

Characterisation and pathogenicity of *Rhizoctonia* isolates associated with black root rot of strawberries in the Western Cape Province, South Africa

A. Botha^A, S. Denman^{A,D,F}, S. C. Lamprecht^B, M. Mazzola^C and P. W. Crous^{A,E}

^ADepartment of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa.

^BARC-PPRI, Private Bag X5017, Stellenbosch 7599, South Africa.

^CUSDA-ARS, 1104 N Western Ave, Wenatchee, WA 98801. USA.

^DPresent address: Forest Research, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, GU10 4LH, United Kingdom.

^ECentraalbureau voor Schimmecultures (CBS), Fungal Biodiversity Centre, PO Box 85167, 3508 AD, Utrecht, Netherlands.

^FCorresponding author; email: sandra.denman@forestry.gsi.gov.uk

Abstract. Black root rot is an important disease of strawberry caused by a complex of fungi including species of *Rhizoctonia*. In this study, the *Rhizoctonia* species and anastomosis groups isolated from diseased strawberries in the Western Cape Province of South Africa were determined and their pathogenicity and relative virulence assessed. Both binucleate and multinucleate types were recovered from diseased roots and identified as *R. fragariae* and *R. solani*, respectively. Anastomosis grouping of the isolates was carried out on a sub-sample using the conventional method of hyphal fusion, and molecular techniques were employed to confirm results of the former. RFLP analysis of the 28S RNA gene was used to further characterise relationships among the isolates of *Rhizoctonia*. The molecular results correlated with those obtained from the conventional methods. In the sub-sample tested, all isolates of *R. solani* were members of Anastomosis Group 6, whereas three AG types were identified among isolates of *R. fragariae*, viz. AG-A, AG-G and AG-I at a relative occurrence of 69%, 25% and 6%, respectively. Pathogenicity trials were conducted on 8-week-old cv. Tiobelle plants. All *Rhizoctonia* isolates tested were pathogenic to strawberry, but *R. solani* (AG 6) was the most virulent causing severe stunting of plants. *R. fragariae* AG-A and AG-G were not as virulent as *R. solani* but also caused stunting. *R. fragariae* AG-I was the least virulent, and did not cause stunting of the plants; however, it incited small, pale, spreading lesions on infected roots. This is the first species confirmation and AG type identification of *Rhizoctonia* taxa causing root rot of strawberries in South Africa.

Introduction

Black root rot is a serious, yield-limiting disease of strawberries that reduces annual production by about 30% in the Western Cape Province of South Africa, and it is currently recognised as the most important root disease of strawberries in this area (Botha *et al.* 2001). This disease has also been identified in Australia (Porter *et al.* 1999), the Netherlands (Klinkenberg 1955), Japan (Watanabe 1977), the UK (Wardlaw 1927) and the USA (Heald 1920; Coons 1924; Wilhelm and Paulus 1980; Yuen *et al.* 1991; Duniway 1998). Despite its significance, the etiology of black root rot has not yet been fully resolved, and appears to vary according to the site on which it occurs. However, in general, it is accepted that a complex of fungal pathogens is the primary cause of this disease (Denman 1994; Maas 1998; Ellis 2000; Botha *et al.* 2001).

Rhizoctonia spp. (frequently divided into anastomosis groups, AG) have consistently been associated with the black root rot complex (Heald 1920; Coons 1924; Herr 1979; La Mondia and Martin 1989; Denman 1994). Two species, *R. fragariae* and *R. solani* are considered the major pathogens contributing to disease development (Ribeiro and Black 1971; La Mondia and Martin 1989; Martin 1988, 2000). In a study carried out in Connecticut, USA, only 2.7% of the isolates were identified as *R. solani* (AG 5), the rest were *R. fragariae*, including AG-G (52.7%), AG-A (27.3%), and AG-I (17.3%) (Martin 1988). Similar results were obtained in California where *R. fragariae* dominated the population of *Rhizoctonia* recovered from strawberries (Martin 2000). However, the ratio of the different AG types differed from those in Connecticut with AG-A dominating (68%) followed by AG-I (21.3%) and AG-G (10.7%).

Although *Rhizoctonia* spp. were isolated from strawberry plants with symptoms of black root rot in the Western Cape Province of South Africa, isolates were not identified to species level and anastomosis groups were not determined (Denman 1994).

Recently, molecular techniques have enabled researchers to identify AG types of *Rhizoctonia* spp. more efficiently. Nuclear encoded ribosomal RNA genes (rDNA) have been shown to differ sufficiently to distinguish between the different anastomosis groups (Cubeta *et al.* 1991; Mazzola 1997; Gonzalez *et al.* 2001).

Although different species and AG types of *Rhizoctonia* have been associated with the black root rot complex of strawberries, little has been recorded about their potential role in this disease complex. Ribeiro and Black (1971) suggested that *R. fragariae* existed as an endophytic mycorrhiza on strawberries and that environmental conditions might affect the pathogenicity of this fungus. Martin (1988) demonstrated that the relative virulence of anastomosis groups of *R. fragariae* differed in a temperature-dependent manner. AG-I was the most virulent at 15°C causing severe disease, but at higher temperatures AG-A and AG-G were more virulent than AG-I.

The aims of this work were to identify the *Rhizoctonia* spp. associated with black root rot of strawberries in the Western Cape Province and to determine their AG types. In addition, studies were conducted to determine the pathogenicity of each species, and relative virulence of the AG types on strawberries.

Methods

Isolation of Rhizoctonia spp.

During 1999–2000, 630 diseased plants were collected from five strawberry farms in the Western Cape Province. Plants were taken to the laboratory in cooler boxes and prepared for isolations immediately. Leaves were removed and the remainder of the material was washed. Plants were surface disinfested for 1 min in 1% sodium hypochlorite (NaOCl) (prepared from commercial bleach) and 30 s in 50% ethanol, then rinsed twice (1 min each) in sterile water and air dried in a laminar flow cabinet. Isolations were made by dissecting three pieces of tissue approximately 5 mm long from the margin between healthy and diseased tissues of the roots and placing them onto a range of culture media. Crowns were split longitudinally, tissue excised from the centres and two pieces per crown plated onto culture media. Media used were 2% V-8 juice agar (V-8 agar; Galindo and Gallegly 1960), PARPH (*Phytophthora* selective medium; Solel and Pinkas 1984), PARP (a medium selective for *Pythium* adapted from PARPH by omitting the hymexazol), water agar (WA; Biolab, Midrand, South Africa), 1.7% corn-meal agar (CMA; Difco) and fresh, homemade, potato-dextrose agar (PDA; 200 g potatoes, 20 g dextrose, 12 g agar in 1 L water). Hyphal tips of fungi emerging from plant material were subcultured and transferred to divided plates containing carnation-leaf agar (CLA; Fisher *et al.* 1982) in one half of the dish and PDA in the other half. Plates were incubated under near-ultraviolet and cool white light with a 12 h photoperiod at 25°C for 3 weeks. Microscopic examination of cultures was carried out and isolates identified as *Rhizoctonia* spp. (according to Sneh *et al.* 1991) were subjected to the staining procedures outlined by Yamamoto and Uchida (1982). AG typing and

molecular confirmation of identifications were carried out according to the methods described by Mazzola (1997).

Nuclear staining of Rhizoctonia spp.

Nuclear staining was performed using a fluorochrome, acridine orange stain, and viewing the stained specimen through a fluorescence microscope (Yamamoto and Uchida 1982). Isolates were cultured on clarified 2% vegetable-juice agar without CaCO₃ (Yamamoto and Uchida 1982). Four sterilised microscope glass cover slips (Marienfeld cover glasses number 1, 22 × 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 were mounted, mycelium side downwards, into the drop of stain on a microscope slide and examined using a Zeiss Axioskop microscope equipped with an epifluorescence 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.

AG typing using conventional methods

A sub-sample of 50 isolates representing 10% of each species was selected randomly and used for the anastomosis tests. In establishing AG types, the unknown isolates and tester strains (Table 1) were paired by plating one unknown isolate opposite a known AG type on 2% WA 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 100× magnification using a light microscope.

Identification using molecular techniques

The anastomosis results of 20 isolates was confirmed using restriction analysis of the 28S rRNA gene of the isolates, according to the following procedure.

DNA extraction. DNA extraction was carried out according to the method described by Mazzola *et al.* (1996). Erlenmeyer flasks (250 mL) containing 50 mL yeast, malt-extract broth (YM + broth) were

Table 1. Source, species and anastomosis type of *Rhizoctonia* tester isolates

Isolate number	Species	AG type
BN4 ^A	<i>R. fragariae</i>	AG-A
C-662	<i>R. fragariae</i>	AG-A
C-653	<i>R. fragariae</i>	AG-G
AV-2	<i>R. fragariae</i>	AG-I
R-43	<i>R. solani</i>	AG1-IC
F56 L	<i>R. solani</i>	AG2-1
RI-164	<i>R. solani</i>	AG2-2IV
Sc124	<i>R. solani</i>	AG 3
AH-1	<i>R. solani</i>	AG 4 HGI
RH-165	<i>R. solani</i>	AG 4 HGII
RH-184	<i>R. solani</i>	AG 5
HAM1-1	<i>R. solani</i>	AG 6 HGI
1529	<i>R. solani</i>	AG 7
SqR1	<i>R. solani</i>	AG 9
91507	<i>R. solani</i>	AG 10
Roth 25	<i>R. solani</i>	AG 11
AI 1-4	<i>R. solani</i>	AG-BI

^AAll isolates were supplied by M. Cubeta of the University of North Carolina, Vernon James Centre, Plymouth.

inoculated with isolates of *Rhizoctonia* and placed on a rotary shaker at 200 rpm until a large mat of mycelium covered the surface of the medium. The YM + broth contained 3 g yeast extract, 3 g malt extract, 5 g peptone and 20 g glucose added to 1 L water. The mycelium was harvested onto a sterile filter paper disk that was placed in a Buchner funnel. The growth medium was gently suctioned from the mycelium, which was then washed with sterile H₂O. Excess H₂O was removed by blotting the filter paper between two sterile paper towels. Approximately 200 mg of mycelium was placed into a 1.5 mL eppendorf tube and lyophilised overnight without closing the caps of the tubes.

Dried mycelium was ground using a pipette tip and resuspended in 600 µL extraction buffer (200 mM Tris-Cl, pH 8.0, 250 mM NaCl, 25 mM EDTA, pH 8.0, and 0.5% non-sterile SDS) and shaken briefly in a vortex machine. After resuspension, the mixture was microfuged for 5 min at maximum speed and the upper aqueous layer removed to a new tube. The DNA was extracted once with 70% volume phenol and 30% volume chloroform. After centrifugation, the extraction was repeated, the upper layer transferred to a new tube and mixed with ca. 55% volume isopropanol to precipitate the DNA.

After precipitation the solution was microfuged for 1 min at maximum speed to pellet the DNA, and the supernatant was carefully removed and discarded. The DNA was washed once with 70% ETOH, microfuged again for 3 min at maximum speed to reform the DNA pellet and the supernatant was again discarded. The DNA was resuspended in 100 µL TE buffer (10 mM Tris-Cl and 1 mM EDTA, pH 8.0) and placed in a heat block at 55°C for 1 h to dry. The DNA was diluted 4:1000 in TE buffer (10 mM Tris-Cl and 0.1 mM EDTA) for use in PCR.

PCR amplification. PCR amplification of DNA was conducted using the two primers LR7 (5'-TACTACCACCAAGATCT-3') and LROR (5'-ACCCGCTCAACTTAAGC-3') (Cubeta *et al.* 1991). A final volume of 25 µL per reaction was used that included 5 µL dilute DNA (10–20 ng), 2 units *Taq* polymerase, 1× manufacturer's reaction buffer with 1.5 µM MgCl₂, and 200 µM of each deoxynucleoside triphosphate. The amplification reactions were conducted in a DNA Thermal Cycler 480 (Perkin-Elmer Corp., Norwalk, CT) using the following reaction conditions, 94°C for 1 min, 49°C for 2 min and 74°C for 3 min, which were repeated for 30 cycles (Cubeta *et al.* 1991).

Restriction enzyme digestion. Restriction enzyme digestion was used to distinguish between the different AG types. Digestion reactions were set up using isolates of known AG type as well as the unknown isolates. PCR products (2 µL) were mixed with one of the following restriction enzymes *Hpa*II, *Hha*I or *Taq*I (10 units; Invitrogen Life Technologies, Carlsbad, CA, USA), sterile distilled water (15 µL) and enzyme reaction buffer (1×) in a total reaction volume of 20 µL. The mixtures were incubated overnight at room temperature (ca. 25°C). Reaction products were then separated on a 1.5% agarose gel in Tris–borate–EDTA buffer. The gel was stained using ethidium bromide and DNA fragments were viewed using an ultraviolet transilluminator.

Pot trials

Pathogenicity tests were carried out in pot trials using 8-week-old strawberry cv. Tiobelle plants. To ensure that plants were pathogen free, hardened-off, tissue-culture plantlets were used. Plantlets were transplanted into sterilised, composted, pine-bark medium and hardened off with glass jars inverted over them for 4 weeks in the glasshouse at 25–35°C. After the hardening off period, glass jars were removed and plants were kept for an additional 4 weeks before pathogenicity trials were carried out.

Sand-bran inoculum was prepared using the method described by Lamprecht *et al.* (1988). Washed river sand (400 g) and wheat bran (20 g) were mixed together in 500 mL Schott bottles and 60 mL sterile water was added. The mixture was autoclaved at 120°C and 1.5 kg/cm²

for 15 min and then left to cool. This process was repeated for three successive days after which each bottle was inoculated with four 5-mm plugs of a single *Rhizoctonia* isolate and incubated at 25°C for 1 week before use.

Pilot trial

A pilot trial was carried out to determine the level of inoculum that would enable differentiation of pathogenicity and relative virulence of the various *Rhizoctonia* isolates. A single isolate of *R. solani* and one of *R. fragariae* were selected randomly and sand-bran inoculum was prepared as described above. One plant was used for each inoculum concentration (1, 5 and 10% mass of inoculum: mass of planting medium) and uninoculated, sterile sand-bran was used in the controls. Inoculum was mixed into sterilised potting medium and 8-week-old cv. Tiobelle plants were planted into the pots. Plants were kept in a growth chamber at 25°C with a 12 h day / night interval for 5 weeks as described by Martin (2000). The experiment was laid out as a complete randomised design. At the end of the trial, plants were removed from the soil and carefully washed. Five roots were selected at random from each plant and the percentage of root necrosis was calculated by measuring the total length of each root and summing the lengths of the lesions formed on each root, and expressing the necrotic area as a percentage of the root length (Martin 1988). Isolations from necrotic areas that developed on roots were made on PDA as described previously.

Pathogenicity trial

Pathogenicity trials were conducted based on the results of the pilot trial, and an inoculum level of 5% was selected. The planting medium of the control plants was amended with 5% sterile sand-bran. Using results of the anastomosis characterisation, seven isolates of *R. fragariae* (STE-U 4612, 4613, 4614, 4615 all type AG-A; STE-U 4616, 4617 both type AG-G and STE-U 4618 type AG-I) and three isolates of *R. solani* (STE-U 4609, 4610 and 4611) were selected for the pathogenicity trial. Three plants were inoculated per isolate and there were three replications per treatment. Tests were carried out as described previously and the percentage root necrosis was assessed as stated above. In addition to recording percentage root rot, the above ground parts were dried at 60°C for 72 h and dry mass was recorded. The entire experiment was conducted twice.

Statistical treatment of data

All data from the pilot trial and the pathogenicity tests were subjected to analysis of variance (ANOVA) within the framework of a general linear model (PROC GLM), which takes into account unbalanced data. Fisher's Least Significance Difference method (LSD) was used to test for significant differences at the 5% confidence level (Lyman-Ott 1993). The data were analysed using SAS version 8.2 (SAS 1999).

Results

Isolation of *Rhizoctonia* spp.

A total of 284 isolates of *Rhizoctonia* spp. was isolated from 44% of the diseased plants surveyed and the highest number of isolates was obtained on WA. Different morphological types were evident on visual inspection. The isolates were divided into two groups; those with very dark brown mycelium and those with white to pale brown mycelium. Overall, 59.3% of the *Rhizoctonia* species isolated were pale coloured and 40.7% were dark brown.

Table 2. Occurrence of species and AG types of *Rhizoctonia* isolated from strawberries in the Western Cape Province

Species	Occurrence (%) ^A	AG type	Occurrence (%) ^B
<i>R. fragariae</i>	59.3	AG-A	69
		AG-G	25
		AG-I	6
<i>R. solani</i>	40.7	AG 6	100

^APercentage based on the number of isolates of each species out of the total number of *Rhizoctonia* isolates obtained.

^BPercentage based on the number of isolates of each AG type out of the total number of isolates of each species of *Rhizoctonia*.

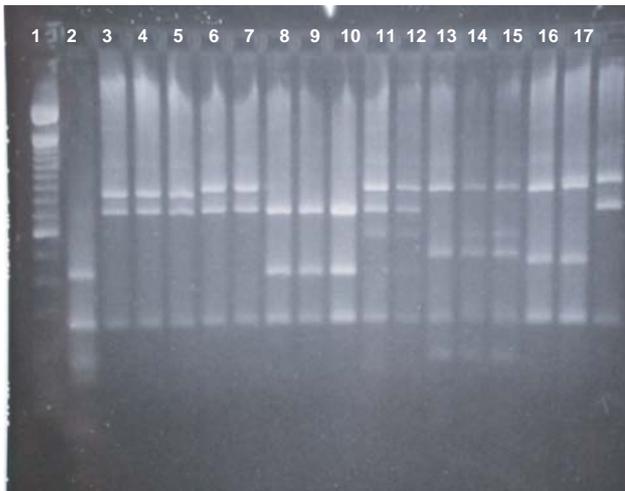


Fig. 1. *Rhizoctonia* isolates digested with a restriction enzyme *Hpa*II. Lane 1: 100-bp ladder, lane 2: F-2 AG 1-1, lane 3: H-18 AG 2-1, lane 4: C-111 AG 2-2, lane 5: C-116 AG 2-2, lane 6: ST-9 AG 3, lane 7: ST-41 AG 3, lane 8: 2-3-2 AG 4, lane 9: 2-5-5 AG 4, lane 10: 6-3-5 AG 4, lane 11: D-1 AG 5, lane 12: E-22-2 AG 5, lane 13: TSH-1-1 AG 6, lanes 14 and 15: unknown isolates (STE-U 5246 and 4611), lane 16: C-1 AG 8, lane 17: S1BS1 AG 8, lane 18: 7-2-2 AG 8. All isolates of known AG type used to compare with the isolates of unknown AG type are maintained in Dr Mazzola's culture collection (USDA-ARS, Wenatchee, USA).

Nuclear staining of *Rhizoctonia* spp.

Nuclear staining revealed that all the dark brown isolates were multinucleate. The main runner hyphae were usually more than 7 µm wide, sclerotia were not differentiated into medulla and rind, and these isolates were, therefore, identified as *R. solani* (Sneh et al. 1991). The pale coloured isolates all had binucleate cells, the main runner hyphae were usually less than 7 µm in diameter and sclerotia formed rarely. Thus, they were identified as *R. fragariae* (Sneh et al. 1991). Of the isolates obtained, 59.3% were *R. fragariae* and 40.7% were *R. solani* (Table 2).

AG typing using conventional methods

All the multinucleate *R. solani* isolates were successfully anastomosed with AG 6, and were, therefore, designated AG 6. Anastomosis tests revealed that there were three AG

groups of *R. fragariae*, viz. AG-A, AG-G and AG-I (Table 2). AG-I was represented at a very low level (6%), AG-A was the most frequently isolated type (69%) whereas 25% of the *R. fragariae* isolates were AG-G.

Identification using molecular techniques

The restriction analysis of the 28S rRNA gene of the isolates confirmed the AG types assigned to the isolates using conventional methods. The banding pattern formed by the restriction enzyme digested DNA is shown in Fig. 1. The two *R. solani* isolates in lanes 14 and 15 had the same banding pattern as the AG 6 isolate in lane 13 (Fig. 1), and were, therefore, identified as AG 6. This process was repeated with all the unknown isolates of both *R. solani* and *R. fragariae* to support the AG types that were determined by analysis of hyphal anastomosis. Polymorphisms were evident in some of the AG-A and AG-G types as also experienced by Mazzola (1997), but the restriction patterns obtained for these isolates conformed to those observed for multiple isolates of the corresponding anastomosis group in North American strains (strains maintained by Mazzola, USDA-ARS, Wenatchee, WA 98801, USA).

Pilot trial

Both *R. solani* and *R. fragariae* were pathogenic to strawberry, causing root lesions, and the pathogens were reisolated from lesions on the roots of plants grown in artificially infested soils. There were no significant differences ($P = 0.05$) in percent root necrosis of plants grown in soils infested with 1% or 5% of *Rhizoctonia* inoculum (Table 3). However, at an inoculum concentration of 10%, the level of root necrosis was significantly higher relative to all other treatments and much of the root system had disintegrated. The pattern of disease severity at the different inoculum levels was similar for both *Rhizoctonia* species. Some degree of root discoloration was evident on some of the control plants, but no pathogenic fungi were isolated from these lesions.

Table 3. Percentage root necrosis formed at different concentrations of *R. fragariae* and *R. solani* inoculum used in the pilot trial

Species	Percentage root necrosis ^A formed at different inoculum concentrations		
	1 (%) ^B	5 (%)	10 (%)
<i>R. fragariae</i>	17 bc ^C	27 bc	76 a
<i>R. solani</i>	22 bc	34 b	58 a
Control	21 bc	7 c	31 bc

^AValues are the mean of five measurements per inoculum concentration.

^BThe percentage inoculum is based on the mass of inoculum as a percentage of the mass of the planting medium.

^CMeans followed by the same letter do not differ significantly ($P = 0.05$).

Table 4. Mean dry mass of the above ground parts of cv. Tiobelle strawberry plants and percentage root necrosis caused by different *Rhizoctonia* species in artificially inoculated soil

Species	AG type	Root necrosis (%) ^A	Standard deviation	Dry mass (g) ^B	Standard deviation
<i>R. fragariae</i>	AG-A	37 ab	28.0	1.81 b ^C	0.47
	AG-G	50 a	32.3	2.00 ab	0.80
	AG-I	21 bc	22.0	2.77 a	0.55
<i>R. solani</i>	AG 6	16 cd	11.5	0.84 c	0.70
Control	—	0 d	00.0	2.70 a	1.08

^AValues are the mean of readings made on five roots per isolate per AG type. Cultures are maintained at the University of Stellenbosch culture collection. Data are pooled over two experiments.

^BValues are the mean of three plants inoculated per isolate of each AG type per experiment.

^CMeans followed by the same letter do not differ significantly ($P = 0.05$).

Pathogenicity trial

R. solani (AG 6) and *R. fragariae* (AG-G, AG-A and AG-I) were pathogenic to strawberry roots causing root necrosis similar to that seen on plants with black root rot symptoms. No root necrosis developed in the control plants (Table 4). The same trends were evident in both experiments and data were therefore pooled. The percentage visible root necrosis caused by *R. solani* was significantly less ($P = 0.05$) than that caused by *R. fragariae*, with the exception of *R. fragariae* (AG-I) (Table 4). The differences in visible root necrosis caused by AG-G and AG-A isolates were not significant, but AG-G caused significantly more root necrosis than *R. fragariae* AG-I.

Root lesions resulting from infection by *R. solani* were small and restricted. Many ring-like lesions occurred along the length of infected roots. In contrast, irrespective of AG type, *R. fragariae* typically incited a single or a few large spreading lesions on strawberry roots. The lesions caused by the AG-I isolates were very pale by comparison with those caused by the other AG types.

Using plant dry mass as an indicator of pathogen virulence, *R. solani* was more virulent than the *R. fragariae* isolates, causing severe stunting, wilting and collapse of young strawberry plants. The mean dry mass of plants inoculated with *R. solani* (AG 6) was significantly lower ($P = 0.05$) than that of any of the other inoculated plants or the controls (Table 4). The dry mass of plants inoculated with *R. fragariae* (AG-A) was significantly lower than that of the control plants or of the AG-I inoculated plants, but was not significantly different from the AG-G inoculated plants (Table 4).

Discussion

This study represents the first attempt to comprehensively characterise the species of *Rhizoctonia* associated with the black root rot complex of strawberries in the Western Cape Province of South Africa. Two species were identified, *R. fragariae* and *R. solani*, and multiple AG types of *R. fragariae* were also confirmed. RFLP analysis of the 28S rRNA gene provided further evidence to support the

identification of the AG types, and these techniques could be readily carried out in the laboratory, whereas interpretation of the anastomosis reaction was not always easy.

Identification of the *Rhizoctonia* spp. and anastomosis groups has made it possible to compare the Western Cape Province situation with that in the USA (Connecticut and California), where black root rot is also a problem. *R. solani* was isolated at a much higher incidence in the former than in the latter areas. Furthermore, *R. solani* AG 6 was present in the Western Cape Province in contrast to *R. solani* AG 5 which was present in both Connecticut and California (Martin 1988, 2000). While in the Western Cape *R. solani* (AG 6) was the most virulent *Rhizoctonia* sp. associated with the black root rot complex, *R. solani* (AG 5) is not considered an important pathogen in root disease of strawberries in Connecticut or California (Martin 1988, 2000).

The binucleate isolates (*R. fragariae*) in the Western Cape Province contained the same three anastomosis groups (AG-A, AG-G, AG-I) as those recorded from the USA. The relative frequencies at which these AG types occurred in the sub-sample may not be representative of the entire area. In the sub-sample, AG-A was the most frequently isolated AG type, which is contrary to that reported from Connecticut where AG-G was the most frequently isolated strain. The ecological significance of the occurrence of the different AG types has yet to be established.

The pathogenicity tests elucidated differences in the severity and morphology of the lesions caused by the two species of *Rhizoctonia*. In the present study, *R. solani* was more virulent than *R. fragariae* causing root necrosis, wilting and severe stunting of plants. Plants inoculated with *R. fragariae* AG-A and AG-G were only slightly stunted although root tip necrosis was clearly evident.

Despite *R. solani* being more virulent than *R. fragariae*, the percentage root necrosis caused by *R. solani* was significantly lower than that caused by *R. fragariae* (AG-A and AG-G). AG-A and AG-G usually caused a single, large lesion at the root tips, which rendered only the basal portion of the root dysfunctional. On the other hand, much smaller lesions were caused by *R. solani*, and more than one lesion

occurred over the length of the root. Frequently, lesions caused by *R. solani* were formed just below the crown area of the plant, causing the roots to break off or become non-functional near the crown. The absorption and transport capacity of the root system would, therefore, be greatly reduced, which would prevent nutrients and water from reaching the above-ground plant parts. This might explain why plants infected with *R. solani* in the pathogenicity tests had a very low dry mass, even though the percentage root necrosis was less than that of plants infected with the *R. fragariae* isolates.

The AG-I isolate caused lesions that were comparable to those induced by *R. solani* as opposed to those caused by the other *R. fragariae* anastomosing groups. However, the lesions caused by AG-I were far less severe and did not occur as close to the crown area as those caused by *R. solani*. It has been shown that AG-I is more virulent under cooler conditions (10–15°C) (LaMondia and Martin 1989; Martin 2000). The tests carried out in the present study were conducted at 25°C and thus the relative virulence observed for the South African AG-I isolates may indicate that environmental conditions will have a significant impact on the interaction between these fungi and the plant host. Similar findings have been reported for other species and anastomosis groups of *Rhizoctonia* (Burton *et al.* 1988; Smiley and Uddin 1993). It will, therefore, be important to establish the effects of temperature on the virulence of the different species and anastomosis groups so that this information can be incorporated into an integrated disease management program.

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