1. CHARACTERISATION OF *RHIZOCTONIA* ASSOCIATED WITH CEREALS, CANOLA, PASTURE AND GRAIN LEGUMES WITH SPECIAL REFERENCE TO THE EFFECT OF CROP ROTATION ON *RHIZOCTONIA* DISEASES

INTRODUCTION

The genus *Rhizoctonia* comprises a collective species complex that includes fungi that do not have any distinctive taxonomic features allowing their classification into any known fungal genus (Ogoshi, 1987; Sneh, Burpee & Ogoshi, 1991; Carling & Sumner, 1992). Therefore, *Rhizoctonia* represents a diverse group of fungi that differs in many significant features, including their sexual stages (teleomorph), asexual stages (anamorph), and morphology (Vilgalys & Cubeta, 1994). The diverse ecological niches occupied by *Rhizoctonia* spp. further exemplify the diversity present in this group. A considerable number of *Rhizoctonia* isolates are saprophytes, whereas others are mycorrhizal on orchids (Carling *et al.*, 1999) or other plants. The genus *Rhizoctonia* also contains significant soilborne plant pathogens, pathogens of flooded crops and pathogens of aerial parts of plants (Sneh *et al.*, 1991).

The important role of many *Rhizoctonia* spp. as plant pathogens and mychorrhizas, has led to the development of several useful methods for identifying specific groups within this diverse genus. The most widely used methods for subdividing *Rhizoctonia* into groups include identification of their nuclear status (multinucleate or binucleate) and anastomosis group (Rovira, Ogoshi & McDonald, 1986; Ogoshi, 1987; Ogoshi, Cook & Bassett, 1990). Anastomosis groupings are classically based on the characterisation of hyphal anastomosis reactions of cultures. However, deoxyribonucleic acid (DNA)-based methods are increasingly being used to identify anastomosis groups (AGs) (Vilgalys & Gonzalez, 1990; Cubeta *et al.*, 1991; Carling, Kuninaga & Brainard, 2002b). Some anastomosis groups are further divided into subgroups based on pathogenicity testing (Roberts & Sivasithamparam, 1986; Ogoshi,

1987), isozyme analysis (Neate & Warcup, 1985; Neate, Cruickshank & Rovira, 1988), cellular fatty acid composition (Stevens-Johnk & Jones, 1993; Priyatmojo *et al*., 2001a), antibody reactions (Thornton *et al*., 2004), internal transcribed spacer sequence variation (Carling *et al.*, 2002b) and morphological characteristics (Sneh *et al.*, 1991; Carling & Sumner, 1992). Identification of *Rhizoctonia* isolates to some taxonomic level is of utmost importance for studying their epidemiology and control in different cropping systems.

Rhizoctonia diseases can cause severe economic losses and therefore require effective control measures (Vilgalys & Cubeta, 1994; Sneh *et al.*, 1996). Various control methods have been investigated including crop rotation (Rovira, 1986; Yang, Kharabanda & McAndrew, 1995), tillage practices (Jarvis & Brennan, 1986; Rovira, 1986; Yang *et al.*, 1995), resistant cultivars (Yang & Verma, 1992), as well as biological (Fiddaman & Rossall, 1995) and chemical control (Kataria & Verma, 1990; Kataria, Verma & Gisi, 1991). Among these, crop rotation and tillage practices have been the subject of many studies worldwide due to their potential to either control or enhance disease symptoms incited by *Rhizoctonia* (MacNish, 1985b; Rovira, 1986; Roget, Neate & Rovira, 1996). Crop rotation is widely used in different parts of the world, since it not only controls weeds and diseases including those caused by *Rhizoctonia*, but also improves soil fertility (Leach & Clapham, 1992; Wessels, 2001). Conservation tillage practices (sowing of crops with reduced tillage, no-tillage or direct drilling) are widely promoted due to the advantages in reduced soil preparation costs, increased availability of annual pastures for grazing, reduction in wind erosion and greater flexibility in farm management (Rovira, 1986; Boer *et al.*, 1991). However, several studies have shown that conservation tillage practices can exacerbate weed problems and increase the severity of diseases caused by various soilborne pathogens including *Rhizoctonia* (MacNish, 1985b; Rovira, 1986; Roget *et al.*, 1996).

In South Africa, particularly in the winter rainfall region of the Western Cape province, crop-pasture rotations have become a relatively stable pattern of land use since the 1970's (Wessels, 2001). The most important rotation crops in this region include canola

(*Brassica napus* L. var. *oleifera* DC), medic (annual *Medicago* spp.), lucerne or alfalfa (*Medicago sativa* L.), clover (*Trifolium* spp.) and lupin (*Lupinus* spp.). In these rotation systems, wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are often grown in rotation with grain and pasture legumes and/or canola (G. A. Agenbag, pers. comm.). The break in cereal/crop production in successive years, by planting a pasture legume, helps to increase the organic nitrogen content of the soil because lucerne, medic and clover have the capability to fix atmospheric nitrogen through the activity of associated Rhizobia (Ladd, Oades & Amato, 1981). The crop-pasture rotations in this region have also been shown to limit the incidence of weeds and diseases that accumulate in a monoculture (Beyers, 2001; Wessels, 2001). Another important crop practice that is becoming more widely used in the cereal producing areas of the Western Cape province is conservation (minimum and no-till) tillage (G. A. Agenbag, pers. comm.).

Worldwide, *Rhizoctonia* spp. have been implicated as soilborne pathogens of all the crops (barley, canola, clover, lucerne, lupin, medics, and wheat) used in cropping systems in the Western Cape province (Samuel & Garret, 1932; Bretag, 1985; Kaminski & Verma, 1985; Weller *et al.*, 1986; Pumphrey *et al.*, 1987; Kronland & Stanghellini, 1988; Kataria & Verma, 1992; Vincelli & Herr, 1992; MacLeod & Sweetingham, 1997). Therefore, *Rhizoctonia* has the potential to limit crop production within these cropping systems. Although, the pathogenicity of South African isolates of *Rhizoctonia* have not been shown on the aforementioned crops, results from many surveys, as well as investigations conducted since 1999 in the Western Cape province, have clearly shown that *Rhizoctonia* spp. are associated with all the crops of interest (barley, canola, clover, lucerne, lupin, medics and wheat) (Lamprecht, De Villers & Janse van Rensburg, 1999; Lamprecht, Auret & Janse van Rensburg 2000a; Lamprecht *et al.*, 2000b, 2001, 2002; Auret, Janse van Rensburg & Lamprecht, 2002). Therefore, the aims of this thesis are to (1) characterise *Rhizoctonia* isolates associated with seven rotation crops used within a crop rotation trial over a four-year period in the Western Cape province, (2) determine the appropriate time during the growth season for isolating *Rhizoctonia* from rotation crops and (3) determine the pathogenicity and relative virulence of

Rhizoctonia AGs on seven rotation crops. Basic knowledge of the *Rhizoctonia* AGs present in the crop-pasture rotation systems is essential for developing sound control measures within crop rotation systems of the Western Cape province.

CHARACTERISATION OF *RHIZOCTONIA* ISOLATES

Higher taxonomic diversity in *Rhizoctonia*

Traditionally fungi have been classified into the genus *Rhizoctonia* based on the absence of other distinctive taxonomic features. In the genus *Rhizoctonia* taxonomists have found it challenging to define species using conventional taxonomic criteria due to high levels of phenotypic variation and inability to fruit most strains of *Rhizoctonia* in culture (Vilgalys & Cubeta, 1994). Consequently, *Rhizoctonia* isolates can belong to several different orders of mostly basidiomycetes (Carling & Sumner, 1992). The teleomorphs of fungi that have the mycelial characteristics of *Rhizoctonia* isolates are assigned to the phylum Basidiomycota, class Basidiomycetes and order Ceratobasidiales (Warcup & Talbot, 1966; Murray & Burpee, 1984; Alexopoulos, Mims & Blackwell, 1996). Within the class Hymenomycetes the most important plant pathogenic *Rhizoctonia* isolates can be classified into three major teleomorphic basidiomycete genera, i.e. the *Rhizoctonia solani* Kühn complex that includes multinucleate species with a *Thanatephorus* (Frank) Donk teleomorph, binucleate *Rhizoctonia* spp. with a *Ceratobasidium* Rogers teleomorph, and multinucleate *Rhizoctonia zeae* Voorhees and *Rhizoctonia oryzae* Ryker & Gooch groups with a *Waitea* Warcup and Talbot teleomorph (Carling & Sumner, 1992).

Nuclear determination

Rhizoctonia isolates can be divided into binucleate and multinucleate groups based on the numbers of nuclei per cell of young vegetative hyphae (Yang *et al.*, 1994a). The best

known multinucleate plant pathogen is *R. solani* of which the teleomorph is *Thanatephorus cucumeris* (Frank) Donk (Spencer & Fox, 1978). For a fungus to be called *R. solani* it must possess a *T. cucumeris* perfect state. The second group of *Rhizocotonia* isolates that are characterised by multinucleate cells are *R. zeae*, *R. oryzae* and a type culture *Waitea circinata* var. *circinata* (Warcup & Talbot) isolated by Warcup and Talbot (1962), that all have teleomorphs in the genus *Waitea* (Warcup & Talbot, 1962; Carling & Sumner, 1992). The anamorphic name of *W. c* .var *circinata* has not yet been assigned, but the teleomorphic name may be useful, although the teleomorph is rarely observed (Carling & Sumner, 1992; Leiner & Carling, 1994). *Rhizoctonia* isolates that are characterised by binucleate cells have a *Ceratobasidium* teleomorph (Spencer & Fox, 1978; Carling & Sumner, 1992).

Anastomosis grouping

Conventional methods. Anastomosis groupings that are based on hyphal anastomosis among isolates having common biological affinities have greatly facilitated the identification of *R. solani* and other *Rhizoctonia* spp. (Parmeter, Whitney & Platt, 1967; Parmeter, Sherwood & Platt, 1969). Conventionally, anastomosis groups are determined by observing whether a hyphal fusion reaction is present between an unknown isolate and a reference isolate of known AG. Anastomosis reactions between hyphae of confronted isolates are assigned to one of four categories, i.e. C0, C1, C2 and C3. A C0 reaction indicates no hyphal fusion and a C3 reaction indicates a self (clonal) anastomosis reaction (Carling *et al.*, 2002b). Pairing of isolates belonging to the same AG results in hyphal fusion (anastomosis), leading to either acceptance (self-pairing) or rejection (somatic incompatibility). Contrarily, pairings between AGs that do not result in hyphal fusion (C0 reaction) suggest greater genetic differences between isolates (i.e., different species, etc.) (Cubeta & Vilgalys, 1997). Unfortunately, determination of relationships based solely on anastomosis behaviour of individual isolates is often uncertain since *Rhizoctonia* can exhibit different types of hyphal fusion within the same AG (Gonzalez *et al.*, 2001). Therefore, molecular-based methods have also been developed to identify and determine diversities within and between AGs (Cubeta & Vilgalys, 1997).

DNA-based molecular analyses of anastomosis groups and genetic variation in *Rhizoctonia*. DNA-based molecular analyses have elucidated some of the genetic and taxonomic relatedness of *Rhizoctonia* isolates, allowing a better understanding of the *Rhizoctonia* species complex, which is still elusive (Gonzalez *et al*., 2001). Initially DNA/DNA hybridization studies were used to determine the relatedness between different *Rhizoctonia* isolates. Restriction fragment length polymorphisms (RFLP) analysis of genomic DNA in conjunction with Southern blot analyses were also used to reveal genetic differences between *Rhizoctonia* isolates (Vilgalys & Cubeta, 1994). Subsequently, polymerase chain reaction (PCR)-based methods [PCR-RFLP, PCR- amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD)] were developed that are less tedious and also require smaller amounts of DNA (Vigalys & Cubeta, 1994; Ceresini *et al*., 2002; Godoy-Lutz *et al.*, 2003). Although molecular studies have greatly advanced our understanding of the genetic structure and variability in *Rhizoctonia* populations, a great deal still needs to be learned about variation in *Rhizoctonia* (Neate & Warcup, 1985; Neate *et al.*, 1988; Pascual *et al.*, 2000).

The most informative DNA-based molecular technique for investigating diversity in *Rhizoctonia* isolates has been sequence analysis of ribosomal ribonucleic acid (rRNA) genes (28S) and the internal transcribed spacer (ITS) region (Gonzalez *et al*., 2001). Analyses of these genes have not only shown the genetic relatedness of *Rhizoctonia* isolates, but have also confirmed some of the anastomosis groupings. Sequence analysis of the 5.8S ribosomal RNA gene of *Rhizoctonia* has not been useful for genetic comparisons in *Rhizoctonia* due to limited sequence variation in this region (Gonzalez *et al.*, 2001). Contrarily, sequencing of the ITS region in *Rhizoctonia* has revealed a high level of variation among *Rhizoctonia* isolates, especially in *R. solani* (Boysen *et al*., 1996; Kuninaga *et al*., 1997; Salazar *et al*., 1999, Salazar, Julian & Rubio, 2000). Gonzalez *et al*. (2001) used sequence analyses of the ITS region as well as part of the 28S rDNA to investigate whether *Thanatephorus* and *Ceratobasidium* represents distinct evolutionary lineages, and if anastomosis groups represent the most fundamental evolutionary units within *R. solani*. ITS rDNA sequence analyses of the

study identified 10 putative genetic groups within 23 isolates of *Ceratobasidium*, suggesting that *Ceratobasidium* may harbour many additional as yet undescribed genetic groups (Gonzalez *et al*., 2001). Important conclusions of the Gonzalez *et al.* (2001) study along with other sequence analyses studies (Gonzalez 1992; Boysen *et al*., 1996, Kuninaga *et al*., 1997; Johanson *et al*., 1998; Salazar *et al*., 1999; 2000) have been that in *R. solani* (1) most AG groups and subgroups represent genetically distinct groups, which support previous separation based on hyphal anastomosis behaviour; (2) certain AGs are polyphyletic; and (3) there is greater taxonomic support for AG subgroups than AG, suggesting that AG does not present the most fundamental evolutionary units within *Thanatephorus* (Gonzalez *et al*., 2001).

Large populations of *Rhizoctonia* in cropping systems have not been studied through sequence analyses of ribosomal RNA genes and/or ITS regions, due to the high cost of sequencing. Therefore, alternative DNA-based methods, mostly based on PCR, including RAPD, PCR-RFLP and rep-PCR have been used to characterise *Rhizoctonia* populations (Vigalys & Cubeta, 1994; Toda, Hyakumachi & Arora, 1999a; Gonzalez *et al*., 2001). PCR-RFLP is one of the molecular techniques that is increasingly being used to characterise *Rhizoctonia* isolates and has the advantage of being able to identify different AGs. PCR-RFLP is also very useful due to the simplicity of the technique, small amounts of DNA required and the high reproducibility of results between laboratories. In *Rhizoctonia*, PCR-RFLP methods have primarily utilised polymorphisms in 28S rDNA and ITS sequences (Pascual *et al.*, 2000; Guillemaut *et al.*, 2003). It is important to note that PCR-RFLP has mostly been used for successful identification of multinucleate *Rhizoctonia* isolates. Comparatively, in binucleate isolates PCR-RFLP methods, although initially thought to be useful (Cubeta and Vilgalys, 1991), were later shown to be much more complex and not specific enough for identification of binucleate isolates (Martin, 2000). Analyses of *Rhizoctonia* populations using RAPD markers also have the advantages of PCR-RFLP, except that RAPD markers are not always reproducible between laboratories, limiting their universal application (Vilgalys & Cubeta, 1994). Table 1 represents the different AGs that have been identified using PCR-RFLP in bi- and multinucleate *Rhizoctonia* isolates.

Pathogenicity testing

Rhizoctonia contains a diverse group of organisms, ranging from saprophytes to plant pathogenic species that vary in their pathogenicity and host range. Therefore, an important characteristic of *Rhizoctonia* isolates, especially to plant pathologists, is their pathogenic or saprophytic nature (Ogoshi, 1987). Knowledge of the variation in pathogenicity within and between AGs is furthermore important for studying the ecology of *Rhizoctonia* diseases.

Several different methods have been used for characterising the pathogenicity of *Rhizoctonia* isolates. These methods vary in the source of inoculum used as well as age of host inoculated. Inoculum of *R. solani* and other species of *Rhizoctonia* can be prepared by growing isolates on autoclaved soil, cornmeal sand, oats, millet or other small grains and other natural materials (Hollins, Jellis & Scott, 1983; Maughan & Barbetti, 1983; Clulow & Wale, 1984; Rovira, 1986; Sweetingham, Cruickshank & Wong, 1986; Weller *et al.,* 1986; Leach & Clapham, 1992; Yang *et al.,* 1994a, b; MacLeod & Sweetingham, 1997; Demirci, 1998; Khangura, Barbetti & Sweetingham, 1999). Infection of hosts from seeds can be accomplished by mixing these inoculum sources with soil and planting the seeds to the appropriate depth into infested soil (Rovira, 1986; MacNish *et al.*, 1995; Demirci, 1998). For crown and stem diseases, the inoculum source may be scattered over or around the base of plants and covered with a shallow layer of soil, some time after plant emergence (Carling & Sumner, 1992). During inoculation studies it is important to use different inoculum densities to establish the influence of inoculum density on disease severity (Houston, 1945; Carling & Sumner, 1992). The inoculum concentration for hosts planted as seeds can range from 0.01 to 4% (vol./vol. or wt/vol.). In addition to the above mentioned inoculum sources, agar plugs from cultures may also be placed in the soil before planting (Murray, 1981; Weller *et al.*, 1986; MacNish *et al.*, 1995) or after emergence directly against stems, crowns, and other wounded or non-wounded plant parts of host plants (Spencer & Fox, 1978).

Various forms of *Rhizoctonia* inoculum have been used in greenhouse bioassay studies on barley, canola, lucerne, lupin, medic and wheat. In pathogenicity studies of *Rhizoctonia* on barley and wheat, agar disks (Murray, 1981; Rovira, 1986; MacNish *et al.*, 1995), millet seeds (Sweetingham *et al.,* 1986), oat kernel (Weller *et al.*, 1986) wheat kernel (Demirci, 1998), and sand and maize meal mixtures were used (Hollins *et al.*, 1983). Agar disks were used for canola (Hwang, Swanson & Evans, 1986), and agar disks and sand-bran inoculum for medic (Lamprecht, Knox-Davies & Marasas, 1988), whereas millet seeds were used for the inoculation of canola seedlings (Khangura *et al.,* 1999). Lupins have been inoculated with agar disks (MacNish *et al.*, 1995) and millet seeds (Sweetingham *et al.,* 1986), whereas barley kernels were used to inoculate lucerne (Carling, Kebler & Leiner, 1986).

Pathogenicity studies most often require the addition of inoculum to pathogen-free soil containing the host plant or seeds. Pathogen-free soil can be obtained by treating soil with aerated steam for 30 min at 60^{o}C, with steam sterilisation if an aerator is not available, or using moist soil heated to 60 – 70^{o}C for 30 min in an oven (Carling & Sumner, 1992). If there is no source of steam, soil can be fumigated in a container with methyl bromide, followed by thorough aeration before use in experiments (Weller *et al.*, 1986). If none of the above sterilisation methods is possible, soil can also be stored several months in closed containers until inoculum levels of soilborne pathogens decline to very low levels, and further soil treatment is unnecessary (Carling & Sumner, 1992). Weller *et al.* (1986) used soil fumigated with methyl bromide for their pathogenicity tests on barley and wheat, whereas Sweetingham *et al.* (1986), Carling *et al.* (1994) and Yang *et al.* (1994a) used pasteurised (60^{o}C for 30 min) sand for lupin and wheat. Soils sterilised by heat (autoclaved at 121^{o}C for 35 min) were also used for cereal and pasture crops (Lipps & Herr, 1982; Rovira, 1986; Rovira *et al.*, 1986). Contrarily, Murray (1981) and Clulow and Wale (1984) used natural soils for pathogenicity tests on cereals that were not known to be pathogen-free. The use of native soil from a natural ecosystem (non-agricultural), which is not likely to possess a pathogen profile capable of inciting disease on the crop plant of interest would be better because certain *Rhizoctonia* spp.

isolates are capable of inciting disease in steamed soil but are incapable of causing disease in native soil (Mazzola, Wong & Cook, 1996).

Several field-grown crops have been artificially inoculated with *Rhizoctonia*. In field studies inoculum grown on whole grain, or grain mixed with infested soil, can be mixed in and around plants or incorporated into soil before planting (LeClerg, 1941; Houston, 1945). Cornmeal-sand inoculum can be scattered over the row and incorporated with a power-driven rototiller before planting (Sumner & Minton, 1989), or placed in-furrow at planting. Inoculum rates may vary from 14-56 kg/ha of cornmeal-sand mix (Sumner & Minton, 1989). Clulow and Wale (1984) used 50 mL inoculum (sand-maizemeal mixture) added just below the soil surface of each plot immediately after sowing in cereal field experiments.

Pathogenicity and virulence can be determined in a number of ways. Post-emergence damping-off can be calculated by counting the number of seedlings emerging from a certain number of sown seeds. Symptoms can also be evaluated on older plants about 2 to 4 weeks after planting (longer with perennial crops), where their roots, hypocotyls, pegs, pods, tubers, stolons, and other plant parts are rated for disease severity, or lesions are counted or measured (Lipps & Herr, 1982; Maughan & Barbetti, 1983; MacNish *et al.,* 1995). Different researchers have used different scales for rating. Ichielevich-Auster *et al.* (1985) rated coleoptile infection of barley and wheat on a 0 to 5 scale (0 = no disease, 1 = 1 – 10%, 2 = 11 – 30%, 3 = 31 – 50%, 4 = 51 – 80% and 5 = the entire coleoptile infected). Rovira *et al.* (1986) assessed the pathogenicity of *Rhizoctonia* isolates on medics and wheat using a root damage rating scale of 0 to 5 (0 = no disease, 1 = 1 0%, 2 = 20%, 3 = 30%, 4 = 50% and 5 >80). MacNish *et al.* (1995) used a 0 to 4 scale (0 = no obvious symptoms, 1 = slight discoloration, 2 = moderate discoloration or extensive but non-girdling lesions, 3 = extensive discoloration of tissue or girdling lesions and 4 = plant dead) to rate both root rot and hypocotyl/coleoptile rot on canola, lupin, wheat and barley. Demirci (1998) rated diseased crown and sub-crown internode tissues of barley and wheat using a 0 to 4 scale [0 = no symptoms, 1 = traces of superficial discolouration, 2 = one or more small lesions (<0.5 cm), 3 = one or more large

lesions (>0.5 cm) and 4 = girding lesions]. Disease severity on the taproot of white clover (*Trifolium repens* L.) was determined on a 0 to 3 scale (Maughan & Barbetti, 1983) (0 = healthy root, 1 = girdle of tap root < 50% rotted, 2 = girdle of tap root > 50% but not completely rotted and 3 = girdle of tap root completely rotted). The use of such rating methods is difficult to replicate between laboratories, therefore methods such as emergence count and plant biomass data are more appropriate. In virulence studies of *Rhizoctonia* real-time PCR and lateral flow device (LFD) methods can also be used for quantification of *Rhizoctonia* mycelium in plant tissue. These methods can also be used as a quick and easy diagnostic test for rapid determination of the presence or absence of *Rhizoctonia* spp. (Lees *et al*., 2002; Thornton *et al*., 2004)

ANASTOMOSIS CLASSIFICATION OF MULTINUCLEATE SPECIES OF *RHIZOCTONIA*

Multinucleate species of *Rhizoctonia* include *R. solani*, *R. zeae* and *R. oryzae*. Isolates of *R. zeae* and *R. oryzae* are classified into one AG group each, WAG-Z and WAG-O respectively (Sneh *et al.*, 1991; Carling *et al.*, 1994; 1999; 2002a). *Rhizoctonia solani* isolates are grouped into fourteen AGs [AG-1 to AG-10 including AG BI (Sneh *et al.*, 1991), AG-11 (Carling *et al.*, 1994), AG-12 (Carling *et al.*, 1999) and AG-13 (Carling *et al.*, 2002a)] based on their anastomosis behaviour.

The *R. solani* complex represents an economically important group of soilborne basidiomycete pathogens that occur on many plant species throughout the world (Sneh *et al.*, 1996). In *R. solani*, somatic incompatibility (or compatibility) is observed most directly at the microscopic level between paired isolates (Sneh *et al.*, 1991). Many AGs, including AG-1, -2, -4, -6, and -9, have been subdivided further into subgroups that differ in one or more biochemical, genetic, or pathogenic characteristics (Laroche, Jabaji-Hare & Charest, 1992; Johnk & Jones, 1993; MacNish, Carling, & Brainard, 1993).

The *Rhizoctonia solani* group (teleomorph: *Thanatephorus cucumeris*)

AG-1. This group has a worldwide distribution and is capable of hyphal fusion only with members of AG-1 (Sneh *et al.*, 1991). According to Sneh *et al.* (1991) and Carling and Sumner (1992) isolates are subdivided into three subgroups based on sclerotial form, DNA base sequence homology, colony morphology and pathogenicity but not according to hyphal fusion:

AG-1-IA (also called type 2 or the sasaki type). Isolates are characterised by large (1- to 3-mm diameter), relatively spherical sclerotia; high DNA base sequence homology (98-100%) with members of AG-1-IA and low homology (50-56%) with members of AG-1-IB. AG-1-IA is an aerial pathogen causing sheath blight of rice (*Oryza sativa* L.) (Anderson, 1982), leaf blight of many hosts and brown patch of turfgrass (*Agrotis palustris* Huds.)(Carling & Sumner, 1992).

AG-1-IB (also called type 1, web blight type or the microsclerotial type). Isolates are characterised by small, irregular-shaped sclerotia; a high DNA base sequence homology (96%) with members of AG-1-IB and low homology (50-56%) with representatives of AG-1-IA. AG-1-IB also is an aerial pathogen, causing web blight (Vincelli & Herr, 1992) and leaf blight of many hosts (Carling & Sumner, 1992).

AG-1-IC (also called Sherwood's AG-1 type 3). Isolates have small (0.2-0.8mm diameter) round-shaped sclerotia. DNA homologies with AG-1-IA and AG-1-IB have not been determined. AG-1-IC is soilborne and causes damping-off in many hosts (Ogoshi, 1987; Sneh *et al.*, 1991; Carling & Sumner, 1992).

AG-2. AG-2 is of worldwide distribution with a substantial amount of heterogeneity, as indicated by its many subgroups (Carling & Sumner, 1992; Carling *et al.,* 2002b). Isolates are capable of hyphal fusion with members of AG-2, AG-BI, and in low frequency with

members of AG-8 (Rovira *et al.*, 1986). *Rhizoctonia solani* AG-2 is subdivided into four subgroups (AG-2-1, AG-2-2, AG-2-3 and AG-2-4). The subgroup AG-2-2 is further divided into three intraspecific groups, AG-2-2 IIIB, AG-2-2 IV and AG-2-2 LP (Carling & Sumner, 1992; Kanematsu & Naito, 1995; Hyakumachi *et al.*, 1998; Carling *et al.*, 2002b). Anastomosis reactions between the subgroups range from strong to very weak "bridging"–type reactions. Consequently, anastomosis reaction alone can generally not provide adequate evidence for placement of an isolate into a subgroup (Carling *et al.*, 2002b). Previously, pathogenicity and nutritional requirements seemed useful for dividing AG-2 subgroups (Carling & Sumner, 1992). However, recent studies found that virulence does not seem to be a useful method for dividing subgroups (Carling *et al.*, 2002b). Group specific primers based on the rDNA-ITS sequences, have been developed for all subgroups and intraspecific groups of AG-2 and have the potential to assist classification of all AG-2 groups (Carling *et al.*, 2002b).

AG-2-1 (also called winter crops type). Hyphal fusion occurs in high frequency (≥50%) with members of AG-2-1, and in a low frequency (<30%) with members of AG-2-2 (Sneh *et al.*, 1991). They exhibit a high DNA base sequence homology (100%) with members of AG-2-1, a low homology (37.6-40.1%) with members of AG-2-2 IIIB, and AG-2-2 IV (43-49%) (Sneh *et al.*, 1991). AG-2-1 is a soilborne pathogen causing damping-off and root rot in many hosts and wire stem in crucifers (Anderson, 1977; Carling & Sumner, 1992).

AG-2-2 IIIB (also called rush type). Isolates are capable of hyphal fusion in high frequency with members of AG-2-2, in low frequency with members of AG-2-1, and cultures can grow at 35°C (Sneh *et al.*, 1991). Isolates exhibit high DNA base sequence homology (98%) with members of AG-2-2 IIIB, lower homology (69-71%) with members of AG-2-2 IV, and very low homology (38-40%) with members of AG-2-1 (Sneh *et al.*, 1991). AG-2-2 IIIB is a soilborne and aerial pathogen inducing damping-off in many hosts, brown patch in turf, and sheath blight of mat rush (*Lomandra filiformis* Labill.) (Carling & Sumner, 1992). Sequence analyses of the rDNA-ITS1 and -ITS2 region have shown a high level of similarity (>90%) among AG-2-2 IIIB, AG-2-2IV and AG-2-2 LP isolates (Carling *et al.*, 2002b).

AG-2-2 IV (often called root rot type). Hyphal fusion occurs in high frequency with members of AG-2-2, and in low frequency with members of AG-2-1, and cultures cannot grow at 35°C (Sneh *et al.*, 1991). Isolates exhibit high DNA base sequence homology (100%) with members of AG-2-2 IV, lower homology (69-71%) with members of AG-2-2 IIIB, and very low homology (43-49%) with members of AG-2-1 (Sneh *et al.*, 1991). AG-2-2 IV is a soilborne and aerial pathogen causing blight and root rot of sugar beet (*Beta vulgaris* L.), root rot in many other crops and large patch in turf (Carling & Sumner, 1992).

AG-2 LP. Hyphal fusion occurs in highest frequency with AG-2-2 IIIB and AG-2-2 IV. The rDNA-ITS1 and -ITS2 regions of AG-2 LP isolates also have the highest similarity to those of AG-2-2 IIIB and AG-2-2 IV (Carling *et al*., 2002b). The cultural characteristics of AG-2-2 LP differ from IIIB and IV, since LP isolates do not show distinct sclerotial formation and zonation, and their mycelia and pigment composition is dark brown. LP isolates are highly virulent on warm-seasoned turf grasses (Hyakumachi *et al.*, 1998).

AG-2-3. Hyphal fusion between members of AG-2-3 is not always strong. Hyphal fusion with all other subsets of AG-2 is a very weak bridging-type anastomosis reaction. The weak anastomosis reactions of AG-2-3 with other AG-2 subgroups as well as rDNA-ITS sequence analyses, suggest that this subgroup may be classified as an independent AG in future studies (Carling *et al*., 2002b). AG-2-3 is pathogenic on soybean, *Vigna angularis* Willd. and bean, *Phaseolus vulgaris* L. (Naito & Kanematsu, 1994).

AG-2-4. AG-2-4 isolates do not show strong hyphal fusion reactions with other AG-2-4 isolates. Hyphal fusion with all other subsets of AG-2 is very weak bridging-type reactions. Similar to AG-2-3, AG-2-4 is also a candidate for obtaining independent AG status based on hyphal fusion reactions as well as rDNA-ITS sequence analyses. According to rDNA-ITS1 and -ITS2 sequence analysis AG-2-4 is most closely related to AG-2-1 (Carling *et al.* 2002b). AG-2-4 has been found pathogenic on carrot (*Daucus carota* L.), corn (*Zea mays* L.), lettuce

(*Lactuca sativa* L.), radish (*Raphanus sativus* L.), sugar beet and cauliflower (*Brassica oleracea* L.) (Carling *et al.*, 2002b; Sumner & Phatak, 2003)

AG-3. Isolates are capable of hyphal fusion with members of AG-3 and AG-BI, and in low frequency with AG-8 (Rovira *et al.*, 1986). Isolates exhibit high DNA base sequence homology (100%) with members of AG-3 (Sneh *et al.*, 1991). Isolates of AG-3 grow more slowly and generally are more tolerant to cool temperatures than isolates of other AGs of *R. solani* and it is a soilborne pathogen (Carling & Sumner, 1992). AG-3 is divided into two subgroups AG-3 PT (potato type) and AG-3 TB (tobacco type), based on DNA-DNA hybridization studies as well as rDNA-ITS sequence analyses (Kuninaga *et al*., 2000; Ceresini *et al.*, 2002; Justesen *et al*., 2003;). Primer pairs specific for the detection of each of the subgroups have been developed (Kuninaga *et al*., 2000). The hosts of AG-3 PT are potato (*Solanum tuberosum* L.) and tomato (*Lycopersicum esculantum* L.), whereas AG-3 TB infects tobacco (*Nicotiana* spp.) (Anderson, 1982; Carling & Sumner, 1992; Kuninaga *et al.*, 2000; Ceresini *et al.*, 2002; Justesen *et al*., 2003).

AG-4 (also called the particola type). Isolates are capable of hyphal fusion with members of AG-4 (Sneh *et al.*, 1991). AG-4 can be subdivided into two groups, HG-I and HG-II, based on sclerotial form and differences in DNA base sequence homology (Vilgalys, 1988; Sneh *et al.*, 1991) but not on anastomosis reactions (Carling & Sumner, 1992). AG-4 is soilborne and cause damping-off of seeds and seedlings, root rot, crown rot, root canker, stem canker and stem blight of older plants over a wide host range worldwide (Anderson, 1982; Carling & Sumner, 1992; Vincelli & Herr, 1992; Kulik & Dery, 1995).

AG-4 HG-I. Isolates are characterized by dark brown sclerotia on PDA; high DNA base sequence homology (89-93%) with members of AG-4 HG-I and low homology (31-48%) with members of AG-4 HG-II (Sneh *et al.*, 1991).

AG-4 HG-II. Isolates are characterized by grey or whitish brown sclerotia on PDA; high DNA base sequence homology (89-100%) with members of AG-4 HG-II and low homology (31-48%) with members of AG-4 HG-I (Sneh *et al.*, 1991).

AG-5. A homogeneous group of soilborne pathogens that can induce root and stem rot of potato, but is generally far less virulent than AG-3 (Sneh *et al.*, 1991; Carling & Sumner, 1992). Isolates of AG-5 are capable of hyphal fusion with members of AG-5 (Sneh *et al.*, 1991; Carling & Sumner, 1992). AG-5 occurs in Europe, Asia and North America (Carling & Sumner, 1992). AG-5 is a major pathogen of white lupin (*Lupinus albus* L.) (Leach & Clapham, 1992).

AG-6. Isolates are capable of hyphal fusion with members of AG-6, and in low frequency with AG-8 and AG-BI of *R. solani* and AG-F of binucleate *Rhizoctonia* spp. (Sneh *et al.*, 1991). There are two subgroups of AG-6, HG-I and GV that can be distinguished from one another based on differences in DNA base sequence homology (Sneh *et al.*, 1991), but not easily with the anastomosis technique (Carling & Sumner, 1992). Although long-considered strict saprophytes, AG-6 has been found to cause root rot of apple (*Malus pumila* P. Mill.) (Mazzola, 1997), strawberry (*Fragaria ananassa* Duch.) (Botha *et al.*, 2003), lucerne (Anderson *et al*., 2004) and wheat (Meyer *et al.*, 1998).

AG-6 HG-I. Isolates are characterized by high DNA base sequence homology (92-98%) with members of AG-6 HG-I and low homology (48-63%) with members of AG-6 GV (Sneh *et al.*, 1991).

AG-6 GV. Isolates exhibit low DNA base sequence homology (48-63%) with members of AG-6 HG-I as well as low homology (55-66%) with members of AG-6 GV. This group is genetically heterogeneous, provisionally divergent (Sneh *et al.*, 1991).

AG-7. Isolates are capable of hyphal fusion only with members of AG-7, and exhibit high DNA base sequence homology (99%) with members of AG-7 (Sneh *et al.*, 1991). They are a soilborne group that can cause minor damage to some vegetable crops (Carling & Sumner, 1992).

AG-8. AG-8 is composed of five zymogram groups (ZG) based on pectic enzyme patterns following polyacrylamide gel electrophoresis (Neate *et al.*, 1988). Isolates are capable of hyphal fusion in high frequency (≥50%) with members of AG-8, and in low frequency (≤30%) with members of AG-3, AG-6, AG-BI (Rovira *et al.*, 1986) and AG-2 (Sneh *et al.*, 1991). AG-8 is a soilborne pathogen that induces bare patch in cereals (Neate & Warcup, 1985). Growth-chamber studies indicate it can cause root rot in potatoes (Carling & Leiner, 1990). It is known to occur in Australia, the Pacific North-western USA (Ogoshi *et al.*, 1990), and the United Kingdom (Burton *et al.*, 1988).

AG-9. Isolates are capable of hyphal fusion only with members of AG-9 (Sneh *et al.*, 1991). AG-9 is found in Alaska and Oregon (Carling, Leiner & Kebler, 1987). It is a weak soilborne pathogen that attacks potatoes and vegetables (Carling & Sumner, 1992). AG-9 is subdivided according to DNA base sequence homology (Carling & Kuninaga, 1990):

AG-9TP. Isolates exhibit high DNA base sequence homology (≥94%) with members of AG-9TP and lower homology (78-87%) with members of AG-9TX (Sneh *et al.*, 1991).

AG-9TX. Isolates exhibit high DNA base sequence homology (≥94%) with members of AG-9TX and lower homology (78-87%) with members of AG-9TP (Sneh *et al.*, 1991).

AG-10. Isolates (not well studied) are capable of hyphal fusion only with members of AG-10 (Sneh *et al.*, 1991; MacNish *et al.*, 1995). AG-10 is known to occur in the Pacific Northwest (USA) in association with small grain crops (Ogoshi *et al.*, 1990). AG-10 isolates are soilborne and, although thought to be principally saprophytic (Carling & Sumner, 1992)

and not pathogenic on a wide range of hosts, it may be a weak pathogen on cruciferous hosts (MacNish *et al.*, 1995).

AG-11. Cause severe hypocotyl rot and damping-off of lupins (Sweetingham *et al.*, 1986) and coleoptile rot in wheat (Sweetingham *et al.*, 1986; Carling *et al.*, 1994). Its growth and pathogenicity are greatly influenced by temperature (Kumar *et al.*, 1999).

AG-12. No bridging anastomosis reactions observed between AG-12 and other AGs of *R. solani*. Mature cultures are dark brown, as are mature sclerotia. Some cultures produce alternating dark- and light-coloured concentric rings, with sclerotia forming in the darker rings. Mycorrhizal isolates of AG-12 do little damage to potato and barley seedlings, moderate damage to head lettuce seedlings, and more extensive damage to seedlings of cauliflower and radish. AG-12 isolates are recovered from mycorrhizal orchid (*Pterostylis acuminata* R.Br.) plants (Carling *et al.*, 1999).

AG-13. Isolates have been associated with diseased roots of field grown cotton (*Gossypium hirsutum* L.) plants in Georgia in the United States. AG-13 isolates do not anastomose with all the tester isolates of AG-1 through AG-12. Mycelium of AG-13 isolates is light brown but darkens as cultures age. Concentric rings are visible after 3 to 4 days of growth but disappear as cultures age and darken. Isolates of AG-13 cause minor or no damage to barley, cauliflower, cotton, lettuce, potato, and radish in laboratory and greenhouse studies (Carling *et al.*, 2002a).

AG-BI. This group is called the "bridging isolate" group and is found in Japan (Carling & Sumner, 1992). Its isolates are capable of anastomosing to some degree with isolates of AG-2, AG-3, AG-6, and AG-8. AG-BI isolates are soilborne and exhibit high DNA base sequence homology (91-97%) with representatives of AG-BI whose pathogenicity is not well documented (Sneh *et al.*, 1991; Carling & Sumner, 1992). Recently, Carling *et al.* (2002b) proposed that AG-BI be included as a subset of AG-2, being designated AG-2 BI.

***Rhizoctonia oryzae*, *R. zeae* and *Waitea circinata* var. *circinata* (teleomorph: *Waitea circinata* Warcup & Talbot)**

Sneh *et al.* (1991) described two anastomosis groups of *W. circinata*, WAG-O and WAG-Z, which correspond to the anamorphs of *R. oryzae* and *R. zeae* respectively. Although isolates of *R. oryzae* tend not to anastomose with isolates of *R. zeae* and *vice versa* (Sneh *et al.*, 1991; Carling & Sumner, 1992), isolates of *W. circianta* [the type originally collected by Warcup & Talbot, (1962)] can anastomose with both, though with a lower fusion frequency (Carling & Sumner, 1992).

Rhizoctonia zeae and *R. oryzae* have various hosts. *Rhizoctonia zeae* causes ear rot (Voorhees, 1934) and root rot of maize (*Zea mays* L.) (Sumner & Bell, 1982), as well as foliar diseases of cereals and brown patch in turfgrass (Martin & Lucas, 1983). *Rhizoctonia oryzae* is the cause of bordered sheath blight of rice, but has been isolated from and may cause disease in many other crops, especially grasses (Carling & Sumner, 1992). The symptom that *R. oryzae* is often associated with is root rotting (Warcup & Talbot, 1962).

Waitea spp. appear to be of worldwide distribution and generally is associated with warm weather crops and conditions, however, they are also isolated frequently from soil in South-central Alaska indicating that there are types adapted to cool soils (Carling & Sumner, 1992). Ogoshi *et al.* (1990) described isolates of *R. oryzae* from the Pacific Northwest that have a lower optimum temperature than most other reported isolates of *R. oryzae*.

ANASTOMOSIS CLASSIFICATION OF BINUCLEATE *RHIZOCTONIA* (*CERATOBASIDIUM* SPECIES)

Various authors have grouped binucleate *Rhizoctonia* isolates into different AGs. Sneh *et al*. (1991) grouped binucleate *Rhizoctonia* isolates into AG-A to S. Lipps & Herr (1982)

established seven *Ceratobasidium* anastomosis groups (CAG-1, -2, -3, -4, -5, -6 and -7) based on hyphal pairings. Nineteen AGs of binucleate *Rhizoctonia* have been reported by various Japanese authors (Ogoshi *et al.*, 1979), including AG-A, -B, -Ba, -Bb, -C, -D, -E, -F, -G, -H, -I, -J, -K, -L, -M, -N, -O, -P, and -Q (Carling & Sumner, 1992). Burpee *et al.* (1980a,b) described seven groups: CAG-1 (=AG-D), CAG-2 (=AG-A), CAG-3 (=AG-E), CAG-4 (=AG-F), CAG-5 (=AG-R), CAG-6 (=AG-E) and CAG-7 (=AG-S) (Sneh *et al.*, 1991). Although binucleate *Rhizoctonia* occurs worldwide, distribution of the various groups is poorly documented; and many groups appear to be saprophytic including AG-C, -H, -K, -L, -N, and -O (Carling & Sumner, 1992). Diseases caused by pathogenic isolates include sharp eye spot of cereals, yellow patch of turf as well as damping-off and root rot in strawberry, sugar beet, vegetables, and many other hosts (Carling & Sumner, 1992).

The classification of anastomosis groups of binucleate *Rhizoctonia* isolates by Sneh *et al.* (1991) is based solely on hyphal fusion reactions, except for the subgroups of AG-B where cultural characteristics are also important. Ten of the AGs (AG-G, -H, -J, -K, -L, -M, -N, -O, -P, and -Q) identified by Sneh *et al.* (1991) only show hyphal fusion with their own members (Sneh *et al.*, 1991). The remaining AGs (AG-A, -B, -C, -D, -E, -F, -I, -R and -S) show some degree of hyphal fusion with members of other AGs, in addition to hyphal fusion with their own members (Table 2). AG-B is the only group that is divided into subgroups [AG-Ba, AG-Bb and AG-B(o)] according to frequency of anastomosis and cultural characteristics (Sneh *et al.*, 1991). Isolates of AG-Ba are capable of hyphal fusion in high frequency (≥50%) with members of AG-Ba and low frequency (<30%) with members of AG-Bb and/or AG-B(o), and have irregular (not spherical) greyish sclerotia (Sneh *et al.*, 1991). Isolates of AG-Bb are capable of hyphal fusion in high frequency (≥50%) with members of AG-Bb and low frequency (<30%) with members of AG-Ba and/or AG-B(o), and have spherical, brown sclerotia (Sneh *et al.*, 1991). Isolates of AG-B(o) are capable of hyphal fusion in low frequency with members of AG-Ba and/or AG-Bb (Sneh *et al.*, 1991). Cultural and morphological characteristics of AG-B(o) isolates are variable, but different from AG-Ba and AG-Bb (Sneh *et al.*, 1991).

CEREAL, CANOLA, PASTURE AND GRAIN LEGUME CROP PRODUCTION IN SOUTH AFRICA

In South Africa, the winter rainfall region in the Western Cape province is the second most important wheat production area and the most important barley production area (G. A. Agenbag, pers. comm.) compared to other provinces of South Africa. In 2003/04 wheat was cultivated on 748 000 ha of land and 1.54 million metric tons were produced. In the same period, 240 000 tons of barley were produced on 84 220 ha of land (Anonymous, 2004). Wheat and barley are planted in monoculture and in rotation with other crops in the winter rainfall area. The most important other rotation crops include canola, clover, lucerne or alfalfa, lupin and medics (G. A. Agenbag, pers. comm.).

Canola, lucerne and lupin are important crops in South Africa, with clover and medic also being included in crop rotation systems. Canola has only recently been planted on a commercial scale in South Africa with a total area of 44 200 ha being cultivated (H. Agenbag, pers. comm.; Mchau, Robberts & Crous, 1996). In the winter rainfall region the importance of canola as an alternative crop is increasing each year comprising more than 98% of total national production (Arkoll & Fouche, 1998; H. Agenbag, pers. comm.). Lupins have been cultivated for many years as a field crop in South Africa, currently covering a total of 30,000 ha annually (H. Agenbag, pers. comm.; Van Jaarsveld, 1985). Lupins have excellent nitrogen fixing qualities, with the potential to fix up to 350 kg of nitrogen per hectare (Keeve, 1998). Lucerne is among the country's most important crops (Loos, 1963). Lucerne hay production is a large industry in South Africa with a national production of 1.6 million tons annually having a value in excess of R 1000 million (Du Toit, 2001). The importance of clover as a legume fodder crop has been recognised in South Africa, although it is rather overshadowed by lucerne (Loos, 1963). Medics are grown in the winter rainfall region of the Western Cape province in rotation with cereals, particularly wheat (Lamprecht, 1989).

RHIZOCTONIA DISEASES ON CROPS USED IN ROTATION SYSTEMS OF THE WESTERN CAPE PROVINCE IN SOUTH AFRICA

Cereals (barley and wheat)

Rhizoctonia spp. have long been recognized as pathogens of barley and wheat, causing significant constraints to yields of these crops (Samuel & Garrett, 1932; Weller *et al.*, 1986; Pumphrey *et al.*, 1987; Smiley, Wilkins & Klepper, 1990). The most common species associated with diseases of barley and wheat are *R. solani*, which inhabits roots, and *R. cerealis* (sharp eyespot) that inhabits stems (Hall, 1986). Roberts and Sivasithamparam (1986) reported that multinucleate isolates of *Rhizoctonia* were the most pathogenic, while binucleate isolates were moderately to mildly pathogenic to cereals.

Several names have been used to describe the disease caused by *Rhizoctonia* on barley and wheat, including Rhizoctonia root rot, Rhizoctonia patch, bare patch or purple patch, and barley stunt disorder (Murray & Nicolson, 1979; Murray, 1981; MacNish, 1983; 1984; 1985b). This is an important disease of barley and wheat in different parts of Australia (Samuel & Garrett, 1932; MacNish, 1983), which include eastern Australia (Samuel, 1928), western Australia (MacNish, 1983; Yang *et al.*, 1994a) and southern Australia (Neate & Warcup, 1985; Rovira & Venn, 1985; Roberts & Sivasithamparam, 1986; Rovira, *et al.*, 1986; Neate *et al.*, 1988). The disease is also important in Canada (Benedict & Mountain, 1956), England (Dillon-Weston & Garrett, 1943; Murray, 1981), Poland (Furgal-Wegrzyciqa, Adamkiak & Adamiak, 1998), Scotland (Murray & Nicolson, 1979; Murray, 1981), Tanzania (Kuwite & Piening, 1998) and the United States (Weller *et al.*, 1986; Pumphrey *et al.*, 1987; Ogoshi *et al.*, 1990; Smiley *et al.*, 1990; Mathieson & Rush, 1991; Rush *et al.*, 1994).

Rhizoctonia bare patch (root rot), caused mainly by AG-8, severely affects the growth of roots and shoots of barley and wheat (Yang *et al.*, 1994a; Macleod & Sweetingham, 1997), and can greatly reduce grain yield (MacNish & Lewis, 1985; Jarvis & Brennan, 1986;

MacNish & Fang, 1987; Brennan & Crabtree, 1989). During severe infection much of the root system is rotted away and appears in the field as patches of stunted or dead plants (Lucas, Smiley & Collins, 1993; Wall *et al.*, 1994), which presumably reflects the distribution and growth of the pathogen mycelium in soil (Weller *et al.*, 1986). Infected roots have brown sunken lesions in which the cortex of the root is collapsed leaving only the stele. The root lesions often girdle the root leaving "pinched-off" pointed brown tips ("spear tip" or "needle point") that give root systems a severely pruned appearance (Weller *et al.*, 1986; Wall *et al.*, 1994). In Australia, barley appears to be more susceptible to this disease than wheat (MacNish, 1985a). It is important to note that although *R. solani* AG-8 is frequently listed as an important pathogen of barley and wheat, this is only true for bare batch of wheat and barley in low rainfall (wheat-fallow) areas. Recent work by Tim Paulitz (pers. comm.) has demonstrated that AG-8 has little or no role in *Rhizoctonia* root rot of wheat and barley in higher rainfall (continuous wheat or rotation) areas of the same region.

Apart from *R. solani* AG-8, several other *Rhizoctonia* groups have been reported as pathogens of barley and wheat roots. A few reports have shown that *R. oryzae* is associated with root rot (bare patch) of wheat and barley (Weller *et al.*, 1986; Ogoshi *et al.*, 1990; Smiley *et al.*, 1990). However, *R. oryzae* is less virulent than *R. solani* AG-8 and is also less often associated with bare patch disease (Ogoshi *et al.*, 1990; Matthew & Brooker, 1991; Lucas *et al.*, 1993; Smiley & Uddin, 1993). Furthermore, *R. oryzae* and *R. solani* AG-8 also attack the host at different stages. *Rhizoctonia oryzae* causes pre-emergence damping-off and reduction in root mass, whereas *R. solani* AG-8 has no effect on seedling emergence, but causes root rot of older plants (Mazzola *et al.*, 1996). *Rhizoctonia solani* AG-4 and AG-5 have also been found to cause damping-off and root rot of wheat (Mathieson & Rush, 1991; Rush *et al.*, 1994; Yang *et al.,* 1994a), and *R. solani* AG-4 was isolated from barley and wheat in Poland (Furgal-Wegrzyciqa *et al.*, 1998). Demirci (1998) reported that *R. solani* AG-4 and AG-11 were highly virulent, with AG-2-1, AG-3, AG-5 and *W. circinata* var. *circinata* being moderately virulent, and AG-I and -K non-pathogenic on barley and wheat in Turkey. In the Pacific Northwest of the U.S.A. apart from AG-8 and *R. oryzae*, 85% of the *R. solani* isolates

recovered from wheat-field soils belonged to AG-3, -4, -5, -9, and –10 (Ogoshi *et al.*, 1990). In western Australia *R. solani* AG-2-1 and AG-2-2 were found in wheat fields in addition to *Rhizoctonia* AG-8 (Roberts & Sivasithamparam, 1986). In Texas (Rush *et al.*, 1994) and Poland (Furgal-Wegrzyciqa *et al.*, 1998), AG-2-2 was also isolated and found pathogenic on barley and wheat. In southern Australia, *R. solani* AG-4 and AG-2 were associated with cereal roots in bare patch disease areas, but AG-8 was the primary pathogen (MacNish, 1985b). Another AG pathogenic on wheat is AG-11 and in Australia this fungus causes coleoptile rot on wheat (Sweetingham *et al.*, 1986; Carling *et al.*, 1994).

Crater disease is a root disease of wheat caused by *R. solani* AG-6 (Meyer *et al*., 1998) and is responsible for crop losses of up to 35% (Scott, Visser & Rufenacht, 1979). *Rhizoctonia solani* AG-6 is the primary causal agent of crater disease of wheat of the Springbok Flats of South Africa (Smith & Wehner, 1986), and also causes patchy stunting of cereals in Tanzania (Carling, Meyer & Brainard, 1996; Meyer *et al.*, 1998).

Sharp eyespot is a common *Rhizoctonia* disease of barley and wheat (Lipps & Herr, 1982; Smiley, 1997) in the temperate regions of the world. *Rhizoctonia cerealis* Van der Hoeven (teleomorph: *Ceratobasidium cereale* D, Murray & L. L. Burpee), a binucleate *Rhizoctonia* species causes sharp eyespot of wheat in China (Shi *et al.*, 2000), Europe (Boerema, Pieters & Hamers, 1992), Germany (Reinecke & Fehrmann, 1979), the Netherlands (Boerema & Verhoeven, 1977), Ohio (Lipps & Herr, 1982), and South Africa (Scott *et al.*, 1979). It was also reported on barley in Ireland (McKay & Loughnane, 1959), in Russia (Dorofeeva, *et al.*, 1996) and in Switzerland (Gindrat *et al.*, 1996). However, in Arkansas (Rush *et al.*, 1994), Canada (Rush *et al.*, 1994) and England (Sterne & Jones, 1978) *R. solani* AG-4, and not *R. cerealis*, is the causal agent of sharp eyespot on wheat. These isolates of AG-4 did not infect roots, but killed seedlings in greenhouse studies (Sterne & Jones, 1978). Similarly, *R. cerealis* that killed seedlings did not typically cause root damage (Sterne & Jones, 1978). *Rhizoctonia cerealis* differs from *R. solani*, in having predominantly binucleate hyphal cells and a relatively slow growth rate (Lipps & Herr, 1982). Thus, two different fungi,

resembling one another in morphological features, can cause similar symptoms on small grains (Lipps & Herr, 1982).

Ogoshi *et al.* (1990) reported several binucleate *Rhizoctonia* isolates, viz. AG-C, AG-E, AG-H, and AG-K, on barley and wheat in the Pacific Northwest but they were not pathogenic to these crops. Similarly, Yang *et al.* (1994a) recorded binucleate *Rhizoctonia* isolates to be non-pathogenic on wheat, and Demirci (1998) showed that binucleate *Rhizoctonia* isolates were non-pathogenic on barley and wheat in Turkey.

In South Africa, surveys of soilborne diseases of wheat and barley in the winter rainfall region of the Western Cape province have shown that *Rhizoctonia* are frequently associated with these crops (Lamprecht *et al.*, 1999, 2000a, 2000b, 2001, 2002). However, the specific species and AGs have not been identified and their pathogenicity have not been determined.

Lupin

A number of *Rhizoctonia* AGs are involved in diseases of lupin. In the USA, Rhizoctonia root rot has been listed as the most widely distributed and destructive disease of lupins (Leach & Clapman, 1992). Leach and Clapman (1992), identified *R. solani* AG-5 as a major pathogen of *L. albus* in the USA, causing reduced nodulation, seed rot, stem nipping, stem lesions, reduced root growth and apical bud mortality. They also reported that AG-1 and AG-4 infected plants, but that these AG types produced only small lesions on stems. Sweetingham *et al.* (1986) identified 11 zymogram groups (ZG) in 140 isolates of *Rhizoctonia* obtained from lupin roots. They showed that isolates of ZG1 and ZG2 caused *Rhizoctonia* patch disease of lupins and ZG3 [= AG-11 (Sweetingham, 1989)] and ZG4 caused hypocotyl rot and damping-off of lupins. Five *Ceratobasidium* groups, one *Waitea* group and ZG5 were only weakly virulent on lupins (Sweetingham *et al*., 1986). *Rhizoctonia solani* AG-8, the causative agent of barley and wheat bare patch disease, has been found to severely affect the growth of roots and shoots of lupins (Weller *et al.*, 1986; Murray & Brown, 1987; MacLeod &

Sweetingham, 1997). *Rhizoctonia solani* AG-10 has been isolated from lupins, but were non-pathogenic when inoculated on lupins (MacNish *et al.*, 1995). Thin binucleate Rhizoctonia (TBR), causes Eradu patch disease, a new potentially serious disease of *Lupinus angustifolius* L. on the sand plain soil of the northern wheat-belt of western Australia (MacLeod & Sweetingham, 1997).

Canola

Many researchers have reported AG-2-1 and AG-4 to be pathogenic on canola, with AG-2-1 being the more pathogenic group (Hwang *et al.*, 1986; Yitbarek, Verma & Morrall, 1987; Teo *et al.*, 1988; Yitbarek *et al.*, 1988). *Rhizoctonia solani* AG-2-1 and AG-4 were found to be the major pathogens causing damping-off and root rot of seedling and adult canola in western Canada (Acharya *et al.*, 1984; Kaminski & Verma, 1985; Hwang *et al.*, 1986; Gugel *et al.*, 1987; Kataria & Verma, 1992). In Australia, AG-2-1 is also the predominant and most highly pathogenic group on canola causing hypocotyl rot and post-emergence damping-off (Khangura *et al.*, 1999). Similarly, in Indiana AG-2-1 was found widely distributed, causing crown rot on canola (Huber *et al.*, 1992). Khangura *et al.* (1999) reported that AG-2-1 is most pathogenic to crucifers, and only mildly virulent on leguminous crops. AG-4 has been reported to cause seedling death of canola in Georgia, USA (Baird, 1996). In addition to AG-2-1 and AG-4, Hwang *et al.* (1986) recorded AG-2-2 on canola in Canada, and Khangura *et al.*, (1999) reported that AG-8 can cause serious root rot of canola in Australia, with binucleate isolates including AG-K only being weakly virulent.

In South Africa, Auret *et al.* (2002) and Lamprecht *et al.* (2001, 2002) reported on the incidence of *Rhizoctonia* spp. on canola hypocotyls, crowns and roots. The *Rhizoctonia* spp. and AGs were not characterised. According to Auret *et al.* (2002) some of the *Rhizoctonia* isolates obtained were pathogenic on canola cultivars Dunkeld and Monty.

Pasture crops (clover, lucerne and medics)

Black root canker of lucerne, caused by *R. solani* AG-4, occurs during the hot summer months in the irrigated desert areas of Arizona and California (Kronland & Stanghellini, 1988). This disease, which was first described in 1943 (Smith, 1943), can cause marked reductions in stands of lucerne seedlings (both pre- and post-emergence) as well as mature plants (Kulik, Dery & Douglass, 1995). In Kentucky, two isolates of *R. solani* AG-1 IB and AG-4 caused web blight and stem canker of lucerne respectively (Vincelli & Herr, 1992). Recently, Anderson *et al.* (2004) also reported AG-6 to be pathogenic on lucerne. Although *Rhizoctonia* spp. have been reported on lucerne in South Africa, the AGs associated with this crop have not yet been characterised (Thompson, 1985).

In Australia, a number of researchers recorded *R. solani* to be pathogenic on medics. Bretag (1985) demonstrated that *R. solani* was amongst the most pathogenic fungi associated with root rot of medics. Mebalds (1987) showed that *R. solani* was pathogenic on *M. truncatula* Gaertn., *M. rugosa* Michx. cv. Desr. and *M. littoralis* Loisel. Barbetti (1989) also showed that *R. solani* obtained from subterranean clover (*T. subterraneum* L.) was highly pathogenic on *M. polymorpha* L. cv. Serena, *M. truncatula* cv. Cyprus and *M. murex* Willd. cv. Zodiac. These researchers unfortunately did not identify the AGs involved. Kulik and Dery (1995) evaluated 27 annual *Medicago* spp. for resistance against *R. solani* AG-4 obtained from lucerne, but could not find resistance amongst these species. In South Africa, Lamprecht *et al.* (1988) only recorded binucleate *Rhizoctonia* isolates from medics in surveys conducted in the winter rainfall region. No information is available on the anastomosis grouping of these strains.

Various *Rhizoctonia* AGs have been found pathogenic on clover. *Rhizoctonia solani* AG-4 was isolated from poor stands of clover in east Texas pastures. The pathogen caused crown discolouration, root lesions, and severe root rot, with only 31% plant survival (Pemberton *et al.,* 1998). In western Australia, *R. solani* AG-2-1 and AG-2-2 were highly

virulent on subterranean clover (Wong, Barbetti & Sivasithamparam, 1985). In the same study, it was shown that *W. circinata*, *R. cerealis* and *Rhizoctonia* spp. AG-C, -F and -K are associated with diseased roots of subterranean clover. AG-K was not pathogenic, whereas *R. cerealis* and AG-F varied in virulence and the *Waitea* sp. caused mild damage to tap roots (Wong *et al.*, 1985). *Rhizoctonia solani* AG-8 is also an important root rotting pathogen of subterranean clover in southern Australia (MacNish *et al.*, 1993). *Rhizoctonia* spp. also caused root rot of white clover (*T. repens* L.) in Australia (Maughan & Barbetti, 1983) and root and crown rot of red clover (*Trifolium pratense* L.) in the USA (Kilpatrick, Hanson & Dickson, 1954a,b). Violet root rot caused by *R. crocorum* (Pers.) D.C. (Ware, 1923; Buddin & Walefield, 1924) and black patch caused by *R. leguminicola* Gough and Elliott (Berkenkamp, 1977) are also listed as diseases of red clover.

EFFECT OF CROP ROTATION ON *RHIZOCTONIA* DISEASES

Attempts to control or reduce *Rhizoctonia* diseases by means of crop rotation have met with varying success (Lee & Rush, 1983; Belmar, Jones & Starr, 1987; Specht & Leach, 1987), probably due to differences in host-specificity between the strains of *R. solani* involved (Shipton, 1977). An alternative to crop rotation is using monoculture systems for controlling *Rhizoctonia* diseases. Various researchers have shown that *Rhizoctonia* diseases can decline in monoculture systems (Henis, Ghaffar & Baker, 1978; Chet & Baker, 1980; Roget, 1995). Therefore, the use of monoculture systems to induce disease decline could be worth investigating in agricultural areas subject to *Rhizoctonia* root rot.

Cereals (barley and wheat)

Due to the wide host range of some *R. solani* AGs, as well as their ability to survive on plant debris, crop rotation as a measure for controlling *R. solani* diseases can be difficult (Rovira & Venn, 1985). Grass roots host *R. solani* and provide inoculum from readily metabolised roots, which may be more infective than inoculum from older particulate organic

matter (Rovira, 1986). The importance of partly decomposed plant residues as the food base for hyphae of *R. solani* has also been demonstrated by Weinhold (1977).

Crop rotation was considered to be an appropriate means to manage crater disease of wheat as *R. solani* AG-6 was only pathogenic toward certain species in the Gramineae (Deacon & Scott, 1985). However, several plant species have been shown to be susceptible to AG-6, and more recently lucerne was added to the list of susceptible hosts (Anderson *et al*., 2004). In addition, work by Meyer and Dyk (2002) indicated that various pasture crops could be susceptible to AG-6 isolates associated with this disease of wheat. Cropping of crater disease soil with sunflower (*Helianthus annuus* L.), maize, grain sorghum (*Sorghum bicolour* (L.) Moensch.), soybean or cotton significantly reduced the inoculum density and viability of *R. solani* in the soil compared to cropping with wheat, indicating that a reduction in crater disease severity by crop rotation is feasible (Smith & Wehner, 1989). Rovira (1986) demonstrated an effect of rotation on bare patch disease of wheat caused by *R. solani* AG-8. The study showed that the area lost to patches in wheat after the annual grass-medic pasture was greater than in wheat after medic, peas (*Pisum sativum* L.) or wheat (Rovira, 1986).

Mazzola, Johnson and Cook (1997) indicated that *R. cerealis* can be a potential obstruction to the establishment of Kentucky bluegrass (*Poa pratensis* L.) fields from seed in the Pacific Northwest. In addition to this, *R. cerealis* can also be a constraint to yield of wheat following bluegrass in rotation. In the United Kingdom it was also found that *R. cerealis* caused significant damage when wheat followed grass in a rotation system (Richardson & Cook, 1985).

Lupin

Lupins are usually planted by minimum tillage in rotation with barley, oats (*Avena sativa* L.) or wheat in western Australia (Brennan & Crabtree, 1989; MacLeod & Sweetingham, 1997). The importance of lupin as a rotation crop and supplier of organic

nitrogen could be greatly reduced in the presence of *R. solani* AG-5 (the main host is potato), because this pathogen causes serious losses in lupin by reducing nodule formation (Leach & Clapham, 1992). Circular patches caused by thin binucleate *Rhizoctonia* have not been observed in wheat crops in the year following affected lupin crops (MacLeod & Sweetingham, 1997). However, patches of less vigorous plants are occasionally seen late in the growing season in barley crops following affected lupins (MacLeod & Sweetingham, 1997). The lack of pathogenicity of AG-3 isolates on lupin indicates that lupin in a potato rotation would not be greatly affected by isolates of *R. solani* that usually attack potato (Leach & Clapham, 1992).

Canola

Crop rotation with a non-host crop could have a significant effect on seedling blight and root rot of canola (Kharbanda & Tewari, 1996). Yang *et al.* (1995) found that rotation of canola with barley for two or more years reduced the population of *R. solani* AG-2-1.

Pastures (clover, lucerne and medics)

Although outbreaks of *Rhizoctonia* stem canker of lucerne were observed in a variety of rotation sequences, most of the severe outbreaks occurred in new seedlings of lucerne where the previous crop was a grass (Vincelli & Herr, 1992). Other than this observation, there is no information concerning the effect of crop rotation on *Rhizoctonia* diseases of these crops.

CONCLUSION

This review emphasises the great diversity in the genus *Rhizoctonia* as well as the wide host range of this genus. Therefore, knowledge of the specific *Rhizoctonia* spp. and AGs, and

their pathogenicity is of utmost importance for developing efficient control measures, especially those involving crop rotation. Information on *Rhizoctonia* diseases of cereals (barley and wheat), oil crops (canola), pasture (clover, lucerne and medics) and grain (lupin) legumes in South Africa is very limited, especially in the Western Cape province. The fact that *Rhizoctonia* has been found pathogenic in many other countries on the plant species used in cropping systems in the winter rainfall area of the Western Cape province, emphasizes the need for investigations into the pathogenicity and cross-pathogenicity of *Rhizoctonia* spp. isolated from various plants utilized in these cropping systems. Information obtained from this study will be essential to the development of sustainable disease management strategies for *Rhizoctonia* diseases in cropping systems employed in the winter rainfall region.

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Table 1. Anastomosis groups (AGs) of bi- and multinucleate *Rhizoctonia* that have been identified using PCR-RFLP.

Isolate type	DNA region used in PCR-RFLP	Restriction enzymes used	AGs distinguished	References
Binucleate	28S rDNA	*Hha*I, *Hpa*II, *Sau3A*I, *Taq*I	AG-A, -Bo, -D, -E, -F, -G, -K, CAG-1, CAG-2, CAG-3, CAG-4, CAG-6, CAG-7	Cubeta *et al.*, 1991
Bi- and Multinucleate	ITS[y], 28S rDNA	*Hha*I, *Hpa*II or *Taq*I	AG-5 and AG-6; AG-A, -G, -I, -J & -Q	Mazzola, 1997
Binucleate	ITS	*Hinf*I, *Hha*I, *Mbo*I	AG-I	Sen *et al.*, 1999
Bi- and Multinucleate	ITS	*Eco*RI, *Hae*III, *Hha*I, *Hinf*I, *Mbo*I	AG-D (I & II), -A, -Ba, -Bb, -C, -E, -F, -G, -I, -K, -L, -O, -P, -Q and AG-1 IC	Toda *et al.*, 1999b
Multinucleate	ITS	*Eco*RI, *Mbo*I, *Hinf*I	AG-1-IA, AG-1-IB, AG-1-IC	Pascual *et al.*, 2000
Multinucleate	ITS	-	AG-1, -2, -3, -4 and -5, and AG-2-1, -2-2, -2-3	Salazar *et al.*, 2000
Multinucleate	ITS	-	AG-6, AG-12	Pope & Carter, 2001
Multinucleate	ITS	*Hae*III, *BSr*DI, *Alu*I, *Taq*I, *Mbo*I, *Eco*RI, *Msp*I	AG-1 and AG-2	Godoy-Lutz *et al.*, 2003
Multinucleate	ITS	Msp I, Taq I	AG-2-2 intraspecific groups IIIB, IV, LP	Hyakumachi *et al.*, 1998
Multinucleate	ITS	*Msp I, Taq I, EcoRI*	AG-2-1, AG-2-2 IIB, AG-2-2 IV	Liu & Sinclair, 1992
Multinucleate	ITS	*Hinf*I, *Hinc*II	AG-4	Priyatmojo *et al.*, 2001b
Bi- and Multinucleate	28S rDNA	*Hpa*II, *Hha*I, *Taq*I	AG-6, AG-A, AG-G and AG-I	Botha *et al.*, 2003
Multinucleate	ITS	*Mse*I, *Ava*II, *Hinc*II, *Mun*I, *Fnu4H*I	AG-1 up to AG-12 and AG-BI	Guillemaunt *et al.*, 2003

[y] ITS = Internal transcribed spacer region

Table 2. Hyphal fusion reactions of binucleate *Rhizoctonia* anastomosis groups according to Sneh *et al.* (1991).

Anastomosis group	Hyphal fusion reaction with
AG-A	AG-A and CAG-2
AG-C	AG-C and sometimes with AG-I
AG-D	AG-D and CAG-1
AG-E	AG-E, CAG-3 and CAG-6
AG-F	AG-F and CAG-4, and in low frequency with AG-6
AG-I	AG-I and occasionally with AG-C
AG-R	AG-R and CAG-5
AG-S	AG-S and CAG-7

2. CHARACTERISATION OF *RHIZOCTONIA* ASSOCIATED WITH ROTATION CROPS IN CROPPING SYSTEMS IN THE WESTERN CAPE PROVINCE

ABSTRACT

Studies were conducted to characterise *Rhizoctonia* populations isolated from barley, canola, clover, lucerne, lupin, annual *Medicago* spp. (medic) and wheat that were cultivated in a four-year (2000-2003) crop rotation trial in the Western Cape province of South Africa. During the four-year sampling period, 428 *Rhizoctonia* isolates were obtained from hypocotyl, coleoptile, crown and root tissue of the seven crops. Nuclear staining of the *Rhizoctonia* isolates revealed the presence of 104 (24%) multinucleate *Rhizoctonia* isolates and 324 (76%) binucleate *Rhizoctonia* isolates. The anastomosis groups (AGs) of isolates were determined by Dr. M. Mazzola (USDA-ARS, Wenatchee, WA, USA), through sequence analyses of the ribosomal deoxyribonucleic acid (rDNA) internal transcribed spacer (ITS) region (unpublished data). The analyses revealed that the multinucleate isolates were comprised of AG-4 HG-II (69%), AG-2-1 (19%), AG-3 (8%), AG-2-2 (2%) and AG-11 (2%). Among the binucleate *Rhizoctonia* species 53% were AG-K, 10% were AG-A, 5% were AG-I and 32% were unidentified binucleate *Rhizoctonia* AGs. Traditional hyphal fusion of representative isolates with tester isolates confirmed these identifications, except for AG-A isolates that did not anastomose with the tester isolate. AG-K was isolated from all crops. Similarly, AG-4 HG-II was also common to all crops except for lucerne. AG-2-2 and AG-11 were only isolated from medic/clover and lupin respectively. AG-2-1 was isolated from all crops except barley and lupin; AG-3 from canola, lupin and wheat; AG-A from all crops except lupin; whereas AG-I was isolated from barley, canola, lupin and wheat. Sampling *Rhizoctonia* at three different times during the growth season showed that the optimal time for isolating *Rhizoctonia* from plants was at the flowering stage (20 – 22 weeks after planting), compared to isolations made at the seedling (4 – 6 weeks after planting) or mid season (12 – 14 weeks after planting) stages. Temperature studies using 28 isolates representing nine AGs showed that AG-2-2, AG-4 HG-II and AG-K had significantly higher optimum growth temperatures than the other AGs.

INTRODUCTION

The genus *Rhizoctonia* is a collective species complex and includes a group of diverse *Basidiomycete* fungi (Sneh *et al.,* 1996). They occupy diverse ecological niches and have interactions with plants that range from pathogenic to symbiotic relationships (Sneh, Burpee & Ogoshi, 1991; Vilgalys & Cubeta, 1994). Plant pathogenic *Rhizoctonia* occurs worldwide and are economically important pathogens on vegetable and field crops, turf grasses, ornamentals, and fruit and forest trees (Anderson, 1982; Adams, 1988; Sneh *et al.*, 1996).

The high levels of phenotypic variation and inability to induce sporulation of most *Rhizoctonia* isolates in culture have challenged the ability of taxonomists to define species using conventional taxonomic criteria (Vilgalys & Cubeta, 1994). Therefore, methods for subdividing *Rhizoctonia* have been developed, and consist of first identifying the nuclear status of isolates as either being uni-, bi- or multinucleate, followed by anastomosis groupings (Rovira, Ogoshi & McDonald, 1986; Ogoshi, 1987; Ogoshi, Cook & Bassett, 1990; Hietala, 1995). Conventionally, anastomosis groups (AGs) have been determined by observing whether a hyphal fusion reaction is present between an unknown isolate and a reference isolate of a known AG. Pairing of isolates belonging to the same AG results in hyphal fusion (anastomosis) leading to either acceptance (self-pairing) or rejection (somatic incompatibility). Strong (C2) anastomosis reactions indicate AG membership (Cubeta & Vilgalys, 1997; Carling *et* al., 2002). Contrarily, pairings between isolates of unrelated AGs result in no hyphal fusion, indicating that they belong to different AGs (Cubeta & Vilgalys, 1997). However, sometimes “bridging” type reactions (less than C2 reaction) are observed between obvious discrete AGs (i.e., AG-3 and AG-2) as well as isolates of the same AG. These types of reactions increase the difficulty in identifying AGs, especially for researchers newly entering the field of *Rhizoctonia* identification (Carling *et al.,* 2002). Therefore, deoxyribonucleic acid (DNA)-based methods that allow typing of a larger number of isolates are increasingly being used to identify anastomosis groups (Vilgalys & Gonzalez, 1990; Cubeta *et al.*, 1991; Gonzalez *et al*., 2001; Carling *et al.*, 2002).

The most widely distributed plant pathogenic multinucleate *Rhizoctonia* group is *R. solani* Kühn (Roberts & Sivasithamparam, 1986). *Rhizoctonia solani* isolates are grouped into fourteen AGs [AG-1 to AG-10 including AG BI (Sneh *et al.*, 1991), AG-11 (Carling *et al.*, 1994), AG-12 (Carling *et al.*, 1999) and AG-13 (Carling *et al.*, 2002)] based on their anastomosis behaviour. Many AGs, including AG-1, -2, -4, -6, and -9, have been subdivided further into subgroups that differ for one or more biochemical, genetic, or pathogenic characteristics (Laroche, Jabaji-Hare & Charest, 1992; Johnk & Jones, 1993; MacNish, Carling, & Brainard, 1993).

Only a small number of binucleate *Rhizoctonia* isolates are plant pathogenic, with the majority being saprophytic or having symbiotic relationships with plants (Sneh *et al.*, 1996). Although binucleate *Rhizoctonia* occurs worldwide, distribution of the various groups is poorly documented with many groups appearing to be saprophytic including AG-C, -H, -K, -L, -N, and -O (Carling & Sumner, 1992). Diseases caused by pathogenic isolates include sharp eye spot of cereals, yellow patch of turf (*Agrotis palustris* Huds.) as well as damping-off and root rot in strawberry (*Fragaria ananassa* Duch.), sugar beet (*Beta vulgaris* L.), vegetables, and many other hosts (Carling & Sumner, 1992). Nineteen binucleate *Rhizoctonia* AGs have been reported by various Japanese authors (Ogoshi *et al.*, 1979), including AG-A, -B, -Ba, -Bb, -C, -D, -E, -F, -G, -H, -I, -J, -K, -L, -M, -N, -O, -P, and -Q (Carling & Sumner, 1992). Burpee *et al.* (1980a,b) further described seven binucleate groups: CAG-1 (=AG-D), CAG-2 (=AG-A), CAG-3 (=AG-E), CAG-4 (=AG-F), CAG-5 (=AG-R), CAG-6 (=AG-E) and CAG-7 (=AG-S). The most commonly used system for classifying binucleate *Rhizoctonia* isolates is the one proposed by Sneh *et al*. (1991), where binucleate *Rhizoctonia* isolates are grouped into AG-A to S.

Rhizoctonia can cause severe economic losses in various cropping systems, and therefore require effective control measures (Vilgalys & Cubeta, 1994; Sneh *et al.*, 1996). Crop rotation is one of the control strategies that have been used against *Rhizoctonia* disease.

Crop rotation not only has the advantage of controlling diseases, but can also control weeds and improve soil fertility (Leach & Clapham, 1992; Wessels, 2001).

In the Western Cape province of South Africa, many of the important agricultural crops that are used in rotation systems are susceptible to *Rhizoctonia* infection based on the reported host range of these pathogens in other countries (Samuel & Garret, 1932; Bretag, 1985; Kaminski & Verma, 1985; Weller *et al.*, 1986; Pumphrey *et al.*, 1987; Kronland & Stanghellini, 1988; Kataria & Verma, 1992; Vincelli & Herr, 1992; MacLeod & Sweetingham, 1997). Important rotation crops used in the Western Cape province include barley (*Hordeum vulgare* L.), canola (*Brassica napus* L. var. *oleifera* DC), clover (*Trifolium* spp.), lucerne (*Medicago sativa* L.), lupins (*Lupinus* spp.), medic (annual *Medicago* spp.) and wheat (*Triticum aestivum* L.). In the Western Cape province, crop-pasture rotations have become a relatively stable pattern of land use since the 1970's (Wessels, 2001). In these rotation systems, barley and wheat are often grown in rotation with grain and pasture legumes and/or canola (G. A. Agenbag, pers. comm.). The break in cereal/crop production in successive years, by planting a pasture legume, helps to increase the organic nitrogen content of the soil because clover, lucerne and medics have the capability to fix atmospheric nitrogen through the activity of associated Rhizobia (Ladd, Oades & Amato, 1981). Lupins also have excellent nitrogen fixing qualities, with the potential to fix up to 350 kg of nitrogen per hectare (Keeve, 1998). In the Western Cape province the crop-pasture rotations have been shown to limit the incidence of weeds and some diseases that accumulate in a monoculture (Beyers, 2001; Wessels, 2001).

In South Africa, very little is known regarding the *Rhizoctonia* groups associated with barley, canola, clover, lucerne, lupin, medics and wheat. *Rhizoctonia solani* AG-6 and *R. cerealis* Van der Hoeven have been shown to cause crater disease of wheat in summer rainfall regions of South Africa (Scott, Visser & Rufenacht, 1979; Smith & Wehner, 1986). Other than these *Rhizoctonia* groups, no anastomosis group characterization of isolates has been conducted on the aforementioned crops in South Africa, although *Rhizoctonia* spp. have been isolated from all the crops of interest (barley, canola, clover, lucerne, lupin, medic and wheat)

(Lamprecht, De Villiers & Janse van Rensburg, 1999; Lamprecht, Auret & Janse van Rensburg 2000a; Lamprecht *et al.*, 2000b, 2001, 2002; Auret, Janse van Rensburg & Lamprecht, 2002). Therefore, the first objective of this chapter was to characterise *Rhizoctonia* isolates obtained from a four-year crop rotation trial at the Tygerhoek experimental farm, Riviersonderend in the Western Cape province. *Rhizoctonia* isolates obtained in the first three years of the rotation trial (2000 to 2002) were obtained from Dr. S. C. Lamprecht (ARC-Plant Protection Research Institute, Stellenbosch, South Africa). In 2003 isolations were made for this study, and *Rhizoctonia* isolates from all years were characterised with regard to nuclear status, AG and the effect of temperature on *in vitro* growth. Anastomosis groups of all isolates were determined by Dr. M. Mazzola through rDNA internal transcribed spacer (ITS) regions sequence analyses. The AG of a subset of isolates was confirmed by conventional hyphal anastomosis testing in this study. The second objective of the study was to determine which time of the season [seedling stage (4 – 6 weeks after planting), mid season (12 – 14 weeks after planting) or flowering stages (20 – 22 weeks after planting)] is optimal for isolating *Rhizoctonia* from crops.

MATERIALS AND METHODS

Experimental site used for sampling and isolation of *Rhizoctonia*

Rhizoctonia isolates were obtained from a crop rotation trial over a four-year period (2000 to 2003) at the Tygerhoek experimental farm, Riviersonderend. The land used for the rotation trial was previously planted to lucerne since 1996. The soil at the experimental site is a sandy loam shale soil and was classified as a Glenrosa soil form (MacVicar *et al.*, 1977) with an average A-horizon depth of 400 mm and a B-horizon of well weathered yellow/red clay above poorly weathered schist.

The rotation trial at Tygerhoek is situated in the southern cereal producing region of the Western Cape province of South Africa at 34°9’ S and 19°54’ E. The region experiences a

Mediterranean climate with hot dry summers and cool moist winters. Longterm (n = 35 years) average annual rainfall is 451 mm of which approximately 60% falls during the period April to September. The mean daily maximum and minimum temperatures for March to October from 2000 to 2003 are presented in Fig. 1 (Agromet Division, ARC–Institute for Soil, Climate and Water, Stellenbosch).

The following crops were included in the trial: canola cv. Varola 50, barley cv. Clipper, alfalfa or lucerne cv. SA Standard, lupin (*Lupinus angustifolius* L.) cv. Wonga, medic (*Medicago truncatula* Gaertn.) cvs Mogul, Parabinga and Sephi/clover (*Trifolium michelianum* Savi.) cv. Patrick mixture and wheat cv. SST57. Four different rotation systems were used: system one (wheat and barley), system two (barley, medic/clover mixture, wheat and medic/clover mixture), system three (lupin, wheat, canola, and barley) and system four (monoculture lucerne). Each experimental plot was 80 x 15 m^2 (0.12 ha). Soils were fertilised annually according to soil analyses. Planting dates for all crops in the four-years were either early May or mid April, except for lucerne that is a perennial crop and was therefore only planted in 2000. The medic/clover mixture also re-established itself in 2002 and 2003. Weeds, pests and foliage diseases were controlled when necessary using commercially available chemicals. Fungicides (bromuconazole and propiconazole) for control of foliage diseases (rust and net blotch) were only applied to barley and wheat.

Sampling of *Rhizoctonia* within the rotation trial

Plants were sampled at three different plant growth stages during the season. Plants were sampled at three sampling times in 2000, 2001 and 2003, i.e. at the seedling (4 - 6 weeks after planting), mid season (12 – 14 weeks after planting) and flowering stages (20 – 22 weeks after planting). In 2002, plants were only sampled at the flowering stages. Sixty plants were collected from ten sampling locations (six plants/location) along a zig-zag (W) pattern through each experimental plot at each sampling time. Plants were placed in cooler boxes immediately after sampling and isolations were performed within one day after sampling.

Isolations from hypocotyl/coleoptile/crown and root tissue

Rhizoctonia isolates collected in the first three years (2000-2002) of the trial were provided by Dr. S. C. Lamprecht (ARC-Plant Protection Research Institute, Stellenbosch, South Africa). In 2003 isolations were conducted in a similar way as in the previous years (2000-2002), where 25 plants were randomly selected from plants collected from each experimental plot at each sampling time. Plants were washed under running tap water to remove adhering soil, and rinsed twice in sterile distilled water. Small pieces of root and hypocotyl or coleoptile or crown tissue were excised and plated onto growth media. Three root and hypocotyl or coleoptile or crown pieces were plated onto each of the following growth media: water agar (WA) (Agar Bacteriological, Biolab Diagnostics, Midrand, South Africa) containing 0.02% novostreptomycin, unamended WA, potato dextrose agar (PDA) (Biolab Diagnostics, Midrand, South Africa) containing 0.02% novostreptomycin, unamended PDA, selective *Fusarium* medium (SFA) modified from Tio *et al.* (1977) by replacing Alisan with 0.05 g/L pentachloronitrobenzene, *Phytophthora* selective medium (PH medium) (Solel & Pinkas, 1982) and *Pythium* selective medium (P medium = PH medium without hymexazol). Another aim of the rotation trial, which was treated in a different study, was to also determine the incidence of *Pythium, Phytophthora* and *Fusarium*. Therefore, isolations were also made on media selective for these organisms, but occasionally also yielded some *Rhizoctonia* isolates. A total of 18 hypocotyl/coleoptile/crown and 18 root pieces were plated for each experimental plot at each sampling time for each crop. The total number of pieces plated for each crop at each sampling time was 864, 288, 144, 288, 516 and 864 for barley, canola, lucerne, lupin, medic/clover mixture and wheat respectively. The total number of pieces plated per crop depended on the number of plots containing the specific crops within the rotation trial.

All fungi that developed were transferred to divided Petri dishes containing carnation leaf agar (Fisher *et al.*, 1982) in one half and PDA containing 0.02% novostreptomycin in the other half. Cultures were incubated under near-ultraviolet and cool white lights with a 12 h

photoperiod for 14 – 28 days. Microscopic examination of cultures was carried out and all isolates identified as *Rhizoctonia* spp. (according to Sneh *et al.,* 1991) were stored on potato carrot agar (10 g potato, 10 g carrot and 12 g agar) slants and/or sterile wheat grain by adapting (excluding vermiculite) the method used by Sneh *et al.* (1986) to prepare inoculum. Cultures on agar slants were stored at room temperature and in a cold room at 10°C, whereas cultures on wheat grain were stored at -20°C.

Nuclear staining

Nuclear staining was performed using a fluorocrome, acridine orange stain and viewing the stained specimen through a fluorescence microscope (Yamamoto & Uchida, 1982). Isolates were cultured on clarified 2% vegetable juice agar without $CaCO_3$ (Yamamoto & Uchida, 1982). Four sterilized microscope glass cover slips (Marenfeld cover glasses number 1, 22 x 22 mm) were placed on the medium next to the inoculum disc and plates were incubated for 48 h at 25°C with a 12 h photoperiod under cool white light. After 48 h the cover slips that were overgrown with mycelium growth were mounted, mycelium side downwards, into the drop of stain on a microscope slide and examined using a Zeiss Axioskop microscope equipped with an epifluorecence condenser and a high-pressure mercury lamp. The Zeiss 02, 06 and 18 filters were used. The nuclei stained bright green and were clearly visible and easy to count when freshly stained. The nuclei of 20 cells per isolate were counted to confirm the nuclear status of each isolate.

DNA extraction and identification of anastomosis group through sequence analyses

DNA was extracted using the protocol described by Lee and Taylor (1990). Four hundred and twenty-eight *Rhizoctonia* isolates were grown on PDA plates at 25°C with a 12 h photoperiod for 5 days to ensure adequate development of mycelia. Fungal mycelia were scraped from plates and transferred to 2.2 mL eppendorf tubes and 1 mL SDS extraction buffer [2% SDS, 50mM Tris-HCl (pH 8), 150mM NaCl, 100mM EDTA (pH 8)] was added.

The eppendorf tubes were boiled for 3 min, placed on ice for 10 min, and incubated at 65°C for 60 – 90 min. Subsequently, 400 µL chloroform: isoamyl alcohol (24:1) and 600 µL phenol were added and mixed by inverting the eppendorf tubes 5 – 10 times, followed by centrifugation for 15 min at 14 000 rpm (rounds per minute), after which the supernatant was transferred to a new tube. This step was repeated once. 10 µL RNase A (boiled for 5 min) was added to the supernatant and the tube was incubated at 37°C for 3 h to overnight. After incubation 5 µL 20% SDS and 10 µL proteinase K were added and incubated for 2 h to overnight at 37°C. The abovementioned chloroform: isoamyl alcohol and phenol steps were repeated, and 50 µL cold 7.5 M NH_4OAc and 600 µL cold isopropanol were added to the supernatant in order to precipitate DNA overnight in a freezer. Following the precipitation period, tubes were centrifuged for 10 min and the supernatant discarded. DNA pellets were washed with 1 mL cold 100% ethanol, followed by another 70% ethanol wash. DNA pellets were left to dry on a bench, and dried DNA was sent to Dr. M. Mazzola (USDA-ARS, Wenatchee, WA, USA) for AG group identification through sequence analyses of the rDNA-ITS region.

Anastomosis group (AG) typing using conventional hyphal fusion reactions

The anastomosis group of 28 representative isolates was confirmed by conventional hyphal anastomosis testing. These isolates (characterised through sequence analyses of the rDNA-ITS region) and tester strains (Table 1) were paired by plating an unknown isolate opposite a known AG type on 2% water agar in Petri dishes. The cultures were incubated under cool white light with a 12 h photoperiod at 25°C. Analysis of hyphal fusion was conducted by examining the zone of hyphal interaction at 40x magnification using a light microscope.

Temperature growth studies

The effect of temperature on *in vitro* radial growth of 28 *Rhizoctonia* isolates, representing nine AGs (Table 2), was studied at seven different temperatures. Mycelial plugs

(0.6 cm in diameter) were transferred from the edge of 5-day-old actively growing colonies to PDA. Cultures were incubated in the dark at 5, 10, 15, 20, 25, 30, or 35°C and colony radius was measured in four directions per each PDA plate after 48 h. Each isolate was replicated three times at each temperature.

Experimental design and statistical analysis

The rotation system experiment was a randomised block design with 20 main plots (treatments) randomly replicated within four blocks. The 20 main plots consisted of a 10 x 2 factorial (ten crop sequences x two tillage practices) with three rotation systems. Three sampling times per year were used as a subplot factor (Little & Hills, 1972). A further sub-subplot was the *Rhizoctonia* groups (binucleate and multinucleate) isolated from the samples. Analysis for each year was done separately because crop sequences differed from one year to the next. Although isolations were done separately from hypocotyls, coleoptiles, crowns, and roots these data were combined for the analysis of variance. The percentage relative incidences of the two *Rhizoctonia* groups (binucleate and multinucleate) were determined out of a possible 36 pieces of plant material plated. Data obtained from lucerne isolations in the rotation system consisting of monoculture lucerne were not subjected to analysis of variance. Appropriate analysis of variance was performed for each separate year (SAS, 1999) and the student's t-LSD were calculated to compare means of significant effects at the 5% significance level.

For the temperature growth studies second order polynomials were fitted on the radial length over temperature for each isolate AG combination and replicates (Snedecor & Cochran, 1967). The optimum temperature and area under each curve were calculated. These measurements were subjected to an appropriate analysis of variance and the means were compared using student's t-LSD at the 5% significance level.

RESULTS

Isolations from hypocotyl/coleoptile/crown and root tissue

A total of 428 *Rhizoctonia* isolates were obtained during the four years of the crop rotation trial. Dr. S. C. Lamprecht provided the 317 *Rhizoctonia* isolates that were isolated from 2000 to 2002, and 111 *Rhizoctonia* isolates were obtained from isolations made in 2003.

Nuclear staining

Nuclear staining of all *Rhizoctonia* isolates (428) was possible, and revealed the presence of 104 (24%) *R. solani* isolates with nuclear numbers ranging from 3 - 12 per young vegetative hyphal cell and 324 (76%) binucleate *Rhizoctonia* species with only two nuclei per young vegetative hyphal cell (Fig. 2).

DNA extraction and identification of anastomosis group through sequence analyses

DNA was successfully extracted from 428 isolates and were of sufficient quality for polymerase chain reaction (PCR) amplification of the rDNA-ITS1 and rDNA-ITS2 region by Dr. M. Mazzola using primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') [White *et al.*, 1990]. The PCR products were first cloned into vector pCR®4-TOPO® (Invitrogen, Carlsbad, CA, USA). Sequencing analyses of the cloned PCR products by Dr. M. Mazzola revealed that the multinucleate *Rhizoctonia* (*R. solani*) isolates were comprised of AG-4 HG-II (69%), AG-2-1 (19%), AG-3 (8%), AG-2-2 (2%) and AG-11 (2%) (Mazzola *et al.*, unpublished data). Among the binucleate *Rhizoctonia* species 53% were AG-K, 10% were AG-A, 5% were AG-I and 32% were unidentified binucleate *Rhizoctonia* AGs (Mazzola *et al.*, unpublished data).

Anastomosis Group (AG) typing using conventional hyphal fusion reactions

All 16 multinucleate *R. solani* isolates were successfully anastomosed with the respective tester isolates and all exhibited a C2 reaction [wall connection obvious membrane contact uncertain; location of reaction site obvious; diameter of anastomosis point less than hyphal diameter; anastomosing and adjacent cells always die (Carling, 1996)]. The multinucleate *Rhizoctonia* isolates that were identified included AG-2-1 (three isolates), AG-2-2 (two isolates), AG-3 (three isolates), AG-4 HG-II (six isolates) and AG-11 (two isolates). AG-I and AG-K were the only binucleate AGs that were successfully anastomosed with the tester isolates. The unidentified binucleate *Rhizoctonia* group failed to anastomose with tester isolates of AG-A, AG-I, AG-D and AG-K.

The identification of AGs through sequence analyses was compared with AG typing through conventional hyphal anastomosis typing. The AG group of isolates as determined through conventional anastomosis typing, corresponded with the identification determined through sequence analyses of the ITS1 and ITS2 region (data not shown). The only exception was three AG-A isolates that did not anastomose with the tester AG-A isolate PPRI 2130 (C-517).

Temperature growth studies

Analysis of variance showed significant differences in the temperatures for optimum growth as well as the growth rates at different temperatures (area under the curve) of the nine AGs (Table 3). Significant differences for these parameters were also recorded for isolates within AGs (Table 3). Isolates within AG-2-2, AG-3, AG-4 HG-II and AG-I differed significantly with regard to the temperature at optimum growth, whereas isolates within AG-2-2, AG-3, AG-4 HG-II, AG-11, AG-A and AG-I differed significantly in their growth rates (area under curve). The appropriate trends (second degree polynomial function) were fitted for

AG x temperature combinations and the result of the regression analyses were $R^2 \geq 77.68\%$ for all AGs (Table 4). These predicted trends are illustrated in Fig. 3.

Rhizoctonia AG-2-2, AG-4 HG-II and AG-K had significantly ($P = 0.05$) higher optimum growth temperatures than the other AGs (Table 4). *Rhizoctonia solani* AG-2-2 had the highest optimum growth temperature (25.9°C), followed by the binucleate *Rhizoctonia* AG-K (23.2°C) and the multinucleate AG-4 HG-II (22.4°C). The lowest temperatures for optimum growth were recorded for the binucleate AGs, viz. AG-A, AG-I and the unidentified group (UNBR) (Table 4). Comparison of the area under the curve (Table 4) indicated that the growth rates of the binucleate AGs (AG-A, -I, -K, UNBR) were significantly lower than the growth rates of the multinucleate groups (AG-2-1, AG-2-2, AG-3, AG-4 HG-II, AG-11).

All AGs grew poorly below 10°C, performed relatively well in the range 15 – 30°C and performed better at 15°C than at 35°C. In the 15 – 30°C range all multinucleate isolates performed better than the binucleate AGs, except for AG-K. *Rhizoctonia solani* AG-2-2 and AG-K had higher growth rates at 35°C than the other AGs (Fig. 3). The *in vitro* growth of *Rhizoctonia* isolates representing nine AGs used in temperature growth studies are shown in Fig. 4.

Incidence of *Rhizoctonia* on rotation crops and effect of sampling time on *Rhizoctonia* incidence

Results on the incidences of the nine AGs identified in this study are given in Table 5. AG-K was the predominant AG followed by the unidentified binucleate group (UNBR) and AG-4 HG-II. Certain AGs viz. AG-2-1, -4 HG-II, -A, -I, -K and UNBR were isolated in all four years, whereas AG-11 was only recorded in 2000, AG-2-2 in 2002 and 2003 and AG-3 in 2000, 2001 and 2003 (Table 5). Most of the anastomosis groups were associated with more than one crop: AG-2-1 with canola, lupin, medic/clover and wheat; AG-2-2 with medic/clover; AG-3 with canola, lupin and wheat; AG-4 HG-II with barley, canola, medic/clover, lupin and

wheat; AG-11 with lupin; AG-A with barley, canola, lucerne, medic/clover and wheat; AG-I with barley, canola, lupin and wheat and AG-K and the unidentified binucleate group with all the crops (Table 5).

Analyses of variance were conducted on the effect of sampling time on the percentage incidences of *Rhizoctonia* groups (binucleate and multinucleate) for each of the four years (Table 6). Sampling time significantly (P = 0.05) affected the frequency of isolation of *Rhizoctonia* groups in 2000 (P = 0.0003) and in 2001 (P = 0.0037), but not in 2003 (P = 0.1104). In all years (2000, 2001 and 2003) the incidence of *Rhizoctonia* was higher at the third sampling time (flowering stage) than at the seedling or mid-season stages with significantly higher incidences at the third sampling time than the other sampling times during 2000 and 2001 (Table 7).

Analyses of variance were conducted on the incidence of bi- and multinucleate *Rhizoctonia* groups over the four years of the trial (Table 6). In all four years the incidence of the two *Rhizoctonia* groups differed significantly [P = 0.0105 (2000), P = 0.0464 (2001), P = 0.0060 (2002) and P = 0.0062 (2003)] (Table 6). Significantly (P = 0.05) higher incidences of binucleate compared to multinucleate isolates were recovered for all the years (Table 8).

DISCUSSION

This study characterised *Rhizoctonia* populations associated with rotation crops (barley, canola, lucerne, lupin, medic/clover and wheat) used in the Western Cape province of South Africa. The study was conducted within one four-year crop rotation trial. The *Rhizoctonia* isolates were characterised with regard to nuclear status, anastomosis group and the effect of temperature on *in vitro* growth. Characterisation of the *Rhizoctonia* populations showed that the majority of *Rhizoctonia* isolates were binucleate (76%) whereas only 24% were multinucleate isolates. Anastomosis group identification of a subset of isolates using both conventional and sequence analyses were mostly in agreement, except for three isolates

of AG-A. Sequence analyses revealed that among the multinucleate isolates, AG-4 HG-II (69%) were most frequently isolated followed by AG-2-1 (19%). *Rhizoctonia* AG-K was the binucleate group that was present at the highest frequency (53%). Studies on the effect of temperature on *in vitro* growth showed that two of the AGs (AG-K and AG-4 HG-II) that were present at high frequency also had the highest optimal growth temperatures. AG-4 HG-II and AG-K were the only AG's associated with all crops, with the exception of AG-4 HG-II from lucerne. Analysis of isolation frequency data over all years of this study indicated that the optimum time for recovery of *Rhizoctonia* isolates was at the third sampling time (flowering stage).

In the crop rotation trial conducted in the Western Cape province binucleate isolates were recovered at a significantly higher frequency than multinucleate isolates. Similarly, in Australia binucleate isolates were isolated at a higher frequency from lupin and cereal seedlings (Sweetingham, Cruickshank & Wong, 1986; MacLeod & Sweetingham, 1997). Contrastingly, a number of researchers isolated higher numbers of multinucleate than binucleate *Rhizoctonia* from barley, canola and wheat. In Canada, Kaminski and Verma (1985) obtained a high number of multinucleate *Rhizoctonia* isolates from seedling and adult canola. A higher number of multinucleate compared to binucleate *Rhizoctonia* isolates were also obtained from barley and wheat in Turkey (Demirci, 1998) and the Pacific Northwest of the USA (Ogoshi *et al.*, 1990), and from wheat in western Australia (Yang *et al*., 1994). The higher incidence of binucleate *Rhizoctonia* isolates in the crop rotational trial in the Western Cape province is interesting since multinucleate *Rhizoctonia* isolates are generally more virulent than binucleate isolates (Sneh *et al.*, 1991). Furthermore, binucleate *Rhizoctonia* AGs have been shown to protect crops against multinucleate *Rhizoctonia* AGs. Binucleate *Rhizoctonia* AG-B(o) isolates have been found to protect sugar beet and tall fescue (*Festuca arundinacea* Schreb.) against multinucleate AG-2-2 (Herr, 1991) and AG-1-IA (Yuen, Craig & Giesler, 1994) respectively, and binucleate AG-G protects potato (*Solanum tuberosum* L.) against AG-3 (Cubeta & Echandi, 1991).

The high incidence of binucleate *Rhizoctonia* isolates in this study could be due the fact that plant materials were not surface disinfested before isolations were made, thus favouring the isolation of bi- versus multinucleate isolates. However, various other investigations (Sweetingham *et al.*, 1986; Ogoshi *et al.*, 1990; and MacLeod and Sweetingham, 1997) that also did not surface disinfest plant materials prior to isolations, obtained more multi- than bi-nucleate isolates. Therefore, it seems unlikely that the absence of surface disinfestation in this study would have influenced the incidence of bi- and multinucleate isolates. The high incidence of bi- versus multinucleate isolates in this study therefore warrants further investigations into the possible protective role of binucleate isolates within crop rotation systems.

The identification of *Rhizoctonia* through sequence analyses has greatly aided the identification of AGs (Gonzalez *et al*., 2001; Carling *et al*., 2002). Amplification and sequence analysis of the nuclear ribosomal internal transcribed spacer (ITS) regions of *Rhizoctonia* isolates recovered in this study showed that there were five multinucleate *Rhizoctonia* AGs (AG-2-1, AG-2-2, AG-3, AG-4 HG-II and AG-11) and four binucleate *Rhizoctonia* AGs (AG-A, AG-I, AG-K and unidentified group) (Mazzola *et al.*, unpublished data). Conventional AG determination of a subset of the isolates through evaluation of hyphal anastomosis reactions, confirmed the identification based on sequence analyses, except for isolates from AG-A that did not show anastomosis with the reference isolates. Only one reference isolate of AG-A was available. Future studies should evaluate whether the isolates designated as AG-A through sequence analyses can anastomose with other reference AG-A isolates.

The frequency of different AGs within bi- and multinucleate *Rhizoctonia* varied. *Rhizoctonia solani* AG-4 HG-II (69% of the multinucleate isolates) followed by AG-2-1 (19% of the multinucleate isolates) were the predominant multinucleate AGs obtained in this study. The other multinucleate AGs, viz. AG-2-2, AG-3 and AG-11 were infrequently isolated. Of the identified binucleate AGs, AG-K (53%) was the predominant AG.

Temperature significantly affected the *in vitro* growth rate of the AGs included in this study. All AGs grew relatively well in the range 15 – 30°C and performed better at 15 than at 35°C. The optimum growth range for AG-2-1 has been reported as being in the range of 20-25°C (Kaminski & Verma, 1985; Ogoshi *et al.,* 1990). Similarly, in this study the optimum growth temperature for AG-2-1 of 21.7°C, is within this range. Kaminski and Verma (1985) found that AG-4 isolates had higher growth rates than AG-2-1 with optimum growth at 26°C. In this study the predicted optimum growth temperature (22.4°C) for AG-4 HG-II is lower than that reported by Kaminski and Verma (1985). However, similar to their results, in this study AG-4 HG II also had significant higher growth rates and optimum growth temperatures than AG-2-1. The differences in *in vitro* optimum growth temperatures between AG-2-1 and AG-4 are also reflected in the fact that AG-4 isolates are more virulent on canola at 20°C than AG-2-1 (Kataria & Verma, 1992). Similarly, it has been found that at lower temperatures (15°C) AG-2-1 caused more damping-off than AG-4 (Teo *et al.*, 1988). In this study the *in vitro* optimum growth temperature for AG-11 was 21.6°C, which agrees with pathogenicity studies done by Kumar *et al.* (1999) showing that AG-11 can only cause very little lupin pre-emergence damping-off and hypocotyl rot at 10°C, and most severe hypocotyl rot at 20 and 25°C.

The binucleate *Rhizoctonia* AG-K was found associated with barley, canola, clover, lucerne, lupin, medic and wheat in this study. In Turkey and the USA isolates of AG-K were also isolated from barley and wheat, and were found to be non pathogenic (Demirci, 1998; Ogoshi *et al.*, 1990). Information on binucleate AGs associated with canola is limited to pathogenicity studies by Khangura *et al.* (1999) indicating that AG-K is only weakly virulent on canola. Wong *et al.* (1985) found that AG-K was not pathogenic on clover.

The unidentified binucleate group of *Rhizoctonia* were isolated from barley, canola, clover, lucerne, lupin, medic and wheat. Previously, Lamprecht, Knox-Davies and Marasas (1988) also obtained binucleate *Rhizoctonia* isolates from medic in surveys conducted in the Western Cape province of South Africa.

The binucleate *Rhizoctonia* AG-A was associated with barley, canola, clover, lucerne, medic and wheat in this study. There are no reports of this AG on the above-mentioned crops in other countries.

The binucleate *Rhizoctonia* isolates of AG-I were associated with barley, canola, lupin and wheat. In Turkey, isolates of AG-I were also isolated from barley and wheat, where is it was found to be non-pathogenic (Demirci, 1998). Only a thin binucleate *Rhizoctonia* (cause of Eradu patch disease in Australia) has been reported as a binucleate *Rhizoctonia* pathogen of lupin (MacLeod & Sweetingham, 1997).

The multinucleate *Rhizoctonia* AG-2-1 was associated with canola, clover, lupin, medic and wheat in this study. In other parts of the world, isolates of AG-2-1 have not only been often associated with canola, but have also been found highly virulent on this crop (Kaminski & Verma; 1985; Hwang, Swanson and Evans, 1986; Gugel *et al.,* 1987; Khangura, Barbetti & Sweetingham, 1999). In Australia isolates of AG-2-1 were associated and found weakly virulent towards lupin and wheat (Sweetingham *et al.* 1986). In Turkey, AG-2-1 was associated with barley and wheat and was moderately virulent on these crops (Demirci, 1998). Isolates of *R. solani* AG-2-1 have also been found pathogenic on clover elsewhere (Wong, Barbetti & Sivasithamparam, 1985).

Rhizoctonia solani AG-2-2 was only isolated from medic in this study. Although AG-2-2 was found associated with wheat in Texas it was not found to be pathogenic on this crop (Rush *et al.*, 1994). Isolates of *R. solani* AG-2-2 were found to be pathogenic on clover in Australia (Wong, Barbetti & Sivasithamparam, 1985). This is the first report on the isolation of AG-2-2 from medic.

In this study *R. solani* AG-3 was associated with canola, lupin and wheat. In Turkey, AG-3 was isolated from barley and wheat, where it was found to be moderately virulent on

these crops (Demirci, 1998). Contrarily, in the USA, isolates of AG-3 were associated with barley and wheat, but were non pathogenic to these crops (Ogoshi *et al.*, 1990).

The mulitinucleate *Rhizoctonia* AG-4 HG-II was associated with barley, canola, clover, lupin, medic and wheat in this study. In Turkey, isolates of AG-4 were also found associated with barley and wheat, and were highly virulent on these crops (Demirci, 1998). Similarly, in Texas, isolates of AG-4 were found associated and pathogenic on wheat (Mathieson & Rush, 1991: Rush *et al.*, 1994). Contrarily, in the USA, isolates of AG-4 were found associated with barley and wheat, but were non pathogenic or only mildly virulent on these crops (Ogoshi *et al.*, 1990). In other parts of the world isolates of AG-4 are also frequently isolated from canola, where it was found to be highly virulent on this crop (Kaminski & Verma; 1985; Hwang, Swanson and Evans, 1986; Gugel *et al.,* 1987; Khangura, Barbetti & Sweetingham, 1999). *Rhizoctonia solani* AG-4 was further also isolated and found to be pathogenic on lupin in the USA (Leach & Clapman, 1992). Although *R. solani* is an important pathogen of medic in Australia (Bretag, 1985; Mebalds, 1987; Barbetti, 1989), the specific AGs involved were not identified. Information on the AGs pathogenic on medics is limited to a study on the resistance of 27 annual *Medicago* spp. to AG-4. this study demonstrated the pathogenicity of AG-4 on different Medicago spp. (Kulik & Dery, 1995). *Rhizoctonia solani* AG-4 has also been found associated with poor stands of clover in Texas (Pemberton *et al.*, 1998). Although *Rhizoctonia solani* AG-4 is the most important *Rhizoctonia* pathogen of lucerne in other countries (Kulik, Dery & Douglas, 1995), it was not isolated from lucerne grown in monoculture in this study.

Rhizoctonia solani AG-11 was only isolated from lupin in this study. In Western Australia and Arkansas isolates of AG-11 were also found associated with lupin, as well as wheat. *Rhizoctonia solani* AG-11 was pathogenic on both of these crops (Carling *et al.*, 1994). In Turkey, AG-11 has also been isolated and found to be highly virulent on barley and wheat (Demirci, 1998).

Several *R. solani* AGs that have previously been found associated with some of the rotations crops were not isolated in the present study. *Rhizoctonia solani* AG-6 that had previously been reported as a pathogen of wheat in South Africa (Scott *et al.*, 1979; Carling, Meyer & Brainard, 1996) was not isolated in this study. *R. solani* AG-8 and *R. oryzae* that have been frequently isolated from cereals and canola in other countries were also not isolated in this study. (MacNish, 1983; Smiley, Wilkins & Klepper, 1990; Furgal-Wegrzyciqa, Adamkiak & Adamiak, 1998; Khangura *et al.*, 1999). Furthermore, *R. cerealis* (AG-D) that had previously been reported as pathogens of wheat in South Africa was not isolated in this study (Scott *et al.*, 1979).

Rhizoctonia was generally more frequently isolated from plants at the flowering than the seedling stage. This increase in incidence from the seedling to the flowering stage was mostly the result of an increase in binucleate *Rhizoctonia* AGs, but it was also evident that AG-2-1 and AG-4 HG-II were more frequently isolated at the flowering than the seedling stage. The higher incidence of *Rhizoctonia* at the flowering stage is most probably partly due to more favourable temperatures for growth of the *Rhizoctonia* AGs during September and October (flowering stage) compared to July and August (mid season) (Fig. 1). In Western Australia, MacLeod and Sweetingham (1997) recorded an increase in the number of *Rhizoctonia* isolates obtained from July (six isolates) to August (32 isolates). There is limited information on the incidence of *Rhizoctonia* AGs at different times during the growing season.

This is the first report of the specified *Rhizoctonia* AGs on barley, canola, clover, lucerne, lupin, medic and wheat in South Africa. Proper seedling establishment of these crops in the Western Cape province has always been problematic, and fungal pathogens including *Rhizoctonia* spp. have been implicated as a contributing factor (Lamprecht *et al.*, 2000a; Auret *et al.*, 2002). Within the crop rotation trial of this study the association of known pathogenic *Rhizoctonia* AGs (AG-2-1 and AG-4) with canola was noticeable and should be further investigated. Furthermore, it will be interesting to determine whether the large number of binucleates associated with the rotation crops have a beneficial association with their hosts.

Another interesting observation of the study was that no multinucleate isolates were isolated from lucerne that has been grown in this region for an extended period in monoculture. This is especially striking considering that AG-4 HG-II, that is a very well known pathogen of lucerne (Kulik, Dery & Douglas, 1995), could not be isolated from the lucerne monoculture plots, even though it was the predominant multinucleate AG (69%) isolated from various other rotations crops used in the study. The absence of AG-4 HG-II from lucerne in this study could be due to the practice of monoculture. Similarly, in sugar beet and wheat, monoculture has resulted in a gradual reduction of disease caused by AG-5 and AG-8 (Hyakumachi, 1999; Mazzola, 2002). Another factor contributing to the absence of AG-4 HGII and other pathogenic multinucleate isolates on lucerne in this study, could be due to the fact that lucerne residues in itself has been found to suppress pathogenic *Rhizoctonia* in beans (Sneh *et al.*, 1996).

Determining *Rhizoctonia* AGs associated with rotation crops is essential for development of effective integrated management strategies. Seed treatments can significantly improve seedling establishment, but it is known that certain *Rhizoctonia* AGs differ in their sensitivity to fungicides (Kataria & Gisi, 1996). Including practices such as seed treatments and crop rotation in management strategies will therefore be much more reliable in the future as the specific AGs involved in disease development have been characterised to some degree. However, before implementing fungicide seed treatment for the control of pathogenic multinucleate isolates, the effect of fungicides on potentially beneficial binucleate isolates should first be established. Since the current study was restricted to one crop rotational trial in one production area, future studies should investigate the association of *Rhizoctonia* in areas with different climatology (higher rainfall in the western than southern production areas during the growing season) to obtain a better understanding of the *Rhizoctonia* AGs associated with rotation crops. This additional knowledge will further aid the development of sound control practices.

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Table 1. *Rhizoctonia* reference cultures used in this study.

Rhizoctonia group	Anastomosis group	Isolate	Collector or supplier
Multinucleate	AG-2-1	H-24	Mazzola, M.
	AG-2-2	455-11	Mazzola, M.
	AG-3	PPRI[z] 2152 (W14L)	PPRI/ (Ogoshi, 1988)
	AG-4	6-3-6	Mazzola, M.
		PPRI 2153 (F 10)	PPRI / (Ogoshi, 1988)
	AG-4 HG-II	RH 165	Mazzola, M.
	AG-11	WAC9938	Barbetti, M.
		WAC10000	Barbetti, M.
		WAC10001	Barbetti, M.
Binucleate	AG-A	PPRI 2130 (C-517)	PPRI / (Ogoshi, 1993)
	AG-D	PPRI 2135 (OR706)	PPRI / (Ogoshi, 1993)
		C-610	Mazzola, M.
	AG-I	AV-2	Mazzola, M.
	AG-K	PPRI 2141 (AC-1)	PPRI / (Ogoshi, 1993)

[z]PPRI – Plant Protection Research Institute (Agricultural Research Council).

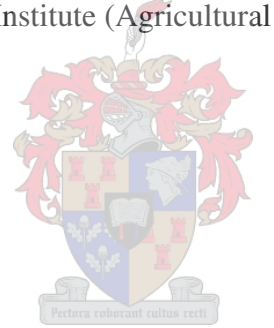

Table 2. *Rhizoctonia* isolates used for conventional hyphal fusion reaction and for growth studies.

Nuclear status	Anastomosis group	Accession number[z]	Origin
Multinucleate	AG-2-1	PPRI 7426	Lupin
		PPRI 7427	Canola
		PPRI 7428	Medic
	AG-2-2	PPRI 7429	Medic
		PPRI 7430	Medic
	AG-3	PPRI 7431	Lupin
		PPRI 7432	Canola
		PPRI 7433	Wheat
	AG-4 HG-II	PPRI 7434	Barley
		PPRI 7435	Wheat
		PPRI 7436	Medic
		PPRI 7437	Barley
		PPRI 7438	Wheat
		PPRI 7439	Medic
	AG-11	PPRI 7440	Lupin
		PPRI 7441	Lupin
Binucleate	AG-A	PPRI 7414	Lucerne
		PPRI 7415	Barley
		PPRI 7416	Medic
	AG-I	PPRI 7420	Barley
		PPRI 7421	Lupin
		PPRI 7422	Wheat
	AG-K	PPRI 7423	Lucerne
		PPRI 7424	Lupin
		PPRI 7425	Canola
	Unidentified *Rhizoctonia*	PPRI 7417	Wheat
		PPRI 7418	Wheat
		PPRI 7419	Wheat

[z]Cultures deposited in the National Collection of Fungi at the ARC-Plant Protection Research Institute in Pretoria, South Africa.

Table 3. Analysis of variance of the second order polynomial functions, temperature at optimum radial growth and total growth area under curve (growth rate) for the nine anastomosis groups (AGs) of *Rhizoctonia*.

		A		B		C		Optimum T[y]		Area under curve	
Source	**Df**	**MS**	**SL**	**MS**	**SL**	**MS**	**SL**	**MS**	**SL**	**MS**	**SL**
Isolate	28	158	<0.0001	4.07	<0.0001	0.002	<0.0001	7.11	<0.0001	46786.01	<0.0001
AG	8	521.96	<0.0001	13.09	<0.0001	0.007	<0.0001	23.18	<0.0001	140888.93	<0.0001
Isolates within AG-2-1	2	5.958	0.0247	0.03	0.1917	0.00003	0.0576	0.51	0.0325	393.31	0.0435
Isolates within AG-2-2	2	11.65	0.0011	0.26	<0.0001	0.00020	<0.0001	1.16	0.0007	1898.61	<0.0001
Isolates within AG-3	2	4.726	0.0512	0.12	0.0051	0.00020	<0.0001	1.08	0.0011	9232.52	<0.0001
Isolates within AG-4 HG-II	5	12.93	<0.0001	0.45	<0.0001	0.00020	<0.0001	0.05	0.8566	6066.72	<0.0001
Isolates within AG-11	1	0.29	0.6623	0.00	0.8749	0.00001	0.3215	0.43	0.0860	3073.77	<0.0001
Isolates within AG-A	2	3.75	0.0926	0.07	0.3074	0.00004	0.0236	0.18	0.2921	428.70	0.0334
Isolates within AG-I	2	52.77	<0.0001	2.54	<0.0001	0.00130	<0.0001	3.41	<0.0001	57060.25	<0.0001
Isolates within AG-K	2	0.63	0.6617	0.00	0.9050	1.98600	<0.0001	0.05	0.7162	357.37	0.0572
Isolates within UNBR[z]	2	12.88	0.0006	0.44	<0.0001	0.00020	<0.0001	0.19	0.2691	5373.93	<0.0001
Isolate (AG)	20	12.48	<0.0001	0.46	<0.0001	0.00020	<0.0001	0.68	<0.0001	9144.84	<0.0001
Experimental Error	58	1.51		0.02		0.00001		0.14		118.83	
Corrected Total	86										

[y]Temperature at optimum growth

[z]Unidentified binucleate *Rhizoctonia*

Table 4. Comparison of second order polynomial function coefficients, temperatures at optimum radial growth and total growth area under the curve (growth rate) for the nine anastomosis groups (AGs) of *Rhizoctonia* following a temperature growth study at seven different temperatures.

Anastomosis group	Quadratic equation ($Y = A + BX + CX^2$)			$(R^2)^w$	Optimum temperature[x]	Area under the curve
	A	B	C			
AG-2-1	-17.48 f[y]	3.75 d	-0.09 f	79.28%	21.65 d	492.18 e
AG-2-2	-15.02 e	3.09 e	-0.06 c	80.93%	25.90 a	549.88 c
AG-3	-17.89 f	3.90 c	-0.09 f	80.45%	21.72 d	521.08 d
AG-4 HG-II	-28.12 h	5.25 a	-0.12 h	77.68%	22.43 c	637.95 a
AG-11	-21.69 g	4.52 b	-0.11 g	83.06%	21.63 d	572.87 b
AG-A	-12.40 c	3.04 e	-0.07 e	87.44%	20.97 e	419.61 g
AG-I	-6.95 a	1.94 g	-0.05 a	79.68%	20.70 e	296.91 i
AG-K	-13.73 d	2.99 e	-0.06 d	79.44%	23.21 b	464.38 f
UNBR[z]	-8.98 b	2.25 f	-0.05 b	81.37%	21.04 e	318.37 h
LSD	**1.158**	**0.141**	**0.004**		**0.350**	**10.286**

[w]Coefficient of determination.
[x]Temperature at optimum growth.
[y]Values followed by the same letter in a column do not differ significantly ($P = 0.05$).
[z]Unidentified binucleate *Rhizoctonia*.

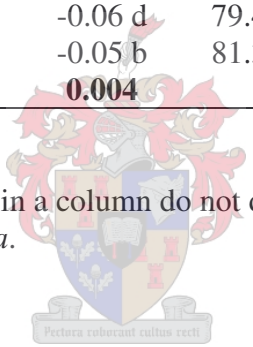

Table 5. Frequency of isolation (number of isolates) of *Rhizoctonia* anastomosis groups (AG) from crops over four years.

Rhizoctonia group	Anastomosis group	Crop[w]	2000	2001	2002[x]	2003	Total
Multinucleate	AG-2-1	B	-[y]	-	-	-	-
		C	5	-	2	3	10
		Luc	-	-	-	-	-
		L	4	1	-	1	6
		M	-	-	2	1	3
		W	-	-	-	1	1
	Total		**9**	**1**	**4**	**6**	**20**
	AG-2-2	B	-	-	-	-	-
		C	-	-	-	-	-
		Luc	-	-	-	-	-
		L	-	-	-	-	-
		M	2	-	-	-	2
		W	-	-	-	-	-
	Total		**2**	**-**	**-**	**-**	**2**
	AG-3	B	-	-	-	-	-
		C	2	-	-	-	2
		Luc	-	-	-	-	-
		L	1	-	-	-	1
		M	-	-	-	-	-
		W	2	1	-	2	5
	Total		**5**	**1**	**-**	**2**	**8**
	AG-4 HG-II	B	1	14	-	2	17
		C	-	7	-	1	8
		Luc	-	-	-	-	-
		L	-	-	-	1	1
		M	11	10	3	8	32
		W	8	1	3	2	14
	Total		**20**	**32**	**6**	**14**	**72**
	AG-11	B	-	-	-	-	-
		C	-	-	-	-	-
		Luc	-	-	-	-	-
		L	2	-	-	-	2
		M	-	-	-	-	-
		W	-	-	-	-	-
	Total		**2**	**-**	**-**	**-**	**2**
	Multi. Total		**38**	**34**	**10**	**22**	**104**

Table 5. Continued

Rhizoctonia group	Anastomosis group	Crop[w]	2000	2001	2002[x]	2003	Total
Binucleate	AG-A	B	-[y]	3	-	-	3
		C	5	-	1	-	6
		Luc	6	2	-	4	12
		L	-	-	-	-	-
		M	1	-	2	-	3
		W	1	3	1	3	8
	Total		**13**	**8**	**4**	**7**	**32**
	AG-I	B	1	-	-	2	3
		C	1	-	1	5	7
		Luc	-	-	-	-	-
		L	2	-	-	-	2
		M	-	-	-	-	-
		W	-	4	1	-	5
	Total		**4**	**4**	**2**	**7**	**17**
	AG-K	B	2	3	-	11	16
		C	13	8	3	1	25
		Luc	5	6	2	-	13
		L	7	6	6	4	23
		M	30	19	22	9	80
		W	3	8	-	3	14
	Total		**60**	**50**	**33**	**28**	**171**
	UNBR[z]	B	2	5	-	2	9
		C	7	2	2	4	15
		Luc	-	-	-	6	6
		L	1	-	-	1	2
		M	11	6	2	30	49
		W	17	2	-	4	23
	Total		**38**	**15**	**4**	**47**	**104**
	Binuc. Total		**115**	**77**	**43**	**89**	**324**
	Overall total		**153**	**111**	**53**	**111**	**428**

[w]B = barley, C = canola, L = lupin, Luc = lucerne, M = medic/clover mixture and W = wheat.
[x]Plants sampled only at third sampling time in 2002.
[y] - = not isolated.
[z]Unidentified binucleate *Rhizoctonia*.

Table 6. Analysis of variance for the effect of sampling time (ST) on the percentage incidence of bi- and multinucleate *Rhizoctonia* groups (RG) in four years.

	2000			2001			2002			2003		
Source	**Df**	**MS**	**SL**	**Df**	**MS**	**SL**	**Df**	**MS**	**SL**	**Df**	**MS**	**SL**
Block	3	13.75	0.1880	3	15.13	0.2451	3	11.93	.	3	21.29	0.2218
ST	2	251.65	0.0003	2	137.25	0.0037	0	.[z]	.	2	35.40	0.1104
Experimental Error (a)	6	6.23	0.6690	6	8.35	0.3341	0	.	.	6	10.88	0.0926
RG	1	144.02	0.0105	1	41.67	0.0464	1	92.69	0.0060	1	111.92	0.0062
ST x RG	2	51.74	0.0662	2	6.08	0.4885	0	.	.	2	9.68	0.3762
Experimental Error (b)	9	13.89	0.1406	9	7.82	0.3803	3	1.90	0.8814	9	8.86	0.1501
Sample Error	938	9.21		936	7.29		312	8.56		936	5.98	
Corrected total	961			959			319			959		

[z]Only one sampling time in 2002.

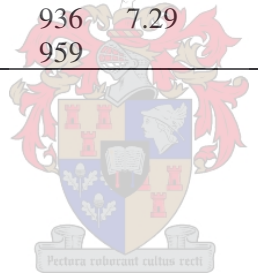

Table 7. Effect of sampling time on the mean percentage incidence of *Rhizoctonia* isolates over four years.

Sampling time[x]	2000	2001	2002	2003
1	0.42 b[y]	0.05 b	-[z]	0.35 a
2	0.17 b	0.40 b	-	0.42 a
3	1.81 a	1.32 a	0.89	0.96 a
LSD	0.482	0.559	-	0.638

[x]Sampling time 1 = seedling stage (4 – 6 weeks after planting), 2 = mid season (12 – 14 weeks after planting) and 3 = flowering stage (20 – 22 weeks after planting).
[y]Means within a year (in a column) followed by the same letter do not differ significantly (P = 0.05)
[z]Plants sampled only at third sampling time in 2002.

Table 8. Mean percentage incidence of bi- and multinucleate *Rhizoctonia* groups over four years.

Rhizoctonia group	2000	2001	2002	2003
Binucleate	1.19 a[z]	0.80 a	1.42 a	0.91 a
Multinucleate	0.42 b	0.38 b	0.35 b	0.23 b
LSD	0.544	0.408	0.490	0.435

[z]Means within a year (in a column) followed by the same letter do not differ significantly (P = 0.05)

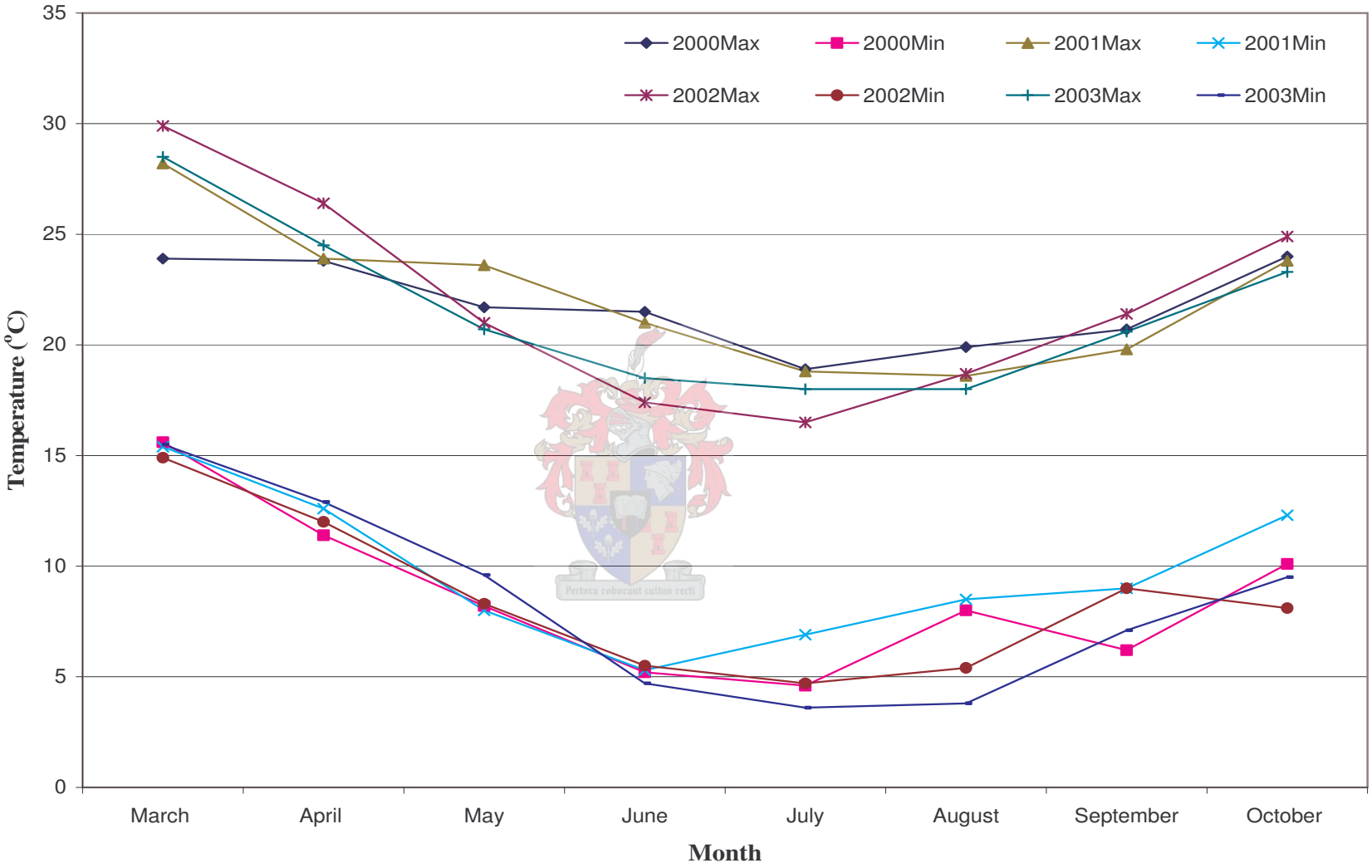

Figure 1. Mean daily maximum and minimum temperatures for March to October of 2000 - 2003 at the Tygerhoek experimental farm.

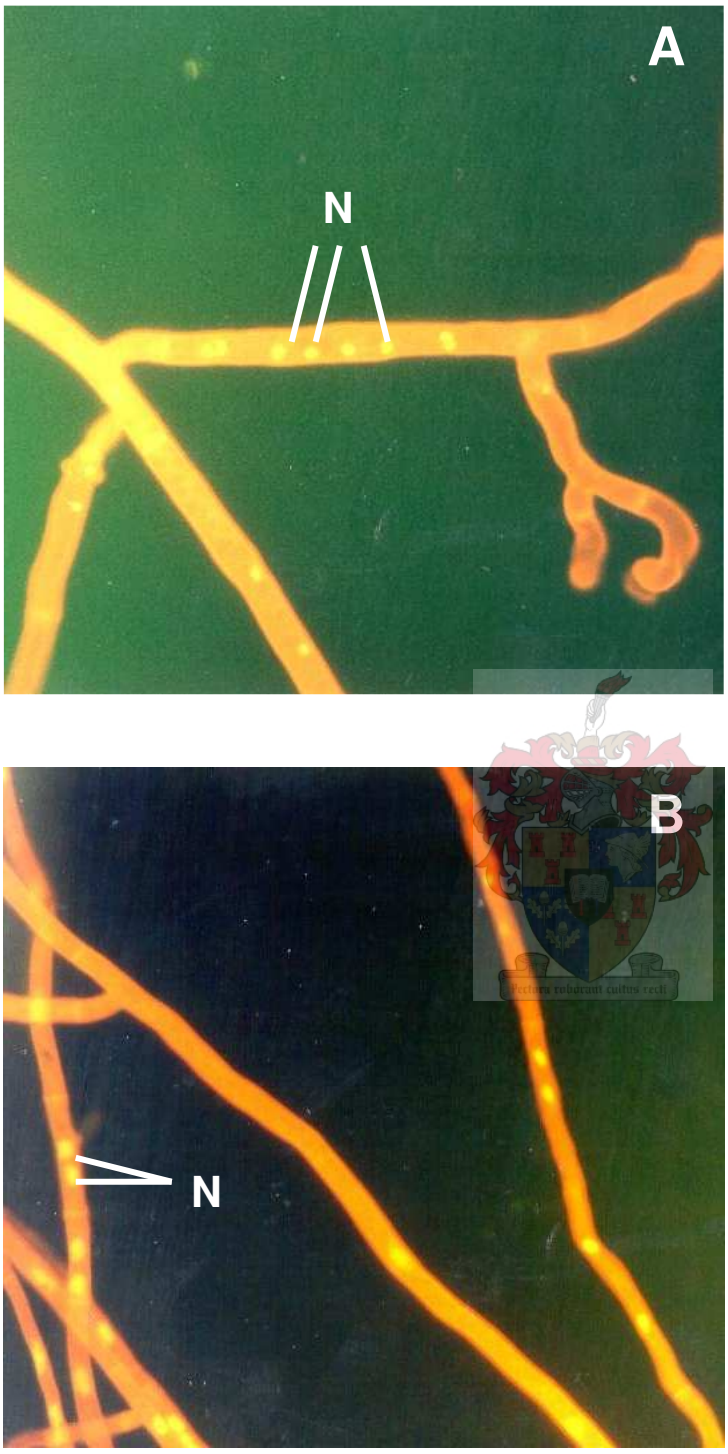

Figure 2. Nuclei (N) in vegetative hyphae of *Rhizoctonia* spp. A) multinucleate species; B) binucleate species.

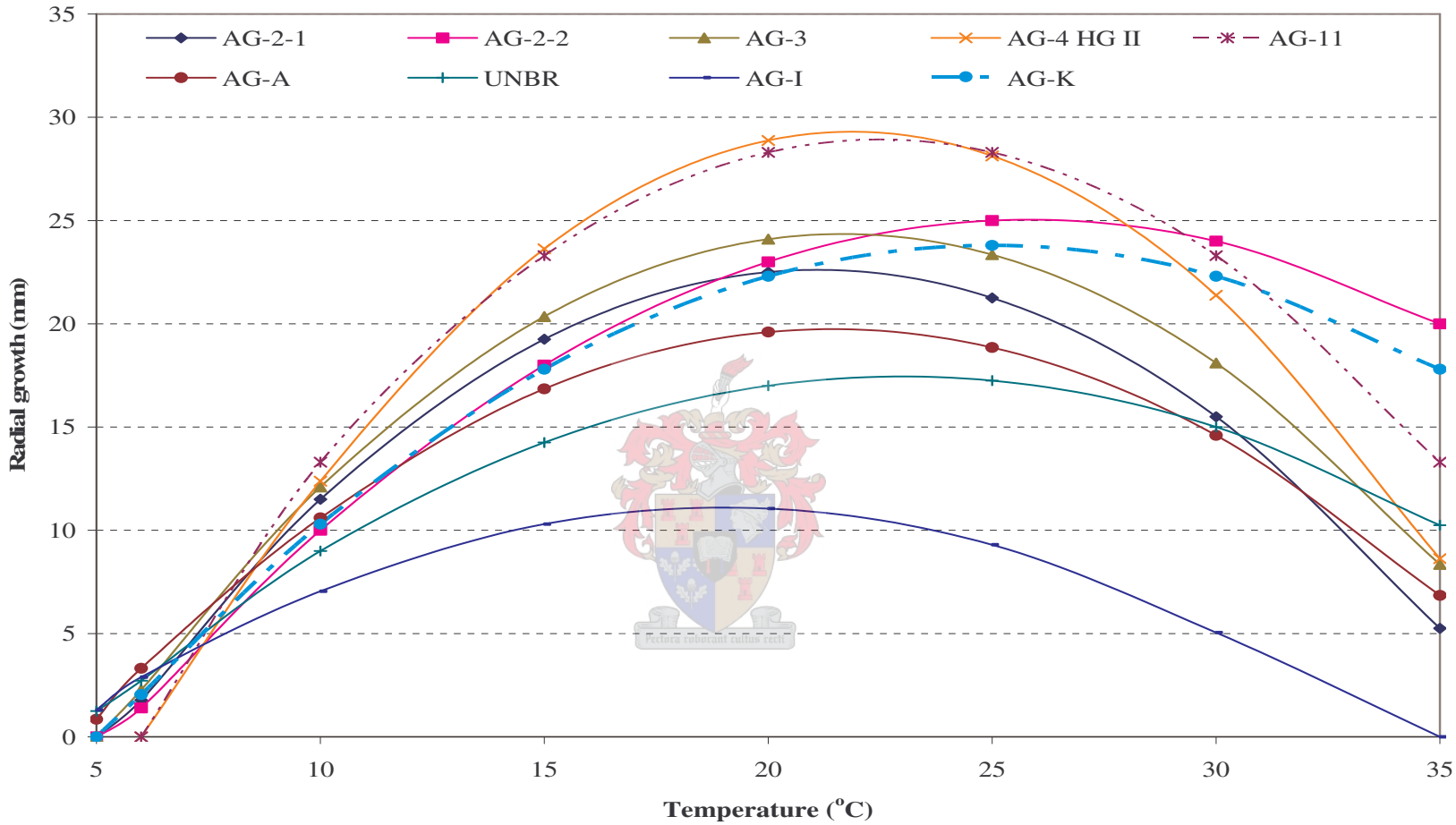

Figure 3. Second order polynomial functions showing the predicted trends of the effect of temperature on radial growth rate of different anastomosis groups of *Rhizoctonia* including the unidentified binucleate *Rhizoctonia* (UNBR) following a temperature growth study at seven different temperatures.

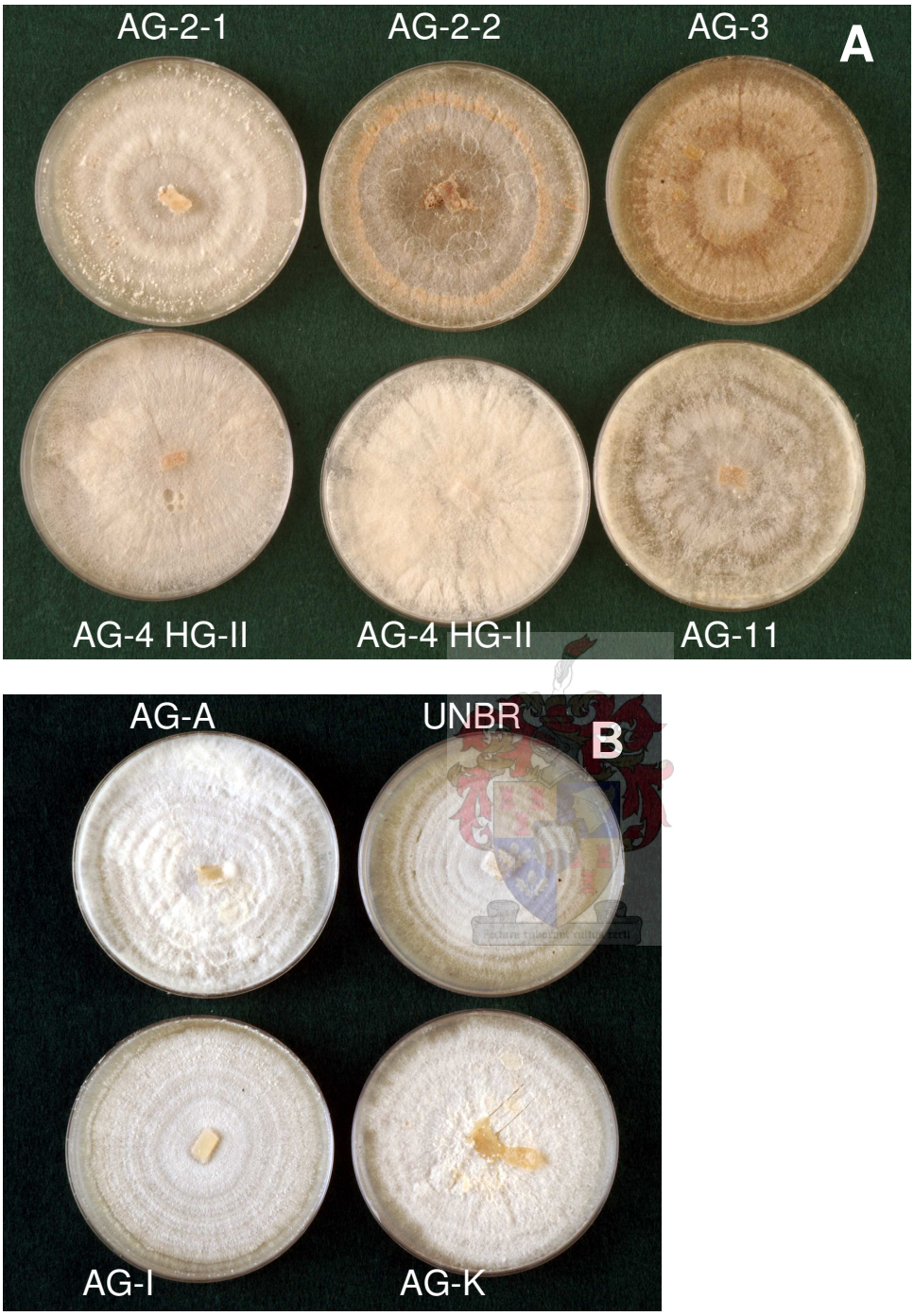

Figure 4. *In vitro* growth of *Rhizoctonia* isolates on PDA after 21 days representing nine anastomosis groups. A) multinucleate groups (AG-2-1, AG-2-2, AG-3, AG-4 HG-II and AG-11); and B) binucleate groups [AG-A, AG-I, AG-K and unidentified binucleate *Rhizoctonia* (UNBR)].

3. PATHOGENICITY OF *RHIZOCTONIA* ON ROTATION CROPS IN THE WESTERN CAPE PROVINCE

ABSTRACT

The pathogenicity and relative virulence of 28 *Rhizoctonia* isolates were evaluated on emerging seedlings as well as 14-day-old seedlings of seven crops (barley, canola, clover, lucerne, lupin, medic and wheat) used within crop rotation systems in the Western Cape province of South Africa. Prior to evaluating pathogenicity and relative virulence of these isolates on emerging seedlings, an inoculum concentration trial was conducted, using three anastomosis groups (AGs) and two crops. The trial indicated that an inoculum concentration of 0.05% mass inoculum/mass planting medium will be appropriate for evaluating pathogenicity and virulence of isolates on emerging seedlings. The inoculum concentration for inoculation of 14-day-old seedlings had previously been established (Lamprecht *et al.*, unpublished data). Pathogenicity tests using 28 *Rhizoctonia* isolates, representing nine AGs, showed that AG-2-2 and AG-4 HG-II were the most virulent AGs on both seedling stages of all crops. The exception was AG-2-2 that could only cause 22% damping-off on emerging clover seedlings, as well as no significant root and hypocotyl symptoms on 14-day-old clover seedlings. AG-4 HG-II was significantly less virulent on barley and wheat than on the other crops, this is due to the fact that the assays were conducted on very young plants in the absence of environmental stress (es). Similarly, AG-2-1 was least virulent on barley, only affecting 14-day-old seedlings, and not being pathogenic at all on wheat. Contrarily, AG-2-1 was highly virulent on both seedling stages of canola. *Rhizoctonia solani* AG-2-1 was further moderately virulent on both seedling stages of medic and lupin, but only weakly virulent on both seedling stages of lucerne. *Rhizoctonia solani* AG-11 was moderate to weakly virulent on both seedling stages of all crops, except barley and wheat. On wheat AG-11 was not pathogenic at all, and on barley only coleoptile lesions were present on 14-day-old seedlings. *Rhizoctonia solani* AG-3 was only weakly virulent on both seedling stages of canola, lupin

and medic. The binucleate AGs, viz., AG-A, AG-I and unidentified binucleate isolates were not considered pathogenic, even though very low disease incidence was present in a few instances. The binucleate *Rhizoctonia* AG-K was the only weakly virulent binucleate group capable of causing significant disease on emerging seedlings as well as 14-day-old inoculated canola, lucerne, lupin and medic seedlings. This is the first comprehensive study on the pathogenicity of different AGs associated with crops used in rotation systems in the Western Cape province of South Africa.

INTRODUCTION

The genus *Rhizoctonia* contains a diverse group of organisms, ranging from saprophytes to plant pathogenic species. Pathogenic members of the genus are economically important constraints to crop production in temperate and tropical agriculture (Sneh *et al.,* 1996). Therefore, worldwide efforts are being made to obtain more information on the variation in virulence, or lack thereof, among *Rhizoctonia* isolates. This knowledge is essential for developing sound management strategies against *Rhizoctonia* diseases, and understanding the ecology of the genus.

The genus *Rhizoctonia* is extremely diverse, traditionally containing all fungi that do not have distinctive taxonomic features (Vilgalys & Cubeta, 1994). Therefore, useful methods have been developed to classify isolates to some taxonomic level. Classification of *Rhizoctonia* isolates is often accomplished by first identifying their nuclear state as either being bi- or multinucleate (Vilgalys & Cubeta, 1994; Yang *et al*., 1994). The most useful system for identifying *Rhizoctonia* isolates is based on anastomosis groups (AGs), which are determined by identifying hyphal anastomosis among isolates (Parmeter, Whitney & Platt, 1967; Parmeter, Sherwood & Platt, 1969). More recently, deoxyribonucleic acid (DNA)-based methods have also been used to classify *Rhizoctonia* into AGs (Gonzalez *et al*., 2001).

The multinucleate *Rhizoctonia solani* Kühn isolates have been studied extensively due to their ability to cause disease on more than 250 plant species under diverse environmental conditions (Wong, Barbetti & Sivasithamparam, 1985). Some *R. solani* isolates have a wide host range, while others show a higher level of host specificity (Anderson, 1982). *Rhizoctonia solani* isolates can attack their annual host while still at the young seedling stage and/or when the host is older. Symptoms incited by *R. solani* can vary from root and crown rot on older plants, to damping-off, hypocotyl and coleoptile rot of young seedlings (Sneh *et al.,* 1996). It is also important to note that these fungi are significant pathogens in perennial cropping systems, such as forest nursery production, perennial tree ecosystems and even natural ecosystems (Mazzola, 1997). Currently, *R. solani* can be divided into fourteen AGs [AG-1 to AG-10 including AG BI (Sneh, Burpee & Ogoshi, 1991), AG-11 (Carling *et al.,* 1994), AG-12 (Carling *et al*., 1999) and AG-13 (Carling, Kuninaga & Brainard, 2002)], some of which are further divided into subgroups.

Similar to multinucleate isolates, binucleate *Rhizoctonia* isolates are also diverse and have been isolated from a wide number of plant species. However, unlike the multinucleate isolates, binucleate isolates are not commonly regarded as highly virulent plant pathogens (Anderson, 1982; Roberts & Sivasithamparam; 1986). Binucleate *Rhizoctonia* isolates have been divided into AG-A to S by Sneh *et al.* (1991).

Rhizoctonia species can be a potential production limiting factor to rotation crops [barley (*Hordeum vulgare* L.), canola (*Brassica napus* L. var. *oleifera* DC), clover (*Trifolium* spp.), lucerne (*Medicago sativa* L.), lupin (*Lupinus* spp.), medic (annual *Medicago* spp.) and wheat (*Triticum aestivum* L.)] that are currently being planted in the Western Cape province of South Africa. *Rhizoctonia* spp. are known to have a significant impact on the health of these crops in other countries (Samuel & Garret, 1932; Bretag, 1985; Kaminski & Verma, 1985; Weller *et al.*, 1986; Pumphrey *et al.*, 1987; Kronland & Stanghellini, 1988; Kataria & Verma, 1992; Vincelli & Herr, 1992; MacLeod & Sweetingham, 1997). For example, *R. solani* has been reported as widely distributed and very destructive on lupin and canola (Acharya *et al.*,

1984; Kaminski & Verma, 1985; Hwang, Swanson & Evans, 1986; Gugel *et al.*, 1987; Yitbarek Verma & Morrall, 1987; Kataria & Verma, 1992; Leach & Clapham, 1992). This is especially a concern since *Rhizoctonia* spp. have often been isolated from all the rotation crops used in the Western Cape province of South Africa (Lamprecht, De Villiers & Janse van Rensburg, 1999; Lamprecht, Auret & Janse van Rensburg 2000a; Lamprecht *et al.*, 2000b, 2001, 2002; Auret, Janse van Rensburg & Lamprecht, 2002). Unfortunately, the pathogenicity and virulence of South African *Rhizoctonia* isolates are unknown on the rotation crops of interest, except for the pathogenicity of *R. solani* AG-6 (Smith & Wehner, 1986) and *R. cerealis* Van der Hoeven (Scott, Visser & Rufenacht, 1979) on wheat.

The main objective of this study was to determine the pathogenicity and relative virulence of *Rhizoctonia* AGs associated with seven rotation crops (barley, canola, clover, lucerne, lupin, medics and wheat) used in the Western Cape province of South Africa (Chapter 2). The *Rhizoctonia* isolates used for pathogenicity testing were previously isolated from a crop rotation trial over a four-year period (2000 to 2003) at the Tygerhoek experimental farm, Riviersonderend, Western Cape province (Chapter 2). Three independent trials were conducted to determine the pathogenicity and relative virulence of isolates on emerging seedlings as well as 14-day-old seedlings. The first trial, an inoculum concentration trial, allowed the establishment of an appropriate inoculum concentration for determining the pathogenicity and relative virulence of *Rhizoctonia* isolates on emerging seedlings. Subsequently, the pathogenicity of 28 isolates representing nine AGs were determined on emerging seedlings of all seven rotation crops. In a third trial the pathogenicity and relative virulence of these isolates were also determined on 14-day-old seedlings. The inoculum concentration for determining pathogenicity and virulence on 14-day-old seedlings had previously been established (Lamprecht *et al.*, unpublished data).

MATERIALS AND METHODS

Isolates

Rhizoctonia isolates used in the study were obtained from the aforementioned trial at the Tygerhoek experimental farm, Riviersonderend, Western Cape province (Chapter 2). Cultures were stored on potato carrot agar slants [10 g potato, 10 g carrot and 12 g agar (Bacteriological, Biolab Diagnostics, Midrand, South Africa)] at 10°C and/or sterile wheat grain in a deep freezer adapting (excluding vermiculite) the method used by Sneh *et al.* (1986) to prepare inoculum. *Rhizoctonia* isolates were all deposited in the Culture Collection of the Plant Protection Research Institute, Pretoria (PPRI). The accession numbers, crop origin and nuclear state of isolates are listed in Table 1. The anastomosis grouping of isolates had previously been determined through sequence analyses of the ribosomal internal transcribed spacer region (ITS) and confirmed by hyphal fusion reactions (Chapter 2).

Inoculum preparation

Millet seed inoculum was used in all three pathogenicity trials (inoculum concentration, emerging seedling and 14-day-old seedling trial). Millet seed inoculum was prepared according to the method described by Strauss and Labuschagne (1995). Millet seeds were soaked in deionised water for 12 h (200 g seed/100 mL in 1 L Schott bottles) and then autoclaved for 20 min at 120°C on two consecutive days. The bottles were shaken before autoclaving and each time after autoclaving. The autoclaved millet seeds were inoculated with 10 (6 mm diam.) mycelial discs punched from 5-day-old *Rhizoctonia* cultures grown on potato dextrose agar (PDA) (Biolab Diagnostics, Midrand, South Africa). Control bottles were inoculated with discs of PDA only. The bottles with inoculated millet seeds were incubated in an incubation room at 25°C for 10 days and shaken every third day to ensure thorough seed colonisation.

Planting media and plant growth conditions

Pathogenicity trials were conducted using 13 cm diam. plastic pots with a capacity of holding 700 g of planting medium. The planting medium was made up of equal quantities of soil [collected from land adjacent to the rotation trial at Tygerhoek (Chapter 2)] perlite and sand, and was pasteurised (30 min at 83°C) 3 days before being mixed with inoculum (inoculum concentration and emerging seedling trial) or sown with seeds (14-day-old seedling trial). Each pot was placed on an inverted saucer, thereby lifting it above shelf level to avoid possible cross contamination from drainage water.

All trials were conducted in a growth room at 15°C night and 25°C day temperatures with Sylvania Gro-Lux lights, a 10 h photoperiod and a relative humidity of 60 to 70%. Pots were watered every third day to field capacity.

Inoculum concentration trial on emerging seedlings

A trial was conducted to determine the appropriate inoculum concentration for evaluating pathogenicity and relative virulence of *Rhizoctonia* isolates on emerging seedlings. Three isolates representing three AGs, viz., AG-2-1 (PPRI 7426), AG-4 HG-II (PPRI 7434) (multinucleate isolates) and AG-K (PPRI 7423) (binucleate isolate) (Table 1) were used. Multinucleate and binucleate isolates were specifically chosen since the multinucleate isolates are known to be highly virulent whereas the binucleate isolates are typically less virulent. Two crops, i.e. wheat and canola were used to represent a monocotyledonous and dicotyledonous crop, respectively.

Millet seed inoculum of each AG was individually mixed with planting media at five different concentrations, viz. 0.005, 0.0158, 0.05, 0.158 and 0.5% mass inoculum/mass planting media (wt/wt). Controls containing millet seed at the five different concentrations that were not inoculated with *Rhizoctonia*, were also included. Millet seed inoculated planting

media were placed in sterile pots and watered until water dripped from the bottom of the pots. The pots were left to stand overnight in the growth room before making ten holes in the soil of each pot using sterile 1 cm diameter doweling rods. Canola (cv. Varola 50) and wheat (cv. SST57) seeds were planted at 50 seeds/pot (5 seeds/hole) in 1.5 and 2 cm holes respectively.

Pathogenicity and relative virulence of the AGs at different inoculum concentrations were determined by calculating the percentage damping-off and percentage reduction in dry mass of seedlings 14 days after planting. Percentage damping-off caused by each AG at a specific inoculum concentration and crop was determined by using the formula: [(Number of emerged seedlings in crop A at x % w/w un-inoculated millet seed – number of emerged seedlings in crop A at x % w/w AG inoculated millet seed) / number of emerged seedlings in crop A at x% w/w un-inoculated millet seed] × 100. The percentage dry mass reduction was determined using a similar formula except that dry mass was used instead of number emerged seedlings. Dry mass was determined by placing the above ground material of each pot in paper bags and drying in an oven at 60°C for 3 days. Re-isolation of the different AGs was carried out by washing plants under running tap water to remove adhering soil. Plants were surface disinfected in 1% sodium hypochlorite, rinsed twice in sterile distilled water, and air-dried in a laminar flow cabinet. Four small pieces of root and crown tissue were excised and plated on PDA.

The experiment used a complete randomised block design. The treatment design for the trial was a 3 x 2 x 5 factorial with three replications per treatment. Factors were three AGs, two crops and five concentrations. An experimental unit consisted of a pot with 50 seeds. The trial was conducted twice, with three replicates per treatment.

Emerging seedling pathogenicity trial

The pathogenicity and virulence of 28 *Rhizoctonia* isolates representing nine AGs (Table 1) were determined on the emerging seedlings of seven crops including canola (cv.

Varola 50), clover (*T. michelianum* Savi cv. Patrick), lucerne (cv. SA Standard), lupin (*L. albus* L. cv. Wonga), medic (*M. truncatula* Gaertn. cv. Sephi), wheat (cv. SST57) and barley (cv. Clipper). Inoculation of planting media was done as described under inoculum concentration trial except that only one inoculum concentration (0.05% w/w) was used. All crops were planted at 50 seeds/pot (5 seeds/hole) except for lupin that was planted at 20 seeds/pot (2 seeds/hole). The planting depth for all crops was 1.5 cm except for barley, lupin and wheat that were planted at 2 cm depth. Controls consisting of plant media amended with 0.05% un-inoculated millet seed/planting media were included for each crop.

Pathogenicity and relative virulence of the AGs on the emerging seedlings of the different crops were determined by calculating the percentage damping-off and percentage reduction in dry mass of seedlings 14 days after planting. Percentage damping-off caused by each AG on a specific crop was determined by using the following formula: [(Number of emerged seedlings in control crop A – number of emerged seedlings in AG inoculated crop A) / number of emerged seedlings in control crop A] × 100. The percentage dry mass reduction was determined using a similar formula except that dry mass was used instead of number emerged seedlings. Dry mass and re-isolation of *Rhizoctonia* was done as previously described.

Experimental design was a randomised block design. The treatment design was a 29 x 7 factorial with two replications per treatment. The factors were 29 (28 isolates and a control) isolates (representing nine AGs) and seven crops. An experimental unit consisted of a pot with 50 seeds for all the crops except lupin where 20 seeds were planted in each pot. The trial was conducted twice.

Fourteen-day-old seedling pathogenicity trial

The 28 *Rhizoctonia* isolates (Table 1) tested for their pathogenicity towards emerging seedlings, were also evaluated for their pathogenicity and virulence toward 14-day-old

seedlings of seven crops (barley, canola, clover, lucerne, lupin, medic, and wheat). Pots were filled with pasteurised planting medium and an empty test tube (17 x 150 mm) was inserted in the centre of each pot. Five planting holes were made around each test tube at 1 cm from the test tube. The same seven crop cultivars used in the emerging seedling pathogenicity trial were also used in the 14-day-old pathogenicity trial. Seed planting depths of the crops were similar to that previously described. The number of seeds planted per hole was five for all crops, except three seeds were planted for lupin. Fourteen days after planting, the test tube was removed from each pot and planting medium amended with 0.5% w/w inoculum was placed in the holes left by the test tubes at 30 g/pot.

Pathogenicity and relative virulence of isolates were determined by evaluating root rot, coleoptile rot (barley and wheat) and hypocotyl rot (canola, clover, lucerne, lupin and medic). Fourteen days after inoculating the seedlings the plants were carefully removed from pots. Ten plants from each pot were used for disease assessments. Root rot was rated according to MacNish *et al.* (1995) on a 0 to 4 scale, with 0 = no obvious symptoms; 1 = slight discolouration or small superficial lesions; 2 = moderate discoloration or extensive but non-girdling lesions; 3 = extensive discolouration of tissue or girdling lesions; and 4 = plant dead. Coleoptile (barley and wheat) and hypocotyl (canola, clover, lupin, lucerne and medic) rot were also rated on a 0 to 4 scale as described for root rot. Re-isolation of *Rhizoctonia* was done as previously described.

The experimental and treatment design for the trial was the same as for the emerging seedling trial, viz., a 29 x 7 factorial with two replications (pots) per treatment. The 29 (28 isolates and a control) isolates represented the nine AGs. An experimental unit consisted of a pot with 25 seeds for all the crops except lupin where 15 seeds were planted in each pot. The trial was conducted twice and utilized a randomised block design.

Statistical analyses

Statistical analyses of all three trials were done similarly. Levene's variance ratio test (Levene, 1960) was performed to test for homogeneity of trial variances between the trial repeats. In these analyses, data of the two independent trials were considered block treatments and the replications within each trial were used as subsamples, providing that Levene's variance ratio test showed homogeneity in trial variance. Data were subjected to analysis of variance (SAS 1999), and the Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). In cases where deviations from normality were due to kurtosis and not skewness, the data were accepted as reliable and the results were interpreted without transformation (Glass, Peckham & Sanders, 1972). Pearson correlations between variables were also calculated.

RESULTS

Effect of inoculum concentration on seedling emergence

Variance for the data from the two independent trials was comparable based on Levene's variance ratio test (Levene, 1960). The error variance ratios for the percentage damping-off was $P = 0.8509$ and $P = 0.9085$ for the percentage reduction in dry mass. Consequently, data from the two trials were combined in all the analyses.

Analyses of variance were conducted on the mean percentage damping-off and percentage dry mass reduction of canola and wheat resulting from different inoculum concentrations of the three AGs. Significant crop x AG x inoculum concentration interactions were recorded for percentage damping-off ($P < 0.0001$) and percentage dry mass reduction ($P < 0.0001$) (Table 2).

The influence of inoculum concentration on disease development incited by the three *Rhizoctonia* AGs on wheat and canola was evaluated using two parameters, i.e. percentage damping-off and percentage dry mass reduction. Correlation analysis showed that there was a very high correlation ($P < 0.0001$, r = 98542) between percentage damping-off and percentage dry mass reduction. Therefore, only percentage damping-off will subsequently be discussed as a measure of pathogenicity and virulence.

The multinucleate *Rhizoctonia solani* isolates (AG-2-1 and AG-4 HG-II) were more virulent on canola than on wheat. Consequently, the extent of damping-off of canola caused by AG-2-1 and AG-4 HG-II could not be distinguished significantly ($P = 0.05$) at high inoculum concentrations, viz., 0.158 and 0.5 %w/w (Fig. 1). Contrarily, on wheat significant ($P = 0.05$) differences in percent damping-off between the multinucleate isolates could be discerned at all inoculum concentrations, except at the lowest inoculum concentration (0.005%) (Fig. 1). Although it seemed that the percentage damping-off on wheat decreased with increasing inoculum concentration, this was not significant (Fig. 1).

Binucleate *Rhizoctonia* AG-K caused low percentages of damping-off of canola as well as wheat at all inoculum concentrations (Fig. 1). Inoculation of wheat seedlings with different concentrations of the binucleate *Rhizoctonia* isolate did not result in a significant increase in percentage damping-off of wheat seedlings. However, on canola a significant increase in percentage damping-off of seedlings was observed with an increase in inoculum concentration of the binucleate *Rhizoctonia* (Fig. 1).

Emerging seedling pathogenicity trial

Levene's variance ratio test (Levene, 1960) showed that variability values in the observations of the two independent trials were of comparable magnitude and that an analysis of combined data of the two trials could be validly carried out. The error variance ratio for the

percentage damping-off was $P = 0.3727$ and $P = 0.5647$ for the percentage reduction in dry mass. Combined data of the two trials were analysed.

Analyses of variance were conducted on the percent damping-off and dry mass reduction of seven crops caused by nine AGs of *Rhizoctonia* spp. Significant crop x AG interactions were recorded for both damping-off ($P < 0.0001$) and dry mass reduction ($P < 0.0001$). There were no significant differences in percentage damping-off ($P < 0.0001$) or percentage dry mass reduction ($P < 0.0001$) caused by isolates within seven of the nine AGs. However, a significant difference was present in percentage damping-off ($P < 0.0001$) and percentage dry mass reduction ($P < 0.0001$) caused by isolates within AG-2-1 and AG-2-2 (Table 3).

Similar to the inoculum concentration trial, a significant positive correlation ($P < 0.0001$, $r = 0.97685$) was present between percentage damping-off and percentage dry mass reduction caused by the different AGs on the seven crops (Table 3). Therefore, pathogenicity and relative virulence of the AGs towards the different crops will only be discussed with regard to damping-off results.

Rhizoctonia solani AG-2-1 caused a significant amount of damping-off on canola, lucerne, lupin and medic (Fig. 2). Canola was most susceptible of all crops with 96% of seedlings failing to emerge. Lucerne had significantly less damping-off than the aforementioned crops when inoculated with AG-2-1 (Fig. 2). Barley, clover and wheat showed no significant damping-off when inoculated with AG-2-1 (Fig. 2).

Rhizoctonia solani AG-2-2 caused significant damping-off (between 22% and 88%) on all seven crops (Fig. 2). Of these crops canola, lupin and medic suffered significantly more damping-off (more than 87%) than the other crops (Fig. 2). Clover and lucerne had the least amount (< 25%) of damping-off when inoculated with AG-2-2 (Fig. 2).

Rhizoctonia solani AG-3 was least virulent of all the multinucleate AGs on all seven crops, only causing significant damping-off on canola, lupin and medic (Fig. 2). Damping-off caused by AG-3 did not exceed 28% among the crop plants examined (Fig. 2).

Rhizoctonia solani AG-4-HG-II caused significant damping-off on all seven crops (Fig. 2). Barley and wheat had significantly less damping-off (less than 22%) than the other crops that all had more than 74% damping–off when inoculated with AG-4 HG-II (Fig. 2).

Rhizoctonia solani AG-11 was moderately virulent on five of the seven crops, inlcuding canola, clover, lucerne, lupin and medic (Fig. 2). All of the crops cited above exhibited significantly more damping-off than the control (Fig. 2).

The binucleate *R. solani* isolates did not cause significant damping-off on most of the crops (Fig. 2). AG-I and AG-A only caused significant damping-off on lupin (7.92%) and canola (8.29%) respectively. *Rhizoctonia* AG-K caused a significant amount of damping-off (between 12 and 22%) on canola, lupin, clover and medic (Fig. 2).

The number of *Rhizoctonia* AG's capable of inciting disease on any specific crop plant varied among those evaluated in these studies (Fig. 2). Barley and wheat were least susceptible, only suffering significant damping-off when inoculated with two AGs (AG-2-2 and AG-4 HG-II). Significant damping-off (between 8 and 75%) of clover was incited by four AGs (AG-2-2, AG-4 HG-II, AG-11 and AG-K). Clover and lucerne were susceptible to four of the AGs, showing between 8 and 84% damping-off. Medic was susceptible to six AGs with more than 22% damping-off caused by all AGs. Lupin (Figs 3, 4) and canola were susceptible to six of the AGs (Fig. 2). Lupin was very susceptible to AG-2-1, AG-2-2 and AG-4 HG-II (Figs 2, 3).

Fourteen-day-old seedling pathogenicity trial

Variances in the data from the two independent trials were comparable based upon Levene's variance ratio test (Levene, 1960). The error ratios [P = 0.2562 (root rot), P = 0.9662 (coleoptile rot) and P = 0.3455 (hypocotyl rot)] indicated that an analysis of the combined data of the two trial repeats could be validly carried out.

Analyses of variance were conducted on root rot of seven crops, on coleoptile rot of barley and wheat, and on hypocotyl rot of canola, clover, lucerne, lupin and medic caused by nine *Rhizoctonia* AGs (Table 4, 5). Significant crop x AG interactions were recorded for root ($P < 0.0001$), coleoptile ($P = 0.0228$) and hypocotyl ($P < 0.0001$) rot. Significant differences in root rot severity caused by isolates within AG-4 HG-II (P = 0.0010) and AG-K (P = 0.0125) and for coleoptile rot caused by isolates within AG-4 HG-II (P = 0.0398) were observed (Tables 4, 5).

Rhizoctonia solani AG-2-1 was pathogenic on 14-day-old inoculated seedlings of canola, lucerne, medic and lupin (Figs 5, 6). Fourteen-day-old canola and lupin seedlings had the highest root rot rating, whereas medic and canola seedlings had the highest hypocotyl rot rating. *Rhizoctonia solani* AG 2-1 was only very weakly virulent or not pathogenic at all to barley, wheat and clover (Figs 5, 6). Lucerne also only showed low root- and hypocotyl rot ratings when inoculated with AG-2-1 (Figs 5, 6).

Rhizoctonia solani AG-2-2 caused significant rot (root, hypocotyl or coleoptile) on 14-day-old inoculated seedlings of all the rotation crops, except clover (Figs 5 – 7). AG-2-2 was weakly virulent to barley and wheat, causing slight coleoptile rot. Fourteen-day-old lupin and medic was most susceptible to AG-2-2, having significantly more root- and hypocotyl or coleoptile rot than lucerne, barley and wheat (Figs 5 – 7).

Rhizoctonia solani AG-3 caused significant root rot on canola and lupin, as well as slight, but significant hypocotyl rot on 14-day-old inoculated medic seedlings (Figs 5, 6).

Rhizoctonia solani AG-4 HG-II caused significant disease (either root-, hypocotyl- or coleoptile rot) on 14-day-old seedlings of all the rotation crops (Figs 5 – 7). Hypocotyl and root rot caused by this AG on lupin seedlings are illustrated in Fig. 8. *Rhizoctonia solani* AG-4 HG-II was only weakly virulent on barley and wheat seedlings causing no significant root rot, and only slight coleoptile rot on both crops (Figs 5, 7).

Rhizoctonia solani AG-11 was mildly virulent on 14-day-old inoculated canola, clover, lucerne, lupin and medic seedlings (Figs 5, 6). *Rhizoctonia solani* AG-11 caused significant root rot on all crops except barley and wheat. Wheat coleoptiles were not susceptible to AG-11. Medic had significant more hypocotyl rot than the other crops (Figs 5 – 7).

The binucleate *Rhizoctonia* isolates did not cause significant above ground symptoms (coleoptile or hypocotyl rot) on 14-day-inoculated seedlings of any of the crops, except for AG-K that caused significant hypocotyl rot on medic (Figs 6, 7). *Rhizocotonia* AG-K also caused significant root rot on 14-day-old canola, lucerne, lupin and medic seedlings (Fig. 5).

Recovery of introduced *Rhizoctonia* AGs from plant hypocotyl, coleoptile and roots

The respective AGs were re-isolated from diseased plant material from all three trials, but not from the control plants. *Rhizoctonia* spp. re-isolated from infected seedlings were compared with the original isolate by studying nuclear number and morphological characteristics. All isolates obtained had characteristics similar to the original cultures.

DISCUSSION

In this study the first attempt was made to characterize the pathogenicity and relative virulence of *Rhizoctonia* associated with rotation crops (barley, canola, clover, lucerne, lupin medic, wheat) used in the Western Cape province of South Africa. The pathogenicity and relative virulence of 28 *Rhizoctonia* isolates (representing nine AGs) previously isolated from rotation crops (Chapter 2) varied on emerging seedlings and 14-day-old seedlings of these crops. *Rhizoctonia solani* AG-4 HG-II and AG-2-2 were pathogenic to emerging seedlings as well as 14-day-old inoculated seedlings of all rotation crops. The only exception was 14-day-old clover seedlings that did not develop significant root and hypocotyl rot after inoculation with AG-2-2. *Rhizoctonia solani* AG-2-1 was highly virulent on canola, and moderately virulent on lupin and medic seedlings. *Rhizoctonia solani* AG-3 and AG-11 were pathogenic on some of the crops, but were clearly not as virulent as AG-4 HG-II and AG-2-2. The binuleate *Rhizoctonia* isolates AG-I and AG-A only caused a very low percentage damping-off (< 8.25%) on emerging lupin and canola seedlings respectively. Binucleate isolates of AG-K were weakly virulent on both seedling stages of some crops. Crop wise barley and wheat were most tolerant to all *Rhizoctonia* AGs, whereas canola seemed most susceptible followed by lupin, medic, lucerne and clover.

One of the important aims of this study was to differentiate the pathogenicity and relative virulence of *Rhizoctonia* AGs on emerging seedlings of rotation crops used in the Western Cape province of South Africa. In order to differentiate pathogenicity and relative virulence of *Rhizoctonia* isolates, knowledge of the effect of inoculum concentration on emerging seedlings is of utmost importance. Inoculum concentrations that are too high can result in an inability to distinguish between highly virulent isolates, whereas use of too low inoculum concentration can result in an inability to distinguish the virulence of weakly virulent isolates. The inoculum concentration trial clearly showed that high inoculum concentrations (0.158% w/w and 0.5% w/w) would not allow differentiation between highly virulent AGs of multinucleate isolates (AG-4 HG-II and AG-2-1) on emerging seedlings of

susceptible crops such as canola. Conversely, at too low inoculum concentrations (0.005% and 0.0158%) the virulence and pathogenicity of weakly virulent binucleate isolates (eg. AG-K) may be difficult to determine on a susceptible crop such as canola, as well as on more resistant crops like wheat. Therefore, the 0.05% wt/wt inoculum concentration was considered most appropriate for determining pathogenicity and relative virulence of *Rhizoctonia* AGs on emerging seedlings of different crops. Since the aim of the study was to determine the relative susceptibility to multiple AG's of *Rhizoctonia* spp. among seven crops within the same cropping system, all seven crops had to be evaluated at the same fungal inoculum concentration.

Various other studies have used different inoculum concentrations than what was used in this study. Pathogenicity studies conducted by Sweetingham *et al.* (1986), Yang *et al.* (1994), MacLeod and Sweetingham (1997) used lower inoculum concentrations than what was used in this study. However, these studies all first allowed establishment of the pathogen for at least two weeks within the planting mix, before planting the various crops. Pathogenicity studies conducted by Botha *et al.* (2003), Sterne and Jones (1978), Demirci (1998) and Ogoshi *et al.* (1990) used a higher inoculum concentration than this study. These differences in inoculum concentration used in previous pathogenicity tests could explain why the pathogenicity of some AGs on certain crops in this study, does not correspond to the pathogenicity of these AGs on certain crops in other studies.

In this study AG-2-2 was pathogenic to emerging seedlings of all crops, as well as 14-day-old seedlings of all seven rotation crops, except clover. The pathogenicity of AG-2-2 on emerging seedlings as well as 14-day old seedlings of almost all the rotation crops suggest that this AG could have a significant impact on crop production in the Western Cape province of South Africa, with clover and wheat being least affected. Information on the incidence and pathogenicity of AG-2-2 on the rotation crops evaluated in this study is limited in other locations. AG-2-2 is divided into three intraspecific groups, viz., AG-2-2 IIIB, AG-2-2 IV and AG-2-2 LP (Carling & Sumner, 1992; Kanematsu & Naito, 1995; Hyakumachi *et al*., 1998;

Carling *et al.*, 2002) and pathogenicity studies do not always indicate which intraspecific group has been studied.

Rhizoctonia solani AG-2-1, one of the other subgroups of AG-2, was found highly virulent on canola in this study. AG-2-1 not only caused more than 95% damping-off of emerging canola seedlings, but also incited significant root and hypocotyl rot on 14-day-old canola seedlings. This is the first report of AG-2-1 being a pathogen of canola in South Africa. In western Canada AG-2-1 is also considered as a very important pathogen of canola causing damping-off and root rot of seedling and adult canola (Acharya *et al.*, 1984; Kaminski & Verma, 1985; Hwang *et al*., 1986; Gugel *et al.*, 1987; Kataria & Verma, 1992). Similarly, in Australia AG-2-1 is also regarded as one of the predominant and most virulent AGs on canola causing hypocotyl rot and post-emergence damping-off (Khangura, Barbetti & Sweetingham, 1999). In concert, the data from these studies indicate that AG-2-1 is an important pathogen limiting canola production on a worldwide basis.

Rhizoctonia solani AG-2-1 was moderately virulent on both seedling stages of lupin and medic in this study. This AG does not seem to be a common pathogen of lupin and medic as no other reports of its pathogenicity on these crops were uncovered. This is also the first report of AG-2-1 as a pathogen of lupin and medic in South Africa, indicating that it could have the potential to reduce crop yields.

Rhizoctonia solani AG-2-1 was not virulent on clover, wheat and barley, but moderately virulent on lucerne in this study. AG-2-1 caused 10.2% damping-off of emerging lucerne seedlings, as well as significant root and hypocotyl rot on 14-day-old inoculated seedlings. Contrarily, Eken and Demirci (2003) reported that AG-2-1 is not pathogenic on lucerne. Barley, clover and wheat did not have significant disease symptoms at the emerging seedling or 14-day-old seedling stage when inoculated with AG-2-1 in this study. However, Wong *et al.* (1985) reported that AG-2-1 is highly virulent to subterranean clover in Western Australia, and Demirci (1998) found AG-2-1 to be moderately virulent on barley and wheat.

Rhizoctonia solani AG-4 has a wide host range causing damping-off of seedlings and root rot, crown rot, root canker, stem canker and stem blight of older plants (Anderson, 1982; Carling & Sumner 1992; Vincelli & Herr 1992; Kulik & Dery, 1995). AG-4 is subdivided into two groups, viz., HG-I and HG-II based on sclerotial form and differences in DNA base sequence homology (Vilgalys, 1988; Sneh *et al.*, 1991). However, these groups are often only referred to as AG-4 when recording pathogenicity.

In this study *R. solani* AG-4 HG-II was highly virulent on lupin, medic, clover, canola and lucerne. *Rhizoctonia solani* AG-4 HG-II caused more than 74% seedling damping-off, as well as significant rot (root- and hypocotyl rot) on 14-day-old seedlings of these crops. *Rhizoctonia solani* AG-4 (subgroups unknown) has also been found pathogenic to lupin, medic, clover, canola and lucerne in other countries. Pemberton *et al.* (1998) reported that *R. solani* AG-4 caused crown discolouration, severe root rot and 69% plant death of clover (*Trifolium vesiculosum* Savi). AG-4 has been reported to cause various symptoms on lucerne including black root canker, pre- and post-emergence damping off of mature plants and stem canker (Vincelli & Herr, 1992; Kulik, Dery & Douglass, 1995; Eken & Demirci, 2003). AG-4 has also been reported as a pathogen of lupin cv. Ultra (Leach & Clapham, 1992) and medic (27 annual *Medicago* spp.) (Kulik & Dery, 1995). However, Leach and Clapham (1992) reported that AG-4 infected lupin (*L. albus*), but that this AG produced only small lesions on stems. *Rhizoctonia solani* AG-4 has been widely reported as a pathogen of canola causing damping-off and root rot on adult canola (Acharya *et al.*, 1984; Kaminski & Verma, 1985; Hwang *et al*., 1986; Gugel *et al.*, 1987; Yitbarek *et al.*, 1987; Teo *et al.*, 1988; Yitbarek *et al.*, 1988; Kataria & Verma, 1990; Kataria & Verma, 1992; Baird, 1996).

Among the rotation crops examined, *Rhizoctonia solani* AG-4 HG-II was least virulent to wheat and barley. AG-4 HG-II did not cause more than 22% damping-off of emerging seedlings, and was only mildly virulent on 14-day-old wheat and barley seedlings causing slight coleoptile rot. Contrary to the results of this study, Ogoshi, Cook & Bassett (1990) found that isolates of AG-4 were not pathogenic at all on barley and wheat. Demirci (1998)

found AG-4 to be highly virulent on barley cv. Tokak and wheat cv. Kirik in Turkey (Demirci, 1998). The fungus was also found to cause damping-off and root rot of wheat in Texas and in Western Australia (Mathieson & Rush, 1991; Rush *et al.*, 1994; Yang *et al.,* 1994). In Arkansas, Canada and England, *R. solani* AG-4 has been reported as the causal agent of sharp eyespot on wheat, causing seedling damping-off but not infecting roots (Sterne & Jones, 1978; Rush *et al.*, 1994). In South Africa, AG-4 has not been associated with sharp eyespot of wheat, with *R. cerealis* (AG-D) being the only identified AG (Scott *et al.*, 1979). Previously, *R. solani* AG-6 was found to be pathogenic on wheat in South Africa causing crater disease (Meyer *et al*., 1998). The fact that AG-4 HG-II was the most prevalent *Rhizoctonia* AG in the trial evaluated at Tygerhoek (Chapter 2), and that it is less virulent on barley and wheat than on the dicotyledonous crops in this study, has important implications for selecting the appropriate cropping systems for the winter rainfall region of the Western Cape province. It is therefore important to rotate dicotyledonous (canola, clover, lucerne, lupin, medic) with monocotyledonous (barley and wheat) crops in the region.

In this study AG-11 was moderate to weakly virulent to emerging seedlings as well as 14-day-old inoculated seedlings of all crops with the exception of barley and wheat. This appears to be the first report of AG-11 as a pathogen of canola, clover, medic and lucerne. Lupin was the most susceptible of all the rotation crops suffering 50% damping-off when inoculated with AG-11. *Rhizoctonia solani* AG-11 (Carling *et al.*, 1994) was first described as a pathogen of lupin in 1986 in Australia (Sweetingham, Cruickshank & Wong, 1986), but there is no information concerning its pathogenicity toward canola, clover, medic and lucerne. In this study AG-11 was not pathogenic on wheat, and only weakly virulent to 14-day-old barley seedlings. Contrarily, Demirci (1998) found AG-11 to be highly virulent on wheat as well as barley in Turkey. Sweetingham *et al.* (1986) and Carling *et al.* (1994) found that AG-11 caused coleoptile rot of wheat in greenhouse and growth-chamber studies. This discrepancy could be due to the fact that Sweetingham *et al.* (1986) first allowed the colonisation and establishment of AG-11 in the growth medium before sowing seeds. Demirci (1998) used

wheat kernels and Carling *et al.* (1994) used agar disks as inoculum source, that were placed in indirect contact with the seeds during planting.

Rhizoctonia solani AG-3 was weakly virulent on emerging as well as 14-day-old inoculated canola, lupin and medic seedlings. This is the first report of AG-3 being pathogenic on lupin, canola and medic seedlings. Only two other studies reported on the virulence of AG-3 to these crops, indicating that AG-3 is not pathogenic on lupin (Leach & Clapham; 1992) and lucerne (Eken & Demirci, 2003). Interestingly, AG-3 is best known as a pathogen of potato (*Solanum tuberosum* L.) causing root and stem rot (Carling & Sumner, 1992).

The binucleate *Rhizoctonia* isolates AG-A and AG-I caused a minor, though statistically significant, incidence of damping-off of emerging seedlings. The significant damping-off caused by AG-I and AG-A on lupin and canola respectively is difficult to interpret. It can be hypothesised that these isolates will most likely not be pathogenic in natural soil ecosystems where inoculum concentrations will most likely be lower than that evaluated in this study. This is especially true considering that, in the inoculum concentration trial, there was a significant increase in the percentage damping-off caused by a binucleate isolate (AG-K) with increasing inoculum concentration on a more susceptible crop like canola. Furthermore, it is very important to consider that in a native soil system, the fungus will interact with a competitive soil microflora that can significantly reduce the capacity of *Rhizoctonia* isolates to incite disease (Smiley & Uddin, 1993; Mazzola, Wong & Cook, 1996). In pathogenicity tests conducted in Turkey, AG-I was not pathogenic on lucerne (cv. Bilensoy) (Eken & Demirci, 2003). Contrarily, in Australia, Khangura *et al.* (1999) reported that some binucleate isolates are pathogenic on canola. Lamprecht, Knox-Davies & Marasas, (1988) reported that an unidentified binucleate *Rhizoctonia* sp. was pathogenic on medic in the winter rainfall region of South Africa.

Rhizoctonia AG-K was the only binucleate AG that caused significant damping-off of emerging seedlings as well as rot on 14-day-old seedlings of canola, lucerne, lupin and medic.

In Turkey AG-K was also found moderately virulent on lucerne (cv. Bilensoy) (Eken & Demirci, 2003). In Australia AG-K was found to be weakly virulent on canola (Khangura *et al.*, 1999). However, in western Australia AG-K was not found pathogenic on subterranean clover (Wong *et al.*, 1985). In this study AG-K was not pathogenic on wheat and barley. Various other studies worldwide also found that AG-K was not pathogenic on wheat and barley (Ogoshi *et al.,* 1990; Yang *et al.*, 1994; Demirci, 1998). Altogether, especially considering the inoculum concentration trial data, the role of AG-K as a pathogen will most likely be influenced by inoculum concentration levels as well as environmental conditions.

One important factor that should be considered when evaluating pathogenicity and relative virulence of *Rhizoctonia* is that pathogenicity will most likely be a function of inoculum concentration, host cultivar susceptibility and environmental conditions. In this study the susceptibility of only one cultivar of each crop was evaluated. However, Smith *et al*. (2003) reported that wheat genotypes differed in their susceptibility to AG-8. Kulik and Dery (1995) further reported slight differences in the susceptibility of *Medicago* spp. to AG-4. Therefore, future studies should evaluate more cultivars of the rotation crops. According to this study inoculum concentration can have a significant effect on disease development, or lack thereof, on emerging seedlings of hosts. Botha *et al.* (2003) also found that inoculum concentration can influence the virulence of *Rhizoctonia* isolates. Therefore, it is important that future studies should be aimed at establishing inoculum concentrations of specific AGs in natural soil ecosystems using techniques such as quantitative real-time polymerase chain reaction (PCR).

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Table 1. *Rhizoctonia* isolates used for pathogenicity tests.

Nuclear status	Anastomosis group	Accession number[z]	Origin
Binucleate	AG-A	PPRI 7414	Lucerne
		PPRI 7415	Barley
		PPRI 7416	Medic
	AG-I	PPRI 7420	Barley
		PPRI 7421	Lupin
		PPRI 7422	Wheat
	AG-K	PPRI 7423	Lucerne
		PPRI 7424	Lupin
		PPRI 7425	Canola
	Unidentified *Rhizoctonia*	PPRI 7417	Wheat
		PPRI 7418	Wheat
		PPRI 7419	Wheat
Multinucleate	AG-2-1	PPRI 7426	Lupin
		PPRI 7427	Canola
		PPRI 7428	Medic
	AG-2-2	PPRI 7429	Medic
		PPRI 7430	Medic
	AG-3	PPRI 7431	Lupin
		PPRI 7432	Canola
		PPRI 7433	Wheat
	AG-4 HG-II	PPRI 7434	Barley
		PPRI 7435	Wheat
		PPRI 7436	Medic
		PPRI 7437	Barley
		PPRI 7438	Wheat
		PPRI 7439	Medic
	AG-11	PPRI 7440	Lupin
		PPRI 7441	Lupin

[z]Cultures deposited in the National Collection of Fungi at the ARC-Plant Protection Research Institute in Pretoria, South Africa.

Table 2. Analysis of variance for the effect of different inoculum concentrations (IP) of three anastomosis groups (AGs) of *Rhizoctonia* species on mean percentage damping-off and dry mass reduction of canola and wheat.

		Damping-off		Dry mass reduction	
Source of variation	**Df**	**MS**	**SL**	**MS**	**SL**
Trial(Block)	1	0.5544	0.9478	74.949	0.4471
AG	2	42053.019	<0.0001	36208.991	<0.0001
Crop	1	74065.403	<0.0001	83094.527	<0.0001
IP	4	5193.411	<0.0001	5533.629	<0.0001
Crop x AG	2	11298.396	<0.0001	11528.031	<0.0001
AG x IP	8	738.072	0.0002	708.145	0.0002
Crop x IP	4	1843.013	<0.0001	1868.662	<0.0001
AG x Crop x IP	8	1903.332	<0.0001	1652.897	<0.0001
Experimental Error	29	127.175		126.168	
Sample Error	120	143.319		150.986	
Corrected total	179				

Table 3. Analysis of variance for the effect of different anastomosis groups (AGs) of *Rhizoctonia* species on mean percentage damping-off and dry mass reduction of seven crops following inoculation of emerging seedlings.

		Damping-off		Dry mass reduction	
Source of variation	**Df**	**MS**	**SL**	**MS**	**SL**
Trial(Block)	1	681	0.0024	71	0.4760
Crop	6	22869	<0.0001	16074	<0.0001
Isolate	28	20162	<0.0001	21370	<0.0001
Isolates within AG-2-1	2	2015	<0.0001	3171	<0.0001
Isolates within AG-2-2	1	3910	<0.0001	2745	<0.0001
Isolates within AG-3	2	29	0.6690	46	0.7193
Isolates within AG-4 HG-II	5	130	0.1139	353	0.0003
Isolates within AG-11	1	106	0.2264	97	0.4028
Isolates within AG-A	2	27	0.6878	15	0.8959
Isolates within AG-I	2	14	0.8235	1	0.9930
Isolates within AG-K	2	53	0.4803	31	0.7947
Isolates within UNBR[x]	2	10	0.8704	52	0.6844
Control	0	-	-	-	-
Between AG	9	61731	<0.0001	65237	<0.0001
Crop X Isolate	168	1249	<0.0001	1139	<0.0001
Crop X AG	54	3519	<0.0001	2933	<0.0001
Remainder	133	216		333	
Experimental Error	202	72		138	
Sample Error	406	49		55	
Corrected total	811				

[x]UNBR (unidentified binucleate *Rhizoctonia*).

Table 4. Analysis of variance for the effect of different anastomosis groups (AGs) of *Rhizoctonia* species on root rot of seven crops following inoculation of fourteen-day-old seedlings.

Source of variation	Df	MS	SL
Trial(Block)	1	0.00399	0.9027
Crop	6	27.32	<0.0001
Isolate	28	8.86	<0.0001
Isolates within AG-2-1	2	0.07	0.7719
Isolates within AG-2-2	1	0.47	0.1885
Isolates within AG-3	2	0.03	0.8950
Isolates within AG-4 HG-II	5	1.16	0.0010
Isolates within AG-11	1	0.43	0.2084
Isolates within AG-A	2	0.18	0.5144
Isolates within AG-I	2	0.15	0.5744
Isolates within AG-K	2	1.21	0.0125
Isolates within UNBR[z]	2	0.05	0.8313
Control	0	-	-
Between AG	9	26.45	<0.0001
Crop x Isolate	168	1.17	<0.0001
Crop x AG	54	2.95	<0.0001
Remainder	133	0.35	0.0412
Experimental Error	202	0.27	
Sample Error	406	0.12	
Corrected total	811		

[z]UNBR (unidentified binucleate *Rhizoctonia*).

Table 5. Analysis of variance for the effect of different anastomosis groups (AGs) of *Rhizoctonia* species on the severity of coleoptile rot of barley and wheat, and hypocotyl rot of canola, clover, lucerne, lupin and medic following inoculation of fourteen-day-old seedlings.

	Coleoptile rot			Hypocotyl rot		
Source of variation	**Df**	**MS**	**SL**	**Df**	**MS**	**SL**
Trial(Block)	1	0.0797	0.6408	1	0.6422	0.1457
Crop	1	10.14	<0.0001	4	18.75	<0.0001
Isolate	28	2.97	<0.0001	28	11.95	<0.0001
Isolates within AG-2-1	2	0.01	0.9724	2	0.75	0.0856
Isolates within AG-2-2	1	0.00	1.0000	1	0.10	0.5648
Isolates within AG-3	2	0.01	0.9724	2	0.77	0.0803
Isolates within AG-4 HG-II	5	0.91	0.0398	5	0.58	0.9461
Isolates within AG-11	1	0.02	0.8138	1	0.02	0.7961
Isolates within AG-A	2	0.07	0.8242	2	0.09	0.7413
Isolates within AG-I	2	0.02	0.9456	2	0.04	0.8756
Isolates within AG-K	2	0.20	0.5743	2	0.04	0.8756
Isolates within UNBR[z]	2	0.004	0.9891	2	0.05	0.8464
Control	0	-	-	0	-	-
Between AG	9	8.66	<0.0001	9	36.46	<0.0001
Crop x Isolate	28	0.51	0.1467	112	1.66	<0.0001
Crop x AG	9	0.86	0.0228	36	4.41	<0.0001
Remainder	38	0.31	0.7038	95	0.35	0.1912
Experimental Error	57	0.36		144	0.30	
Sample Error	116	0.10		290	0.17	
Corrected total	231			579		

[z]UNBR (unidentified binucleate *Rhizoctonia*).

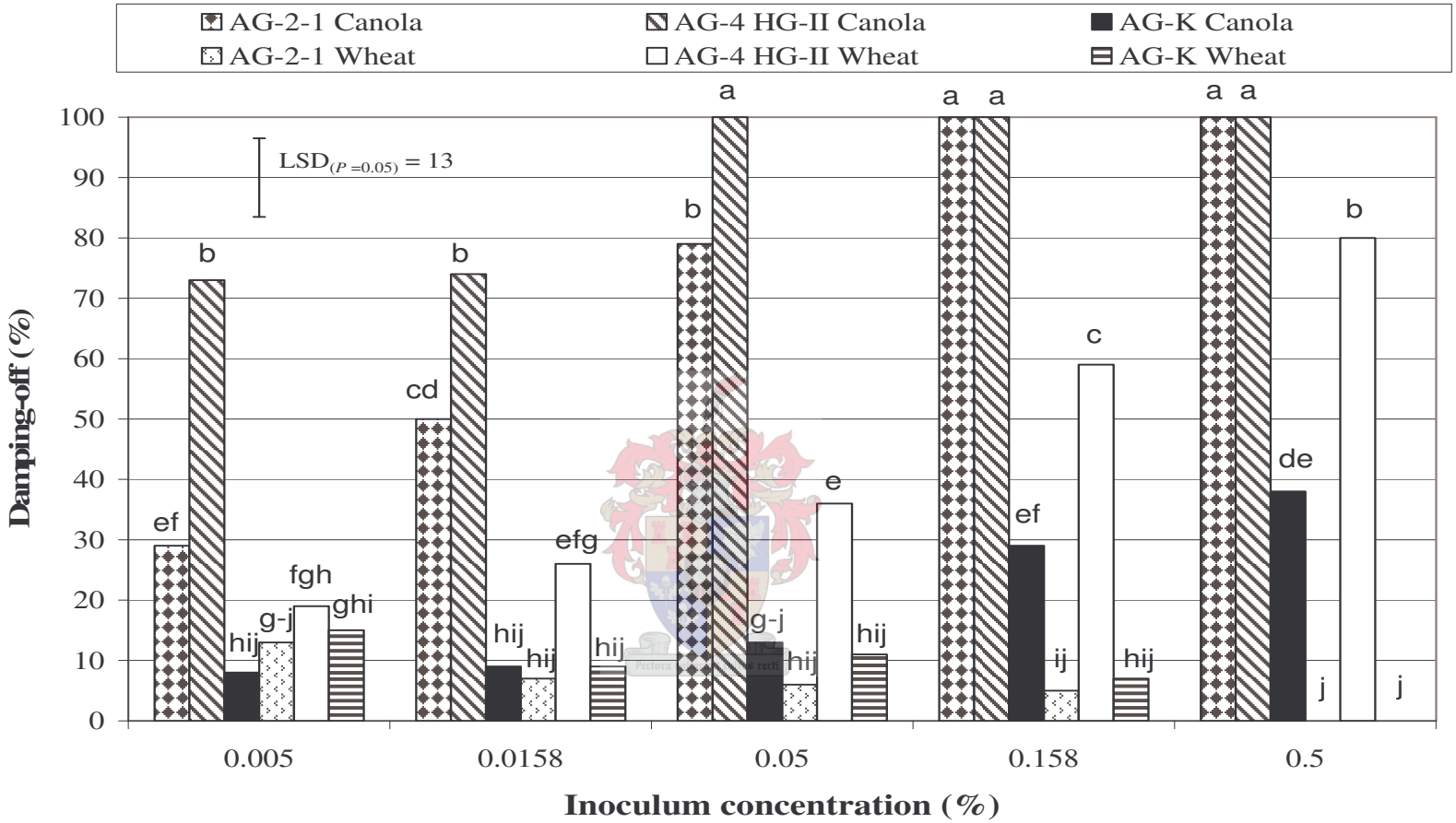

Figure 1. The influence of inoculum concentration on the mean percentage damping-off of emerging canola and wheat seedlings caused by different anastomosis groups (AGs) of *Rhizocotnia* spp. Values are the mean percent damping-off of two replications and data are pooled over two experiments.

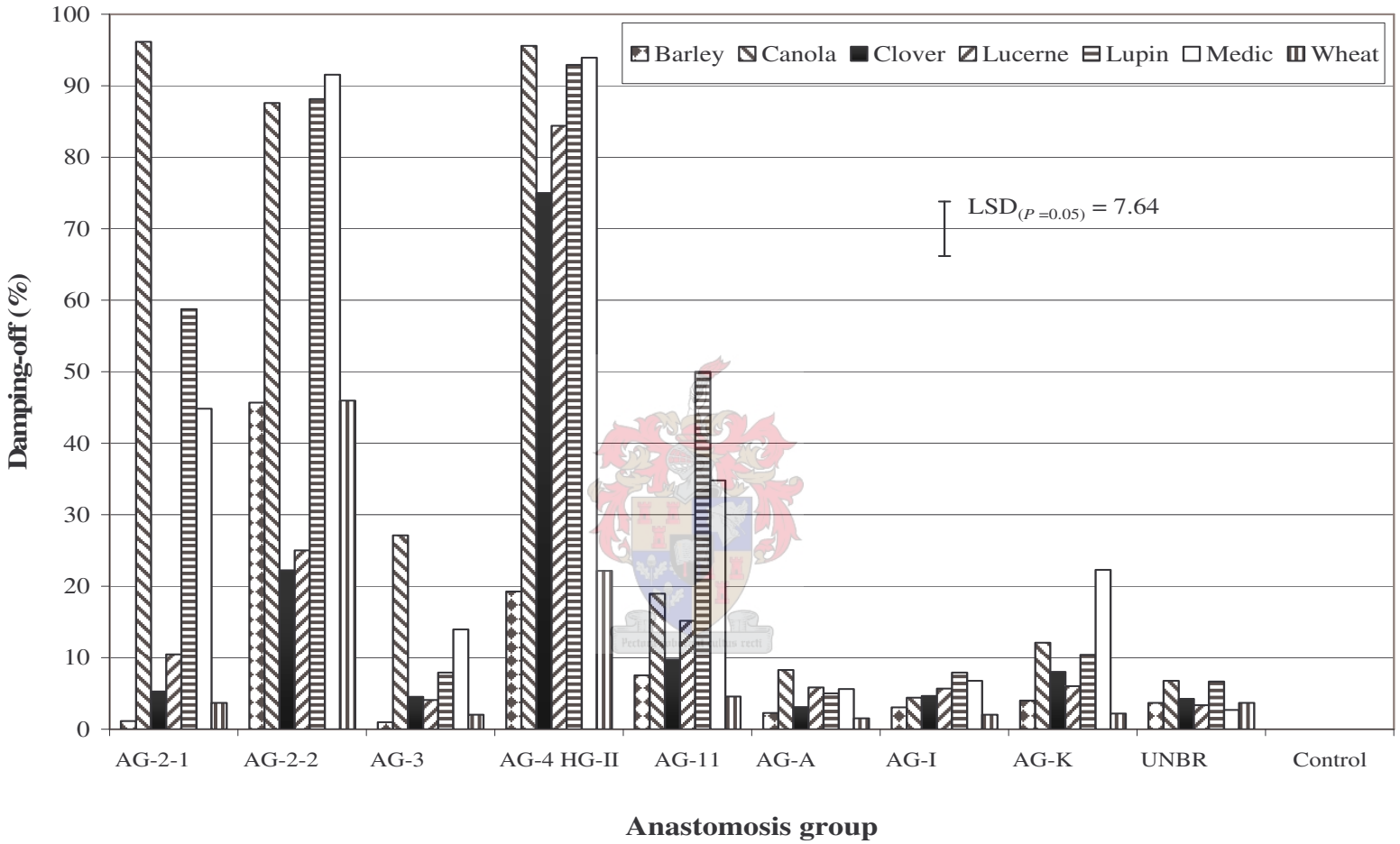

Figure 2. Mean percentage damping-off of emerging seedlings caused by different anastomosis groups (AGs) including UNBR (unidentified binucleate *Rhizoctonia*) of *Rhizoctonia* species in artificially inoculated steam pasteurised soil. Values are the mean percent damping-off of two replications. Data are pooled over two experiments.

Figure 3. Survival of lupin seedlings 14 days after planting in soil artificially infested with multinucleate *Rhizoctonia* A) AG-2-1, AG-2-2 and AG-3; B) AG-4 HG-II and AG-11.

Figure 4. Survival of lupin seedlings 14 days after planting in soil artificially infested with binucleate *Rhizoctonia* AG-A, UNBR (unidentified binucleate *Rhizoctonia*), AG-I and AG-K.

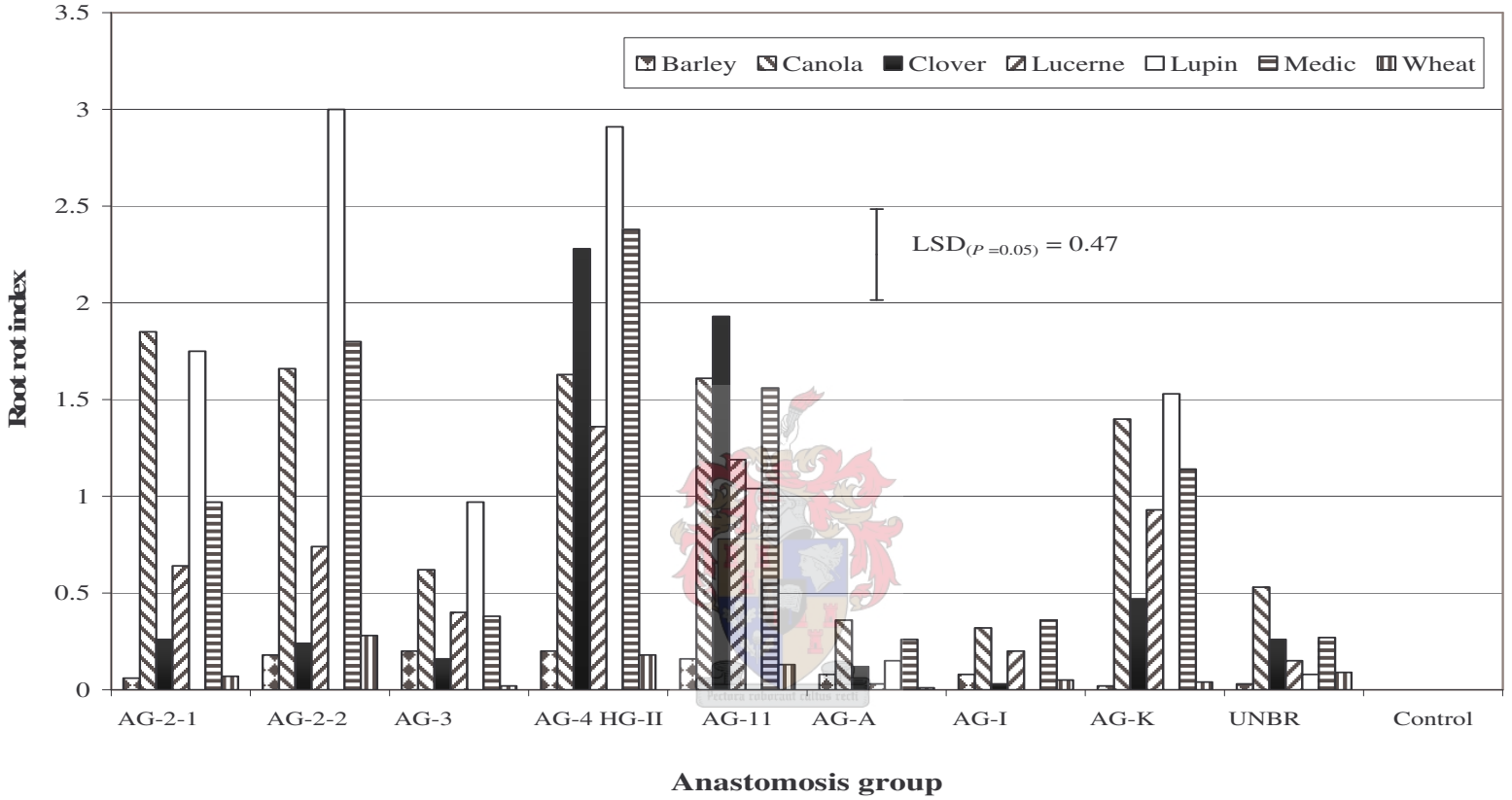

Figure 5. Mean root rot index of 14-day-old seedlings caused by different anastomosis groups (AGs) including UNBR (unidentified binucleate *Rhizoctonia*) of *Rhizoctonia* species in artificially inoculated steam pasteurised soil. Values are the mean root rot index of two replications and data are pooled over two experiments. The value index was a 0 to 4 scale, with 0 = no obvious symptoms; 1 = slight discolouration or small superficial lesions; 2 = moderate discolouration or extensive but non-girdling lesions; 3 = extensive discolouration of tissue or girdling lesions; and 4 = plant dead.

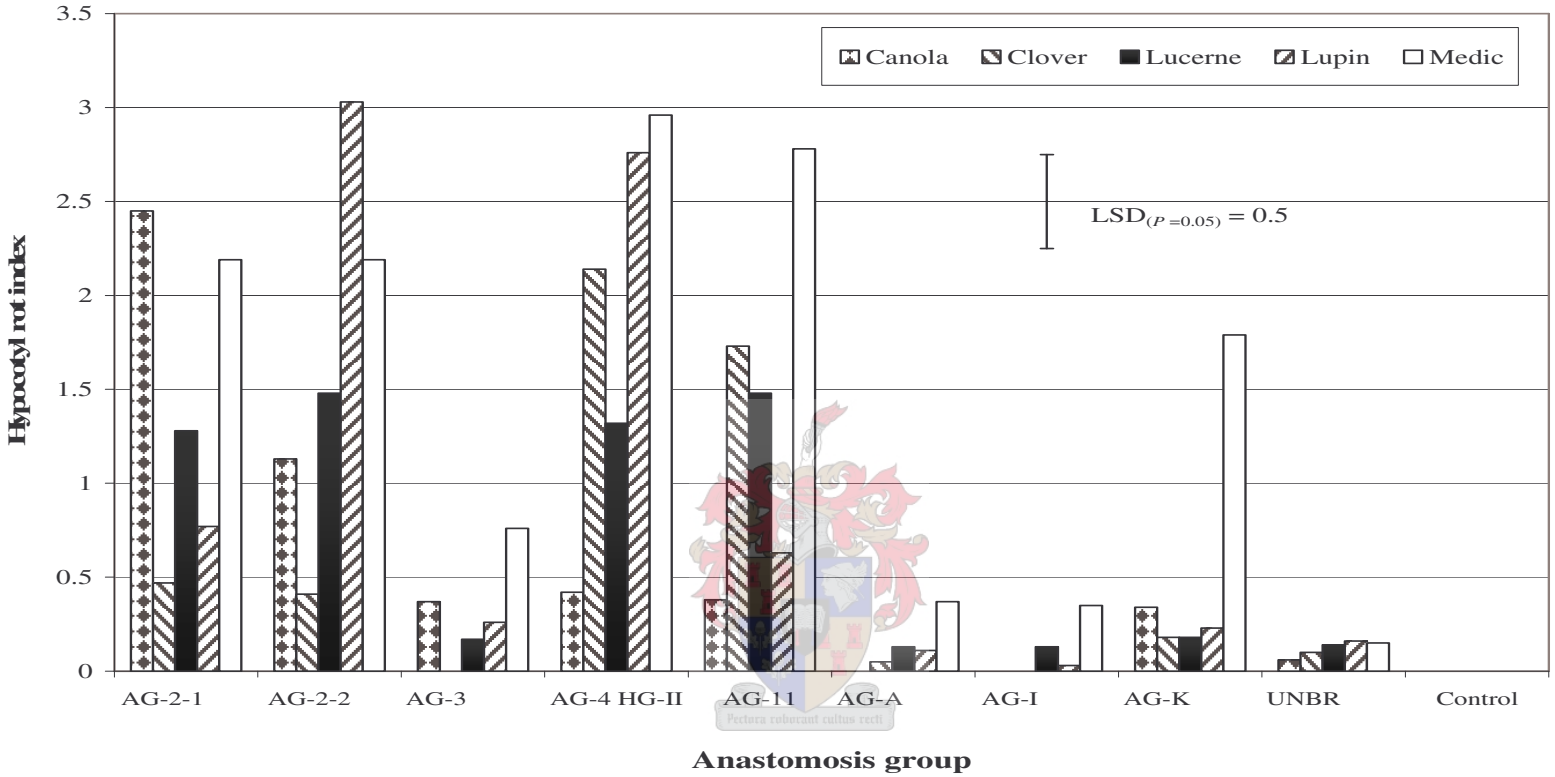

Figure 6. Mean hypocotyl rot index of 14-day-old seedlings caused by different anastomosis groups (AGs) including UNBR (unidentified binucleate *Rhizoctonia*) of *Rhizoctonia* species in artificially inoculated steam pasteurised soil. Values are the mean hypocotyl rot index of two replications and data are pooled over two experiments. The value index was a 0 to 4 scale, with 0 = no obvious symptoms; 1 = slight discolouration or small superficial lesions; 2 = moderate discolouration or extensive but non-girdling lesions; 3 = extensive discolouration of tissue or girdling lesions; and 4 = plant dead.

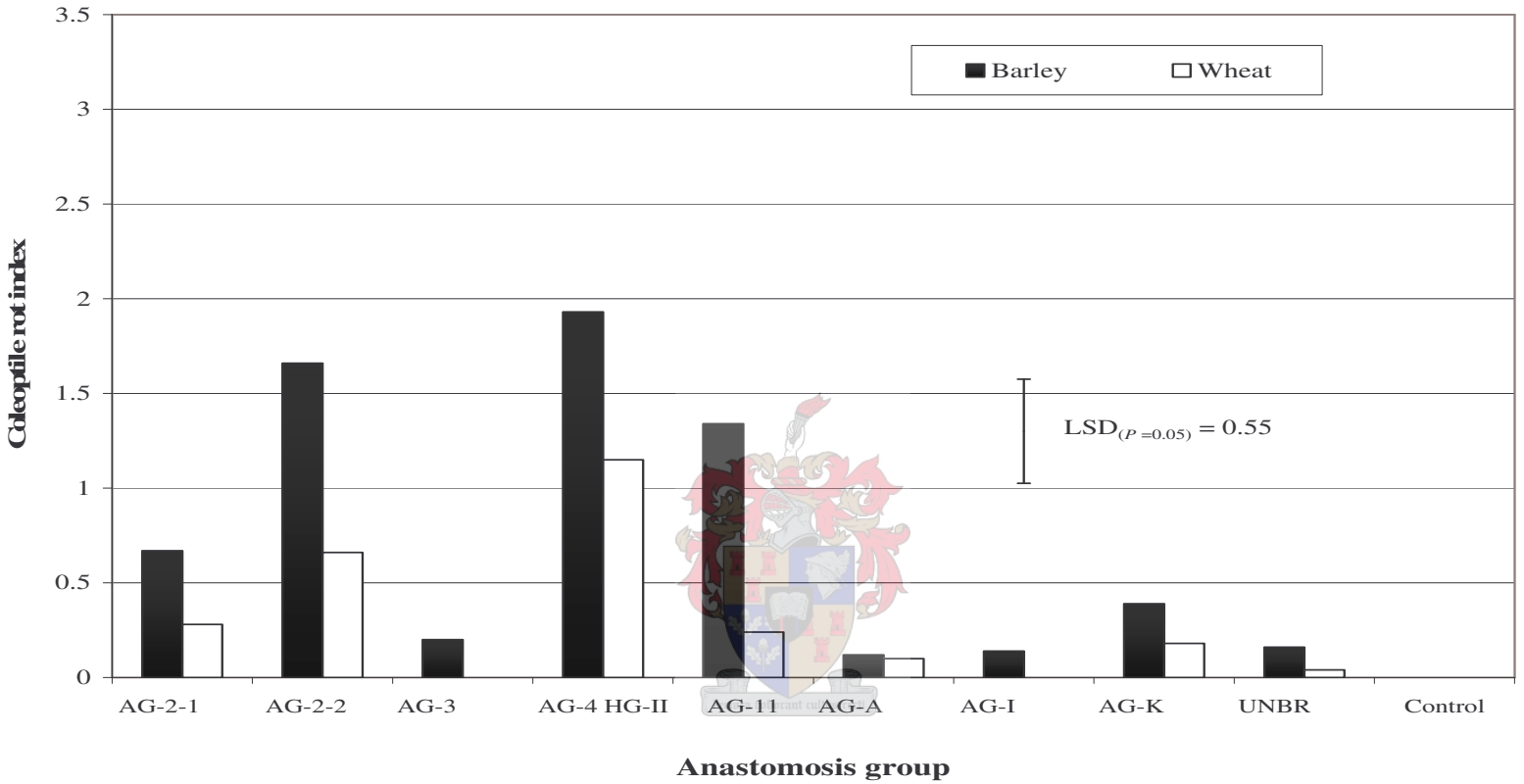

Figure 7. Mean coleoptile rot index of 14-day-old barley and wheat seedlings caused by different anastomosis groups (AGs) including UNBR (unidentified binucleate *Rhizoctonia*) of *Rhizoctonia* species in artificially inoculated steam pasteurised soil. Values are the mean coleoptile rot index of two replications and data are pooled over two experiments. The value index was a 0 to 4 scale, with 0 = no obvious symptoms; 1 = slight discolouration or small superficial lesions; 2 = moderate discolouration or extensive but non-girdling lesions; 3 = extensive discolouration of tissue or girdling lesions; and 4 = plant dead.

Figure 8. Hypocotyl and root rot of lupin seedlings caused by *Rhizoctonia solani* AG-4 HG-II