Martin, 1989). A greater number of isolates were collected in warm environments at low elevations, than in cool environments at high elevations (Anguiz & Martin, 1989; Carling & Leiner, 1990a).

AG-5 is associated with stem and root canker and black scurf (Bandy *et al*., 1984; Balali *et al*., 1995). It can also damage roots, stems, and stolons, but generally is less aggressive and inflicts less damage than AG-3 (Carling & Leiner, 1990a; Bains & Bisht, 1995). AG-5 tends to be more damaging at 21 °C than at 10 °C (Carling & Leiner, 1990a; Balali *et al*., 1995).

AG-7 was isolated from sclerotia on potato tubers. It caused superficial discoloration of shoots at 10 and 21 °C and lesions on roots at 10 °C. It is less damaging to potato than AG-3, and appears to be more aggressive at lower temperatures (10 °C) (Carling *et al*., 1998).

AG-8 mainly attacks root tissue of potato (Carling & Leiner, 1990a; Hide & Firmager, 1990; Balali *et al*., 1995), although limited damage to stolons can occur (Carling & Leiner, 1990a). Nevertheless, Carling & Leiner (1990a) have shown that AG-8 can be as damaging to potato roots as AG-3. Infection by AG-8 has not been observed in the field, but growth chamber evidence indicates that it may pose a threat to potatoes cultivated commercially (Banville *et al*., 1996). Most main roots were pruned off and a large number of lateral roots developed, resulting in an unusually fibrous root system (Hide & Firmager, 1990). Like AG-5, AG-8 tends to be more virulent at higher temperatures (Carling & Leiner, 1990a).

AG-9 isolated from potato stem and root segments and soil in which potatoes grew, caused only small lesions on potato sprouts (Carling *et al*., 1987).

Infection and disease development in potato

R. solani colonises the below-ground potato plant surface in response to root and shoot exudates (Jeger *et al*., 1996). It proliferates on the root/stolon system to form an extensive network of anastomosing hyphae. During the colonising phase the host plant remains symptomless as long as infection structures are not formed. The early steps of infection are initiated by successive branching of runner hyphae resulting in the formation of short swollen cells giving rise to infection cushions (Hofman & Jongebloed, 1988; Keijer, 1996). Infection cushions are believed to be prerequisite to inducing stem and stolon lesions (Keijer *et al*., 1997) and serve as additional food base for further colonisation of the plant. AG-3 forms relative small infection cushions as condensed areas in a network of interconnecting hyphae (Keijer, 1996). As colonisation of plant tissue is restricted to the part directly under the infection cushion, *R. solani* does not colonise sprout tissue progressively and the size of lesions caused by infection is proportional to the size of the infection cushions on the plant surface (Hofman & Jongebloed, 1988). Thin infection hyphae arising from the infection cushions penetrate the underlying plant tissue and lesions on potato sprouts are only formed after penetration from infection cushions and not from appressoria or entry through stomata or wounds (Hofman & Jongebloed, 1988). The infection process is both mechanical and enzymatic, the enzymes involved being DNAse, RNAse, lipase, α-amylase, cellulose, chitinase, pectinase, pectin lyase, β-glucanase, protease and urease (Bertagnolli *et al.,* 1996).

The two most common symptoms caused by *R. solani* on potato are black scurf (presence of sclerotia on tubers) and stem canker (occurring as brown, necrotic lesions on stems and stolons below the soil surface). Hymenia of the teleomorph may form near the soil surface on aerial stems. The hymenia do not cause damage to the plant but basidiospores contained in them may serve as source of subsequent infections (Banville *et al*., 1996). Other manifestations of infection include poor and uneven stands; premature dying; pruned stolons and sprouts; lesions on roots, stems and stolons; rosette appearance; girdled stems; necrosis in the stem-end of tubers; russeting of skin; and cracked and malformed tubers (Carling *et al*., 1989; Hide *et al*., 1992). Infected plants generally produce either a large number of small (<3 cm diameter) progeny tubers, or a few oversize tubers (Banville, 1989). Tubers can form in leaf axils of severely infected plants (Hartill, 1989). Severe

stem and stolon attacks decrease fresh yield, dry matter yield and dry matter content of tubers and increase the number of deformed and small tubers, whereas the effect on haulm yield and stem number is comparatively small (Scholte, 1989). Some reports (e.g. Gudmestad *et al.*, 1999) nevertheless indicate that moderate infection can improve yield and increase gross income per hectare, whereas tuber and soil inoculation with *R. solani* in the greenhouse has been shown to increase the yield of marketable tuber relative to the control (Stack *et al*., 1999).

Both soilborne and tuberborne inoculum are important in disease development. Severity of stem canker depends on the initial inoculum concentration, whereas severity of black scurf is determined by the inoculum present at the end of the growing season (Scholte, 1992). Soilborne inoculum mainly contributes to stolon and root damage and black scurf on progeny tubers, while tuberborne inoculum affects sprout emergence, causes stolon damage and represents the stem canker phase of the disease (Adams *et al*., 1980; Frank & Leach, 1980). Tuberborne inoculum is nearest to the developing shoots and is of greater importance in causing stem canker than soilborne inoculum, which may reach the shoots only when they have become more resistant to infection after emergence (Van Emden, 1965).

Development of the sclerotia is related to tuber maturity and the health of the root and stolon systems. Few sclerotia form on tubers until the onset of plant senescence when, as the tubers mature and roots and stolons decay, sclerotia develop rapidly and extensively (Spencer & Fox, 1979a; Mulder *et al*., 1992). It appears that tuber exudates stimulate black scurf development (Spencer & Fox, 1978; Dijst, 1985, 1990) by mobilising the mycelial resources into sclerotia (Christias & Lockwood, 1973). Maximum sclerotium development occurs 3-4 weeks after vine killing and does not significantly increased thereafter (Gudmestad *et al*., 1979), although new development of sclerotia on tubers in damp storage have been reported (Spencer & Fox, 1979b; Adams *et al*., 1980).

Sclerotia are usually limited to the tuber surface, but can sometimes penetrate the periderm to the cortex (Spencer & Fox, 1979b). Schaal (1939) found mycelium of *R. solani* capable of intercellularly invading several cell layers of the periderm and cork layer. The depth and extent of penetration could increase during storage, particularly under damp conditions (Spencer & Fox, 1979b; Hide & Boorer, 1991).

Occurrence and survival in soil

R. solani survives mainly as tuberborne sclerotia, but also as sclerotia and thick-walled brown hyphae associated with crop residues or detached in the soil (Boosalis & Scharen 1959; Sneh *et al*., 1966; Papavizas, 1968; Jeger *et al*., 1996). Approximately 74 % of sugar beet debris examined by Boosalis & Scharen (1959) contained active sclerotia. Hyphae of *R. solani* were also found on tubers free of visible sclerotia (Hide *et al*., 1973; Wicks *et al*., 1996).

Like most soilborne diseases, potato rhizoctoniasis occurs patchily (Jager & Velvis, 1995; Gilligan *et al*., 1996). Activity of *R. solani* is mostly confined to the upper 10 cm of soil in field plots (Papavizas *et al*., 1975; Naiki & Ui, 1977; Elango, 1986; Otten & Gilligan, 1998). Propagules are frequently aggregated in soil as a result of pathogenic or saprophytic colonisation of plants and fresh organic matter, and the density of active biomass drops sharply with increasing distance from the source of inoculum (Otten & Gilligan, 1998).

Soil factors that can affect the incidence and development of soilborne plant diseases include moisture, temperature, texture, pH, atmospheric composition, organic matter content, and the presence of agricultural chemicals in the soil. Black scurf and stem canker is more severe in dry (45 % water-holding capacity) than in wet (75 % and 90 % water-holding capacity) soils (Hide & Firmager, 1989; Lootsma & Scolte, 1997), and also more acute and less manageable in sandy than in loamy soils (Jager *et al*., 1991). It may be due to a richer load of antagonists in loamy soils than in sandy soils (Jager & Velvis, 1983). Excess moisture early in the season inhibited growth of the pathogen (Blair, 1943), but late in the season it increased the number of sclerotia produced on tubers due to enhanced exudation (Schaal, 1935).

Increased CO_2 levels in soil leads to a decrease in growth and sclerotium production by *R. solani* (Durbin, 1959; Harris *et al*., 2003). Growth of *R. solani* can be promoted by forced aeration of the soil (Blair, 1943). Aeration of the soil plays a more important role in influencing growth of *R. solani* than moisture or soil nutrients (Allington, 1936; Blair, 1943; Sanford, 1956; Dijst, 1990), and air-filled pore volume therefore is the dominant factor dictating fungal spread (Otten & Gilligan, 1998; Harris *et al*., 2003). Otten *et al*. (1999) showed that a tortuous and discontinuous air-filled pore space significantly reduces spread of *R. solani*. The ability of *R. solani* to colonise bulk soil thus depends not only on the pore size, but also on the connectivity and tortuosity of the air-filled pore volume.

Wide variation exists within the species *R. solani* both as to pathogenicity and saprophytic growth habits (Sanford, 1938; Blair, 1943). Some forms of the fungus have better ability to survive saprophytically in soil than others and this feature is not necessarily correlated with lower parasitic ability. Three categories for estimating populations of *R. solani* in soil can be distinguished. The first is competitive saprophytic ability, the second inoculum density, and the third inoculum potential. To isolate all viable propagules that occur in soil in a specific area, techniques representative of all three categories should be used.

Saprophytic ability: Techniques that have successfully been used to isolate *R. solani* in its saprophytic state include immersion tube (Chesters, 1940; Mueller & Durrell, 1957; Martinson, 1963), plate profile (Andersen & Huber, 1965) plating of plant debris particles (Boosalis & Scharen, 1959; Weinhold, 1977) and baiting with plant tissue segments (Sanford, 1952; Papavizas & Davey, 1959, 1961; Davey & Papvizas, 1962).

Inoculum density: Inoculum density could be determined by soil fractionationing and direct plating of soil pellets. Propagules of *R. solani* are usually recovered from soil in relatively low numbers (0.1 - 10 propagules g^{-1} soil) (Ko & Hora, 1971; Weinhold, 1977; Clark *et al*., 1978; Henis *et al*., 1978). Leach *et al*. (1993) nevertheless found that 0.01 propagules g^{-1} of dry soil were sufficient to induce sprout nipping and stem lesions in potato.

Inoculum potential: Inoculum potential is a measure of the ability of a soilborne organism to cause disease in a susceptible host under certain conditions, i.e. to act as inoculum. Studies and techniques measuring the inoculum potential of *R. solani* in field soil have in reality estimated only the population of the fungus in soil, which comprises saprophytic and pathogenic isolates. In order to measure the true inoculum potential of *R. solani* in a given soil sample, it need to be established that all isolates of the fungus in the sample are pathogenic. This is achieved by planting susceptible host plants in samples of the soil and in due course doing an estimate of disease (Davey & Papavizas, 1962; Menzies, 1963; Sneh *et al*., 1966).

Much emphasis is nowadays placed on the development of biochemical, serological and molecular techniques for the detection and quantifying *R. solani* propagules in plants and soil (MacDonald *et al*., 1990; Thornton *et al*., 1993; Neate & Schneider, 1996; Thornton, 1996; Otten *et al*., 1997; Bounou *et al.,* 1999; Lees *et al*., 2002; Weerasena *et al*., 2004). Molecular techniques have the advantages that the degree of specificity can be chosen, the techniques can be quick and can detect specific AG's and subgroups more accurately than biochemical, serological or conventional methods (Neate & Schneider, 1996).

Host range

R. solani AG-3 was isolated from root and stem segments of the weeds, *Chenopodium album* L., *Diplotaxis eurocoides* L., *Oxalis latifolia* H.B.K., *Solanum nigrum* L. and *Sorghum halepense* (L.) Pers. in potato fields in Spain (El Bakali *et al.*, 2000). AG-3 isolates were also retrieved from barley (*Hordeum vulgare* Pers.) (Murray, 1981), flax (*Linum* spp.) (Anderson, 1977), sugar beet (*Beta vulgaris* L.) (Windels & Nabben, 1989) and tobacco (*Nicotiana tabacum* L.) (Meyer *et al*., 1990; Reeleder *et al*., 1996).

In inoculation studies in Alaska, Carling *et al*. (1986) found the roots of brinjal (*Solanum melongena* L.), buckwheat (*Fagopyrum esculentum* Moench), carrot (*Daucus carota* L.), cauliflower (*Brassica oleracea* L. var. *botrytis* L.), cornspurrey (*Spergula arvensis* L.), dandelion (*Taraxacum officinalis* Weber), fireweed (*Epilobium angustifolium* L.), hairgrass (*Deschampsia beringensis* Hultén), lucerne (*Medicago sativa* L.), oats (*Avena sativa* L.), radish (*Raphanus sativus* L.), sweet clover (*Melilotus officinalis* (L.) Lam.), tobacco, tomato (*Lycopersicon esculentum* Mill.) and wheat (*Triticum aestivum* L.) to support mycelium and sclerotia of *R. solani* AG-3. Bean (*Phaseolus vulgaris* L.), weeping love-grass (*Eragrostis curvula* Nees), lettuce (*Lactuca sativa* L.), maize (*Zea mays* L.), onion (*Allium cepa* L.), sunflower (*Helianthus anuus* L.) and wheat became infected when planted to soil infested with *R. solani* AG-3 in a greenhouse (Du Plessis, 1999).

R. solani was isolated from 30 wild plant specimens representing 12 species from potato fields in the Netherlands, *viz. Capsella bursa-pastoris* (L.) Medik., *Cirsium arvense* (L.) Scop., *Elytrichia repens* (L.) Nevski, *Fumaria officinalis* L., *Geranium molle* L., *Lolium perenne* L, *Matricaria recutita* L., *Polygonum aviculare* L., *Polygonum convolvulus* L., *Polygonum persicaria* L., *Solanum nigrum* L. and *Stellaria media* (L.) Cirillo (Jager *et al*., 1982). Retrieval of *R. solani* from the weeds varied from 1.3 to 11.9 %. Of these isolates, 62 % proved to be pathogenic to potato sprouts. Some weed species seemed more frequently colonised by *R. solani* than others, particularly *S. nigrum*, *E. repens* and *M. recutita*. In a separate survey in Colorado, Oshima *et al*. (1963) found the weeds, *Amaranthus retroflexus* L., *Chenopodium album* L., *Chenopodium barlandieri* L. and *Portulaca olerecea* L. collected from potato fields, to be frequently invaded by *Rhizoctonia* spp.

Control

Control of potato rhizoctoniasis relies primarily on the use of *Rhizoctonia*-free seed tubers because of their importance in disseminating the pathogen and adding to the pool of soilborne inoculum. However, planting disease-free seed in new ground does not guarantee disease-free progeny as the new ground could be infested with potato pathogens through the movement of stock and implements or wind-blown dust from existing cropping areas (De Boer & Curtis, 2000), or may contain vegetation naturally sustaining populations of the pathogen. Control of *R. solani* can also be achieved with agrochemicals, biological agents, heat treatment, cultural practices, etc.

Chemical control

Various fungicides have been evaluated as seed or soil treatment against black scurf and stem canker, e.g. azoxystrobin, benomyl, carbendazim, fluazinam, fludioxonil, imazalil, iprodione, mepronil, pencycuron, propiconazole, quintozene (PCNB), thiabendazole, thiram and tolclofos-methyl (Davis *et al*., 1971; Singh *et al*., 1972; Chand & Logan, 1982; Leach & Murdoch, 1985; Sumner, 1987; Jager *et al*., 1991; Olaya *et al*., 1994; Wicks *et al*., 1995; Du Plessis, 1999; Virgen-Calleros *et al*., 2000), but mostly provided varying and inconsistent control. Fungicides presently registered in South Africa for use against black scurf and/or stem canker include dichlorophen, fludioxonil, imazalil + iprodione, pencycuron, quintozene; thiabendazole, thiram and tolclofos-methyl (Nel *et al*., 2003).

R. solani has acquired resistance to both protectant organic fungicides such as captan, dichlone, maneb, quintozene, thiram and tolclofos-methyl (Shatla & Sinclair, 1963; Elsaid & Sinclair, 1964; Meyer & Parmeter, 1968; Van Bruggen & Arneson, 1984) and to systemic fungicides such as benomyl, carboxin, dichlozoline, oxycarboxin, thiophanate-methyl and 2-(thiocyanomathylthio)-benzothiazole (TCMTB) (Martin *et al*., 1984). In most cases the resistance was temporary and possibly due to enzymatic adaptation (Elsaid & Sinclair, 1964). For quintozene and TCMTB, however, the resistance remained stable (Shatla & Sinclair, 1963) and ensued as a consequence of genetic changes.

Control of *R. solani* in infested field soil with methyl bromide fumigation is highly effective but tends to aggravate disease derived from infected tubers (Du Plessis, 1999). Alternatives to methyl bromide, such as methyl iodide, metam-sodium, dichloropropene, chloropicrin, 1,2-dibromo-3-chloropropane and dazomet have been evaluated and found to be equally effective (Ashworth *et al*., 1964; Ohr *et al*., 1996; Csinos *et al*., 1997).

Biological control

Various reports refer to biological control of *R. solani* on potato. Suppression of the pathogen has been achieved or implicated with various fungi and bacteria (Table 1). Besides fungi and bacteria, mycophagous amoebae, nematodes (*Aphelenchus avenae* Bastian and *Aphelenchoides composticola* Franklin) and microarthropods (Acarina and Collembola) have considerable potential for natural suppression of diseases caused by *R. solani* and other soilborne pathogens (Bollen *et al*., 1991; Curl & Lartey, 1996; Lootsma & Scholte, 1997). Like the microflora, their populations respond to pesticides, soil fertility, organic matter, cultivation practices and crop species, hence affording opportunities for manipulation of agrosystems to their benefit and detrimental to the pathogen.

Unfortunately, most of these biocontrol agents have one or more limitations when applied in the field. These constrains include temperature restrictions (Mulder *et al*., 1990), inability of the agent to provide control with high cropping frequencies (Murdoch & Leach, 1993) and incompatibility with chemicals employed in integrated control, e.g. sensitivity of *V. biguttatum* to carbendazim, benomyl, iprodione, thiabendazole and quintozene (Jager, 1987). Contrary to the incompatibility with chemicals, the application of *V. biguttatum* conidia in green-crop-lifting or green-crop-harvesting has lead to predictable and relevant control of black scurf and an appreciable reduction in the viability of tuberborne sclerotia (Mulder *et al*., 1992; Jeger *et al*., 1996).

Thermal control

Hot water treatment of potato tubers was shown to reduce infection by fungi, viruses and bacteria without adversely affecting the tubers (Upreti & Nagaich, 1968; Hide, 1975; Mackay & Shipton, 1983; Dashwood *et al*., 1991; Ranganna *et al*., 1998). Exposing naturally-infected tubers for five minutes to hot water at 55 °C (Dashwood *et al*., 1991) or 10 minutes to 55 °C (Mackay & Shipton, 1983) effectively reduced *R. solani* inoculum. Soil solarisation, although effectively controlling *R. solani* in infested field soil (Katan, 1981), is too expensive on a large scale. Solarised soils are frequently more suppressive and less conducive to certain soilborne pathogens than nonsolarised soils (Greenberger *et al*., 1987).

Table 1. Biological control agents effective against potato rhizoctoniasis, *Rhizoctonia solani in vitro* or *R. solani* on other crops

Reported on	Biocontrol agent	Reference
Potato rhizoctoniasis		
Fungi	Binucleate *Rhizoctonia* spp.	Escande & Eshandi, 1991a, b; Ross *et al*., 1998
	Cylindrocarpon destructans (Zinser.) Scholten	Chand & Logan, 1984
	Cylindrocarpon olivaceum Wollenweber	Chand & Logan, 1984
	Epicoccum nigrum Link	Chand & Logan, 1984
	Fusarium culmorum (Wm. G. Sm.) Sacc.	Chand & Logan, 1984
	Fusarium verticillioides (Sacc.) Nirenberg	Chand & Logan, 1984
	Gliocladium deliquescens Sopp.	Chand & Logan, 1984
	Gliocladium roseum Bain.	Jager *et al*., 1979; Schmiedeknecht, 1993
	Gliocladium virens Miller, Giddens & Foster	Beagle-Ristaino & Papvizas, 1985; Lewis & Papavizas, 1985; Lumsden *et al*., 1992
	Hypovirulent *R. solani* isolates	Bandy & Tavantzis, 1990; Sneh, 1996
	Laetisaria arvalis Burds.	Chet & Baker, 1981; Murdoch & Leach, 1993; Jeger *et al*., 1996
	Penicillium aurantiogriseum Dierckx	Chand & Logan, 1984
	Penicillium fluorescens nom. nub.	Chand & Logan, 1984
	Stachybotrys elegans (Pidopl.) W. Gams	Benyagoub *et al*., 1994, 1996
	Trichoderma hamatum (Bonord.) Bainier	Beagle-Ristaino & Papavizas, 1985; Lewis & Papavizas, 1985
	Trichoderma harzianum Rifai	Elad *et al*., 1980; Beagle-Ristaino & Papavizas, 1985

Table 1. Continued

Reported on	Biocontrol agent	Reference
	Trichoderma viride Pers.	Beagle-Ristaino & Papavizas, 1985
	Trichothecium roseum (Pers.): Fr. Link	Chand & Logan, 1984
	Verticillium biguttatum W. Gams	Velvis & Jager, 1983; Van der Boogert & Saat, 1991; Wicks *et al*., 1995, 1996
Bacteria	*Bacillus* spp.	Schmiedeknecht, 1993
	Bacillus cereus	Lewis & Kulik, 1996
	Bacillus subtilis	Loeffler *et al*., 1986
	Pseudomonas spp.	Geels & Schippers, 1983
	Streptomyces spp.	Chand & Logan, 1984
	Streptomyces hygroscopicus subsp. *limoneus*	Iwasa *et al*., 1971
***In vitro* or other crops**		
Fungi	*Botryotrichum piluliferum* Sacc. & March.	Turhan, 1990
	Cladorrhinum sp.	Lewis & Papavizas, 1988
	Coniothyrium sporulosum (W. Gams & Domsch) Aa	Turhan, 1990
	Coniothyrium minitans Campbell	Lewis & Papavizas, 1988
	Dendrostilbella sp.	Lewis & Papavizas, 1988
	Dicyma olivacea (Emoto et Tubaki) Arx	Turhan, 1990
	Gliocladium catenulatum Gilm. & Abbott	Turhan, 1990
	Schizophyllum commune Fr.: Fr.	Chiu & Tzean, 1995
	Stachylidium bicolor Link	Turhan, 1990
	Stachybotrys chartarum (Ehrenb. ex Link) Hughes	Turhan, 1990

Table 1. Continued

Reported on	Biocontrol agent	Reference
	Talaromyces flavus (Klöcker) Stolk & Samson	Boosalis, 1956
	Verticillium chlamydosporium Gaddard	Turhan, 1990
	Verticillium tenerum (Nees ex Pers.) Link	Turhan, 1990
Bacteria	*Aeromonas caviae*	Inbar & Chet, 1991
	Bacillus subtilis	Hwang & Chakravarty, 1992
	Enterobacter agglomerans	Chernin *et al*., 1995
	Enterobacter cloacae	Kwok *et al*., 1987
	Flavobacterium balustinum	Kwok *et al*., 1987
	Janthinobacterium lividum	Kwok *et al*., 1987
	Nostoc muscorum	De Caire *et al*., 1990
	Pseudomonas cepacia	De Freitas & Germida, 1991; Fridlender *et al*., 1993
	Pseudomonas fluorescens	Kwok *et al*., 1987
	Pseudomonas putida	Kwok *et al*., 1987; De Freitas & Germida, 1991
	Pseudomonas stutzeri	Kwok *et al*., 1987
	Serratia marcescens	Ordentlich *et al*., 1987, 1988
	Stenotrophomonas maltophilia	Kwok *et al*., 1987
	Streptomyces hygroscopicus var. *geldanus*	Rothrock & Gottlieb, 1984

Cultural control

The value of cropping practices to control soilborne diseases has been recognised long before fungicides and fumigants were commonly available. Agronomic factors such as crop rotation, plant material, cultivar, soil management, tillage, irrigation, pesticide application, haulm destruction, harvesting, crop residues, volunteer plants and storage all have a profound influence on the incidence and severity of potato rhizoctoniasis (Jeger *et al*., 1996).

Rotations of 3-5 years are often necessary to effectively reduce losses caused by *R. solani*.

The frequency with which potatoes are cultivated has a greater effect on black scurf incidence than crop rotation as such (Scholte, 1992; Honeycutt *et al*., 1996). It has nevertheless been established that the disease is aggravated by rotation with certain legumes, sugar-beet or broccoli (*Brassica oleracea* L. var. *italica* Plenck) (Baker & Martinson, 1970). Results from different crop rotation programmes vary greatly regarding their effect on *Rhizoctonia* disease incidence on potato (Frank & Murphy, 1977; Specht & Leach, 1987; Leach *et al*., 1993; Johnston *et al.*, 1994; Honeycutt *et al*., 1996; Lootsma & Scholte, 1996; Carter *et al*., 2003; Peters *et al*., 2003) and management of potato rhizoctoniasis with crop rotation alone is unlikely (Conway, 1996). Various other plant species (including weeds) have been shown to sustain *R. solani* (Jager *et al*., 1982; Carling *et al*., 1986; Du Plessis, 1999) and should be considered in crop rotation and weed control.

Attempts at controlling black scurf by tillage showed chisel and mouldboard ploughing to a depth of 25-30 cm to reduce disease (Sumner *et al*., 1981; Leach *et al*., 1993). Subsoiling to reduce soil compaction, to a depth of 35 cm for three consecutive years had no effect on the vertical distribution of *R. solani* (Hussey & Roncadori, 1977). Practices that favour rapid emergence like shallow planting or using greened seed tubers seem to restrict stem canker infection (Carling & Leiner, 1990b; Jeger *et al*., 1996) owing to the greater resistance to infection of mature than immature tissue such as emerging spouts and stolons. Firman & Allen (1995) showed that an increase in plant density resulted in an increased severity of black scurf on progeny tubers.

Harvesting methods that are used in potato production can affect the level of black scurf (Dijst *et al*., 1986). Dijst (1985) suggested that early haulm killing promotes development of sclerotia. Advancing the killing of vines does not lead to a rapid disintegration of the roots. The initially fully functional root system continues to function as water pump for about a week (Dijst, 1985). As evaporation through the foliage ceases, tubers serve as a sink for the water surplus, consequently increasing in mass and commencing leakage. However, the use of herbicides and other chemicals to kill potato shoots just before harvest time can also lead to increased incidence and severity of black scurf on potato tubers (Mulder *et al*., 1992). Green-crop-harvesting (harvesting the immature crop mechanically and returning the tubers to the soil for curing before final harvesting two to four weeks later) and immature-crop-harvesting (pulling haulms and collecting the tubers by hand) often result in low levels of black scurf (Mulder *et al*., 1992; Lootsma & Scholte, 1996). Green-crop-harvesting has the additional advantage of allowing the application of chemicals or antagonists with the first lifting of the tubers, resulting in increased control of black scurf (Mulder *et al*., 1992).

Some foreign potato varieties, e.g. Portage, Mainestay, AC Belmont and AC Brador, are moderately resistant to *Rhizoctonia* attack (Reeves *et al*., 1995, 1997; Tarn *et al*., 1995a, b), but varietal resistance is not regarded as a solution to black scurf and stem canker. Significant genetic variation nevertheless exists among cultivars in response to *R. solani* infection. Environmental and soil conditions can significantly affect cultivar response to the pathogen (Leach & Webb, 1993).

High levels of nitrogen and phosphorus in soil enhance sclerotium formation and disease severity, probably due to more nutritious tuber exudates (Allington, 1936; Sanford, 1956; Papavizas & Davey, 1961; Scholte, 1992). Disease potential is also increased by deficiencies in potassium, sodium and calcium (Baker & Martinson, 1970). It therefore stands to reason that disease can be reduced by fertilisation, although the survival of *R. solani* in artificially infested soil was shown to be little affected by soil fertility (Das & Western, 1959). Other procedures have also been employed to alter the nutrient and microbial status of soils. Barnyard manure and rye crops (Blodgett, 1940), soil supplementation with groundnut and mustard oilcake, sawdust amendment integrated with NPK fertiliser (Singh *et al*., 1972), oat straw and soybean hay enriched with ammonium nitrate (Davey & Papavizas, 1963) and chitin soil incorporation decreased *Rhizoctonia* populations, probably due to the stimulation of antagonistic organisms or increase in microbial activity that increases competition for carbon and nitrogen, limits oxygen in microsites and produces antimicrobial compounds (Sneh & Henis, 1972; Conway, 1996).

Compost-amended soil has been found to be suppressive against nematodes, bacteria and soilborne fungi in various cropping systems (Hoitink & Fahy, 1986), although an increase of disease due to compost application has also been demonstrated (Nelson *et al*., 1983; Tuitert *et al*., 1998). The variation in suppressiveness to *R. solani* was ascribed to compost maturity, with immature compost generally being condusive (Nelson *et al*., 1983; Tuitert *et al*., 1998). Antagonist enrichment of composts increases the reliability of disease suppressiveness of the composts towards *R. solani* (Postma *et al*., 2003).

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CHAPTER 3

ANASTOMOSIS GROUPING OF *RHIZOCTONIA SOLANI* ASSOCIATED WITH POTATO RHIZOCTONIASIS IN SOUTH AFRICA

ABSTRACT

Rhizoctonia solani was isolated from 28 plant and 56 soil samples collected between 1999 and 2002 from seven of the 14 main potato production regions in South Africa, and screened for hyphal anastomosis with tester strains AG-1 to AG-10 of the pathogen. Of the 411 *R. solani* isolates from tubers with black scurf symptoms, 408 (99.3 %) were AG-3 and three AG-5. Symptomless tubers yielded only two AG-3 and three AG-5 isolates. Of the 39 isolates from infected stems and roots, 32 (82.1 %) were AG-3, five AG-4 and two AG-5. A total of 127 isolates were retrieved from soil, 86 (67.7 %) of them belonging to AG-3, 28 to AG-4, seven to AG-5, and three to AG-7 and AG-8, respectively. *R. solani* AG-3 was present in all the regions, AG-4 in five, AG-5 in two, and AG-7 and AG-8 in one region each. Two of the regions yielded only AG-3 and the remaining five between two and four AGs each. Baiting with beet seed proved to be more sensitive for detecting and reisolating *R. solani* AG-3 in artificially infested soil than wet sieving, soil pelleting, baiting with brinjal, potato, tobacco and tomato stem segments, or trapping with blue lupin seedlings, whereas soil pelleting yielded the greatest diversity of AGs from field soil. *In vitro* screening of selected isolates for virulence on sprouts of Up-to-Date potato plants indicated that AG-3 was the most virulent, with isolates from sclerotia on tubers and lesions on stems more aggressive than those from symptomless tubers or soil. AG-4 and AG-5 caused significantly less damage than AG-3, whereas none of the AG-7 and AG-8 isolates tested showed any virulence.

INTRODUCTION

Rhizoctoniasis of potato (*Solanum tuberosum* L.) caused by *Rhizoctonia solani* J.G. Kühn (teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk) was first reported in South Africa by Doidge (1918) and has since become one of the commonest diseases affecting production of the crop in the country (Doidge, 1950; Doidge *et al*., 1953; Crous *et al*., 2000). *R. solani* anastomosis group (AG) 3 is globally regarded as main cause of the disease complex (Carling & Leiner, 1986; Bandy *et al*., 1988; Bains & Bisht, 1995). Preliminary observations by Du Plessis (1999) indicated a similar scenario in South Africa,

though subsequent isolations from local potato plant and soil samples occasionally yielded isolates of *R. solani* that did not anastomose with AG-3 (unpublished data).

Besides AG-3, several other AGs have been reported to cause damage to potato plants, e.g. AG-1 (Carling & Leiner, 1990a), AG-2 (subgroups -1 and -2) (Chand & Logan, 1983; Carling & Leiner, 1986), AG-4 (Suresh & Mall, 1982; Anguiz & Martin, 1989; Gudmestad *et al*., 1989; Balali *et al*., 1995), AG-5 (Bandy *et al*., 1984; Bains & Bisht, 1995), AG-7 (Carling *et al*., 1998), AG-8 (Balali *et al*., 1995) and AG-9 (Carling *et al*., 1987). Considering that the above AGs differ in virulence, ecology, host range, temperature preferences and control, particularly by means of crop rotation (Carling *et al*., 1987, 1998; Ogoshi, 1987; Anquiz & Martin, 1989; Carling & Leiner, 1990a; Hide & Firmager, 1990; Bains & Bisht, 1995; Balali *et al*., 1995; Banville *et al*., 1996), a more comprehensive survey of AGs associated with potato in South Africa obviously is indicated. This report describes the evaluation of techniques for retrieving *R. solani* associated with potato from soil, and the isolation, AG-characterisation and pathogenicity of *R. solani* isolates from seven potato-production regions in South Africa. A synopsis of the results has been published (Truter & Wehner, 2004).

MATERIALS AND METHODS

Evaluation of detection/isolation techniques

Inoculum was prepared by culturing an isolate of *R. solani* AG-3 (Rs44 from a potato tuber, Gauteng, South Africa) for five weeks at room temperature on a sterile maize meal:soil mixture (1:10 m m^{-1}). The colonised maize meal:soil was incorporated into tyndallised (90 °C for 60 minutes on three consecutive days) sand:loam (1:1 v v^{-1}) at rates of 10^{-1} to 10^{-10}, with unamended sand:loam as control. Moisture content of the sand:loam was maintained at *ca*. half field-capacity with sterile tap water for 48 hours prior to evaluating the following detection/isolation techniques.

Wet sieving

Five air-dried samples of 50 g each from each inoculum concentration were wet-sieved as described by De Beer (1965). Each sample was placed in an 1 l beaker and suspended with a jet of tap water. Heavier soil particles were allowed to settle for 10 seconds and the supernatant containing hyphae and debris was decanted through a series of sieves with mesh sizes of 2000, 1000, 425, 106, 53 and 25 µm, respectively. Washing and decanting through the sieves were continued until most of the organic matter was displaced from the

sand:loam fraction onto the sieves. The residue on the 25 µm sieve was washed into a Petri dish with 10 ml of water and the number of mycelial fragments in 25 microscope fields were counted under a stereomicroscope at 50x magnification.

Soil pelleting

One-hundred pellets, each weighing approximately 0.1 g, from each of five samples of each inoculum concentration were plated on water agar (WA), ten pellets per plate, according to the soil pelleting method of Henis *et al*. (1978). Plates were inspected for *Rhizoctonia*-like growth after incubation for two to three days at room temperature and the isolates retrieved were transferred to potato-dextrose agar (PDA) plates for confirmation of their anastomosis grouping.

Baiting

Autoclaved beet (*Beta vulgaris* L.) seed, and stem segments from brinjal (*Solanum melongena* L.), potato, tobacco (*Nicotiana tabacum* L.) and tomato (*Lycopersicon esculentum* Mill.) plants were used as baits. Mature stems were cut into 10 mm segments, dried for 48 hours at 50 °C, and stored at room temperature until used (maximum of 4 weeks). All plant material was moistened and autoclaved for 20 minutes at 121 °C on two consecutive days. For each inoculum concentration, 50 units of each type of bait were intermixed with 50 g of inoculum in each of five 250 ml plastic cups. The cups were covered with lids and incubated at room temperature. After three days, the bait material was recovered, washed in running tap water for five minutes, surface-disinfested for two minutes in 3 % sodium hypochlorite, rinsed with sterile tap water, blot-dried aseptically, and plated on WA supplemented with 50 mg l^{-1} rifampicin. Plates were examined for *Rhizoctonia*-like growth and isolates retrieved were transferred to PDA for anastomosis grouping.

Trapping

Blue lupin (*Lupinus angustifolius* L.) seeds were surface-disinfested for three minutes in 3 % sodium hypochlorite, rinsed with sterile tap water and germinated in moist, autoclaved perlite. After three days, seeds with a primary root of *ca*. 30 mm in length were selected and planted to sand:loam containing the various inoculum concentrations of *R. solani*. For each concentration, five 350 ml plastic pots filled with sand:loam inoculum were each planted to three germinated seeds. Pots were maintained at approximately half field-capacity in a greenhouse at 18-30 °C. Seedlings were harvested after two weeks and the symptoms on roots and stems were recorded. Stems and roots were cut into segments *ca*. 5 and 10 mm long, respectively. Segments were surface-disinfested for two minutes in 3 %

sodium hypochlorite, rinsed in sterile tap water, blot-dried aseptically, and plated on WA with rifampicin. After incubation for two days at room temperature, hyphae resembling those of *R. solani* were transferred to PDA for anastomosis grouping.

Survey of *R. solani* anastomosis groups in South Africa

A total of 28 plant and 56 soil samples were collected between 1999 and 2002 from seven of the 14 main potato-production areas in South Africa, *viz*. Eastern Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, Northern Cape and Sandveld. *R. solani* was isolated from the samples and characterised as follows:

Isolation from plant material

Tubers with and without symptoms of black scurf from Eastern Free State, Gauteng, KwaZulu-Natal, Mpumalanga, Northern Cape and Sandveld were washed clean with tap water, surface-disinfested for two minutes in 3 % sodium hypochlorite, rinsed with sterile tap water, and allowed to air-dry on a laminar-flow bench. Approximately 15 sclerotia were aseptically removed from each tuber and plated on WA. Colonies developing from the sclerotia after incubation for two to three days at room temperature were isolated on PDA.

Naturally-infected potato stems and roots from Eastern Free State, KwaZulu-Natal and Northern Cape were washed clean with tap water, cut into segments *ca*. 5 and 10 mm long, respectively, surface-disinfested for one minute in 3 % sodium hypochlorite, rinsed in sterile tap water, and blot-dried aseptically. Segments were plated on PDA with rifampicin and monitored for *Rhizoctonia*-like growth after two to three days at room temperature. Isolates retrieved were transferred to PDA.

Isolation from soil

Soil samples of approximately 8 kg each from Gauteng, KwaZulu-Natal, Northern Cape and Sandveld were air-dried and passed through a 2 mm mesh sieve to collect plant material. Debris material (roots, stems, leaves, etc.) was cut into 5 mm segments, surface-disinfested for one minute in 1 % sodium hypochlorite, rinsed in sterile tap water and blot-dried aseptically. Fifty segments from each sample were plated on WA with rifampicin. After incubation for two to four days at room temperature, colonies resembling those of *R. solani* were transferred to PDA.

The above soil samples, as well as samples from Eastern Free State and Limpopo, were also subjected to pelleting, baiting with beetroot seed and tomato stem segments, and trapping with lupin seedlings for the isolation of *R. solani* as described above. For pelleting,

200 pellets from each sample were plated, whereas trapping and baiting were done in triplicate.

Anastomosis group typing

AG-identities were determined according to the method of Carling *et al*. (1987). *R. solani* isolates from artificially-infested soil were tested with the original Rs44, and those from naturally-infested soil and potato plants with tester strains of AG-1 to AG-10 (ATCC 42127, 46138, 62803, 62804, 62805, 66159, 76104, 76130, and PPRI 3525, 3526). Isolates were paired at room temperature with the tester strains on 3x1.5 cm PDA-coated cellophane rectangles on 1.5 % WA in Petri dishes. After 48-72 hours, each cellophane rectangle was transferred to a microscope slide, stained with lactofuchsin, and examined under 400x magnification for hyphal anastomosis. Anastomosis reactions were classified from C0 to C3, C0 representing no reaction and C3 a response resembling auto-anastomosis (Carling, 1996). Anastomosis was considered positive when C2 or C3 fusion occurred at at least five sites.

Hyphal diameter and nuclear number

Twenty randomly selected AG-3 isolates were grown in triplicate for 72 hours at 25 °C on PDA-coated cellophane rectangles on WA. The mycelium was stained with acridine orange (Yamamoto & Uchida, 1982) and examined under a fluorescence microscope at 400x magnification. Hyphal diameter was determined by measuring 20 cells per isolate per plate at right angles to the longitudinal cell wall. Nuclei were counted in 20 cells per plate of each of the above isolates in microscope fields where nuclei and septa were clearly discernible.

Induction of teleomorph

Induction of sporulation by the above 20 AG-3 isolates was attempted on 1.5 % WA, 2 % V8-juice agar (20 ml V8-juice and 18 g agar l^{-1}), and by means of the soil overlay technique (Ogoshi, 1976). Three plates per isolate were included for each of the three methods. Incubation occurred at 25 °C and cultures were examined every second or third day for 21 days for the presence of hymenia, basidia and basidiospores.

Virulence

Virulence of 40 randomly selected *R. solani* isolates from potato plants and soil, representing AG-3 (18 isolates), AG-4 (8), AG-5 (8), AG-7 (3) and AG-8 (3), was assessed on Up-to-Date potato plants in the greenhouse. Inoculum was prepared by culturing the isolates for 21 days at 25 °C on moist sterilised maize meal:sand (1:10 m/m). Surface-disinfested (2 % formaldehyde for two minutes) seed tubers were sprouted at room

temperature. When sprouting had commenced, tubers were planted, three per pot and about 100 mm deep, to sterile sand:soil (1:3 v/v) in 4 l plastic pots. The tubers were covered with 30 mm of sterile sand:soil, followed by a 10 mm layer of inoculum and a further 50 mm of sterile sand:soil. Three pots were used per isolate. Control pots received sterile maize meal:sand instead of inoculum. Pots were randomly arranged in a greenhouse at 16-28 °C and were watered when required. Twenty-eight days after planting, the plants were harvested, washed clean, and examined for lesions on the stems. Disease severity was calculated according to a 0-4 rating scale (Carling & Leiner, 1990a), where 0 = no damage, no lesions; 1 = minor damage, one to several lesions less than 5 mm long; 2 = intermediate damage, lesions longer than 5 mm, girdling of some tissue; 3 = major damage, large lesions, girdling and death of most tissue; 4 = most tissue dead. Koch's postulates were confirmed by reisolation from infected tissue and pairing of the isolates with relevant tester strains.

Statistical analysis

Data pertaining to the evaluation of detection/isolation techniques and determination of virulence were analysed statistically according to GenStat (2000). Analysis of variance was used to test for differences between variables and means were separated by means of Fisher's protected *t*-test least significant difference. The geographic distribution of AGs and proportions of the various disease rating categories were analysed by chi-square at 5 % level of significance.

RESULTS

Evaluation of detection/isolation techniques

Of the baiting and trapping techniques evaluated, baiting with beet seed proved to be the most sensitive method for detecting and reisolating *R. solani* AG-3 in artificially infested soil (Table 1). Although not statistically comparable, wet sieving was as effective as the beet seed method for establishing the presence of low densities of *R. solani*, but did not readily facilitate the isolation of hyphae for confirmation of their identity. Soil pelleting and baiting with brinjal and tobacco stem segments were less sensitive than the above methods, but superior to trapping with lupin seedlings.

Table 1. Comparative sensitivity of different techniques for detecting *Rhizoctonia solani* AG-3 in artificially infested soil

Inoculum dilution level	Detection or isolation method[a]								
			Percentage colonisation of plant material[b]						
	Wet sieving (hyphae g^{-1} soil)[c]	Soil pelleting (propagules g^{-1} soil)[d]	Beetroot seed[e]	Brinjal stem segments[e]	Potato stem segments[e]	Tobacco stem segments[e]	Tomato stem segments[e]	Lupin crowns[f]	Lupin roots[g]
0	0	0	0	0	0	0	0	0	0
10^{-1}	758.5	10.5	99.6 a	96.4 a	99.2 a	97.2 a	98.8 a	47.6 b	8.8 c
10^{-2}	186.1	10.1	82.4 a	70.8 b	73.6 b	60.4 c	69.6 b	10.0 d	1.1 e
10^{-3}	30.5	4.1	36.8 a	17.6 bc	25.2 b	14.4 c	20.4 bc	5.2 d	0 d
10^{-4}	7.6	1.3	22.4 a	10.4 b	12.4 b	8.4 b	12.0 b	0 c	0 c
10^{-5}	0.8	0.1	14.4 a	5.2 b	6.8 b	2.0 c	4.4 bc	0 d	0 d
10^{-6}	0	0	5.6 a	0 c	3.2 ab	0 c	2.4 bc	0 c	0 c
10^{-7}	0.3	0	2.8	0	0	0	0	0	0
10^{-8}	0	0	0	0	0	0	0	0	0
10^{-9}	0	0	0	0	0	0	0	0	0
10^{-10}	0	0	0	0	0	0	0	0	0

[a] Mean of five replicates.

[b] Values in rows followed by the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$).

[c] Each replicate comprised 25 microscope fields.

[d] Each replicate comprised 100 soil pellets.

[e] Each replicate comprised 50 seeds/plant tissue segments.

[f] Each replicate comprised 50 crown segments.

[g] Each replicate comprised 75 root segments.

Survey of *R. solani* anastomosis groups in South Africa

Isolation from plant material

A total of 455 isolates from the 28 potato plant samples conformed to the species description of *R. solani* (Table 2). Of these, 411 were isolated from symptomatic potato tubers, 408 (99.3 %) of them belonging to AG-3 and three (0.7 %) to AG-5. Stems and roots produced 39 isolates, 32 (82.1 %) AG-3, five (12.8 %) AG-4 and two (5.1 %) AG-5. Symptomless tubers yielded only five isolates of which two were AG-3 and three AG-5. AG-3 was isolated from all six the regions surveyed, and AG-4 and AG-5 from two each, *viz.* Eastern Free State and Northern Cape, and Gauteng and KwaZulu-Natal, respectively.

Isolation from soil

The 56 soil samples yielded 127 *R. solani* isolates (Table 3). Eighty-six (67.7 %) of these were AG-3, 28 (22.0 %) AG-4, seven (5.5 %) AG-5, and three (2.4 %) AG-7 and AG-8, respectively. Soil from Gauteng produced the greatest variety of AGs, *viz*. four (AGs 3, 4, 5, 8), followed by Eastern Free State (AGs 3, 4, 7), KwaZulu-Natal (AGs 3, 4, 5), and Northern Cape and Limpopo (AGs 3, 4). Sandveld soil samples were void of retrievable *R. solani* propagules. Of the various isolation techniques that were employed, soil pelleting provided the greatest diversity of isolates (AGs 3, 4, 5, 7, 8), followed by beet seed (AGs 3, 4, 5), lupin seedlings and tomato stem segments (AGs 3, 4) and debris particles (AG-4 only). Besides the above multinucleate isolates, binucleate *Rhizoctonia*-like fungi were often isolated from soil with soil pelleting.

No significant differences in geographic distribution of the various AGs in soil, plants or tubers were evident according to chi-square analysis of data.

Hyphal diameter and nuclear number

Mean hyphal diameter of the 20 AG-3 isolates was 7.8 ± 2.7 µm, and the mean number of nuclei 4.8 (range 3-12).

Induction of teleomorph

None of the isolates produced a hymenium or basidiospores on any of the media or soil overlay plates.

Virulence

Sclerotial, stem and soil isolates of *R. solani* AG-3 caused significantly more damage to potato sprouts than isolates of AG-4, -5, -7 and -8, regardless of their origin (Table 4).

Table 2. Anastomosis groups of *Rhizoctonia solani* isolated from potato plants and tubers from six potato-production regions in South Africa

Type of plant material	***R. solani* ananstomosis group**[a]					
	Eastern Free State	Gauteng	KwaZulu-Natal	Mpumalanga	Northern Cape	Sandveld
Tubers with black scurf symptoms	AG-3 (26)	AG-3 (75) AG-5 (2)	AG-3 (143) AG-5 (1)	AG-3 (42)	AG-3 (92)	AG-3 (30)
Symptomless tubers	0	AG-5 (1)	AG-3 (2) AG-5 (2)	0	0	0
Infected stems and roots	AG-3 (13) AG-4 (2)	ND[b]	AG-3 (19) AG-5 (2)	ND	AG-4 (3)	ND

[a] Values in brackets represent the number of isolates.

[b] ND = Not determined.

Table 3. Anastomosis groups of *Rhizoctonia solani* isolated from soil in six potato-production regions in South Africa

Isolation method	***R. solani* ananstomosis group**[a]					
	Eastern Free State	Gauteng	KwaZulu-Natal	Northern Cape	Limpopo	Sandveld
Beetroot seed[b]	AG-3 (4)	AG-3 (3)	AG-3 (28) AG-5 (3)	AG-3 (2)	AG-3 (4) AG-4 (2)	0
Debris particles[c]	ND[d]	0	AG-4 (1)	0	ND	0
Lupin seedlings[e]	ND	AG-3 (1) AG-4 (2)	AG-3 (11)	ND	ND	0
Soil pelleting[f]	AG-3 (1) AG-4 (1) AG-7 (3)	AG-3 (2) AG-4 (1) AG-5 (2) AG-8 (3)	AG-3 (17) AG-5 (2)	AG-3 (2) AG-4 (4)	AG-3 (4) AG-4 (15)	0
Tomato stem segments[b]	0	0	AG-3 (7)	0	AG-4 (2)	0

[a] Values in brackets represent the number of isolates.

[b] Isolates retrieved from 50 seeds/tissue segments in each of three sub-samples from each soil sample.

[c] Isolates retrieved from 50 debris segments.

[d] ND = Not determined.

[e] Isolates retrieved from 30 crown and 45 root segments in each of three sub-samples from each soil sample.

[f] Isolates retrieved from 200 soil pellets.

Table 4. Virulence rating on potato sprouts exposed to isolates of *Rhizoctonia solani* collected from potato plants and soil in South Africa

Anastomosis group	Origin of isolates	No. of isolates	Disease index[a]	
			Mean[b]	Variance
3	Sclerotia	11	2.27 a	0.51
	Symptomless tubers	1	0.67 bc	0.33
	Stem lesions	3	2.26 a	0.40
	Soil	3	1.04 b	0.54
4	Stem lesions	5	0.16 c	0.18
	Soil	3	0 c	0
5	Sclerotia	3	0.33 c	0.17
	Symptomless tubers	2	0 c	0
	Soil	3	0.07 c	0.60
7	Soil	3	0 c	0
8	Soil	3	0 c	0

[a] Disease index: 0 = no lesions; 1 = one to several lesions less than 5 mm long; 2 = lesions more than 5 mm long, girdling of some tissue; 3 = large lesions, girdling and death to most tissue; 4 = all tissue dead (Carling & Leiner, 1990a).

[b] Values followed by the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.05$).

Within AG-3, isolates from sclerotia and stem lesions were significantly more virulent than those from soil or symptomless tubers. Chi-square analysis indicated no significant differences in virulence within AGs between isolates from different regions.

DISCUSSION

Techniques for the estimation and isolation of soil populations of *R. solani* have been compared in various studies. Immersion plate isolation, for instance, was found to be superior to plating on medium (Thornton, 1956), buckwheat stem piece baiting to bean seedling trapping (Papavizas & Davey, 1959), bean segment colonisation to immersion tube isolation and seedling infection (Sneh *et al*., 1966), elutriation to wet sieving (Clark *et al*., 1978), soil debris isolation to beet seed colonisation (Roberts & Herr, 1979), and soil pelleting to wet sieving and beet seed baiting (Van Bruggen & Arneson, 1986; Kinney *et al*., 1992). None of the above reports, however, specified the anastomosis group/s involved and the present investigation is therefore the first to provide evidence in this regard. Results indicated that beet seed baiting and trapping with blue lupin seedlings were the most and least sensitive for detecting propagules of *R. solani* AG-3 in soil, respectively, whereas soil pelleting yielded the greatest diversity of AGs and was the only technique facilitating the isolation of AG-7 and AG-8. Only a few previous reports have described the isolation of *R. solani* from potato soils, but most relied on soil pelleting for the purpose. Using this technique, Bandy *et al*. (1984) isolated AGs-1, -2, -3, -4 and -5, as well as unidentified multinucleate and binucleate *Rhizoctonia*-like fungi from potato soils in Maine, while Carling & Leiner (1990b) found 48 % of the isolates from Alaskan potato soils retrieved by pelleting not to be AG-3. By comparison, sieve screening of potato soils in South Australia yielded AGs-3, -4 and -8, besides binucleates, but no AG-5, notwithstanding the latter AG being present on tubers and in stem canker lesions (Balali *et al*. 1995). Regarding the relative insensitivity of blue lupin trapping for isolating AG-3, it is interesting to note that AG-3 does not infect the closely related white lupin, *Lupinus albus* L., although this species is highly susceptible to infection by AG-5 and, to a lesser extent, AG-4 (Leach & Clapham, 1992). In the present study, roots and crowns of the blue lupin seedlings evidently were infected by AG-3 and AG-4, but not by AG-5, even though beet seed baiting and soil pelleting indicated the presence of AG-5 in the relevant soil samples.

The South African survey comprised 28 plant and 56 soil samples, and yielded 582 isolates of *R. solani*. For the uninformed, this may seem as hardly representative of the local potato industry and, compared to the 21546 samples screened by El Bakali *et al*. (2000) in Catalonia, North East Spain, even as grossly inadequate. Quantitatively it nevertheless exceeded most

similar surveys in other parts of the world, e.g. 313 isolates from 10 field sites in South Australia (Balali *et al*., 1995), 307 from 24 fields in Maine (Bandy *et al*., 1988), 288 from 10 fields in Alaska (Carling & Leiner, 1986), 74 from 13 sites in Alaska and Oregon (Carling *et al*., 1987), 68 from 15 fields in Central Mexico (Virgen-Calleros *et al*., 2000), 64 from 29 fields in Alberta (Bains & Bisht, 1995), and 60 from 25 fields in Maine (Bandy *et al*., 1984). Chi-square analysis furthermore indicated no significant differences in the geographic distribution of the various AGs or in virulence within AGs from different regions. It is therefore unlikely that additional sampling would provide a conclusion different from the present.

In accordance with the majority of reports, *R. solani* AG-3 proved to be the dominant anastomosis group associated with potato in South Africa. It comprised almost 68 % of isolates from potato soils, which is considerably higher than the 19 %, 43 % and 52 % reported from Maine, South Australia and Alaska, respectively (Bandy *et al*., 1984; Carling & Leiner, 1990b; Balali *et al*., 1995). The incidence of 97.6 % of AG-3 on or in plant material (99.0 % on tubers and 82.1 % in stems and roots) is also relatively high, compared for instance to 100 % in Catalonia, North-East Spain (El Bakali *et al.*, 2000), 99.3 % in Canada (Otrysko *et al*., 1985), 96 % in Japan (Abe & Tsuboki, 1978), 95.6 % in Northern Ireland (Chand & Logan, 1983), 95.4 % in Maine (Bandy *et al*., 1988), 94.7 % and 73.7 % in Alaska (Carling & Leiner, 1986, 1990b), 91.2 % in South Australia (Balali *et al*., 1995), 85 % in the cool highlands of Peru (Anguiz & Martin, 1989), 84 % in India (Suresh & Mall, 1982), 76.6 % in Alberta (Bains & Bisht, 1995), 73.5 % in Central Mexico (Virgen-Calleros *et al*., 2000), 40 % in China (Chang & Tu, 1980), and 0 % in the warmer coastal regions of Peru (Anguiz & Martin, 1989).

Reports from which it had been possible to deduce the incidence of AGs on tubers relative to stems, stolons and roots indicated a greater prevalence of AG-3 on tubers, e.g. 100 % versus 10 % (Anguiz & Martin, 1989), 100 % versus 82.1 % (Bandy *et al*., 1988), 99.3 % versus 76.6 % (Otrysko *et al*., 1985; Bains & Bisht, 1995), 97.7 % versus 88.9 % (Balali *et al*., 1995), and 97.3 % versus 67.6 % (Carling & Leiner, 1986). These ratios, and the present incidence of 99.0 % versus 82.1 %, support the belief that the black scurf phase of potato rhizoctoniasis is almost exclusively due to AG-3 and that formation of sclerotia on tubers by other AGs occurs only incidentally. Besides AG-3, potato tubers in the present study also yielded three (0.7 %) AG-5 isolates. Non-AG-3 anastomosis groups of *R. solani* that have previously been isolated from sclerotia on tubers include AG-2-1 (Chand & Logan, 1983; Carling & Leiner, 1986; 1990b), AG-4 (Balali *et al*., 1995), AG-5 (Abe & Tsuboki, 1978; Balali *et al*., 1995) and AG-7 (Carling *et al*., 1998). Of these, only AG-5 has thus far been shown to produce sclerotia upon reinoculation onto potato tubers (Abe & Tsuboki, 1978; Balali *et al*., 1995). Although the

sclerotium-inducing capacity of AG-5 isolates from tubers was not investigated in the present study, it seems reasonable to assume that AG-5 also causes black scurf symptoms in South Africa when present in the soil, as for instance in Gauteng and Kwazulu-Natal. AG-7, albeit present in soil from the Eastern Free State, could not be isolated from any of the corresponding tuber samples. Although the latter AG is not uncommon in soil, it has been isolated from potato only once, *viz.* tuberborne sclerotia collected in the Toluca Valley in Mexico, but caused only superficial discoloration to shoots upon artificial inoculation onto potato plants (Carling *et al*., 1998). Isolates screened in the present study were avirulent.

Due to the infrequent occurrence of stem canker in South Africa (Doidge *et al*., 1953), relatively few symptomatic stem and root samples were available for collection, hence restricting the number of lesion isolates of *R. solani* that could be obtained. Although AG-3 dominated in lesions, isolations also produced AG-4 or AG-5, and in the Northern Cape comprised only AG-4. Regions yielding lesion isolates of AG-4 or AG-5 invariably contained soil populations of either or both these AGs. AG-4 and AG-5 are commonly isolated from lesioned potato stems, stolons and roots and are capable of causing significant damage to these organs (Bandy *et al*., 1984, 1988; Bolkan & Ribeiro, 1985; Anguiz & Martin, 1989; Carling & Leiner, 1990a; Bains & Bisht, 1995; Balali *et al*., 1995). They do, however, seem to be more prevalent and virulent at higher temperatures (Anguiz & Martin, 1989; Carling & Leiner, 1990a; Balali *et al*., 1995). It should nevertheless be noted that a nonpathogenic isolate of AG-4 has been reported to induce a significant increase in the leaf, shoot and tuber mass of potato in field experiments (Sneh *et al*., 1986). Other AGs that have previously been recorded from lesions on potato plants, *viz.* AG-1, AG-2-1, AG-2-2 and AG-9 could not be isolated from any of the stem, root, tuber or soil samples in the present study, but are in any case not known to cause significant damage to potato (Carling & Leiner, 1990a). Of greater potential concern is the presence of AG-8 in potato soil from Gauteng. AG-8 has thus far been isolated from potato plants only once (Balali *et al*., 1995), and the three soil isolates screened in the present study showed no virulence to potato sprouts. However, isolates of AG-8 from wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) have been shown to aggressively invade potato roots, causing severe cankers and pruning of the feeder roots (Hide & Firmager, 1989; Carling & Leiner, 1990a; Balali *et al*., 1995).

Besides being the anastomosis group most commonly associated with potato, AG-3 is also considered to be the most virulent (Carling & Leiner, 1990a), as evidenced by the present results. Isolates of AG-3 can, however, range in virulence from highly aggressive to nonpathogenic (Carling & Leiner, 1990b; Bains & Bisht, 1995). Various earlier reports indicated that *R. solani* isolates from tuberborne sclerotia were of low virulence (Sanford,

1937, 1938; Person, 1945; James & McKenzie, 1972). Conversely, Hill & Anderson (1989) reported that isolates of AG-3 from below-ground stem lesions were less virulent than those from stolons, tuberborne sclerotia and hymenia. Carling & Leiner (1990b), on the other hand, showed that the virulence of AG-3 isolates from sclerotia can be similar to that of isolates collected from other sources on the plant, including lesioned stems. Carling & Leiner (1990b) furthermore found the mean virulence of AG-3 isolates collected from soil to be equal to that of isolates from hymenia, stem lesions and sclerotia. Results of the present study are in agreement with Carling & Leiner (1990b) that sclerotial and stem lesions of AG-3 do not differ in virulence, though isolates from soil in South Africa were less virulent than those from stems and tuberborne sclerotia. The study by Carling & Leiner (1990b) was done at 10 °C since AG-3 is known to be more virulent at lower (10-15.5 °C) than at higher (21.1 °C) temperatures, unlike AG-4 and AG-5 which prefer a relatively warm environment (Carling & Leiner, 1990a). Bains & Bisht (1995) nevertheless reported AG-3 to be more virulent on potato stems than AG-4 and AG-5 at 18-24 °C, while Anguiz & Martin (1989) observed damping-off due to AG-3 to be about 50 % higher at 18-24 °C than at 9-18 °C. The significantly higher virulence exhibited by AG-3 than by AG-4 and AG-5 at 16-28 °C (≈ *ca*. 22 °C) in the present study supports the findings of the latter authors that the pathogenic capacity of some strains of AG-3 is not restrained at higher temperatures, and indicates that local populations of AG-3 may be adapted to the relatively warm climate in South Africa.

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CHAPTER 4

THERMAL AND CHEMICAL INACTIVATION OF *RHIZOCTONIA SOLANI* ASSOCIATED WITH POTATO RHIZOCTONIASIS

ABSTRACT

Hot water dipping and treatment with disinfectants were evaluated as alternatives to fungicides for inactivating inoculum of *Rhizoctonia solani* on potato seed tubers. Significant inhibition of mycelial viability of *R. solani* AG-3 was achieved by dipping agar plugs colonised by the fungus for 4 minutes or longer in water at 50 °C, or for 1 minute or longer in water at 55 °C, but complete inhibition was only evident after exposing the mycelium to water at 55 °C for a period of 4 minutes or more. Total inhibition of sclerotial viability on naturally-infected potato tubers was attained by submersing the tubers in water at 50, 55, 60 and 65 °C for 16, 8, 4 and 4 minutes, respectively. The progeny of naturally-infected seed tubers was rendered free of infection by dipping the seed tubers in water at 55 °C for 8 or 16 minutes, 60 °C for 6 minutes, or 65 °C for 4 minutes. However, exposure to thermal treatments more severe than 55 °C for 8 minutes progressively increased tuber mortality. Of 20 disinfectants that were tested, only OA5 DP, an organic tin complex, inhibited mycelial growth of *R. solani* completely, although significant inhibition was evident with most of the other disinfectants. OA5 DP also proved to be the most effective disinfectant for killing sclerotia of the pathogen and rendered the progeny of seed tubers treated with it free of infection, but exhibited acute phytotoxicity towards the tubers. Significant control without any phytotoxicity was obtained with the didecyl dimethyl ammonium chloride compound, Sporekill. Tolclofos-methyl was the only fungicide that gave total control of potato rhizoctoniasis, whereas seed tuber treatment with fludioxonil, kresoxim-methyl and metam-sodium significantly reduced disease severity and incidence in the progeny.

INTRODUCTION

Tuberborne inoculum of *Rhizoctonia solani* J. G. Kühn is responsible for early-season attack of young potato (*Solanum tuberosum* L.) sprouts and stolons, and the introduction of the pathogen into disease-free soil (Adams *et al*., 1980; Frank & Leach, 1980; Jeger *et al*., 1996). Although tuber treatment with fungicides such as benomyl, carbendazim, fluazinam, fludioxonil, iprodione, mepronil, pencycuron, propiconazole, quintozene, thiabendazole, thiophanate-methyl, thiram and tolclofos-methyl can inactivate propagules of *R. solani* on

seed tubers (Wright, 1968; Davis *et al*., 1971; Singh *et al*., 1972; Davis, 1973; Chand & Logan, 1982; Hide & Cayley, 1982; Cother, 1983; Leach & Murdoch, 1985; Martin & Torres, 1986; Hide & Read, 1991; Jager *et al*., 1991; Anonymous, 1995; Leadbitter *et al*., 1995; Wicks *et al*., 1995, 1996; Du Plessis, 1999; De Boer, 2000; Stevenson, 2000; Virgen-Calleros *et al*., 2000), control often tends to be variable and inconsistent (Wicks *et al*., 1996). The practice furthermore is not a sound approach from a global perspective and provides no more control than using disease-free seed tubers (Powelson *et al*., 1993).

For the above reasons, various alternatives to conventional fungicides have been evaluated for eradicating *R. solani* from seed tubers. The most effective treatment thus far involves submersing of tubers for 1 to 2 hours in "standard corrosive sublimate" (0.3 % mercuric chloride). However, even in the old days this practice has been confined to tubers destined for seed production (Dana, 1925) and today is prohibited due to the persistence in the environment and cumulative toxicity of mercurous compounds. Dipping tubers in formaldehyde solution is also highly effective (Dana, 1925; Carling *et al*., 1989) and is commonly used to disinfect tubers for experimental purposes, though commercial utilisation of the compound is restricted by its acutely irritating effect on mucous membranes and toxicity to virtually all forms of life (Buckle, 1981; Leach & Murdoch, 1985). Probably the least hazardous procedure is to expose seed tubers to hot water at a temperature and for a time sufficient to inactivate inoculum of the pathogen without affecting sprouting of the tubers (Hide, 1975; MacKay & Shipton, 1983; Burnett *et al*., 1988; Dashwood *et al*., 1991; Edwards & De Boer, 2000). Quatenary ammonium compounds (QACs) were used by Letal (1977) against potato ring rot and black leg bacteria, and much emphasis is nowadays being placed on the utilisation of QACs and other disinfectants for sanitising potato tubers and storage facilities (Edwards & De Boer, 2000; Morgan & Wicks, 2000; Stevenson, 2000). Disinfectants, as a rule, are relatively benign multi-purpose sanitisers with various applications in the food industry, including the suppression of plant pathogenic organisms (Tomlinson & Faithfull, 1979, 1980; Bancroft *et al*., 1984; Hoy & Ogawa, 1984; Brown, 1987; Dave, 1987; Stanghellini & Tomlinson, 1987; Reyes, 1992; Spotts & Cervantes, 1994; Boshoff & Korsten, 1996; Stanghellini *et al*., 1996), and have been reported effective against *R. solani* (Edwards & De Boer, 2000) and other basidiomycetous fungi such as *Marasmius oreades* (Bolton ex Fr.) Fr. (Blenis *et al*., 1997).

Potato seed producers in South Africa rely almost exclusively on the use of fungicides for the management of tuberborne inoculum of *R. solani*, and very little work has been done on alternative strategies. This report describes the evaluation of heat treatment and surfactants for rendering potato tubers free of rhizoctoniasis inoculum and also provides evidence

regarding the efficacy of some new-generation chemical phytoprotectants and conventional fungicides not presently registered (Nel *et al*., 2003) on potato in the country.

MATERIALS AND METHODS

Hot water dip treatment

In vitro thermal inactivation of *Rhizoctonia solani*

Eight *R. solani* anastomosis group (AG) 3 isolates from naturally infected potato tubers, *viz.* Rs1001 and Rs1002 from Gauteng, Rs2001 and Rs2002 from KwaZulu-Natal, Rs3001 and Rs3002 from the Northern Cape, and Rs4001 and Rs4002 from the Western Cape, were selected to determine the most effective temperature and exposure time for inhibiting mycelial and sclerotial viability of the pathogen. For mycelial inhibition, 6 mm discs were cut from the colony periphery of seven-day-old cultures of the isolates on potato-dextrose agar (PDA) and immersed for 1, 2, 4, 6, 8 and 16 minutes in water at 25, 40, 50 and 55 °C, respectively. After exposure, the discs were retrieved, excess water drained off and plated, five per plate and 20 per treatment per isolate, on PDA. Discs from which mycelium developed after 3 days incubation at 25 °C were recorded.

For suppression of sclerotial viability, naturally black scurf-infected BP1 potato tubers with visible sclerotia covering 25-35 % of the tuber surface were immersed in water at 25, 50, 55, 60 and 65 °C for the same periods as above. After exposure, tubers were allowed to air-dry on a laminar-flow bench. For each treatment, 50 small (<2 mm) and 50 large (2-5 mm) sclerotia were aseptically removed from *ca*. 15 tubers and plated, five per plate, on water agar (WA). Sclerotia that germinated were recorded after 3 days incubation at 25 °C. Both the above experiments were repeated twice.

Greenhouse evaluation of hot water treatment

Temperature treatments that provided the greatest *in vitro* inactivation of sclerotia, as well as two higher temperatures, were evaluated with naturally black scurf-infected Up-to-Date and BP1 potato tubers in separate greenhouse experiments. In both experiments the temperature treatments involved 25 °C for 16 minutes, 50 °C for 8 and 16 minutes, 55 °C for 4, 6, 8 and 16 minutes, 60 °C for 4 and 6 minutes, and 65 °C for 2 and 4 minutes. Tubers were dipped in the water for the respective durations, air-dried on a laminar-flow bench, and planted 100 mm deep in tyndallised (80 °C for 1 hour on three consecutive days) sand:loam mix (1:1 v/v) (pH 6.2) in 4 l pots, assigning 10 replicate pots with one tuber in each to each treatment. Pots were randomly arranged in a greenhouse of which the temperature ranged between 29 °C

(day) and 16 °C (night), and 31 °C (day) and 16 °C (night) for experiment with Up-to-Date and BP1, respectively, and were watered when required. After three months the haulms were cut at soil level and the tubers left for two weeks to allow skin-setting, after which they were harvested and the yield and black scurf index (BI) of the progeny tuber determined according to the formula of Lootsma & Scholte (1996):

$BI = 100 \times (0n_1 + 0.25n_2 + 0.5n_3 + 0.75n_4 + n_5)/n_{total}$

Where n = number or tubers in each of the following categories (n_1-n_5)

n_1 = no sclerotia on tubers

n_2 = 1-25% of tuber surface covered with sclerotia

n_3 = 26-50% of tuber surface covered with sclerotia

n_4 = 51-75% of tuber surface covered with sclerotia

n_5 = 76-100% of tuber surface covered with sclerotia

Agrochemicals

In vitro screening of agrochemicals

Mycelial inhibition

Twenty disinfectants (Table 1) and 9 fungicides (Table 2) were added at 0.1 % and 100 ppm active ingredient (a.i.), respectively, to autoclaved PDA at *ca*. 45 °C. The registered fungicides, tolclofos-methyl and fludioxonil, and unamended PDA were included as references. The amended and unamended PDA were poured into 90-mm-diameter Petri dishes (15 ml per dish), allowed to solidify and inoculated, five plates per treatment per isolate, with a 6 mm disc from a 7-day-old PDA culture of *R. solani* isolates Rs1001, Rs1002, Rs2002, Rs3001, Rs3002, Rs4001 and Rs4002, respectively. Radial growth was recorded after incubation for 5 days at 25 °C. The experiment was conducted three times.

Inhibition of sclerotial germination

The agrochemicals that provided the greatest inhibition of mycelial growth were selected for this purpose. These included 14 disinfectants, *viz*. Agral 90, Bladwett 9, Citowett, Eco sanitizer, Extent, Fitosan, Frigate, G49, OA5 DP, Sanawett, Solitaire, Sporekill, Terminator and Tinsem, and five fungicides, *viz*. fludioxonil, kresoxim-methyl, metam-sodium, tebuconazole and tolclofos-methyl. Naturally black scurf-infected BP1 tubers, with visible sclerotia covering 25-35 % of the tuber surface, were dipped for 5 minutes in 0.1 % and 100 ppm solutions/suspensions of the disinfectants and fungicides, respectively. After treatment the tubers were air-dried on a laminar-flow bench and, when dry, 50 small (<2 mm) and 50 large (2-5 mm) sclerotia were aseptically excised from *ca*. 15 tubers from each treatment and

Table 1. Disinfectants screened for activity against *Rhizoctonia solani*

Surfactant	Chemical character	Ionic activity	Supplier
Agral 90	90% m/m alkaryl polyglycol ether	Nonionic	Kynoch chemicals
BP Agripron Super	Emulsifiable mineral oil plus surfactant	Nonionic	Agricura
Biofilm	Alkyl aryl polyoxyethylene sorbitan mono-oleate (POE), free and combined fatty acids, glycol ethers, dialkyl benzenedicarboxylate	Nonionic	Plaaskem
Bladwett 9	Alkylated phenyl-ethylene oxide condensate	Nonionic	Plaaskem
Citowett	Alkylaryl POE	Nonionic	BASF
Commodobuff	Organic acid and alkali	Ionic	Villa Crop Protection
Eco sanitizer	5% glutaraldehyde	Unknown	BTC
Extent	Detergent	Unknown	Diversylever
Fitosan (F 10)	Household detergent	Cationic	Health and Hygiene
Frigate	Fatty amine ethoxylate	Weakly cationic	ISK Biotech
G 49	Blend of surfactants	Nonionic/anionic	Agricura
Latron B-1956	Modified tallow gliserol alkyd harpon	Nonionic	Schering
OA5 DP	Organic tin complex	Cationic	Ocean Agriculture
Purogene + activator	Chlorine dioxide	Nonionic	BTC
Sanawett 90	Unknown	Unknown	Sanachem
Solitaire	Polyether-polymethylsiloxane-copolymer and vegetable oil	Nonionic	SAFAGRIC
Sporekill	Didecyl dimethyl ammoniumchloride	Nonionic	Hygrotech Seed
Terminator	Dimethyl didecyl ammoniumchloride	Nonionic	Zeneca Agrochemicals
Tinsem	QAC N alkyl dimetyl ammonium compound	Unknown	Ocean Agriculture
Tronic	Alkylaryl POE glycols, mixed petroleum distillates, alkylamine acetate, alkylaryl sulphonates, polyhydric alcohol.	Mixture of cationic, anionic and nonionic	Plaaskem

Table 2. Fungicides screened for activity against *Rhizoctonia solani*

Common name	Trade name	Chemical class	Active ingredient content	Formulation	Supplier
Azoxystrobin	Heritage	Methoxyacylate	500 g kg^{-1}	WG	Syngenta
Fludioxonil	Celest	Cyanopyrrole	100 g l^{-1}	FS	Syngenta
Furalaxyl	Fongarid	Acylalanine	250 g kg^{-1}	WP	Syngenta
Imazalil	Fungazil	Imidazole	800 g l^{-1}	EC	Dow AgroSciences
Kresoxim-methyl	Stroby	Strobilurine	500 g kg^{-1}	WG	BASF
Metam-sodium	Herbifume	Methyl isothiocyanate precursor	510 g l^{-1}	SL	Plaaskem
Phosphorous acid	Phytex	Phosphorous acid	320 g l^{-1}	SL	Horticura
Tebuconazole	Folicur	Triazole	250 g l^{-1}	EW	Bayer
Tolclofos-methyl	Rizolex	Organophoshate ester	500 g kg^{-1}	WP	Philagro

plated, five per plate, on WA. Sclerotial germination was recorded after 3 days incubation at 25 °C. The experiment was repeated twice.

Greenhouse screening of agrochemicals

In vivo tests were conducted with the eight chemicals that suppressed sclerotial viability the most effectively, *viz*. OA5 DP, Sporekill and Terminator applied at 0.1 % a.i., and fludioxonil, kresoxim-methyl, metam-sodium, tebuconazole and tolclofos-methyl applied at 100 ppm a.i. Naturally black scurf-infected BP1 tubers with visible sclerotia covering 25-35 % of the tuber surface were dipped in the various chemical solutions/suspensions for 5 minutes, drained and air-dried on a laminar-flow bench. Twelve tubers from each treatment were planted, 100 mm deep and one per pot, to tyndallised (80 °C for 1 hour on three consecutive days) sand:loam mix (1:1 v/v) (pH 6.4) in 4 l pots. The pots were randomly arranged in a greenhouse of which the temperature ranged between 32 °C (day) and 18 °C (night), and were watered when required. After three months the haulms were cut at soil level and the tubers left for two weeks to allow skin-setting before they were harvested. Yield and BI were determined as described above.

Statistical analysis

Data were analysed according to GenStat (2000). Analysis of variance was used to test for differences between treatments and treatment means were separated by Fisher's protected *t*-test least significant difference. Data pertaining to inhibition of sclerotial germination by chemicals had to be angularly transformed to stabilise treatment variances.

RESULTS

Hot water dip treatment

In vitro thermal inactivation of *Rhizoctonia solani*

Significant inhibition of mycelial viability was achieved by dipping in water at 50 °C for 4 minutes or longer and 55 °C for 1 minute or longer (Fig. 1). Total inhibition, however, was only evident after exposure of the mycelium to 55 °C for a period of 4 minutes or more, although the inhibition obtained by dipping in water at 50 °C for 16 minutes (99 %) was statistically as effective. Exposure of the mycelium to 25 °C and 40 °C did not result in any reduction in viability. Sclerotia of *R. solani* AG-3 were more resistant to heat than the mycelium, but not as much as expected. Total inhibition of sclerotial viability was attained with temperatures of 50, 55, 60 and 65 °C for 16, 8, 4 and 4 minutes, respectively, regardless of the size of the

Table 3. Effect of hot water treatment on the viability of sclerotia of *Rhizoctonia solani* on BP1 potato seed tubers naturally infected with the pathogen

Sclerotium size (mm)	Exposure time (minutes)	Percentage inhibition of sclerotial germination [a]				
		Water temperature (°C)				
		25	50	55	60	65
< 2	1	0 c	0 Dc	2.0 Dc	10.7 Cb	30.7 Ba
	2	0 e	6.7 Dd	31.3 Cc	79.3 Bb	92.7 Aa
	4	0 d	68.7 Cc	84.0 Bb	100 Aa	100 Aa
	6	0 c	84.0 Bb	100 Aa	100 Aa	100 Aa
	8	0 c	91.3 ABb	100 Aa	100 Aa	100 Aa
	16	0 b	100 Aa	100 Aa	100 Aa	100 Aa
2 – 5	1	0 b	0 Eb	0 Db	4.0 Cb	24.7 Ca
	2	0 d	4.0 Ed	19.3 Cc	76.0 Bb	90.0 Ba
	4	0 d	29.3 Dc	43.3 Bb	100 Aa	100 Aa
	6	0 c	71.3 Cb	91.3 Aa	100 Aa	100 Aa
	8	0 c	86.0 Bb	100 Aa	100 Aa	100 Aa
	16	0 b	100 Aa	100 Aa	100 Aa	100 Aa

[a] Mean of 50 sclerotia from *ca*. 15 tubers assessed in each of three separate experiments; values followed by the same letter in columns (upper case) and rows (lower case) within sclerotium size do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$).

sclerotia (Table 3). Exposure of small sclerotia to 55 °C for 6 minutes also resulted in complete loss of viability. Overall, smaller sclerotia were slightly more sensitive to hot water treatment than the larger ones, the minimum time to obtain significant suppression at 50, 55, 60 and 65 °C being 2, 2, 1 and 1 minutes in the case of small sclerotia, and 4, 2, 2 and 1 minutes with large sclerotia, respectively.

Greenhouse evaluation of hot water treatment

BP1 and Up-to-Date tubers responded similarly to hot water treatment and results of the two experiments were therefore combined for statistical analysis. Compared to what can be regarded as the control (25 °C for 16 minutes), all heat treatments evaluated significantly reduced BI in the progeny, with total control evident in the case of 55 °C for 8 and 16 minutes, 60 °C for 6 minutes and 65 °C for 4 minutes (Table 4). Exposure of seed tubers to 25 °C or 50 °C had no effect on their viability and yield. Subjecting them to higher temperatures, e.g. 55 °C for 8 and 16 minutes, 60 °C for 4 and 6 minutes, and 65 °C for 2 and 4 minutes, resulted in a mortality of between 5 and 40 %, with significant reduction in progeny yield associated with a mortality rate of 20 % or higher. Yield per pot with progeny tubers nevertheless did not differ significantly between treatments, although seed tubers that survived 65 °C for 4 minutes yielded 23.4 % less progeny mass than the overall mean (data not tabulated).

Agrochemicals

In vitro screening of agrochemicals

Only one disinfectant, OA5 DP, resulted in 100 % inhibition of mycelial growth of *R. solani*, although it did not differ significantly in efficacy from Bladwett 9 (85 %), Agral 90 (82 %), Citowett (81 %) and Frigate (81 %) (Fig. 2). Inhibition achieved by the other disinfectants ranged from a significant 77 % (Sanawett 90) to 45 % (Latron B-1956) to a non-significant 28, 20 and 6 % (Commodobuff, Biofilm and Purogene + activator, respectively). Fludioxonil, tolclofos-methyl and tebuconazole were the most effective fungicides, the first two compounds suppressing mycelial growth completely (Fig. 3). Significant inhibition was also evident with metam-sodium, kresoxim-methyl, imazalil, azoxystrobin and phosphorus acid, in that order, but not with furalaxyl.

Viability of small sclerotia was totally inhibited by fludioxonil, OA5 DP and tolclofos-methyl, but 100 % inhibition of large sclerotia could only be achieved with the latter compound (Table 5). All compounds tested nevertheless significantly inhibited sclerotial germination relative to the control, the minimum inhibition recorded being 14.7 and 11.3 % of small and large sclerotia,

Table 4. Effect of hot water treatment of BP1 and Up-to-Date potato seed tubers naturally infected with *Rhizoctonia solani* on black scurf index and yield of the progeny

Exposure time (minutes)	BI (yield in g pot^{-1} / percentage seed tubers not sprouting)[a]				
	25 °C	50 °C	55 °C	60 °C	65 °C
2	ND[b]	ND	ND	ND	6.3 cd[c] (46.1 / 10)
4	ND	ND	11.1 c (55.3 / 0)	4.5 de (42.2 / 5)	0 e (22.6* / 40)
6	ND	ND	9.2 cd (52.8 / 0)	0 e (37.1* / 20)	ND
8	ND	18.0 b (51.8 / 0)	0 e (44.2 / 5)	ND	ND
16	36.4 a (55.0 / 0)	7.2 cd (52.2 / 0)	0 e (43.9 / 10)	ND	ND

[a] BI = black scurf index determined according to Loostma & Scholte (1996); mean of 10 replicates assessed in each of two separate experiments with BP1 and Up-to-Date seed tubers, respectively; values followed by the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$).

[b] ND = Not determined.

* Differs significantly from other yield values according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$).

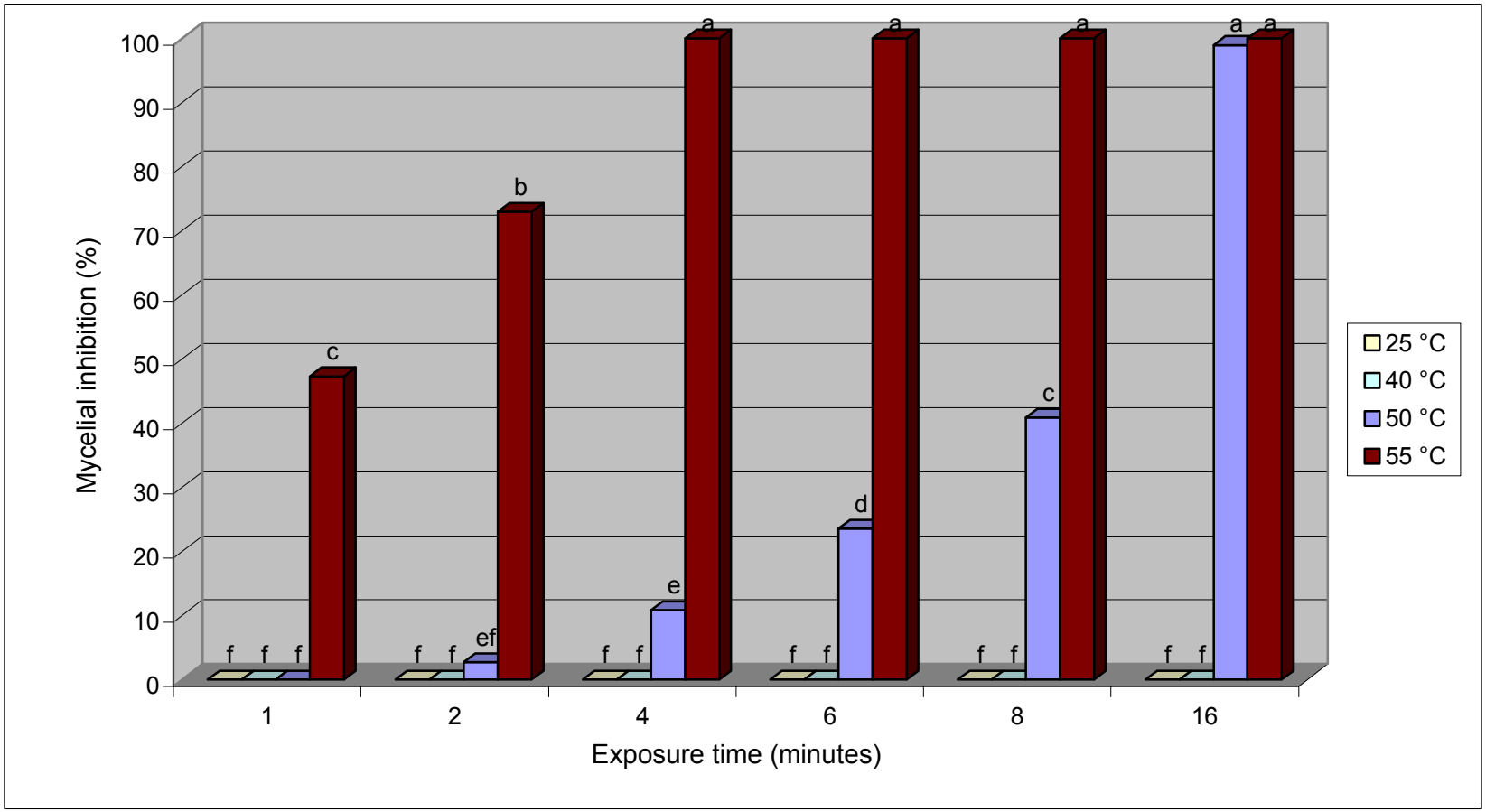

Figure 1. Inhibition of *Rhizoctonia solani* AG-3 mycelium on agar plugs exposed to water at different temperatures. Mycelial inhibition refers to the percentage agar plugs from which no fungal growth was evident after treatment. Data represent the mean of eight *R. solani* isolates, each from which 20 agar plugs with mycelium were assessed in each of three separate experiments. Bars with the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$).

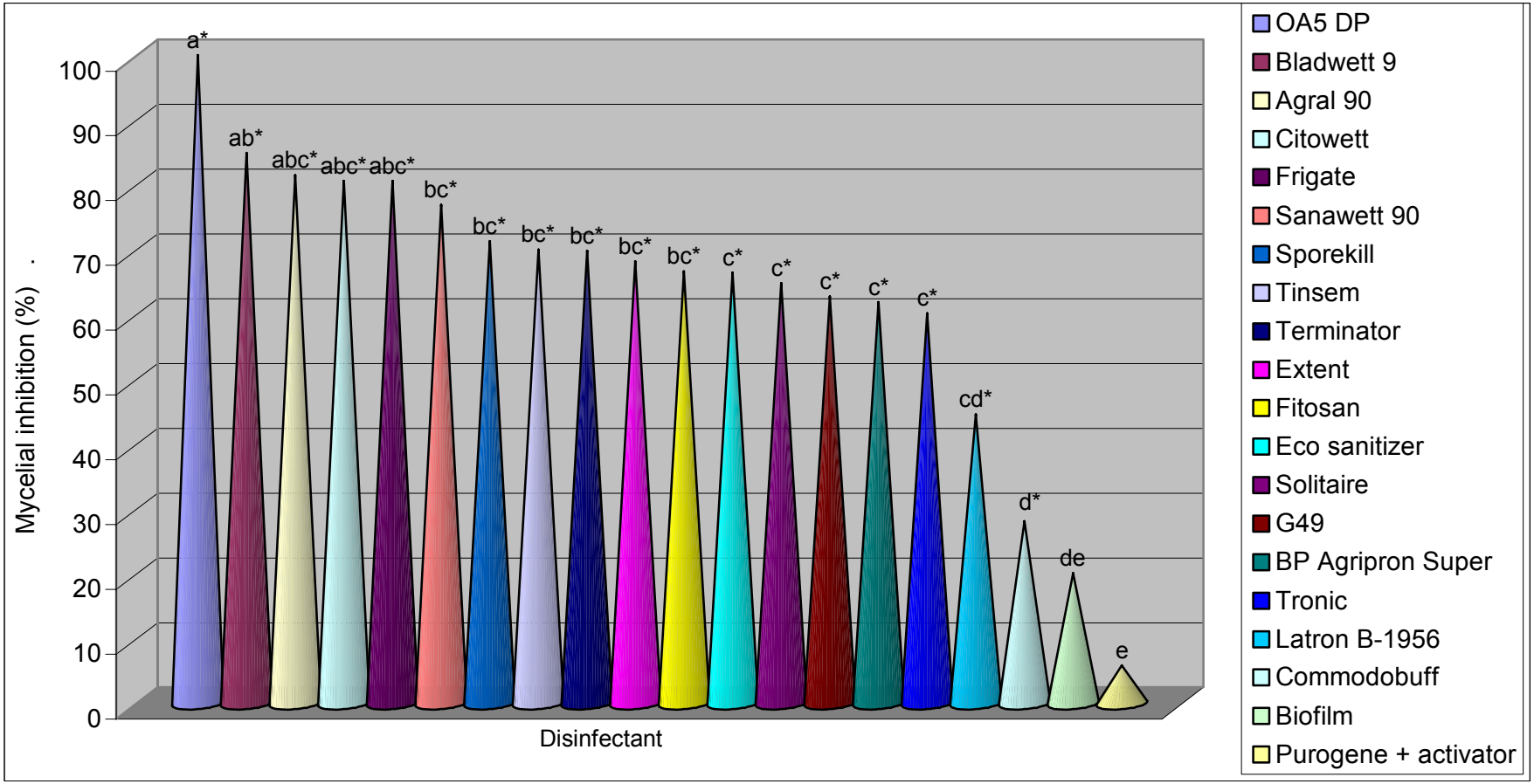

Figure 2. Mycelial inhibition of *Rhizoctonia solani* AG-3 on potato-dextrose agar amended with disinfectants at 0.1% a.i. (v/v). Mycelial inhibition refers to the percentage reduction in colony diameter relative to the control. Data represent the mean of eight isolates of *R. solani*, each of which were assessed on five replicate plates in each of three separate experiments. Bars with the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$). *Significantly different from the control according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$).

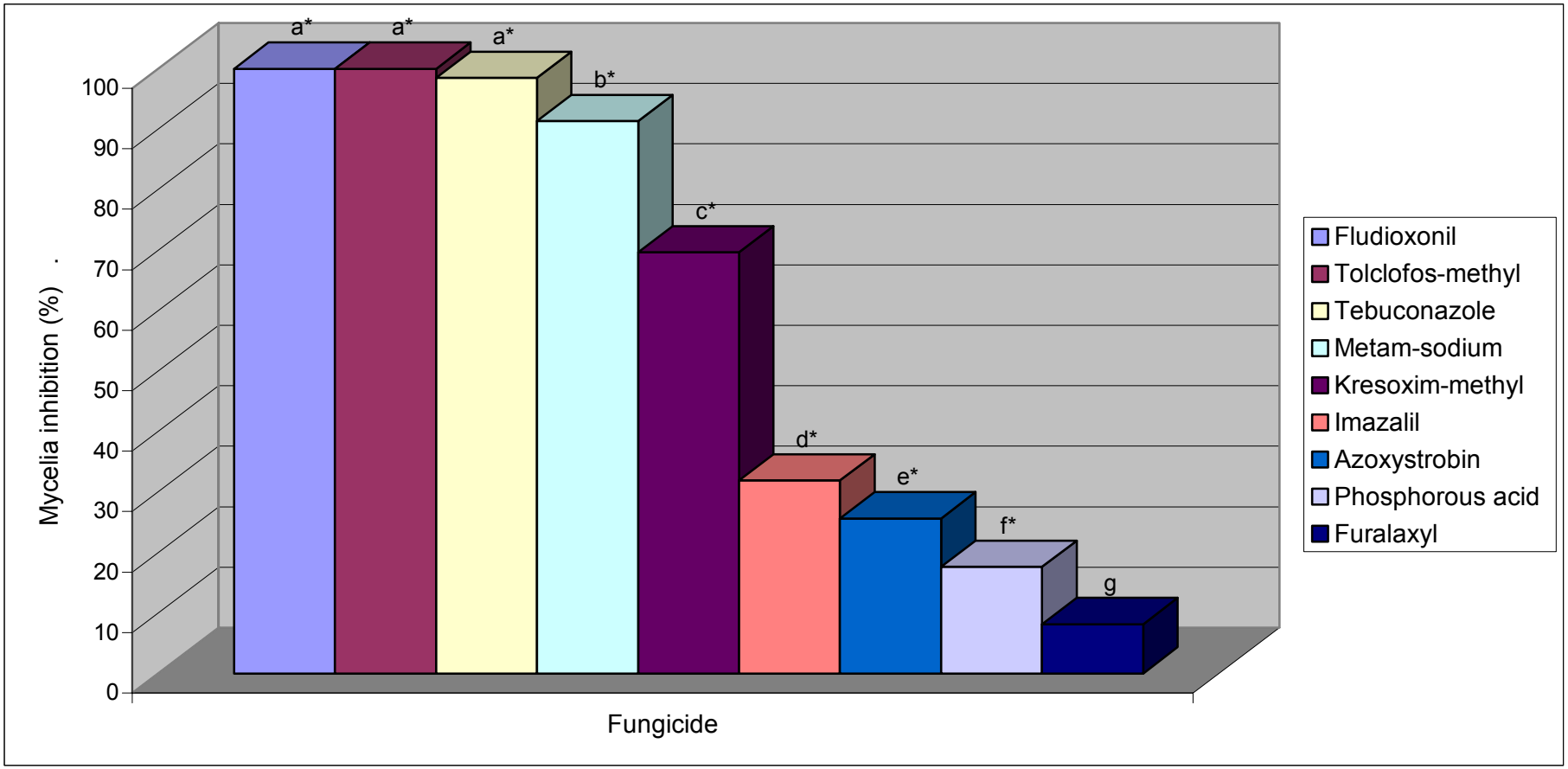

Figure 3. Mycelial inhibition of *Rhizoctonia solani* AG-3 on potato-dextrose agar amended with fungicides at 100 ppm a.i. Mycelial inhibition refers to the percentage reduction in colony diameter relative to the control. Data represents the mean of eight isolates of R. solani, each of which were assessed on five replicate plates in each of three separate experiments. Bars with the same letter do not differ significantly according to Fisher's protected *t*-test least significant ($P \leq 0.001$). *Significantly different from the control according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$).

Table 5. Effect of chemical treatment of BP1 potato seed tubers naturally infected with *Rhizoctonia solani* on the viability of sclerotia of the pathogen

Treatment[a]		Inhibition (%)[b]	
		<2 mm sclerotia	2 – 5 mm sclerotia
Fungicides	Fludioxonil	100 a	94.7 b
	Kresoxim-methyl	76.7 d	70.7 e
	Metam-sodium	93.3 b	86.7 c
	Tebuconazole	74.7 d	67.3 e
	Tolclofos-methyl	100 a	100 a
Disinfectants	Agral 90	25.3 ij[b]	22.0 ij
	Bladwett 9	30.7 h	24.0 ij
	Citowett	18.7 j	14.7 k
	Eco sanitizer	18.0 j	14.0 kl
	Extent	15.3 k	14.0 kl
	Fitosan	26.0 l	22.0 ij
	Frigate	26.0 l	21.3 ij
	G49	20.0 j	18.0 j
	OA5 DP	100 a	93.3 b
	Sanawett 90	18.7 j	15.3 k
	Solitaire	14.7 kl	11.3 l
	Sporekill	43.3 f	39.3 g
	Terminator	39.3 g	34.7 g
	Tinsem	20.0 j	18.7 j
	Control	0.7 m	0 m

[a] Fungicides applied for 5 minutes at 100 ppm and disinfectants at 0.1 % a.i. (v/v).

[b] Mean of 50 sclerotia from *ca*. 15 tubers assessed in each of three separate experiments; values followed by the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.05$). Angular transformation of percentages were used to stabilise treatment variances.

respectively, by Solitaire. On average, fungicides were considerably more effective than the disinfectants. Mean inhibition of small sclerotia achieved by fungicides was 89.7 % compared to 29.7 % by disinfectants, and that of large sclerotia 83.9 % and 25.9 %, respectively.

Greenhouse screening of agrochemicals

Total control of black scurf was obtained with tolclofos-methyl and OA5 DP (Table 6). A significant reduction in BI was also evident with fludioxonil, kresoxim-methyl, metam-sodium and Sporekill, but not with tebuconazole and Terminator, despite the latter two compounds reducing BI by about 50 %. No significant phytotoxicity was evident, except with OA5 DP, which caused a 42 % seed tuber mortality and a consequent 60.4 % reduction in progeny yield.

DISCUSSION

Of the two alternatives to fungicides that were evaluated in this study, *viz*. hot water treatment and disinfectants, the former proved to be the most effective for eradicating inoculum of *R. solani* from potato seed tubers without injury them. *R. solani* as a species is relatively sensitive to elevated temperatures, with thermal death points of 50-52.5 °C for 5 minutes and 50 °C for 10 minutes having been recorded for various isolates (Bollen, 1969; Sherwood, 1970; Pullman *et al*., 1981). Differences in sensitivity are mostly ascribed to natural variance between populations from different climates (Harikrishnan & Yang, 2004), though strains associated with potato appear to be comparatively resistant. Mackay & Shipton (1983), for instance, found 55 °C for 10 minutes sufficient to inactivate some, but not all, Scottish potato isolates of *R. solani* on agar plate cultures containing mycelium and sclerotia. A separate study by Dashwood *et al*. (1991), also in Scotland, indicated a survival rate of 100 % for mycelium and sclerotia of a potato isolate of *R. solani* after exposure to 50 °C for 5 minutes, and about 80 % and 40 % after 55 °C and 57 °C for 5 minutes, respectively, and showed *R. solani* to be more resistant to heat than other potato pathogens such as *Colletotrichum coccodes* (Wallr.) S. Hughes, *Helminthosporium solani* Dur. & Mont., *Phoma exigua* Desm. var. *foveata* (Fisher) Boerema and *Polyscytalum pustulans* (M.N. Owen & Wakef.) M.B. Ellis. Total killing of local isolates of *R. solani* AG-3 could not be achieved by exposing them to 50 °C for periods of up to 16 minutes, surprisingly because the mycelium, and not the sclerotia, exhibited some resistance to the treatment. The minimum thermal treatment for eradicating *R. solani* AG-3 from potato tubers in South Africa therefore is 55 °C for 8 minutes, which indicates that local isolates of the pathogen are as, or slightly less, resistant to heat than the ones tested by Mackay & Shipton (1983) and Dashwood *et al*. (1991), but nonetheless more resistant than *R. solani* in general.

Table 6. Black scurf index and yield of progeny tubers of seed tubers treated with chemicals

Treatment[a]		BI [b,c]	Progeny tuber yield (g pot^{-1})[c]
Fungicides	Fludioxonil	4.5 b	48.8 a
	Kresoxim-methyl	6.4 b	39.2 a
	Metam-sodium	5.3 b	39.7 a
	Tebuconazole	12.5 ab	48.5 a
	Tolclofos-methyl	0 b	40.9 a
Disinfectants	OA5 DP	0 b	18.4 b
	Sporekill	8.2 b	40.0 a
	Terminator	13.0 ab	43.9 a
	Control	25.9 a	46.5 a

[a] Fungicides applied for 5 minutes at 100 ppm and disinfectants at 0.1 % a.i. (v/v).

[b] BI = black scurf index determined according to Lootsma & Scholte (1996).

[c] Mean of 12 replicates; values in columns followed by the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$).

A temperature of 55 °C for at least 8 minutes also proved to be the minimum for providing a black scurf-free progeny without any adverse effect on yield. The 100 % control achieved by this treatment compares favourably with the 89 % and 80 % reduction in tuber eye infection and percentage tubers with black scurf reported by Hide (1975) and Mackay & Shipton (1983), respectively, for the progeny of seed tubers exposed to 55 °C for 10 minutes, though lack of total control in the above two instances could have been due to reinfection of the progeny by soilborne inoculum of the pathogen. It should nevertheless be noted that total killing of the superficial inoculum on seed tubers does not necessarily guarantee a disease-free progeny, even when the treated tubers are planted to a substrate free of the pathogen. In the present study, for instance, 100 % mortality of mycelium and sclerotia was achieved after exposure to 60 °C for 4 minutes, but the progeny still developed some black scurf symptoms in tyndallised sand:loam mix.

Dipping of ware potatoes in hot water at 57.5 °C for 20 to 30 minutes is an effective means of reducing spoilage by organisms such as *Erwinia carotovora* subsp. *carotovora* and *Fusarium solani* (Mart.) Appel & Wollenw. without adversely affecting the quality of the tubers (Ranganna *et al*., 1998). However, although potato cultivars apparently differ in sensitivity to

hot water treatment (Burnett *et al*., 1988), 55 °C seems to be the highest temperature that can be tolerated by seed tubers, and only for a limited period, without affecting sprouting and subsequent yield. Blodgett (1923) found tubers of various potato varieties incapable of surviving 24 minutes in circulating hot water at 55.5 °C, or 4.3 to 12 minutes in water at 60 °C. Hide (1975) reported that 27 % and 70 % of King Edward tubers did not develop sprouts after submersing them in hot water at 55 °C for 10 and 15 minutes, respectively. Upreti & Nagaich (1968), on the other hand, observed no effect on sprouting after exposing Up-to-Date, Kufri Kisan and Kufri Red tubers to 55 °C for 10 minutes, and a maximum of 20 % inhibition after exposure for 15 minutes to the same temperature. Mackay & Shipton (1983) similarly noted a reduction of only 5 % in the number of Pentland Javelin tubers that sprouted after hot water treatment at 55 °C for 10 minutes, though a significant 32 % reduction in the mean number of eyes containing actively growing sprouts was evident. BP1 and Up-to-Date, which represent 42 % and 21 % of registered seed potato plantings in South Africa, respectively (Nortje *et al*., 2000), do not appear to be particularly resistant or susceptible to heat, but it is clear that their yield will be reduced if they are subjected to thermal treatments effectively eliminating *R. solani* that are more severe than 55 °C for 8 minutes, with the possible exception of 55 °C for 16 minutes.

Reports on the utilisation of disinfectants in potato production are limited. Letal (1977) reported mercuric chloride, chlorine bleach and formaldehyde to be the most effective of eight disinfectants tested against *E. carotovora* subsp. *atroseptica* and *Corynebacterium sepedonicum* on metal, wood and burlap surfaces, whereas Morgan & Wicks (2000) found various sanitising agents, including Sporekill and chlorine dioxide, to reduce the levels of *E. carotovora* subsp. *carotovora* and *atroseptica* and *E. chrysanthemi* in wash water in a potato washing plant. Stevenson (2000) mentioned that chlorine dioxide and other similar products applied to tubers entering storage and/or injected into the air handling system during storage are being marketed in the USA for the management of silver scurf. Ironically, Edwards & De Boer (2000) found *H. solani* by far the most resistant of eight potato pathogens they tested *in vitro* against 12 disinfectants, including chlorine dioxide. Most of the other pathogens, which included *R. solani*, were killed by the disinfectants within two and a half minutes while conidia of *H. solani* remained viable after exposure for 20 minutes. Be that as it may, disinfectants seldom provide total control of fungal diseases, despite often having pronounced *in vitro* inhibitory activity against the causal fungi, e.g. chlorine dioxide was effective against *E. carotovora*, *Fusarium* spp. and *H. solani* at low concentrations in laboratory studies, while the extent of disease in potato tubers, inoculated or naturally infected, were generally unaffected by chlorine dioxide treatments (Olsen *et al.,* 2003). In a separate study, the surfactant Nacconol, was effective in controlling tomato (*Lycopersicon esculentum* Mill.) decay

pathogens such as *Botrytis cinerea* Pers.: Fr., *Geotrichum candidum* Link, *Phytophthora nicotiana* Breda de Haan and *Rhizopus stolonifer* (Ehrenb.: Fr) Vuill., *in vitro*, but provided inconsistent control in the pack-house with phytotoxicity evident in some treatments (Hoy & Ogawa, 1984). The present investigation was no exception to the rule. Although 18 of the 20 disinfectants tested exhibited significant *in vitro* activity towards *R. solani*, only one (OA5 DP) provided total control of the disease on progeny tubers. Unfortunately OA5 DP also proved to be highly phytotoxic and can therefore not be recommended for use as tuber treatment against potato rhizoctoniasis. It is nevertheless clear from the results that the efficacy of a disinfectant for rendering potato tubers free of viable inoculum of *R. solani* depends on its capacity to kill sclerotia of the pathogen. Further work with such compounds, e.g. Sporekill and Terminator, at higher concentrations and/or longer exposure times, is thus indicated.

Fungicides included in this study have been selected primarily for the following reasons: (i) fludioxonil and tolclofos-methyl because the are registered against *R. solani* on potato in South Africa (Nel *et al*., 2003) and have proven activity against the pathogen (Anonymous, 1995; Leadbitter *et al*., 1995; Du Plessis, 1999; De Boer, 2000; Stevenson, 2000); (ii) furalaxyl on account of its pronounced activity against various soil pathogens, including *Rhizoctonia* spp. (MacLeod, 2001); (iii) imazalil to warrant the registration of Rhapsodie (imazalil + iprodione) (Nel *et al*., 2003), costing R495 per litre, rather than iprodione only at R250 per litre, for the control of black scurf and stem canker in South Africa; (iv) tebuconazole because of previous conflicting reports regarding its effectivity against *R. solani* (Brenneman *et al*., 1991; Sumner *et al*., 1991); (v) azoxystrobin and kresoxim-methyl by virtue of their biotic derivation and reported activity against *R. solani* (Zens *et al*., 1998; Kiewnick *et al*., 2001); (vi) phosphorous acid because it effectively controlled crater disease of wheat (*Triticum aestivum* L.), caused by *R. solani* AG-6 in South Africa (Wehner *et al*., 1987); and (vii) metam-sodium because it is widely used as soil fumigant to control soilborne diseases of potato (Powelson & Rowe, 1993) and other vegetables (Sumner & Phatak, 1988; Czinos *et al*., 2000), but highly prone to accelerated biodegradation when applied to soil, even after one application (Warton *et al*., 2001; Di Primo *et al*., 2003).

Azoxystrobin, imazalil and phosphorous acid were excluded from further study after the initial testing since they showed relatively little *in vitro* inhibition of mycelial growth of *R. solani*, and furalaxyl because it had no activity. The weak activity of phosphorous acid on *R. solani* was anticipated as phosphonates primarily act by inducing or stimulating host defence responses in plants (Bompeix *et al*., 1980; Raynal *et al*., 1980; Guest, 1984). The mediocre performance by imazalil was also not surprising as Cayley *et al*. (1983), Hide *et al*. (1987) and De Boer (2000) have previously found it ineffective or variable in efficacy against potato rhizoctoniasis,

and its registration in combination with iprodione for control of the disease in South Africa (Nel *et al*., 2003) therefore does not seem justified. Azoxystrobin and furalaxyl, however, did not measure up to their reported *in vitro* suppressiveness towards *R. solani* (Frank & Sanders, 1994; Kiewnick *et al*., 2001), indicating that AG-3 strains (or at least the local ones) are relatively resistant to these compounds. Tolerance of AG-3 to tebuconazole could also have been the reason why this fungicide provided relatively poor control of black scurf on progeny tubers, despite being highly effective against *Rhizoctonia* on various other crops (Kiewnick *et al*., 2001; Mocioni *et al*., 2003). Of the fungicides not registered for use against potato rhizoctoniasis in South Africa that significantly reduced the disease on progeny tubers in this study, only kresoxim-methyl has previously been found effective against *R. solani* (Zens *et al*., 1998). As far as could be established, this is the first time that metam-sodium has been tested as tuber treatment. This mode of application should be investigated further as it could provide an environmentally-compatible means of reducing tuberborne inoculum of *R. solani* and other pathogens without the risk of inducing enhanced biodegradation of the compound in soil.

Considering that tolclofos-methyl was the only registered fungicide that provided total control of potato rhizoctoniasis without adversely affecting the progeny yield, this compound should be preferred to fludioxonil for the purpose of managing the disease. There also seems no purpose for combining it with thiram, as recommended by Nel *et al*. (2003). A major advantage of tolclofos-methyl is that it is highly effective against all major pathogenic *Rhizoctonia* species and AGs and exhibits little or no selectivity within these fungi (Ohtsaki & Fujinami, 1982; Kataria & Verma, 1991; Kataria *et al*., 1991; Olaya *et al*., 1994; Anonymous, 1995). Insensitivity or tolerance to tolclofos-methyl among indigenous *Rhizoctonia* populations is also very rare. At R1.40 per litre applied product, tolclofos-methyl is one of the more affordable fungicides on the market and, being agonomycete-specific (Csinos, 1985; Montealegre & Henriquez, 1990), particularly suited for inclusion in integrated control programmes directed at potato rhizoctoniasis (Van Boogert & Luttikholt, 2004). It has a low toxicity (LD_{50} in rats = 5000 mg kg^{-1}; Tomlin, 2003) but, being an organophosphate with known persistence in soil, not the most desirable from an environmental perspective. Fludioxonil, on the other hand, is chemically related to pyrrolnitrin, a secondary metabolite produced by a number of *Pseudomonas* species (Arima *et al*., 1964; Roitman *et al*., 1990), and with a LD_{50} of >5000 mg kg^{-1} for rats (Tomlin, 2003), not only as safe as tolclofos-methyl, but environmentally more benign. At R4.28 per litre applied product, fludioxonil is rather expensive, but has activity against a wide range of organisms (Olaya *et al*., 1994). It can therefore be used for the simultaneous control of various pathogens besides *R. solani*, e.g. *C. coccodes*, against which it is registered in South Africa (Nel *et al*., 2003), *Streptomyces scabiei* (Wilson *et al*., 1999; Pung & Cross, 2000), *Fusarium* spp. and *H. solani* (Bains *et al*.,

2002). This broad spectrum of biocidal activity would, however, preclude the use of fludioxonil in most integrated disease control strategies.

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CHAPER 5

ECO-COMPATIBLE CONTROL OF SOILBORNE INOCULUM OF *RHIZOCTONIA SOLANI* ASSOCIATED WITH POTATO RHIZOCTONIASIS

ABTRACT

Various biocontrol agents, types of plant tissue, compost, manure, biotically derived fungicides, and a compound inducing systemic resistance in plants, were evaluated *in vitro* and/or in the greenhouse for the control of potato rhizoctoniasis caused by *Rhizoctonia solani*. Significant inhibition of mycelial growth of the five *R. solani* anastomosis groups (AGs) associated with the disease in South Africa was achieved with volatiles from *Bidens formosa*, *Bidens pilosa*, *Brassica napus*, *Brassica oleracea* var. *capitata*, *Raphanus sativus*, *Sinapsis alba* and *Tagetes minuta* shoots and roots and *Datura stramonium* shoots, the antagonists *Azospirillum brasilense*, *Bacillus subtilis* and *Trichoderma harzianum*, and the fungicides azoxystrobin and kresoxim-methyl. The five AGs varied in sensitivity towards the various treatments, though AG-8 on average was the most sensitive and AG-7 the least. Overall, the greatest inhibition of mycelial growth was evident with kresoxim-methyl, followed by volatiles from roots and shoots of *B. napus*, *B. oleracea* var. *capitata*, *R. sativus*, *S. alba*, and *T. minuta*. When artificially inoculated into soil, populations of *R. solani* AG-3 declined within eight days by 9.8 % and 32.7 % in unsterilised sand-loam and clay-loam soil, respectively, but increased by 12.3 % in previously sterilised sand-loam soil. Amendment of the artificially infested soils with the biocontrol formulation Trykocide™ (*T. harzianum*) eradicated the pathogen. Significant reductions in pathogen populations were also evident in soils amended with azoxystrobin, kresoxim-methyl, Maxiflo™ (*A. brasilense*), Avogreen™ (*B. subtilis*), cattle, chicken and sheep manure, citrus and mango waste compost, composted kraal manure, and shoot tissue of *B. napus*, *B. oleracea* var. *capitata*, *R. sativus*, *S. alba* and *T. minuta*. Trykocide™ also provided total control of stem canker in soil artificially infested with *R. solani* AG-3, albeit not significantly more than kresoxim-methyl, azoxystrobin, sheep manure or *B. napus* and *B. oleracea* var. *capitata* shoot tissue. Efficacy of the latter three treatments did not differ significantly from that of acibenzolar-*s*-methyl and mango waste compost.

INTRODUCTION

Soilborne inoculum of *Rhizoctonia solani* J.G. Kühn is the main cause of black scurf on potato (*Solanum tuberosum* L.) tubers and also contributes to stolon and root damage (Hide *et al*.,

1973; Frank & Leach, 1980). Propagules of the pathogen can persist in soil almost indefinitely in the absence of a host (Coley-Smith, 1979). Inadvertent infestation or reinfestation of the soil can also occur through the planting of seed tubers symptomlessly infected by *R. solani* (Hide *et al*., 1973; Wicks *et al*., 1996; Du Plessis, 1999) and, in countries such as South Africa, as a result of lenient certification specifications (Republic of South Africa, 1998). Where soil becomes the predominant source of inoculum, seed treatment with fungicides obviously becomes less effective (Hide & Read, 1991; De Boer, 2000).

Traditionally, control of soilborne populations of *R. solani* has been attempted through soil fumigation or application of fungicides to soil. However, there is increasing evidence that soil fumigation actually aggravates the disease (Read & Hide, 1995; Du Plessis, 1999; Stevenson, 2000), whereas the efficacy of antirhizoctonial fungicides are greatly influenced by variables such as soil type, pH, temperature and moisture, as well as by the host species (Kataria & Grover, 1976; Jager *et al*., 1991). Applying fumigants and fungicides to soil furthermore is a costly endeavour and, with potato rhizoctoniasis, can be particularly wasteful due to the irregular and patchy nature of the disease (Jager & Velvis, 1989, 1995; Harris *et al*., 2003).

Because of the above considerations and the apparent unattainability of real varietal resistance against potato rhizoctoniasis (Banville *et al*., 1996; Bains *et al*., 2002), research directed at reducing soilborne inoculum of *R. solani* in potato fields has focused mainly on crop rotation (Frank & Murphy, 1977; Specht & Leach, 1987; Hide & Read, 1991; Powelson *et al*., 1993; Honeycutt *et al*., 1996; Carter *et al*., 2003) and tillage (Leach *et al*., 1993; Peters *et al*., 2003). There are, however, various alternatives that could be considered for this purpose. Perhaps the area most actively researched is currently in the field of biological control. Various bacteria (Meshram, 1984; Tanii *et al*., 1990; Schmiedeknecht, 1993; Berg *et al*., 2004), fungi (Beagle-Ristaino & Papavizas, 1985; Jager & Velvis, 1985, 1986; Murdoch & Leach, 1993; Schmiedeknecht, 1993), hypovirulent or non-pathogenic strains of *R. solani* (Bandy & Tavantzis, 1990; Tsror *et al*., 2001), non-pathogenic binucleate *Rhizoctonia*-like species (Escande & Echandi, 1991a, b), as well as microfaunal predators (Bollen *et al*., 1991; Lootsma & Scholte, 1997, 1998; Scholte & Lootsma, 1998) have been studied with respect to controlling rhizoctoniasis in field-grown potatoes. Other options include soil solarisation (Grinstein *et al*., 1979; Elad *et al*., 1980; Abdul-Rahman & Katan, 1987), organic soil amendments (Weber, 1977), biotically-derived biocides (Iwasa *et al*., 1971) and soil incorporation of residues of certain plant species, particularly brassicaceous crops (Harding & Wicks, 2000), the latter procedure being referred to as biofumigation (Brown & Morra, 1997).

Potato growers in South Africa seem amenable to biological control and biofumigation, apparently because of the local market presently being inundated with biocontrol formulations and biofumigation already been proven effective in the country against diseases such as common scab and *Verticillium* wilt (Gouws & Mienie, 2000; Millard, 2003). This report provides preliminary results on the control of potato rhizoctoniasis by means of the above two strategies, as well as on the efficacy in this regard of organic soil amendments, residues of non-brassicaceous plants with potential disease-suppressive activity, biotically-derived fungicides, and a compound inducing systemic resistance in plants.

MATERIALS AND METHODS

In vitro evaluation

Isolates

Five *R. solani* isolates representative of the anastomosis groups (AGs) associated with potato rhizoctoniasis in South Africa (Truter & Wehner, 2004) were selected for evaluation purposes (Table 1). Stock cultures of the isolates were maintained as colonised agar discs in sterile distilled water (SDW) at 10 °C.

Plant material

The 10 plant species listed in Table 2 were reared from seed in potting soil in a greenhouse. At vegetative maturity, i.e. just prior to flowering, plants of each species were removed from the soil, their roots washed free of soil and separated from the shoots. The roots and shoots were frozen at -20 °C, freeze-dried, ground with a mortar and pestle, and passed through a 1 mm mesh sieve. Freeze-dried material was stored in sealed containers at 25 °C for one to four weeks before being used.

Plugs (6 mm in diameter) of actively growing colonies of the above five *R. solani* isolates on potato-dextrose agar (PDA) were placed centrally on freshly prepared PDA plates, which were kept in a laminar flow cabinet at 25 °C for a maximum of one hour prior to further assessment. Freeze-dried plant tissue (0.25 g) was transferred to small plastic vessels, 30 mm in diameter and 5 mm deep. Each vessel with plant tissue was positioned inside the inverted lid of an inoculated PDA plate. SDW (1.5 ml) was then added to the tissue in each vessel to induce hydrolysis and the bottom of the inoculated plate was replaced, upside down, into the lid. Plates were sealed with two layers of Parafilm™ and incubated 25 °C. Plastic vessels in the control plates received SDW only. Five replicate plates were included for each treatment and colony diameters were measured after five days.

Table 1. *Rhizoctonia solani* isolates included in the study

Anastomosis group	Isolate code	Region collected from	Source	Virulence[a]
AG-3	Rs2379	Gauteng	Sclerotium from potato tuber	+
AG-4	Rs2047	Limpopo	Symptomatic potato stem tissue	+
AG-5	Rs041	Gauteng	Sclerotium from potato tuber	+
AG-7	Rs043	Free State	Potato soil	-
AG-8	Rs045	Gauteng	Potato soil	-

[a] Virulent (+) or avirulent (-) towards potato in *in vitro* tests (Chapter 3).

Table 2. Plant species tested for inhibition of *Rhizoctonia solani*

Plant family	Scientific name	Common name	Source of seed
Asteraceae	*Bidens formosa* (Bonato) Sch. Bip.	Cosmos	Straathof's Seeds
	Bidens pilosa L.	Common blackjack	Field, Pretoria
	Tagetes minuta L.	Tall khaki weed	Field, Pretoria
Brassicaceae	*Brassica napus* L.	Rape cv. English Giant	Mayford Seeds
	Brassica oleracea L. var. *capitata* L.	Cabbage cv. Drumhead	Mayford Seeds
	Capsella bursa-pastoris (L.) Medic.	Shepherd's purse	Garden, Pretoria
	Raphanus sativus L.	Radish cv. Red silk	Mayford Seeds
	Sinapsis alba L.	White mustard	Straathof's Seeds
Solanaceae	*Datura stramonium* L.	Thorn apple	Field, Pretoria
	Physalis angulata L.	Wild gooseberry	Field, Pretoria

Antagonists

Three commercial liquid antagonist formulations were evaluated *in vitro*. Avogreen™ (*Bacillus subtilis*, Stimuplant) and Maxiflo™ (*Azospirillum brasilense*, Axiom Bio-products) were plated on STD 1 medium, and Trykocide™ (*Trichoderma harzianum* Rifai, Axiom Bio-products) on PDA. Pure cultures were prepared from the developing colonies and tested in dual culture for

inhibition of the five *R. solani* isolates. The following procedure was followed with *B. subtilis* and *A. brasilense*: plugs (6 mm in diameter) of actively growing colonies of the various *R. solani* isolates on PDA were placed centrally on nutrient agar plates. The plates were incubated for 24 hours at 25 °C, whereafter a loopful of antagonist cells was streaked 35 mm from the plug on three sides of the plate, each streak *ca*. 20 mm long (Fig. 1A). Control plates were inoculated with *R. solani* only. Five replicates were used per treatment and colony radius of the *R. solani* isolates were measured from the inoculation point towards the middle of each streaked antagonists, with three measurements per plate, after incubation for 72 hours at 25 °C. With *T. harzianum*, 6-mm-diameter plugs from actively growing colonies of the antagonist and *R. solani* were placed at opposite sides of PDA plates (Fig. 1B). Control plates were inoculated with *R. solani* only. Five replicates were included for each treatment and the colony radius of *R. solani* in the direction of the antagonist was measured after incubation for 72 hours at 25 °C.

Biotically-derived fungicides

Kresoxim-methyl and azoxystrobin were added to autoclaved, cooled (*ca*. 45 °C) PDA toa concentration of 10 ppm active ingredient (a.i.). The amended PDA was poured into 90-mm-diameter Petri dishes, allowed to solidify and inoculated centrally in quintuplicate with a 6 mm agar plug from a 7-day-old culture of each of the five *R. solani* isolates. Unamended PDA served as control. Radial growth of the colonies was determined after incubation for five days at 25 °C.

Soil treatment

Inoculation of soil

A sand-loam soil (pH 6.7) was collected from Gauteng and a clay-loam (pH 5.8) from KwaZulu-Natal. Some of the soil from Gauteng was sterilised by autoclaving for 40 minutes at 121 °C on two consecutive days and allowed two weeks to detoxify. Autoclaved maize meal-soil (1:10 m/m) was inoculated with an isolate of *R. solani* AG-3 (Rs2379) and incubated for four weeks at room temperature, whereafter the inoculum was incorporated into each of the above soils at 1:100 (m/m). The artificially infested soils were left undisturbed for 24 hours before further treatment.

Soil amendment

Each of the above *R. solani*-infested soils was amended with the following: (i) freeze-dried shoot tissue of *B. napus*, *B. oleracea* var. *capitata*, *R. sativus*, *S. alba* and *T. minuta* mixed into the soil at 1:100 (m/v), (ii) citrus waste compost from Hall and Sons in Nelspruit,

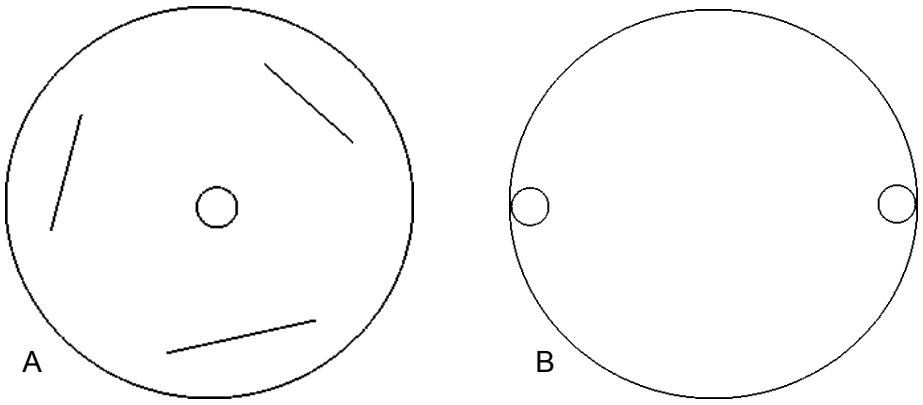

Figure 1. Diagrammatic illustration of the *in vitro* assay of antagonists against *Rhizoctonia solani*. A) Bacterial antagonists streaked 35 mm from the central fungal plug on three sides of the plate. B) Fungal plugs at opposite sides of the plate.

composted kraal manure from Just Nature Organic Products at Onderstepoort, mango waste compost from Bavaria near Hoedspruit, and cattle, chicken and sheep manure from ARC-Irene in Irene mixed into the soil at 1:10 (m/m), (iii) Avogreen™ (2.5 ml l^{-1}), Maxiflo™ (5 ml l^{-1}) and Trykocide™ (10 ml l^{-1}) drenched into the soil at 125 ml l^{-1}, (iv) azoxystrobin (10 ppm a.i.) and kresoxim-methyl (10 ppm a.i.) drenched into the soil at 125 ml l^{-1}. The variously amended soils were dispensed in quintuplicate into 1.2 l pots, with unamended artificially infested soils serving as control. Pots were randomly arranged in a greenhouse at 14-28 °C and moisture content of the soils was maintained at half field-capacity with tap water.

Assessment of survival of *R. solani*

Viability of *R. solani* was determined by means of soil pelleting (Henis *et al*., 1978) immediately prior to, and 1, 2, 4 and 8 days after soil amendment. On each occasion, 100 soil pellets from each replicate were plated, 10 per plate, on water agar and the plates incubated at 25 °C. Survival was calculated as the percentage soil pellets yielding growth of *R. solani* after incubation for 48 hours.

***In vivo* evaluation**

Inoculum of *R. solani* AG-3 was prepared by seeding flasks containing moist autoclaved maize meal-sand (1:10 m/m) with isolate Rs2379 and incubating the flasks for 21 days at 25 °C. The inoculum was incorporated into the clay-loam soil from KwaZulu-Natal at 1:200 (m/m). Twenty-four hours after artificial infestation, the soil was amended with freeze-dried shoots of *B. napus* and *B. oleracea* var. *capitata* at 1:100 (m/v), and mango waste compost and sheep manure at 1:10 (m/m). The variously amended soils were each dispensed into 10 4 l capacity

plastic pots. A further 50 pots were also filled with infested soil. Three surface-disinfested (2 % formaldehyde for 2 minutes) BP1 potato seed tubers were planted *ca*. 50 mm deep in each pot. After planting, 10 of the pots with previously unamended soil were each drenched with 500 ml Trykocide^{TM} (10 ml l^{-1}), azoxystrobin (10 ppm a.i.), kresoxim-methyl (10 ppm a.i.) and acibenzolar-*s*-methyl (10 ppm a.i.), respectively. The remaining 10 pots served as control. Pots were randomly arranged in a greenhouse at 19-28 °C and were watered when required. Plants were harvested 28 days after planting, washed free of debris and examined for lesions on the stems. Disease severity was calculated according to Carling & Leiner (1990), based on the following damage categories: 0 = no damage, no lesions; 1 = minor damage, one to several lesions less than 5 mm long; 2 = intermediate damage, lesions longer than 5 mm, girdling of some tissue; 3 = major damage, large lesions, girdling and death of most tissue; 4 = all tissue dead.

Statistical analysis

Data were analysed using GenStat (2000). Analysis of variance was used to test for differences between treatments and treatment means were separated by Fisher's protected *t*-test least significant difference.

RESULTS

Overall, the greatest inhibition of mycelial growth of *R. solani* was achieved with kresoxim-methyl, followed by volatiles from roots and shoots of *B. napus*, *B. oleracea* var. *capitata*, *S. alba*, *R. sativus* and *T. minuta* (Table 3). Except for *D. stramonium* roots and *C. bursa-pastoris* and *P. angulata* roots and shoots, all the other treatments nevertheless resulted in significant inhibition of mycelial growth. Of the three plant families involved, greatest inhibition was provided by the *Brassicaceae*, followed by the *Asteraceae*. Within the *Asteraceae*, shoot volatiles were the most effective, but no distinct pattern was evident in the *Brassicaceae*. *B. subtilis* was the most effective antagonist, with *A. brasilense* second best. Kresoxim-methyl provided greater inhibition than azoxystrobin.

The different *R. solani* AGs varied in sensitivity towards the various treatments, although AG-8 on average was the most sensitive and AG-7 the least. AG-5 was significantly more sensitive than the other AGs to *B. pilosa* and *S. alba* shoot volatiles and *T. minuta* root volatiles, and AG-8 to *R. sativus* shoot volatiles, azoxystrobin and kresoxim-methyl. AG-4 was significantly the least sensitive to *A. brasilense* and *B. subtilis*, and AG-7 to *S. alba* shoot volatiles,

Table 3. *In vitro* sensitivity of different *Rhizoctonia solani* anastomosis groups (AGs) to plant volatiles, antagonists and fungicides

Treatment	Tissue	Percentage inhibition of mycelial growth[a]					
		AG-3	AG-4	AG-5	AG-7	AG-8	Mean[b]
Plant volatiles							
Bidens formosa	s[c]	12.7 Ck	22.0 Ah	19.5 ABij	11.6 Cl	15.7 BCg	**16.3** m
	r	2.6 Clm	7.9 Bjk	13.0 Ak	6.3 BCj	10.5 ABI	**8.0** o
Bidens pilosa	s	18.4 Bj	16.6 BI	21.9 Ahi	15.8 Bh	17.2 Bg	**18.0** l
	r	17.4 Aj	10.4 Dj	16.2 ABjk	12.0 CDI	14.5 BCgh	**14.1** n
Brassica napus	s	55.8 ABb	49.9 CDb	52.0 BCab	56.9 Aa	47.9 Db	**52.5** c
	r	53.2 BCb	59.9 Aa	55.5 ABa	59.3 Aa	49.9 Cb	**55.6** b
Brassica oleracea	s	29.7 Bfg	39.4 Acd	41.5 Acd	32.9 Bcde	38.0 Acd	**36.3** e
var. *capitata*	r	40.7 Ac	39.9 ABc	39.7 ABd	33.4 Ccd	36.0 BCd	**37.9** d
Capsella bursa-	s	3.2 Alm	4.1 Alm	1.5 Am	2.8 Akl	2.8 Ajk	2.9 q
pastoris	r	1.1 Amn	1.5 Am	1.5 Am	1.3 Al	2.5 Ajk	1.6 qr
Datura stramonium	s	3.4 Blm	6.8 Akl	8.1 Al	7.6 Aj	5.7 ABj	**6.3** p
	r	2.3 Am	4.4 Alm	1.6 Am	4.5 Ajk	1.9 Ajk	2.9 q
Physalis angulata	s	1.6 Amn	3.8 Alm	0.7 Am	2.5 Akl	2.2 Ajk	2.1 q
	r	-1.8 An	1.6 Am	-0.7 Am	0.8 Al	1.1 Ak	0.2 r
Raphanus sativus	s	23.2 BChi	19.8 Chi	25.7 Bfg	21.0 Cg	38.5 Acd	**25.6** i
	r	35.8 Ade	25.8 Cg	23.5 Cfgh	30.6 Bdef	37.7 Acd	**30.7** g
Sinapsis alba	s	36.1 Bd	34.1 Be	43.9 AcA	29.88 Cef	37.7 Bcd	**36.4** e
	r	26.9 Cgh	34.9 ABe	39.9 Ad	35.25 Ac	29.5 BCe	**33.3** f

Table 3. Continued.

Treatment	Tissue	Percentage inhibition of mycelial growth[a]					
		AG-3	AG-4	AG-5	AG-7	AG-8	Mean[b]
Tagetes minuta	s	33.4 Adef	29.5 Af	31.8 Ae	27.8 Af	29.4 Ae	**30.4** g
	r	20.8 Cij	27.3 Bfg	32.0 Ae	21.0 Cg	22.6 Cf	**24.7** i
Antagonists							
Azospirillum brasilense		23.0 Afg	6.7 Dkl	27.1 Af	12.3 Cl	22.4 Bf	**19.7** k
Bacillus subtilus		32.2 Aef	9.3 Ejk	23.2 Cgh	17.7 Dh	27.3 Be	**21.9** j
Trichoderma harzianum		6.1 Dl	26.8 Afg	15.3 Bk	28.9 Af	10.8 Chi	**17.6** lm
Fungicides							
Azoxystrobin		28.1 Cg	36.4 Bde	21.0 Dhi	16.8 Eh	41.2 Ac	**28.7** h
Kresoxim-methyl		61.1 Ba	60.3 Ba	51.9 Cb	42.5 Db	75.7 Aa	**58.3** a

[a] Mean of five replicates; values in rows (upper case) and columns (lower case) followed by the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.05$).

[b] Values in bold differ significantly from the control according to Fisher's protected *t*-test least significant difference ($P \leq 0.05$).

[c] s= shoot; r = root.

azoxystrobin and kresoxim-methyl. AG-3 was the most sensitive of all the AGs to *B. subtilis*, and the least to *T. harzianum*. It also exhibited relatively high sensitivity towards *B. pilosa*, *B. oleracea* var. *capitata* and *R. sativus* root volatiles, *B. napus* shoot volatiles and *A. brasilense*, and relatively low sensitivity towards *B. formosa* shoot and root volatiles and *D. stramonium* shoot volatiles. No significant differences between AGs were evident in sensitivity towards *T. minuta* shoot volatiles and, as could be expected, any volatiles possibly released by the *C. bursa-pastoris* and *P. angulata* shoot and root, and *D. stramonium* root tissues.

Inoculum of *R. solani* AG-3 declined by 9.8 % over the eight-day period observations were made in unamended unsterilised sand-loam soil artificially infested with the pathogen and by 32.7 % in the unsterilised clay-loam soil, but increased by 12.3 % in sterilised sand-loam (data not tabulated). All treatments progressively inhibited the pathogen in all three the soils (Tables 4-6). However, except for *B. napus* shoot tissue, Trykocide™ and kresoxim-methyl, the extent of inhibition was greater in sterilised sand-loam than in the two unsterilised soils (Table 7). Trykocide™ was significantly the most effective treatment and provided total control in all three soils. Azoxystrobin, kresoxim-methyl and shoot tissue of *B. napus* and *B. oleraceae* var. *capitata* rated second-best. As a class, fungicides afforded the greatest inhibition, followed by plant tissue, biocontrol agents, manure and compost. Within these classes, the plant-derived composts generally were superior to composted kraal manure, sheep manure better than cattle manure, and *B. napus* and *B. oleracea* var. *capitata* more effective than *R. sativus* and *S. alba* in unsterilised soil. *T. minuta* was as effective as *S. alba* in sterilised and unsterilised sand-loam, but not in the clay-loam soil. Trykocide™ obviously was the most effective biocontrol formulation. Azoxystrobin and kresoxim-methyl did not differ in efficacy.

Only minor stem damage was evident after 28 days on stems of the potato plants in artificially infested soil (Fig. 2). All eight the treatments evaluated nevertheless significantly reduced severity of the disease. Trycocide™ provided total control, albeit not significantly more than kresoxim-methyl, azoxystrobin, *B. napus*, sheep manure and *B. oleracea* var. *capitata*. Efficacy of the last three treatments did not differ significantly from that of acibenzolar-*s*-methyl or mango waste compost.

Table 4. Survival of *Rhizoctonia solani* AG-3 over time in artificially infested sterilised sand-loam amended with composts, manures, freeze-dried plant material, biocontrol agents or fungicides

Treatment	Percentage inhibition of *R. solani* in soil over time (days)[a]			
	1	2	4	8
Composts				
Citrus waste compost	26.9 ef	63.0 bcd	62.7 g	67.4 hi
Mango waste compost	34.0 de	31.9 i	61.7 gh	64.3 i
Composted kraal manure	27.9 ef	28.8 i	55.3 hi	57.8 i
Manures				
Cattle	14.4 gh	30.2 i	61.3 gh	71.2 gh
Chicken	25.7 f	46.4 fg	58.1 ghi	78.7 ef
Sheep	37.2 cd	62.3 cd	74.9 de	84.1 d
Freeze-dried plant shoots				
Brassica napus	27.4 ef	43.2 gh	88.7 b	89.7 c
Brassica oleracea var. *capitata*	33.0 de	55.8 de	84.8 bc	83.9 d
Raphanus sativus	27.2 ef	64.8 bc	83.3 bc	82.3 de
Sinapsis alba	35.3 d	63.0 bcd	70.3 ef	75.3 fg
Tagetes minuta	46.5 ab	61.7 cd	64.1 fg	73.4 g
Biocontrol agents				
Avogreen™	12.7 h	36.1 hi	60.2 ghi	73.4 g
Maxiflo™	20.8 fg	43.2 gh	54.2 i	63.0 i
Trykocide™	8.1 h	86.2 a	100 a	100 a
Fungicides				
Azoxystrobin	43.2 bc	52.8 ef	73.8 de	95.5 ab
Kresoxim-methyl	51.6 a	70.8 b	78.3 cd	91.8 bc

[a] Mean of five replicates; values in columns followed by the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$).

Table 5. Survival of *Rhizoctonia solani* AG-3 over time in artificially infested sand-loam amended with composts, manures, freeze-dried plant material, biocontrol agents or fungicides

Treatment	Percentage inhibition of *R. solani* in soil over time (days)[a]			
	1	2	4	8
Composts				
Citrus waste compost	9.4 def	43.0 c	40.8 de	59.9 de
Mango waste compost	19.6 bc	40.1 cd	43.7 de	52.9 efg
Composted kraal manure	3.2 f	47.7 c	39.2 de	31.9 h
Manures				
Cattle	5.2 ef	12.2 e	33.2 ef	51.7 fg
Chicken	29.5 a	48.7 bc	48.8 cd	63.2 cd
Sheep	25.0 ab	31.0 de	47.2 cd	67.8 c
Freeze-dried plant shoots				
Brassica napus	27.4 a	47.3 c	84.1 a	85.6 b
Brassica oleracea var. *capitata*	14.7 cd	65.2 a	64.5 b	83.3 b
Raphanus sativus	11.9 de	57.0 ab	67.4 b	65.0 cd
Sinapsis alba	10.8 de	48.4 bc	58.0 bc	59.1 def
Tagetes minuta	15.4 cd	40.5 c	45.3 d	53.7 efg
Biocontrol agents				
Avogreen™	14.9 cd	24.7 e	41.2 de	48.7 g
Maxiflo™	5.6 ef	28.4 e	26.4 f	41.0 h
Trykocide™	13.2 cd	63.5 a	93.2 a	100 a
Fungicides				
Azoxystrobin	26.7 ab	40.6 c	58.8 bc	85.1 b
Kresoxim-methyl	23.3 ab	43.9 c	67.2 b	89.3 b

[a] Mean of five replicates; values in columns followed by the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$).

Table 6. Survival of *Rhizoctonia solani* AG-3 over time in artificially infested clay-loam amended with composts, manures, freeze-dried plant material, biocontrol agents or fungicides

Treatment	Percentage inhibition of *R. solani* in soil over time (days)[a]			
	1	2	4	8
Composts				
Citrus waste compost	10.5 fg	20.5 e	3.6 i	36.6 h
Mango waste compost	27.1 abc	46.9 c	41.8 f	53.5 def
Composted kraal manure	3.5 g	23.6 de	-0.5 i	19.3 i
Manures				
Cattle	16.5 def	29.7 d	22.3 h	51.8 f
Chicken	26.1 abc	38.8 c	45.9 ef	54.0 def
Sheep	14.6 ef	25.4 de	54.2 cde	63.3 cd
Freeze-dried plant shoots				
Brassica napus	34.1 a	68.8 a	82.8 ab	83.2 b
Brassica oleracea var. *capitata*	33.1 a	71.9 a	77.8 b	73.8 bc
Raphanus sativus	18.8 cdef	64.9 ab	60.9 c	62.9 cde
Sinapsis alba	18.2 cdef	46.9 c	49.3 def	52.0 ef
Tagetes minuta	23.9 bcd	22.2 de	32.1 g	30.2 hi
Biocontrol agents				
Avogreen™	20.5 cde	40.2 c	41.9 f	47.8 fg
Maxiflo™	20.8 cde	21.4 e	25.3 gh	37.6 gh
Trykocide™	12.1 efg	58.0 b	87.8 a	100 a
Fungicides				
Azoxystrobin	11.2 fg	22.1 de	55.9 cd	81.4 b
Kresoxim-methyl	32.6 ab	42.4 c	52.8 de	84.1 b

[a] Mean of five replicates; values in columns followed by the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$).

Table 7. Inhibition of *Rhizoctonia solani* AG-3 after eight days in three artificially infested soils amended with composts, manures, freeze-dried plant material, biocontrol agents or fungicides

Treatment	Percentage inhibition of *R. solani*[a]		
	Sand-loam	Sterilised sand-loam	Clay-loam
Composts			
Citrus waste compost	59.9 Bcd[b]	67.4 Ad	36.6 Cf
Mango waste compost	52.9 Bd	64.3 Ae	53.5 Be
Composted kraal manure	31.9 Bf	57.8 Ae	19.3 Cg
Mean	48.2 B	63.2 A	36.5 B
Manures			
Cattle	51.7 Bd	71.2 Ade	51.8 Be
Chicken	63.2 Bcd	78.7 Acd	54.0 Ce
Sheep	67.8 Bc	84.1 Abc	63.3 Bd
Mean	60.9 B	78.0 A	56.4 B
Freeze-dried plant shoots			
Brassica napus	85.6 Ab	89.7 Abc	83.2 Ab
Brassica oleracea var. *capitata*	83.3 Ab	83.9 Abc	73.8 Bc
Raphanus sativus	65.0 Bcd	82.3 Ac	62.9 Bd
Sinapsis alba	59.1 Bd	75.3 Acd	52.0 Be
Tagetes minuta	53.7 Bd	73.4 Ad	30.2 Cf
Mean	69.3 B	80.9 A	60.4 B
Biocontrol agents			
Avogreen™	48.7 Bde	73.4 Ad	47.8 Bde
Maxiflo™	41.0 Be	63.0 Ae	37.6 Bf
Trykocide™	100 Aa	100 Aa	100 Aa
Mean	63.2 B	78.8 A	61.8 B
Fungicides			
Azoxystrobin	85.1 Bb	95.5 Ab	81.4 Bbc
Kresoxim-methyl	89.3 Ab	91.8 Ab	84.1 Ab
Mean	87.2 A	93.7 A	82.8 A

[a] Mean of five replicates; values in rows (upper case) and columns (lower case) followed by the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.001$).

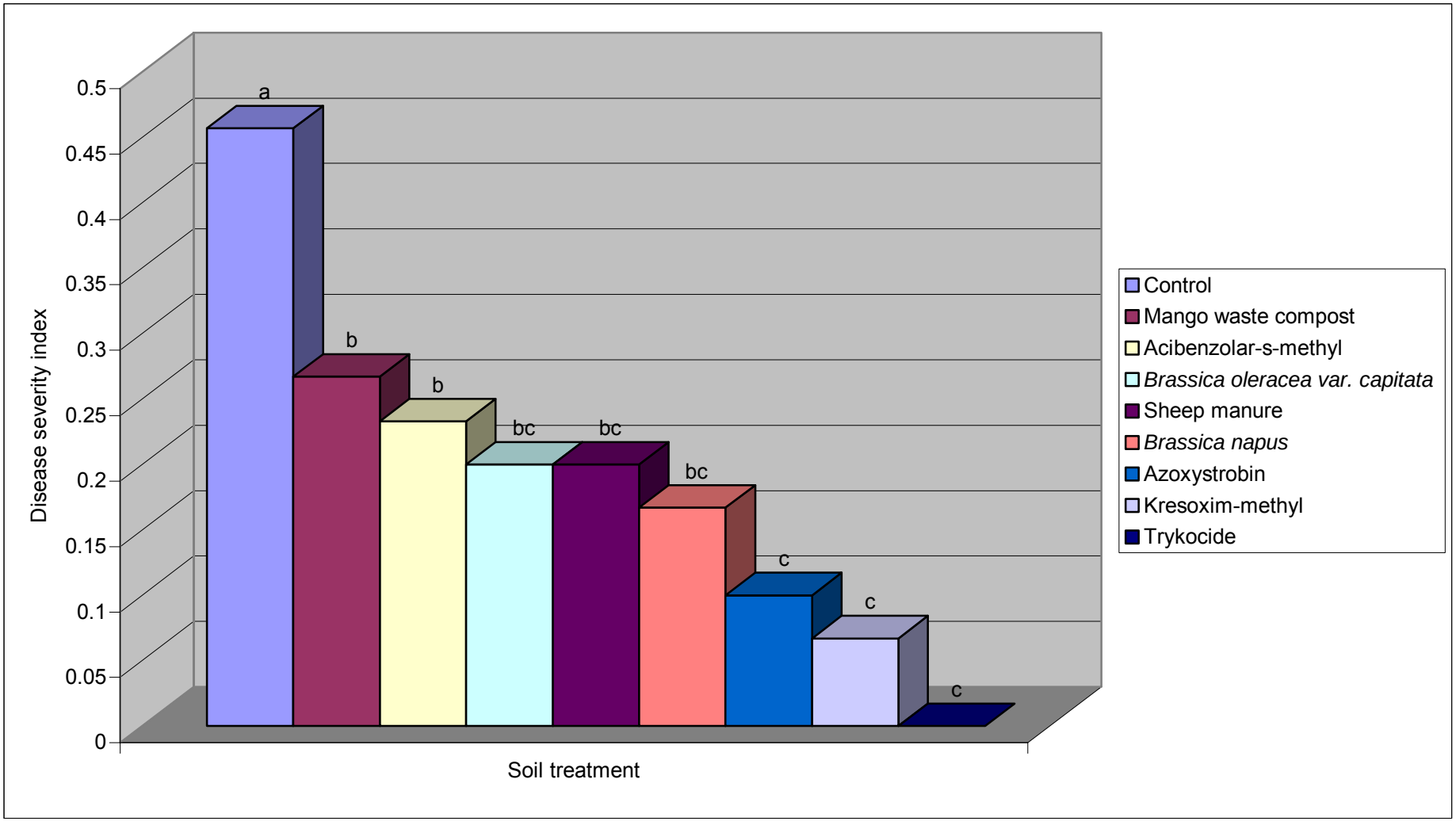

Figure 2. Effect of soil amendment with mango waste compost, sheep manure, freeze-dried *Brassica* shoot residues, Trykocide^{TM} and fungicides on the severity of stem canker, caused by *Rhizoctonia solani* AG-3, on BP1 potato plants.

DISCUSSION

Although tolclofos-methyl is not registered as a soil treatment against potato rhizoctoniasis in South Africa (Nel *et al*., 2003), it is known to effectively control the disease when applied as a soil drench (Jager *et al*., 1991). The compound has nevertheless been excluded from the present study as it was considered inappropriate to compare mostly "trust to luck" treatments with such a highly-effective antirhizoctonial fungicide. This reasoning, however, proved to be fallacious, particularly in view of the excellent control achieved with Trykocide™. According to the suppliers, Trykocide™ is recommended as soil treatment for the control of *R. solani* and *Alternaria solani* Sorauer on potato, as well as *Fusarium* spp. on tomato (*Lycopersicon esculentum* Mill.), *Alternaria*, *Phytophthora* and *Pythium* on tobacco (*Nicotiana tabacum* L.) and *Botrytis* on grapevine (*Vitis vinifera* L.). It apparently has been granted registration against these diseases, though this could not be confirmed.

T. harzianum, the active component in Trykocide™, is one of the oldest and most frequently evaluated biocontrol agents against *Rhizoctonia* on various crops (Henis *et al*., 1978; Elad *et al*., 1980, 1981; Chet *et al*., 1982), including potato (Elad *et al*., 1980; Beagle-Ristaino & Papavizas, 1985; Tsror *et al*., 2001). Antagonism by *T. harzianum* depends on mycoparasitism rather than antibiosis, as evident from the relatively slight inhibition of mycelial growth of *R. solani*, particularly AG-3, in dual culture in the present study. Mycoparasitism by *T. harzianum* is a complex process, involving recognition of the host, attachment to the mycelium, coiling round the hyphae, partial degradation of the cell wall and penetration of the host mycelium (Elad *et al*., 1983a, b; Benhamou & Chet, 1993). Cell wall degradation is achieved by six chitin-induced chitinolytic enzymes comprising two ß-1,4-N-acetylglucoseaminidases and four endochitinases, all of which are required for effective parasitism (Haran *et al*., 1995). Once the host mycelium has been penetrated, additional extracellular enzymes such as lipases and proteases are produced to induce degradation of the cell contents (Elad *et al*., 1982). When attacked, hyphae of the susceptible host respond with rapid vacuolation, collapse and disintegration (Chet *et al*., 1981). Besides being an aggressive mycoparasite, *T. harzianum* is also known to enhance plant growth in the absence of any pathogens, probably by producing plant growth-promoting metabolites in the rhizosphere (Chang *et al*., 1986; Windham *et al*., 1986; Kleifeld & Chet, 1992). It has, however, been observed to parasitise endomycorrhizal fungi (Rousseaeu *et al*., 1996), on which potato seems to be particularly dependent for optimal growth (Gerdemann, 1968).

According to Adams (1990), *T. harzianum* has potential for broad-spectrum control of fungal pathogens, but cannot be applied cost-effectively because of the excessive amounts of

formulated product needed to obtain disease control. In the present study Trykocide™ was applied at an effective concentration of 1.25 x 10^4 cfu ml^{-1} soil. The recommended application rate of the product, however, is 500 l ha^{-1} of a 1:10 suspension. Even when assumed that this rate refers to in-furrow application, the effective concentration of *T. harzianum* in field soils treated with Trykocide™ would be about 20 cfu ml^{-1}, i.e. 625 times lower than in the present experiments. Nevertheless, considering the efficacy of Trykocide™ against *R. solani* in unsterilised soils in the present study, and the broad spectrum of activity of *T. harzianum*, further investigation of the dosage requirements, soil colonisation capacity, range of pathogens affected and effect on beneficial organisms of this biocontrol formulation in different soils is surely warranted.

B. subtilis, like *T. harzianum*, has been included in many biocontrol studies, and has proven activity against *R. solani* on potato (Loeffler *et al*., 1986). Antagonism by *B. subtilis* occurs through antibiosis and the species is known to produce various antimycotic and antibacterial metabolites (Cook & Baker, 1983). Avogreen™, the commercial formulation of *B. subtilis* evaluated in this study, ensued from work done at the Department of Microbiology and Plant Pathology at the University of Pretoria (Korsten *et al*., 1997). It is neither registered nor recommended for the control of potato rhizoctoniasis, but the results indicate that it has potential in this regard. Maxiflo™ is recommended for improving plant growth as its active component, *A. brasilense*, is a well-known plant growth-promoting rhizobacterial species (Smith *et al*., 1984; Horemans *et al*., 1986; Okon & Kapulnik, 1986; Bashan & Levanony, 1990). Plant growth-promoting rhizobacteria also have potential as biocontrol agents for various soilborne diseases (Kloepper, 1992) through mechanisms such as the production of iron-chelating siderophores (Schippers *et al*., 1987), antibiotics (Weller, 1988) and HCN (Voisard *et al*., 1989), which reduce pathogen populations, compete for energy-yielding nutrients (Elad & Chet, 1987) and induce plant resistance (Okon & Kapulnik, 1986). *Azospirillum* is not known to produce antibiotics but has been reported to inhibit sclerotia of *R. solani* by depletion of nutrients (Gupta *et al*., 1995). The significant suppression of mycelial growth of *R. solani* by *A. brasilense* in the present study suggests that it may indeed possess some direct antimycotic activity.

Of the two solanaceous plant species evaluated, *P. angulata* and *D. stramonium*, only the latter significantly inhibited mycelial growth of *R. solani*. It was nevertheless interesting to note that AG-3 was the anastomosis group least affected by shoot volatiles of the weed, indicating that *D. stramonium* will have little, if any, direct suppressive effect on potato rhizoctoniasis in South Africa. *D. stramonium* is well-known for being toxic to humans and animals due to the presence of the alkaloid hyoscyamine in its leaves (Henderson & Anderson, 1966) and has

been shown to have some nematicidal activity (Oduor-Owino, 2003), but no previous report could be traced describing its effect on microorganisms or its susceptibility to infection by *R. solani*.

Biofumigation once again proved to be an effective means of disease control. The biocidal action of brassicaceous plants is ascribed to the release of isothiocyanates, thiocyanates, nitriles, epinitriles and sulphides with fungistatic, fungicidal, bactericidal, nematicidal and/or insecticidal activity when, upon disruption of the plant tissue, glucosinolates (GSLs) contained in vacuoles within the plant cells are hydrolysed by the enzyme myrosinase (α-thioglucosidase glucohydrolase) present in cell walls, endoplastic reticulum, Golgi vesicles and mitochondria (Brown & Morra, 1997; Vaughn, 1999). The type and quality of GSLs vary considerably between plants of different families, between plants in the same family, within a particular species and within organs of an individual plant (Clossais-Besnard & Larher, 1991; Brown & Morra, 1997; Kushad *et al*., 2004). GSL concentrations furthermore fluctuate depending on tissue type, stage of development, and environmental factors such as plant spacing, moisture regime and nutrient availability (Brown & Morra, 1997), making comparisons between separate studies difficult. Overall inhibition of mycelial growth and survival in soil of *R. solani* achieved with brassicaceous volatiles in the present study nevertheless compares favourably with that reported by Lewis & Papavizas (1974), Kirkegaard *et al*. (1996) and Harding & Wicks (2000). More importantly, however, is that it has been shown for the first time that soil incorporation of *B. napus* and *B. oleracea* var. *capitata* residues can actually reduce the severity of stem canker on potato. Of more academic interest is the fact that the brassicaceous weed, *C. bursa-pastoris*, had no activity against *R. solani*. Seed of *C. bursa-pastoris* is known to contain significant amounts of GSLs (Daxenbichler *et al*., 1991), but these seem to have been either absent or not hydrolysed in the root and shoot tissue tested in the present study.

When considering biofumigation as a disease control option, cognisance should be taken of the fact that livestock poisoning has been commonly reported when animals are fed excessive brassicaceous plant material (Kingsbury, 1964). It is furthermore known that extracts of *Brassica* species are inhibitory to endomycorrhizal fungi (Vierheilig & Ocampo, 1990; Schreiner & Koide, 1993a, b) and that the *Brassicaceae* do not establish symbiosis with mycorrhizal endophytes (Gerdemann, 1968; Glenn *et al*., 1985, 1988). The generally lower efficacy of brassicaceous plant material in unsterilised than in sterilised soil observed in the present study also suggests the existence, or rapid development of accelerated microbial degradation of isothiocyanates, similar to the situation with metam-sodium (Warton *et al*., 2001; Di Primo *et al*., 2003), in local soils. Most importantly, however, is the notoriety of GLS-

containing plants to have a negative impact on successive plant communities or those growing in close proximity (Brown & Morra, 1997). Indeed, a study by Leach *et al*. (1993) showed potatoes after broccoli (*Brassica oleracea* L. var. *italica* Plenck) to have a significantly lower yield than after oats (*Avena sativa* L.), buckwheat (*Fagopyrum esculentum* Moench), pea (*Pisum sativum* L.) or lupin (*Lupinus* sp.), and also a very high incidence of secondary tuber growth. The possibility furthermore exists that amendment with brassicaceous residues could actually increase disease caused by *R. solani* in some soils (Papavizas, 1966) or that planting of such crops could selectively enhance populations of *R. solani* AG-4, to which they are hosts (Kuramae *et al*., 2003; Yang *et al*., 2004).

The three *Asteraceae* species screened in the study significantly suppressed *R. solani*, but only *T. minuta* provided inhibition comparable to that achieved with the brassicaceous crops. *T. minuta* and related species have been reported inhibitory towards various plant pests and pathogens (Ross *et al*., 1981; Cook & Baker, 1983; Kimpinski & Arsenault, 1994; Penna *et al*., 1994; Weaver *et al*., 1994; Zygadlo *et al*., 1994; Oduor-Owino, 2003), also in South Africa (Van Biljon *et al*., 2004). The biocidal activity of *Tagetes* and other genera in the *Asteraceae*, including *Bidens*, is ascribed to the presence of thiophenes in these plants which repel, act as toxic substances or have antinutritional effects on herbivores, hence protecting the plants against herbivory (Harborne, 1991). Thiophenes are sulphurous heterocyclic compounds derived from polyacetylenes through a complex process of metabolic steps (Arroo *et al*., 1995), and may be stored in plant tissue or released into the soil (Tang *et al*., 1987). They are known to be toxic to nematodes, insects, fungi, bacteria and viruses (Chan *et al*., 1975; Gommers *et al*., 1980; Cook & Baker, 1983; Champagne *et al*., 1984; Hudson *et al*., 1986; Tereschuk *et al*., 1997; Kéïta *et al*., 2000). Although farmers locally still regard *T. minuta* as a weed, it actually has considerable commercial value as source of volatile oils used in perfumes and as flavour components in many food products (Janick & Simon, 1993; Mohamed *et al*., 2000), and therefore can be cultivated quite profitably. It is also used in folk medicine as antimicrobial, antihelminthic, diuretic and antispasmodic (Amat, 1983), and furthermore has application as mosquito repellent (Seyoum *et al*., 2002). Unfortunately, thiophenes are also phytotoxic and *T. minuta* is notorious for having pronounced allelopathic effects on various commercial crop species (Campbell *et al*., 1982; Rice, 1984; Meissner *et al*., 1986; Kaul & Bedi, 1995; Scrivanti *et al*., 2003).

Organic amendments such as manure and compost could either have a positive or negative effect on *Rhizoctonia* diseases depending on the material used and its state of decomposition (Van Bruggen *et al*., 1996). Fresh amendments generally increase inoculum density and disease severity (Wall, 1984), whereas partially decomposed or composted organic matter

mostly suppress the pathogen (Chung *et al*., 1988). There are, however, exceptions to the rule. Voland & Epstein (1994), for instance, found composted cattle manure to increase *Rhizoctonia* root rot of bean (*Phaseolus vulgaris* L.) seedlings over noncomposted manure, analogous to the situation in the present study. Nevertheless, the cost and logistics of adequately treating commercial potato fields with organic residues, whether composted or not, purely for the purpose of disease control, do not seem justified. Unlike the other treatments included in this study, however, incorporation of manure and compost into soil has the advantage of providing nutrients for plant growth, conserving moisture, improving soil structure, restricting nitrate pollution of groundwater, and limiting the soil's heat absorption capacity, hence reducing fluctuations in soil temperature (Handreck & Black, 1984; Turney & Menge, 1993).

In vitro screening in this study confirmed the results of Chapter 4 that kresoxim-methyl is about twice as inhibitory to mycelial growth of *R. solani* AG-3 as azoxystrobin. In the above chapter it was speculated that AG-3 could be resistant or tolerant to azoxystrobin, but the present results indicate that AG-3 has intermediate sensitivity towards the compound, compared to AG-4, -5, -7 and -8. It is nonetheless important to note that the five AGs that were tested differed in sensitivity to both azoxystrobin and kresoxim-methyl, though none was completely resistant. Due to their broad spectrum of activity, these two fungicides are nowadays receiving much attention in plant disease control (Gullino, 2000), including diseases affecting potato (Stevenson, 2000). Both proved to be quite effective as soil treatments in the present study, but neither is likely to provide the same control as seed treatment with tolclofos-methyl or fludioxonil in the previous chapter. Azoxystrobin has furthermore been shown to be incompatible with the biocontrol agent *Verticillium biguttatum* W. Gams (Van den Boogert & Luttikholt, 2004). More work is needed with acibenzolar-*s*-methyl, but at least it proved capable of inducing some resistance to infection by *R. solani* in potato plants.

An important aspect that emerged from this study is the differences in survival rate of *R. solani*, and in efficacy of most of the treatments, in different soils. The enhanced survival of the pathogen and greater efficacy of amendments in sterilised soil were expected, but the differences observed between the unsteriled sand-loam from Gauteng and clay-loam from KwaZulu-Natal indicate the existence of conduciveness and suppressiveness in South African potato soils towards both the rhizoctoniasis pathogen and the treatments evaluated. More comprehensive studies in this regard could be of great value to the potato industry.

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ETIOLOGY AND ALTERNATIVE CONTROL OF POTATO RHIZOCTONIASIS IN SOUTH AFRICA

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RESUMÉ

Rhizoctoniasis of potato, caused by various anastomosis groups (AGs) of the fungus *Rhizoctonia solani*, occurs in all parts of the world where potatoes are grown. In South Africa, losses attributed to the disease, albeit varying in significance between regions and seasons, are a major constraint to profitable production of the crop. Producers rely almost exclusively on fungicides for controlling the disease. This study aimed at elucidating the etiology of potato rhizoctoniasis in the country and evaluating alternative control strategies. The results indicated the following:

Most (99.3 %) of the *R. solani* isolates from symptomatic tubers collected at seven of the 14 potato production regions in South Africa belonged to AG-3, and 0.7 % to AG-5. Of the isolates from infected stems and roots, 82.1 % were AG-3, 12.8 % AG-4, and 5.1 % AG-5. Isolations from soil yielded 67.7 % AG-3, 22.0 % AG-4, 5.5 % AG-5, and 2.4 % of each of AG-7 and AG-8. Baiting with beet seed proved to be more accurate for detecting *R. solani* AG-3 in artificially infested soil than wet sieving, soil pelleting, baiting with brinjal, potato, tobacco and tomato stem segments, or trapping with blue lupin seedlings, whereas soil pelleting yielded the greatest diversity of AGs from field soil. *In vitro* screening of the various AGs showed that AG-3 isolates were the most virulent.

Significant inhibition of mycelial viability of *R. solani* AG-3 was achieved by dipping agar plugs colonised by the fungus for 4 minutes or longer in water at 50 °C, or for 1 minute or longer in water at 55 °C, but complete inhibition was only evident after exposing the mycelium to water at 55 °C for a period of 4 minutes or more. Total inhibition of sclerotial viability on naturally-infected potato tubers was attained by submersing the tubers in water at 50, 55, 60 and 65 °C

for 16, 8, 4 and 4 minutes, respectively. The progeny of naturally-infected seed tubers was rendered free of infection by dipping the tubers in water at 55 °C for 8 minutes, 60 °C for 6 minutes, or 65 °C for 4 minutes. However, thermal treatments exceeding 55 °C for 8 minutes progressively increased tuber mortality. Of 20 disinfectants that were tested, only OA5 DP, an organic tin complex, inhibited mycelial growth of *R. solani* completely, although significant inhibition was evident with most of the other compounds. OA5 DP also proved to be the most effective disinfectant for killing sclerotia of the pathogen on seed tubers and rendering the progeny free of infection, but exhibited acute phytotoxicity towards the tubers. Significant control without any phytotoxicity was obtained with the didecyl dimethyl ammonium chloride compound, Sporekill. Tolclofos-methyl was the only fungicide that gave total control of potato rhizoctoniasis, whereas seed tuber treatment with fludioxonil, kresoxim-methyl and metam-sodium significantly reduced disease severity and incidence in the progeny.

The antagonists *Azospirillum brasilense*, *Bacillus subtilis* and *Trichoderma harzianum*, the fungicides azoxystrobin and kresoxim-methyl, and volatiles from *Bidens formosa*, *Bidens pilosa*, *Brassica napus*, *Brassica oleracea* var. *capitata*, *Raphanus sativus*, *Sinapsis alba*, *Tagetes minuta* and *Datura stramonium* root and/or shoot tissue significantly suppressed mycelial growth of the five *R. solani* AGs associated with potato rhizoctoniasis in South Africa. When artificially inoculated into soil, populations of *R. solani* AG-3 declined within eight days by 9.8 % and 32.7 % in unsterilised sand-loam and clay-loam soil, respectively, but increased by 12.3 % in previously sterilised sand-loam soil. Amendment of the artificially infested soils with the biocontrol formulation Trykocide™ (*T. harzianum*) eradicated the pathogen. Significant reductions in pathogen populations were also evident in soils amended with azoxystrobin, kresoxim-methyl, Maxiflo™ (*A. brasilense*), Avogreen™ (*B. subtilis*), cattle, chicken and sheep manure, citrus and mango waste compost, composted kraal manure, and shoot tissue of *B. napus*, *B. oleracea* var. *capitata*, *R. sativus*, *S. alba* and *T. minuta*. Trykocide™ provided total control of stem canker in soil artificially infested with *R. solani* AG-3, whereas kresoxim-methyl, azoxystrobin, sheep manure, *B. napus* and *B. oleracea* var. *capitata* shoot tissue, acibenzolar-*s*-methyl and mango waste compost reduced the disease significantly.

ETIOLOGIE EN ALTERNATIEWE BEHEER VAN AARTAPPEL-RHIZOCTONIASE IN SUID-AFRIKA

deur

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SAMEVATTING

Rhizoctoniase van aartappels word deur verskeie anastomose-groepe (AGe) van die swam *Rhizoctonia solani* veroorsaak en kom voor in alle dele van die wêreld waar die gewas verbou word. Verliese teweeggebring deur die siekte, alhowel wisselend in omvang van gebied tot gebied en seisoen tot seisoen, plaas 'n ernstige stremming op winsgewende aartappelproduksie in Suid-Afrika. Produsente maak byna uitsluitlik staat op die gebruik van swamdoders om die siekte te beheer. Hierdie studie het ten doel gehad die uitklaring van die etiologie van aartappel-rhizoctoniase in Suid-Afrika en evaluasie van alternatiewe beheerstrategieë. Die ondersoek het die volgende aan die lig gebring:

Meeste (99.3 %) van die *R. solani*-isolate wat van simptomatiese moere versamel is in sewe van die 14 aartappel-produksiegebiede in Suid-Afrika het behoort tot AG-3 en 0.7 % tot AG-5. Van die isolate vanaf geïnfekteerde stingels en wortels was 82.1 % AG-3, 12.8 % AG-4 en 5.1 % AG-5. Isolasies uit grond het 67.7 % AG-3, 22.0 % AG-4, 5.5 % AG-5 en 2.4 % van beide AG-7 en AG-8 opgelewer. Beetsaad-lokaas was meer sensitief vir die opsporing van AG-3 in kunsmatig-besmette grond as natsif, grondverpilling en herwinning uit eiervrug-, aartappel-, tabak- en tamatiestingelsegmente of bloulupiensaailinge. Grondverpilling het die grootste verskeidenheid van AGe vanuit natuurlik-besmette grond gelewer. *In vitro* toetse het getoon dat isolate van AG-3 die virulentste van die onderskeie AGe is.

Betekenisvolle onderdrukking van die lewenskragtigheid van *R. solani* AG-3 miselium is verkry deur agarblokkies gekoloniseer deur die swam vir 4 minute of langer in water by 50 °C te dompel, of vir 1 minuut of langer in water by 55 °C, maar totale onderdrukking is slegs bereik deur onderdompeling in water by 55 °C vir 'n tydperk van 4 minute of langer. Volkome

onderdrukking van die kiemkragtigheid van sklerotiums op natuurlik-besmette moere is bewerkstellig deur die moere vir 16, 8, 4 en 4 minute in water by onderskeidelik 50, 55, 60 en 65 °C te dompel. Die nageslag van natuurlik-besmette moere was vry van infeksie na onderdompeling van die moere in water by 55 °C vir 8 minute, 60 °C vir 6 minute, of 65 °C vir 4 minute. Hittebehandelings strawwer as 55 °C vir 8 minute het progressief die afsterwe van moere tot gevolg gehad. Van 20 ontsmettingsmiddels wat getoets is, het slegs een, die organiese tinkompleks OA5 DP, miselêre groei van *R. solani* geheel en al onderdruk, alhoewel betekenisvolle onderdrukking verkry is met meeste van die ander middels. OA5 DP was ook die doeltreffendste ontsmettingsmiddel vir die doding van sklerotiums van die patogeen op moere en daarstelling van 'n nageslag vry van besmettings, maar het akute fitotoksisiteit teenoor die moere getoon. Betekenisvolle beheer sonder enige fitotoksisiteit is verkry met die didesiel-ammoniumchloriedverbinding, Sporekill. Tolklofos-metiel was die enigste swamdoder wat volkome beheer gegee het van aartappel-rhizoctoniase, terwyl moerbehandeling met fludioskonil, kresoksim-metiel em metam-natrium die felheid en voorkoms van die siekte in die nageslag betekenisvol verminder het.

Die antagoniste *Azospirillum brasilense*, *Bacillus subtilis* en *Trichoderma harzianum*, die swamdoders azoksistrobin en kresoksim-metiel, en vlugtige stowwe vrygestel deur wortel- en/of loofweefsel van *Bidens formosa*, *Bidens pilosa*, *Brassica napus*, *Brassica oleracea* var. *capitata*, *Raphanus sativus*, *Sinapsis alba*, *Tagetes minuta* en *Datura stramonium*, het miselêre groei van die vyf *R. solani* AGe geassosieer met aartappel-rhizoctoniase in Suid-Afrika betekenisvol onderdruk. Die getalle van *R. solani* AG-3 het binne agt dae na kunsmatige besmetting afgeneem met 9.8 % en 32.7 % in ongesteriliseerde sand-leem en klei-leem grond, onderskeidelik, maar toegeneem met 12.3 % in gesteriliseerde sand-leem. Toevoeging van die biobeheer-formulasie Trykocide® (*T. harzianum*) tot kunsmatig-besmette grond het die patogeen uitgewis. Betekenisvolle verminderings in patogeengetalle is teweeggebring deur grondtoevoegings van azoksistrobin, kresoksim-metiel, Maxiflo® (*A. brasilense*), Avogreen® (*B. subtilis*), bees-, hoender- en skaapmis, kompos berei van sitrus- en mango-afval, gekomposteerde kraalmis, en loofweefsel van *B. napus*, *B. oleracea* var. *capitata*, *R. sativus*, *S. alba* en *T. minuta*. Trykocide® het ook volkome beheer gegee van stamkanker in grond kunsmatig besmet met *R. solani* AG-3, terwyl kresoksim-metiel, azoksistrobin, skaapmis, *B. napus* en *B. oleracea* var. *capitata* loofweefsel, acibensolar-*s*-metiel en mango-afval kompos die siekte betekenisvol verminder het.