***Rhizoctonia* Anastomosis Groups Associated with Diseased Rooibos Seedlings and the Potential of Compost as Soil Amendment for Disease Suppression**

Y. T. Tewoldemedhin and **S. C. Lamprecht**, ARC-Plant Protection Research Institute, Private Bag X5017, Stellenbosch, 7599, Western Cape, South Africa; and **M. Mazzola**, United States Department of Agriculture–Agricultural Research Service, Tree Fruit Research Laboratory, Wenatchee, WA 98801

Abstract

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Rhizoctonia spp. associated with rooibos in the Western Cape province of South Africa were recovered during the 2008 season by planting seedlings in rhizosphere soils collected from 14 rooibos nurseries. In all, 75 *Rhizoctonia* isolates were obtained, of which 67 were multinucleate and 8 were binucleate *Rhizoctonia* spp. The identity of these isolates to anastomosis group (AG) was determined through sequence analysis of the ribosomal DNA internal transcribed spacer region. The collection of multinucleate isolates included representatives of AG-2-2 (67%), AG-4 HGI (14%), AG-11 (5%), and *R. zeae* (3%). Binucleate AGs included AG-Bo (4%) and AG-K (4%) and an unidentified binucleate *Rhizoctonia* (3%). *Rhizoctonia solani* AG-2-2 was the most widely distributed species of *Rhizoctonia* detected among the 11 nurseries sampled.

All AGs recovered from rooibos have been previously reported on crop plants in South Africa, with the exception of *R. zeae*. However, this is the first study to classify the *Rhizoctonia* AGs recovered from rooibos. In glasshouse bioassays, the most virulent *Rhizoctonia* AGs on rooibos and lupin were AG-2-2, AG-4 HGI, and AG-11. Although plant damage was less than that observed for lupin and rooibos, oat was significantly affected by AG-2-2 and AG-4 HGI. Two composts sourced from independent suppliers were evaluated for disease suppression under glasshouse conditions. Compost amendment suppressed damping-off by most *R. solani* AGs, except for AG-4 HGI. Furthermore, within AG-2-2, suppression by compost was isolate specific.

Rooibos (*Aspalathus linearis* (N. L. Burm.) R. Dahlgr.), a leguminous perennial plant, grows naturally in the Cederberg Mountains of the Western Cape province of South Africa. This indigenous plant is grown for its health benefits and the demands for its herbal tea (Joubert et al. 2008). Domesticated production of rooibos starts at nurseries by growing seedlings from rooibos seed for approximately 4 months prior to transplanting into commercial rooibos fields. A complex of soilborne pathogens, including *Rhizoctonia* spp., significantly limits seedling production in rooibos nurseries, where losses of 50% or more can occur on seedlings not treated with proper fungicides (Bahramisharif et al. 2014).

The worldwide occurrence of *Rhizoctonia* spp. as pathogens on a number of crops has been well documented (Sneh et al. 1996). In South Africa, *Rhizoctonia* spp. have been recorded in a number of field crops, and previous work showed that the population of *Rhizoctonia* spp. is composed of diverse anastomosis groups (AGs) within the consortia of bi- and multinucleate species (Lamprecht et al. 2011; Tewoldemedhin et al. 2006). *Rhizoctonia solani* was previously reported as a causal agent of damping-off and crown rot of rooibos seedlings in nurseries (Engelbrecht et al. 1983; Janse van Rensburg and Lamprecht 1997); however, delineation of AGs among the *Rhizoctonia* isolates was not determined. In order to develop an effective management strategy for control of damping-off in organic rooibos nursery systems, it is essential to characterize the *Rhizoctonia* AGs that are responsible for damping-off and crown rot of rooibos seedlings. Although several registered fungicides are used to control seedling diseases of rooibos, at present there is a significant need for development of practical and economically viable measures for control of these diseases in organic nurseries.

Rooibos growers in South Africa use different management strategies to control soilborne diseases of rooibos. Under nursery conditions, growers use crop rotation as a cultural practice to reduce soilborne disease pressure. There is a vast array of literature on the effects of crop rotation on diseases of field crops (Lamprecht et al. 2006; Schaafsma et al. 2005). Studying crop rotation as a management strategy against Fusarium crown rot of wheat in the Western Cape Province of South Africa, Lamprecht et al. (2006) demonstrated that different nonhost crops differ in their efficacy to break disease cycles. Although rooibos is rotated with crops such as oat (*Avena sativa* L.) and lupin (*Lupinus angustifolius* L.), the relative efficacy of these crops to reduce rooibos diseases caused by *Rhizoctonia* spp. is unknown. Of significance is the observation that *Rhizoctonia* spp. can cause soilborne diseases of lupin and oat (Lamprecht et al. 2011; Neate 1989); however, the capacity of these species to incite disease of rooibos has not previously been examined. In a recent study, Bahramisharif et al. (2014) showed that *Pythium* spp. that are highly virulent on rooibos are less virulent on lupin and oat.

Cultural practices have been reported to have a direct effect on soil microbial communities which, in turn, can function to suppress the incidence of soilborne plant diseases (Abawi and Widmer 2000; Mazzola 2004). Application of organic amendment to soil is a cultural practice commonly reported to have potential for control of soilborne diseases (De Ceuster and Hoitink 1999). Organic substrates influence soil structure and moisture but may also modify the composition of soil microflora in a way that induces soil suppressiveness against soilborne pathogens (De Ceuster and Hoitink 1999). Compost has long been recognized as capable of providing some degree of soilborne disease control (Hoitink and Fahy 1986). Compost contains its own complex of microflora which, upon addition to soil, has potential to introduce a microbial community that may be antagonistic to existing soilborne pathogens (De Ceuster and Hoitink 1999). Therefore, the aim of this study was to (i) characterize the AGs of *Rhizoctonia* associated with diseased rooibos seedlings in nurseries, (ii) investigate the pathogenicity and virulence of the identified AGs against rooibos and on the rotation crops lupin and oat, and (iii) determine whether damping-off of rooibos caused by *Rhizoctonia* AGs

Corresponding author: Y. T. Tewoldemedhin;
E-mail: tewoldemedhin@arc.agric.za

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could be suppressed by compost under controlled environment conditions.

Materials and Methods

Isolations. Soil was collected from the rhizosphere of rooibos plants at 14 different nurseries located in the major rooibos production areas in the Western Cape province. Soil was placed in pots (13 cm in diameter) and planted with 50 seeds/pot with four replicates per nursery soil. Pots were incubated in a glasshouse at day and night temperatures of 26 and 18°C, respectively. Damped-off seedlings and seedlings with crown rot were removed from the pots for isolations. Roots and crowns of symptomatic seedlings were washed and surface disinfested in 1% sodium hypochlorite (NaOCl), rinsed twice in sterile distilled water, and allowed to dry in a laminar flow cabinet. Segments of diseased root and crown tissue were excised and plated onto each of the following growth media: water agar (WA), water agar with 0.02% novostreptomycin (WA+), and potato dextrose agar with 0.02% novostreptomycin (PDA+). Twelve segments of plant material (six root and six crown) were plated per seedling. All fungi emerging from plant tissues were transferred to carnation leaf agar plates (Fisher et al. 1982). Mycelial growth possessing cultural characteristics typical of *Rhizoctonia* spp. was purified by hyphal tipping (Sneh et al. 1996). All resulting *Rhizoctonia* cultures were stored on potato-carrot agar slants in a cold room at 10°C and isolates were also stored on sterile wheat kernels in a freezer at -18°C.

Molecular AG typing of *Rhizoctonia* isolates. DNA was extracted from lysed cells of 5-day-old *Rhizoctonia* mycelia, with slight adaptation, using the protocol of Lee and Taylor (1990). Polymerase chain reaction (PCR) amplification and sequencing of the internal transcribed spacer (ITS) regions of *Rhizoctonia* DNA was conducted using the primer set ITS4 (5'-TCCTCCGCTTATTGATATGC-3')/ITS5 (5'-GGAAGTAAAGTCGTAACAAGG-3'), as described by Tewoldemedhin et al. (2006). A nBLAST search was performed on all the sequence data on National Center for Biotechnology Information GenBank to determine the most closely related AG according to Carling et al. (2002).

Pathogenicity bioassay. Twenty-six representative isolates of *Rhizoctonia* spp. and AGs recovered from rooibos were evaluated for pathogenicity on rooibos as well as the potential rotation crops lupin and oat. The selected isolates included AG-2-2 (12 isolates), AG-4 HGI (3 isolates), AG-11 (3 isolates), *R. zeae* (2 isolates), AG-Bo (2 isolates), AG-K (2 isolates), and the unidentified binucleate *Rhizoctonia* (UNBR; 2 isolates). The pathogenicity trial was conducted in a glasshouse (day and night temperatures of 28 and 18°C, respectively) in 13-cm-diameter pots. The planting medium was composed of equal volumes of soil, perlite, and sand; pasteurized at 83°C for 30 min; and mixed with inoculum after 3 days. Sand-bran inoculum of *Rhizoctonia* spp. was prepared according to Lamprecht (1986) and was introduced into the growth medium at a concentration of 0.05% (wt/wt), whereas the control consisted of uninoculated sand-bran. In all, 50 rooibos and oat and 20 lupin seed were planted per pot and watered every alternate day to field capacity. The percent plant survival was calculated 2 weeks after planting in order to determine pathogenicity and relative virulence of each isolate. To fulfill Koch's postulates, reisolation was made by plating 5-mm pieces of tissue excised from symptomatic crown and root tissues on PDA+. The bioassay was repeated and each bioassay was conducted using a randomized complete block design with three block replicates for each treatment.

Compost bioassay. Glasshouse trials were conducted to evaluate the disease-suppressive effect of two composts (A and B; Table 1) caused by the 26 *Rhizoctonia* isolates utilized in the pathogenicity assays noted above. Sand-bran inoculum was prepared for each of the 26 isolates and was introduced into plant growth medium as described above. The assays were conducted in pasteurized planting medium (sand/soil/perlite [1:1:1]) and in pasteurized planting medium amended with 25% (vol/vol) of either compost A or B, as described by Bahramisharif et al. (2013), with the controls receiving only noninoculated 0.05% (wt/wt) sand-bran. The trial was conducted in a glasshouse, with each treatment containing three replicates in

a complete randomized block design, and the trial was repeated. Disease suppression in response to compost application was determined by calculating the percent rooibos seedling survival 2 weeks after planting.

Statistical analysis. Levene's variance ratio test was performed to test for homogeneity of trial variances between the trial repeats. Data of the two independent trials were considered block treatments providing that Levene's variance ratio test showed homogeneity in trial variance. Data were also subjected to analysis of variance (ANOVA) using SAS statistical software (version 9.2; SAS Institute Inc.), and the Shapiro-Wilk test was performed to test for normality. 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 et al. 1972). The Student's *t* least significant difference was calculated at the 5% confidence level to compare treatment means for significant effects.

Results

Isolation of *Rhizoctonia* spp. and determination of AG.

Seventy-five *Rhizoctonia* isolates were obtained from diseased rooibos seedlings planted in soils collected from 14 nurseries. Most nurseries only contained one *Rhizoctonia* AG. Only four nurseries contained two different *Rhizoctonia* groups, whereas one nursery contained four different *Rhizoctonia* groups. Of the 75 isolates characterized, 67 were multinucleate and 8 were binucleate. The multinucleate isolates included AG-2-2 (67%), AG-4 HGI (14%), AG 11 (5%), and *R. zeae* (3%). Binucleate AGs included AG-Bo (4%), AG-K (4%), and the UNBR (3%). *R. solani* AG-2-2 was recovered from 11 nurseries, AG-4 HGI from 3, AG-11 from 2 and *R. zeae* isolates from 1 each. Binucleate *Rhizoctonia* AG-K and AG-Bo were recovered from one nursery each.

Pathogenicity. Variance of the percent seedling survival data from the two independent trials was comparable for the pathogenicity and cross-pathogenicity trials based on Levene's variance ratio test. The error variance ratio for percent survival was $P = 0.2861$. Consequently, data from the two trials were combined in all analyses. The ANOVA on percent survival indicated a significant interaction of isolate-crop and AG type-crop ($P < 0.0001$; Table 2). The most virulent *Rhizoctonia* AGs on rooibos were AG-2-2, AG-4 HGI, and AG-11 whereas, on lupin and oat, AG-2-2 and AG-4 HGI were the most virulent. In addition, *R. zeae* and AG-K were also pathogenic on rooibos and *R. zeae* on oat (Table 3). The effect of individual isolates on seedling survival for each crop species is shown in Table 4. All isolates of AG-4 HGI caused a significant reduction in survival for all crops tested. In addition, all AG-2-2 isolates caused a significant reduction in survival of lupin and rooibos, while all isolates of this AG group, with the exception of two (PPRI14706 and PPRI14710), reduced survival of oat seedlings. Although all three AG-11 isolates

Table 1. Chemical characteristics of the two composts used in this study

Parameter	Compost A	Compost B
pH (KCL)	7.60	6.40
Resistance (Ohm)	90	100
Moisture (%)	42.70	53.80
Density (kg/m ³)	289.90	598.80
N (%)	0.76	1.43
P (%)	0.15	0.27
K (%)	0.07	1.07
Ca (%)	0.29	0.54
Mg (%)	0.07	0.22
Na (mg/kg)	232.91	1591.22
Mn (mg/kg)	80.27	117.59
Cu (mg/kg)	4.00	0.95
Fe (mg/kg)	294.18	343.75
Zn (mg/kg)	111.21	103.70
B (mg/kg)	7.89	10.73
C (%)	13.57	27.82
Ash (%)	74.60	45.40

significantly reduced survival of rooibos seedlings, only one isolate (PPRI14715) reduced survival of oat and no isolate significantly reduced lupin survival. Both *R. zeae* isolates reduced rooibos seedling survival but only one isolate (PPRI14718) caused mortality of oat seedlings. Lupin survival was not affected by *R. zeae*. Although none of the binucleate *Rhizoctonia* AG types and isolates tested were pathogenic on lupin or oat, both isolates of AG-K tested and one isolate (PPRI14721) of AG-Bo were pathogenic on rooibos. In addition, the uncharacterized binucleate isolates were non-pathogenic on all crops (Table 4).

Compost. The error variance ratio of the rooibos seedling survival data for the compost trial was $P = 0.0005$. Therefore, weighted analysis was conducted in order to combine the two trials and data analyses were conducted on the combined data of the two trials (John and Quenouille 1977). ANOVA on percent seedling survival showed that there were significant isolate–compost and AG type–compost interactions ($P < 0.0001$) (Table 2).

Individual isolates within an AG type differed in their susceptibility to suppression in response to compost amendment of infested soil (Table 5). Compost amendment to soils inoculated with isolates of AG-4 HGI had no significant effect on disease development because seedling survival did not differ significantly compared with the no-amendment, pathogen-infested control treatment. However, both composts significantly suppressed disease in soils caused by AG-11 and AG-K. In addition, compost amendment improved rooibos seedling survival in soils inoculated with the majority of the AG-2-2 isolates. Amendment with either compost A or compost B had no significant effect on seedling survival in soils inoculated with two isolates (R8050G and PPRI14708) of AG-2-2. Amendment with compost B had no significant effect on survival of rooibos when inoculated with two additional isolates of AG-2-2, PPRI14709 and PPRI14710. Thus, in total, 33% of the AG-2-2 isolates were not suppressed by compost B.

Compost amendment significantly improved survival of rooibos seedlings planted in soils inoculated with *R. zeae* isolate PPRI14718, whereas only compost A significantly improved survival of rooibos seedlings planted in soil infested with *R. zeae* isolate PPRI14719. With the exception of isolates of AG-K and isolate PPRI14721 (AG-Bo), compost A amendment had no significant effect on survival of rooibos seedlings planted in soils inoculated with binucleate *Rhizoctonia* spp. However, compost B resulted in the one

nonpathogenic AG-Bo isolate (PPRI14720) becoming pathogenic, because seedling survival was significantly reduced compared with the uninoculated control (Table 5).

Discussion

A diverse collection of *Rhizoctonia* spp. were recovered from rooibos seedlings cultivated in soils obtained from multiple rooibos nursery soils in the Western Cape province of South Africa. Therefore, the characterization of these isolates will be representative of the species or subgroups of *Rhizoctonia* associated with diseases of rooibos seedlings in nurseries. The majority of isolates obtained from rooibos seedlings were multinucleate *Rhizoctonia* spp., a group that contains important plant pathogens with a wide host range (Sneh et al. 1996). The most widespread multinucleate AG in rooibos nurseries was AG-2-2, occurring in approximately 78% of nurseries, followed by AG-4 HGI and AG-11 that only occurred in 21 and 14% of the nurseries, respectively.

Although this is the first report of *Rhizoctonia* spp. and *R. solani* AGs associated with rooibos, each of these individual *Rhizoctonia* groups, with the exception of *R. zeae* (to the best of our knowledge)

Table 3. Mean percent survival of lupin, oat, and rooibos seedlings grown in soil artificially infested with different anastomosis groups (AGs) of *Rhizoctonia* under glasshouse conditions

AG, species	Survival (%) ^y		
	Lupin	Oat	Rooibos
Control	70.0 d–g	87.3 ab	88.3 a
AG-2-2	31.9 hi	59.4 g	6.6 jk
AG-4 HGI	11.6 j	40.5 h	0.0 k
AG-11	60.0 g	81.4 abc	5.4 jk
<i>Rhizoctonia zeae</i>	70.4 c–g	65.3 efg	26.8 i
AG-Bo	76.6 b–e	88.3 a	78.1 a–d
AG-K	67.0 d–g	86.6 ab	26.8 i
UNBR ^z	65.0 fg	88.3 a	76.0 b–f

^y Means in columns and rows followed by the same letter do not differ significantly at $P = 0.05$; Student's *t* least significant difference ($P = 0.05$) = 11.35. Seedling survival was evaluated 2 weeks after planting seed. Values are the mean of three replicates, and data that were pooled over two trials.

^z UNBR = unidentified binucleate *Rhizoctonia*.

Table 2. Analysis of variance for the effect of different anastomosis groups (AGs) of *Rhizoctonia* on mean percent survival of lupin, oat, and rooibos seedlings, and the effect of two composts (A and B) on mean percent survival of rooibos seedlings in planting media amended and unamended with composts and inoculated with different *Rhizoctonia* AGs

Source ^z	Pathogenicity			Compost		
	DF	MS	SL	DF	MS	SL
Rep (trial)	5	4,382.87	<0.0001	5	13.68	<0.0001
Isolates	26	7,982.99	<0.0001	26	96.48	<0.0631
AG type	7	27,551.56	<0.0001	7	350.17	<0.0001
Isolates within AG-11	2	185.19	0.4737	2	443.19	<0.0001
Isolates within AG-2-2	11	450.88	0.0484	11	405.39	<0.0001
Isolates within AG-4 HGI	2	106.24	0.6512	2	3.85	0.1374
Isolates within AG-K	1	420.25	0.1932	1	300.44	<0.0001
Isolates within AG-Bo	1	529.0	0.1444	1	81.0	<0.0001
Isolates within <i>Rhizoctonia zeae</i>	1	8,130.03	<0.0001	1	1,600.0	<0.0001
Isolates within UNBR	1	75.11	0.5819	1	106.78	<0.0001
Crop	2	83,201.54	<0.0001	...	...	...
AG type × crop	14	3,312.83	<0.0001	...	...	...
Isolate × crop	52	1,193.78	<0.0001	...	...	...
Error	400	213.58	...	...	...	...
Corrected total	485	...	...	...	...	...
Compost	...	...	...	2	216.91	<0.0001
AG type × compost	...	...	...	14	11.31	<0.0001
Isolate × compost	...	...	...	52	5.67	<0.0001
Error	...	...	...	400	1.93	...
Corrected total	...	...	...	485	...	...

^z UNBR = unidentified binucleate *Rhizoctonia*.

have previously been recorded on crops in South Africa (Lamprecht et al. 2011; Tewoldemedhin et al. 2006; Truter and Wehner 2004). *R. solani* AG-2-2 has been recorded on annual *Medicago* spp. (medic) (Tewoldemedhin et al. 2006) and lupin (Lamprecht et al. 2011). Members of this AG were reported to be pathogenic on six field crops, including barley, canola, clover, lucerne, lupin, medic, and wheat, in South Africa (Tewoldemedhin et al. 2006). Lamprecht et al. (2011) also demonstrated this AG to be more virulent on lupin than canola. In the United States, it was reported that AG-2-2 was pathogenic against a number of crops, including dry bean, flax, maize, mustard, soybean, sugar bean, and sunflower (Nelson et al. 1996). It is important to note that AG 2-2 is subdivided into AG-2-2 IIIB, AG-2-2 IV, and AG-2-2 LP (Carling et al. 2002) but the subgroup associated with rooibos remains to be determined.

R. solani AG-4 and its subgroup AG-4 HGII were previously isolated from a number of crops in South Africa. Truter and Wehner (2004) isolated AG-4 from potato and Tewoldemedhin et al. (2006) characterized AG-4 HGII from barley, canola, lupin, medic, and wheat in South Africa. Tewoldemedhin et al. (2006) reported that AG-4 HGII was pathogenic, causing damping-off on the above-mentioned crops as well as on clover and lucerne, but it was not as virulent on barley and wheat as on the other crops. It is well documented that *R. solani* AG-4 is a pathogen of canola and lucerne in other parts of the world (Vincelli and Herr 1992).

R. solani AG-11 was previously isolated only from lupin in South Africa. Pathogenicity tests showed that it is moderately virulent on canola, clover, lucerne, lupin, and medic, causing 50% or less damping-off and moderate root rot on these crops. However, it was not pathogenic on barley and wheat cultivars tested (Tewoldemedhin et al. 2006). Of the multinucleate *Rhizoctonia* spp. characterized in

this study, only *R. zeae* has not been recorded in South Africa previously.

Only two binucleate *Rhizoctonia* AGs, AG-Bo and K, and the UNBR were obtained from rooibos seedlings. In a previous study, Tewoldemedhin et al. (2006) reported AG-K as the predominant binucleate *Rhizoctonia* sp. associated with rotation crops commonly employed within wheat production in the Western Cape province. Pathogenicity studies showed this AG to cause significant reduction in survival and hypocotyl or root rot of canola, lucerne, lupin, and medic. It has also been reported as weakly to moderately virulent toward canola in Australia (Khangura et al. 1999). Lamprecht et al. (2011) isolated AG-Bo from both canola and lupin in South Africa but this AG was not pathogenic on either canola or lupin (Lamprecht et al. 2011).

Lupin is an important crop in the Western Cape province of South Africa. However, in this region, lupin seedling establishment is a concern and soilborne diseases are among the major factors threatening the expansion and viability of lupin production. The *Rhizoctonia* AGs isolated from lupin in the Western Cape province were characterized by Tewoldemedhin et al. (2006). A range of *Rhizoctonia* AGs were isolated, with AG-2-1, AG-2-2, AG-3, AG-4 HGII, and AG-11 found to be pathogenic on lupin (Lamprecht et al. 2011; Tewoldemedhin et al. 2006). The current study showed that *Rhizoctonia* AGs that were isolated and found pathogenic on rooibos such as AG-2-2 and AG-4 HGI are also pathogenic on lupin but the rooibos AG-11 isolates were nonpathogenic on lupin. The isolation of *Rhizoctonia* AGs from rooibos seedlings that have been shown to cause severe disease problems on lupin has important implications for crop rotation in rooibos nurseries or the use of lupin as a green manure crop in organic rooibos nurseries. There are no

Table 4. Mean percent survival of lupin, oat, and rooibos seedlings grown in soil artificially infested with isolates of different anastomosis groups (AGs) of *Rhizoctonia* under glasshouse conditions

AG, species	Isolate	Survival (%) ^y		
		Lupin	Oat	Rooibos
Control	Control	70.0	87.3	88.3
AG2-2	PPRI14701	20.8	54.0	10.6
	PPRI14702	20.8	48.3	8.3
	PPRI14703	39.1	56.6	3.0
	PPRI14704	28.3	57.0	1.6
	PPRI14705	37.5	67.6	13.3
	PPRI14706	47.5	74.0	2.0
	PPRI14707	40.0	41.3	11.3
	PPRI14708	33.3	70.3	10.0
	PPRI14709	26.6	44.0	11.3
	PPRI14710	32.5	77.6	0.0
AG4-HGI	PPRI14711	27.5	64.6	1.0
	R8151G	29.1	58.0	7.6
	PPRI14712	10.0	47.0	0.0
	PPRI14713	14.1	41.6	0.0
AG-11	PPRI14714	10.8	33.0	0.0
	PPRI14715	65.0	70.3	6.0
	PPRI14716	58.3	89.6	10.0
<i>Rhizoctonia zeae</i>	PPRI14717	56.6	84.3	0.3
	PPRI14718	67.5	45.0	5.0
	PPRI14719	73.3	85.6	48.6
AG-Bo	PPRI14720	76.6	89.0	89.0
	PPRI14721	76.6	87.6	67.3
AG-K	PPRI14722	72.5	92.3	26.0
	PPRI14723	61.6	81.0	27.6
UNBR ^z	PPRI14724	70.0	86.6	77.0
	PPRI14725	60.0	90.0	75.0

^y Seedling survival was evaluated 2 weeks after planting seed. Values are the mean of three replicates, and data were pooled over two trials. Least significant difference ($P = 0.05$) = 16.58. Values are the mean of three replicates, and data were pooled over two trials.

^z UNBR = unidentified binucleate *Rhizoctonia*.

Table 5. Mean percent survival of rooibos seedlings grown in planting media amended and unamended with composts (A and B) and artificially infested with isolates of different anastomosis groups (AGs) of *Rhizoctonia* under glasshouse conditions

AG, species	Isolate	Plant survival in soil type (%) ^y		
		Unamended	Compost A	Compost B
Control	Control	91.6	94.6	89.5
AG2-2	PPRI14701	10.6	26.8	16.0
	PPRI14702	8.2	31.3	27.3
	PPRI14703	2.9	25.9	40.3
	PPRI14704	1.6	51.2	23.1
	PPRI14705	13.5	49.4	34.7
	PPRI14706	1.9	37.2	34.5
	PPRI14707	11.5	33.1	30.3
	PPRI14708	10.0	21.3	21.5
	PPRI14709	11.4	42.7	9.7
	PPRI14710	0.0	27.8	17.2
AG4-HGI	PPRI14711	0.9	33.6	27.0
	R8151G	7.4	36.8	34.6
	PPRI14712	0.0	0.0	5.1
	PPRI14713	0.0	0.0	4.4
AG-11	PPRI14714	0.0	4.1	2.8
	PPRI14715	6.1	36.5	45.8
	PPRI14716	10.2	63.4	35.5
<i>Rhizoctonia zeae</i>	PPRI14717	0.3	43.1	36.4
	PPRI14718	4.9	74.3	53.9
	PPRI14719	48.7	76.2	47.6
AG-Bo	PPRI14720	89.0	86.5	67.5
	PPRI14721	66.3	93.3	74.0
AG-K	PPRI14722	25.1	64.4	65.6
	PPRI14723	27.0	64.1	46.8
UNBR ^z	PPRI14724	76.3	92.9	78.1
	PPRI14725	74.3	86.9	75.7

^y Seedling survival was evaluated 2 weeks after planting seed. Values are the mean of three replicates, and data were pooled over two trials. Least significant difference ($P = 0.05$) = 17.83. Values are the mean of three replicates, and data were pooled over two trials.

^z UNBR = unidentified binucleate *Rhizoctonia*.

previous reports concerning *Rhizoctonia* spp. associated with oat in South Africa, a crop that is often grown in rotation with rooibos in nurseries. In this study, isolates of AG-2-2, AG-4 HGI, and *R. zeae* recovered from rooibos were found to be pathogenic on oat and none of the binucleate *Rhizoctonia* tested were pathogenic on lupin or oat.

The phenomenon of within- and between-AG variation in susceptibility to suppression by compost amendment observed in this study is in agreement with the recent study that evaluated the effect of compost on damping-off of rooibos seedlings caused by *Pythium* spp. (Bahramisharif et al. 2013). It was found that compost application suppressed the incidence of disease caused by two *Pythium* spp. (*Pythium mamillatum* and *P. pyribolum*) whereas only 38% of *P. irregulare* isolates tested were suppressed due to compost application. A similar phenomenon was observed in the current study, where only 67% of AG-2-2 isolates were suppressed by compost B, and none of the AG-4 HGI isolates were suppressed by either of the composts. It is also of concern that the one AG-Bo isolate that was identified as a nonpathogen of rooibos was able to cause significant damping-off when seedlings were grown in compost B. This phenomenon of within- and between-species variation makes the application of compost difficult for control of damping-off of rooibos seedlings caused by soilborne pathogens, as was also noted by Bahramisharif et al. (2013). Previous reports indicated that, in the presence of bark compost in planting medium, a number of soilborne pathogens (*Fusarium* spp., *Pythium* spp., and *R. solani*) are unable to cause disease (Hoitink et al. 1997). In the field, composts have also been shown to suppress several diseases, including brown patch (*R. solani*) of turf grass (Noble and Coventry 2005). However, compost induced disease suppression when evaluated under field conditions has yielded inconsistent levels of disease control compared with controlled environment trials (Noble and Coventry 2005).

There are few published reports concerning the potential of compost to suppress disease caused by specific AG types of *Rhizoctonia*. Diab et al. (2003) reported that suppression of preemergence damping-off of impatiens was obtained by mixing 20% composted swine waste in a potting mix infested with *R. solani* AG-4. van der Gaag et al. (2007) showed that, of the 12 composts evaluated, 9 composts significantly induced suppressiveness of disease incited by *R. solani* AG-2-1 on radish. In general, evidence points to the involvement of a consortia of microbial antagonists, rather than any single microorganism, in compost-based suppression of disease (Millner et al. 2004). Furthermore, the biological component of compost has been reported as a primary mechanism by which disease suppression is attained in response to such an amendment. It has been shown that, when composted material is subjected to sterilization, it generally loses its ability to suppress disease (Noble and Coventry 2005). Soil chemical factors have been previously shown to contribute to disease suppression; however, based on the factors studied, chemical attributes appear to function inconsistently in the control of soilborne plant diseases caused by *Rhizoctonia* spp. (Scheuerell et al. 2005; Termorshuizen et al. 2007).

The current study is the first report of *Rhizoctonia* spp. and AGs associated with rooibos. It was demonstrated that *R. solani* AG-2-2 is the predominant *Rhizoctonia* pathogen of rooibos. This AG was also found to be virulent on the two rotation crops, lupin and oat, used by farmers in rooibos production systems. This has important implications for the use of precrops or green manure crops prior to the establishment of rooibos nurseries. With regards to susceptibility of rotation crop *Rhizoctonia* pathogens of rooibos, oat is likely to serve as a better precrop prior to establishment of rooibos nurseries than lupin. Because suppression of damping-off of rooibos seedlings by compost soil amendment is variable within and between AGs and the effectivity is low (<50% seedling survival), it has to be coupled with other cultural practices or control methods in order to render an effective and sustainable suppressive effect. In this regard, Bahramisharif et al. (2013) showed that the use of compost was effective when it was applied together with nonpathogenic *Pythium* spp.

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