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Evaluation of strategies for the control of canola and lupin seedling diseases caused by *Rhizoctonia* anastomosis groups

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Abstract Several methods with potential for the management of *Rhizoctonia* diseases of canola and lupin including plant resistance, fungicide seed treatment and biological control using binucleate *Rhizoctonia* anastomosis groups (AGs) were evaluated under glasshouse conditions. Screening included the examination of resistance of eight canola and eight lupin cultivars/selections to damping-off and hypocotyl/root rot caused by the multinucleate *Rhizoctonia solani* AG-2-1, 2–2, 4 and 11. All canola cultivars were highly susceptible to AG-2-1, but Rocket, Spectrum and 44C11 were more resistant than the other cultivars. Spectrum and 44C73 were also more resistant to AG-4 than the other canola cultivars. On lupin, *R. solani* AG-2-2 and 4 were most virulent, and the cultivar Cedara 6150 and selection E16 were most resistant to AG-2-2; Cedara 6150, E16, Mandelup and Quilinock were more resistant to AG-4 than the other cultivars/selections. The *Lupinus luteus* selections, E80.1.1.2 and E82.1.1 were most susceptible to AG-2-2, 4 and 11. Seed treatment with the fungicides Cruiser OSR (a.i. difenconazole, fludioxonil, metalaxyl-M, thiamethoxam) and SA-combination (a.i. iprodione, metalaxyl, thiram) significantly increased survival of canola and lupin seedlings, decreased hypocotyl/root rot and improved the percentage of healthy seedlings, with the SA-combination being significantly more effective than Cruiser OSR. Application of the binucleate *Rhizoctonia* AGs (A, Bo, K and I) significantly increased the survival of lupin seedlings inoculated with *R. solani* AG-2-2 and 4, and AG-I and K significantly improved survival of canola in the presence of AG-4. This is the first report of the potential of binucleate AGs to protect canola and lupin seedlings against infection by multinucleate AGs.

Keywords Binucleate · Multinucleate · Protective effect · Resistance · Seed treatment

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Introduction

Rhizoctonia anastomosis groups (AGs) are regarded as economically important pathogens of canola (*Brassica napus* L. var. *oleifera* DC) and lupin (*Lupinus* spp.) in Australia, Canada and the USA (Kaminski and Verma 1985; Hwang et al. 1986; Gugel et al. 1987; Huber et al. 1992; Khangura et al.

1999; Chang et al. 2005). These pathogens cause damping-off, hypocotyl, root and crown rot, and it has been reported that *Rhizoctonia solani* can cause significant yield losses (Verma 1996; Khangura et al. 1999; Klein-Gebbinck and Woods 2002; Paulitz and Okubara 2006).

Canola and lupin are important crops in South Africa that are grown mostly in rotations that include barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) in the Western Cape Province (De Villiers 2004; Agenbag 2008). There are many problems with seedling establishment of canola and lupin in this production area and the expansion and ultimate survival of these crops are threatened by soilborne diseases. A complex of organisms viz., *Fusarium*, *Pythium* and *Rhizoctonia* spp. were shown to be responsible for damping-off and contributed to poor crop establishment (De Villiers et al. 2006). Recently, Tewoldemedhin et al. (2006) characterized the *Rhizoctonia* AG isolated during three growth seasons (2000–2003) from canola and lupin as well as from the plant species used in rotation systems with these crops in the southern production area of the Western Cape. *Rhizoctonia* AG A, I, K, 2–1, 3 and 4-HGII were isolated from canola and AG I, K, 2–1, 3, 4-HGII and 11 from lupin. In more recent studies conducted in both the southern (Tygerhoek) and western (Langgewens) production areas, AG 2–1, 2–2 and 11 were recovered from both areas, but AG-2-1 was isolated only from canola and AG-11 only from lupin. It was also shown that planting of lupin favoured the incidence of binucleate *Rhizoctonia* AGs (Lamprecht et al. unpublished).

The aims of this study were a) to evaluate canola and lupin cultivars for resistance to *R. solani* AG-2-1, 2–2, 4 and 11 at the seedling stage, b) to determine the effect of fungicide seed treatments on damping-off and health of canola and lupin seedlings, and c) to determine whether binucleate AGs can protect canola and lupin seedlings from infection by pathogenic multinucleate AGs.

Material and methods

Bioassay conditions

Three isolates each of four multinucleate *R. solani* AGs (AG-2-1, 2–2, 4 and 11) and two isolates each of four binucleate *Rhizoctonia* AGs (AG-A, Bo, K and I) were included in this study (Table 1). *R. solani* isolates were used in all trials, whereas the binucleate isolates were only included in the trial evaluating their capacity to limit infection of canola and lupin by the multinucleate AG.

Sand-bran inoculum, used in all the trials, was prepared using the method described by Lamprecht (1986). Washed sand (400 g) and wheat bran (20 g) were mixed together in 500 ml Schott bottles and 60 ml distilled water was added. The mixture was autoclaved for 1 h on the first day at 120°C and 1.5 kg cm^{-2}. This process was repeated on two consecutive days, but the mixture was then autoclaved for only 30 min each time. On the fourth day each bottle was inoculated with 10 5-mm diameter plugs of a single *Rhizoctonia* culture grown on water agar and incubated at 22°C for 10 days before use. The bottles were shaken at 4 and 7 days after inoculation to ensure uniform colonization of the sand-bran mixture. An inoculum concentration of 0.05% inoculum/planting media (wet wt/wet wt) was employed in all the trials unless otherwise stated.

The trials were conducted in 13 cm diameter plastic pots with a holding capacity of 700 g of planting medium. The planting medium was made up of equal quantities of soil, perlite and sand, and was pasteurized (30 min at 83°C) 3 days before being mixed with the inoculum and sown with seed. After inoculation of the medium, the pots were transferred to the glasshouse and watered until water flowed from the bottom. The pots were left to stand overnight in the glasshouse before making 10 holes to a soil depth of 1.5 (canola) or 2 (lupin) cm in each pot. Sterile 1 cm diameter doweling rods, one per pot, were used for this purpose. Five canola seeds and two lupin seeds were planted in each hole giving a total of 50 canola and 20 lupin seeds per pot. In all trials an experimental unit consisted of a pot with 50 seeds for canola and 20 seeds for lupin. All trials were conducted in a glasshouse employing a 15°C night and 25°C day temperature. Pots were watered every second day to field capacity.

Cultivar reaction

Eight cultivars/selections each of canola and lupin were evaluated for their reaction to all isolates of the four multinucleate AGs listed in Table 1.

Table 1 *Rhizoctonia* anastomosis groups and isolates included in this study

Rhizoctonia group	Anastomosis group (AG)	Isolate[a]	Accession number[b]
Binucleate	AG-A	**M6691C**	PPRI 10359
		M7134E	PPRI 10360
	AG-Bo	**M6660I**	PPRI 10361
		M6730E	PPRI 10362
	AG-I	**M7004A**	PPRI 10363
		M7118B	PPRI 10364
	AG-K	**M7048B**	PPRI 10365
		M7446B	PPRI 10366
Multinucleate	AG-2-1	**M1979AL**	PPRI 7426
		M6713A	PPRI 10368
		M7161D	PPRI 10369
	AG-2-2	M6705B	PPRI 10370
		M7144P	PPRI 10371
		M7152C	PPRI 10372
	AG-4	**M2279AN**	PPRI 7434
		M3378I	PPRI 7439
		M4339AJ	PPRI 7435
	AG-11	**M1984P**	PPRI 7440
		M6684G	PPRI 10377
		M7123B	PPRI 10378

[a] Isolates in bold were used in the trial to evaluate the protective effect of binucleate towards multinucleate *Rhizoctonia* anastomosis groups

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

Canola cultivars included the conventional cultivars Comet, Outback, Spectrum and 44C11, the Clearfield cultivars Rocket and 44C73, and triazine tolerant Thunder and Tornado. The lupin species and cultivars/selections included were Cedara 6150 and E16 (*L. albus)*, Mandelup, Tanjil, Quilinock and E42 (*L. angustifolius),* and E80.1.1.2 and E82.1.1 *(L. luteus)*.

The method used to asses the trial was similar in all experiments. The number of surviving seedlings and the number of seedlings with hypocotyl/root rot were counted 14 days after planting. The percent survival, seedlings with hypocotyl/root rot and healthy seedlings were calculated and adjusted against controls for each cultivar/selection. Data for the comparison of seed treatments and the protective effect of binucleate AGs were not adjusted for the control.

The experiment used a complete randomized design structure. The experimental design was a factorial design with eight cultivars/selections, five inoculum treatments (control and four *Rhizoctonia* AG) and three isolates within each AG with four replicates per treatment. The entire trial was repeated.

Seed treatment

The chemical seed treatments Cruiser OSR (a.i. difenconazole, fludioxonil, metalaxyl-M, thiamethoxam) and SA-combination (a.i. iprodione, metalaxyl, thiram) tested by De Villiers et al. (2006) were evaluated for their capacity to control seedling damping-off. The SA-combination formulation used in this study differed from that used by De Villiers et al. (2006) in that it did not include the insecticide Gaucho (imidachloprid). Seed of the canola cvs Muster, Rocket and Thunder and lupin cvs/selections Cedara, E82.1.1 and Mandelup were treated with either Cruiser or SA-combination. Cruiser was applied at 3.75 ml/250 g of canola seed and at 1.5 ml/200 g of lupin seed. The individual components of the SA-combination treatment applied were Apron (metalaxyl) at 0.25 g (0.0815 g a.i)/250 g canola and 0.1 g (0.0326 g a.i.)/200 g lupin seed, Thiulin (thiram) at 1.0 g (0.5 g a. i)/250 g canola and 0.4 g (0.2 g a.i.)/200 g lupin seed, Rovral (iprodione) at 3.92 ml (0.9975 g a. i)/250 g canola and 1.568 ml (0.399 g a.i.)/200 g lupin seed. Rovral was applied first and containers shaken until the solution was spread evenly before the Apron and

Thiulin were added in no particular order. Treated seeds were dried under a fume cabinet for 4 h and planted the next day in planting medium inoculated with individual *R. solani* AG as described above. The trial was evaluated 21 days after planting to allow for hypocotyl/root rot to develop in plants grown from treated seed.

The experiment used a complete randomized design structure. The treatment design for the trial was a factorial design with three cultivars, three seed treatments (control, Cruiser and SA-combination), five AG (control, AG-2-1, 2–2, 4 and 11) and four isolates (control and three isolates) within each AG. There were four replicates per treatment. The entire trial was repeated.

Protective effect of binucleate *Rhizoctonia* AG

Canola cv. Thunder and the lupin cv. Tanjil were used in this trial. Two isolates of each multi- (AG-2-1, 2–2, 4 and 11) and binucleate (AG-A, Bo, K and I) AG indicated in bold in Table 1 were tested. Pots were inoculated with one isolate each of a multi- and binucleate AG in all possible combinations at a ratio of 1:1 or 1:10 (multinucleate:binucleate), where 1 represents 0.05% inoculum/planting media (wt/wt) and 10 represents 0.5% inoculum/planting media (wt/wt). The trial was assessed 21 days after planting.

The experiment used a complete randomized design structure. The experimental design was a factorial design with five multinucleate (control, AG-2-1, 2–2, 4 and 11) and five binucleate AG (control, AG-A, Bo, I and K) treatments. There were two isolates within each AG and the two binucleate isolates were used as replicates. The entire trial was repeated.

Statistical analyses

The statistical analysis of data was conducted similarly for all trials. Levene's ratio test for homogeneity was performed to test for trial variances between repeats (Levene 1960). 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 of trial variance. Data were subjected to analysis of variance using SAS (version 9.3; SAS Institute, Inc., SAS Campus Drive, Cary, WC) and the Shapiro-Wilk test was performed to test for normality (Shapiro and Wilk 1965). In cases where deviations from normality were due to kurtosis and not skewness, data were accepted as reliable and the results were interpreted without transformation (Glass et al. 1972). Student's *t*-test and the protected least significant differences (LSD) were calculated to compare means at the 5% significance level.

Results

Cultivar reaction

Canola Canola cultivars were highly susceptible to AG-2-1 and 4, and only marginally or not susceptible to AG-2-2 and 11 (Fig. 1). Seedling survival in AG-2-1 infested growth medium varied from 51.3% (Rocket) to 68.7% (44C73), and seedlings with hypocotyl/root rot ranged from 83.4% (44C11) to 100% (Tornado). Rocket, Spectrum and 44C11 had a significantly ($P \leq 0.05$) lower incidence of hypocotyl/root rot and a significantly higher percentage of healthy seedlings than the other cultivars, however all cultivars were highly susceptible to AG-2-1. Among those examined, cultivars Spectrum and 44C73 exhibited a significantly ($P \leq 0.05$) higher percentage of healthy seedlings than the other cultivars when grown in the presence of AG-4. Comet and 44C73 were less affected by AG-2-2 as demonstrated by significantly higher percent seedling survival, but there were no significant differences in the reaction of canola cultivars when grown in medium infested with AG-11 (Fig. 1).

Lupin Among *R. solani* isolates, those within AG-2-2 and AG-4 were most virulent toward lupin, resulting in significantly ($P \leq 0.05$) reduced seedling survival rates and increased incidence of hypocotyl/root rot (Fig. 2). In the presence of AG-2-2, Cedara 6150 and E16 had fewer seedlings exhibiting symptoms of hypocotyl/root rot and significantly more healthy seedlings compared to other lupin cultivars. Tanjil, Quilinock and E82.1.1 were the most susceptible to AG-2-2. Cedara 6150, E16, Mandelup and Quilinock were least affected by AG-4, and E80.1.1.2 and 82.1.1 were most affected. All cultivars of *L. albus* and *L. angustifolius* all exhibited a high level of tolerance to AG-11. In contrast, the two *L. luteus*

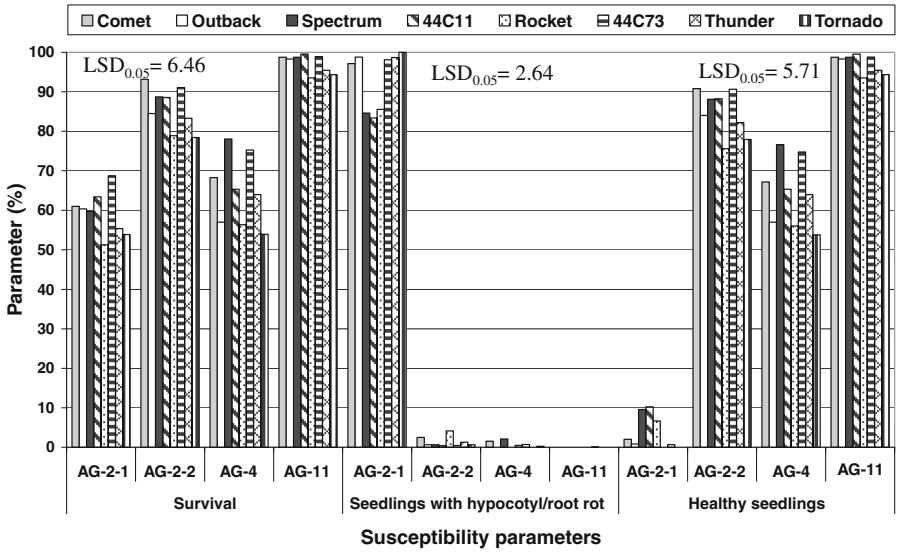

Fig. 1 Susceptibility of canola cultivars to *Rhizoctonia solani* anastomosis groups as defined by percent survival, hypocotyl/root rot, and healthy seedlings 14 days after planting in infested soil.% Survival = (Number of seedlings that survived/50)×100; % Seedlings with hypocotyl/root rot = (Number of seedlings with hypocotyl/root rot out of the number of seedlings that survived)×100;% Healthy seedlings = (Number of seedlings that survived minus number of seedlings with hypocotyl/root rot/50)×100. LSDs for the three different measured parameters (survival, seedlings with hypocotyl/root rot, healthy seedlings) are given at the top of the column

cultivars, E80.1.1.2 and E82.1.1 were highly susceptible to AG-11, exhibiting significantly lower levels of seedling survival and percent healthy seedlings (Fig. 2).

Seed treatment

Canola Significant cultivar x AG x seed treatment interactions were detected for the percent seedling survival ($P<0.0001$), seedlings with hypocotyl/root rot ($P=0.0013$) and healthy seedlings ($P<0.0001$). The Cruiser OSR and SA-combination fungicide seed treatments resulted in significantly ($P\leq0.05$) greater seedling survival in soil inoculated with AG-2-1, 2–2 and 4 (Fig. 3a). Although not in all cases, application of Cruiser OSR resulted in lower seedling survival rates than SA-combination, especially in soils infested with AG-2-1 or AG-4, and higher hypocotyl/root rot levels incited by AG-2-1 compared to SA-combination (Fig. 3b). The percent healthy seedlings for seed treated with Cruiser OSR and planted in soil inoculated with AG-2-1 varied from 16.8 to 22.8% and for seed treated with SA-combination from 64.8 to 90.1%. The percent healthy seedlings for Cruiser OSR-treated seed planted in soil infested with AG-4 ranged from 78.1 to 87.6% and for seed treated with SA-combination from 89.8 to 96.8% (Fig. 3c).

Lupin Significant cultivar x AG x seed treatment interactions were also present for the percent lupin seedling survival ($P<0.0001$), seedlings with hypocotyl/root rot ($P<0.0001$) and healthy seedlings ($P<0.0001$). In most cases, percent lupin seedling survival in soils inoculated with AG-2-2 or AG-4 was significantly ($P\leq0.05$) greater in response to seed treatment with Cruiser OSR or SA-combination (Fig. 4a). Mandelup seedling survial in soil infested with AG-2-2 and treated with Cruiser OSR and SA-combination was not significantly greater. The two seed treatments significantly improved survival of E82.1.1, and SA-combination significantly improved survival of Mandelup in soil inoculated with AG-11 (Fig. 4a). Cruiser OSR and SA-combination also significantly reduced the number of seedlings with hypocotyl/root rot for all three cultivars in soil inoculated with AG-2-2 and of cultivars E82.1.1 and Mandelup in soil inoculated with AG-4 (Fig. 4b). In soil inoculated with AG-2-2, the Cruiser OSR and SA-combination treatments significantly increased percent healthy seedlings relative to the no treatment control, with SA-combination superior to Cruiser

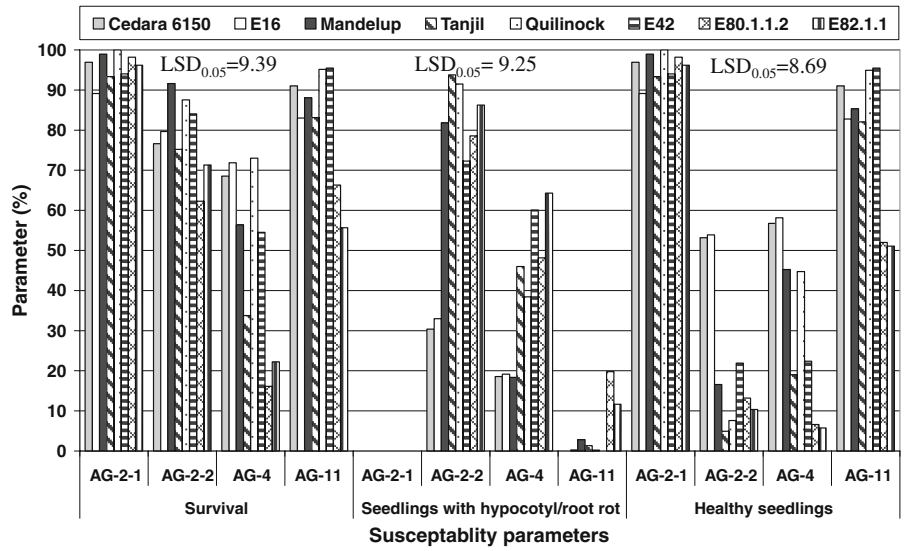

Fig. 2 Susceptibility of lupin cultivars/selections to *Rhizoctonia solani* anastomosis groups as defined by percent survival, hypocotyl/root rot, and healthy seedlings 14 days after planting in infested soil.% Survival = (Number of seedlings that survived/20)×100;% Seedlings with hypocotyl/root rot = (Number of seedlings with hypocotyl/root rot out of the number of seedlings that survived)×100;% Healthy seedlings = (Number of seedlings that survived minus number of seedlings with hypocotyl/root rot/20)×100. LSDs for the three different measured parameters (survival, seedlings with hypocotyl/root rot, healthy seedlings) are given at the top of the column

OSR when used in conjunction with the cultivars E82.1.1 and Mandelup (Fig. 4c).

Protective effect of binucleate AG

Canola Percent canola seedling survival ($P<0.0001$), seedlings with hypocotyl/root rot ($P<0.0001$) and healthy seedlings ($P<0.0001$) varied significantly among the multinucleate/binucleate AG soil treatments. However, these differences were primarily among multinucleate AGs regardless of their combination with binucleate AGs. Although the combination of the multinucleate AGs with binucleate AGs resulted in a numerical increase in seedling survival compared to the control, the difference was not significant ($P>0.05$) with the exception of AG A, I and K, which resulted in significantly greater seedling survival in the presence of AG-4. The combination of AG-A with AG-4 also resulted in a significantly greater percentage of healthy seedlings (Fig. 5).

Lupin Significant multinucleate AG + binucleate AG combination x ratio interactions were found for percent lupin seedling survival ($P<0.0001$), seedlings with hypocotyl/root rot ($P=0.0002$) and healthy seedlings ($P<0.0001$). The combination of AG-2-2 with low and high ratios of each of the binucleate AGs resulted in significantly ($P\leq0.05$) greater seedling survival compared to AG-2-2 in the nil treatment (Fig. 6a). The high ratio of binucleate isolates in combination with AG-2-2 in each instance resulted in significantly greater seedling survival relative to survival attained with the corresponding binucleate strain applied at the lower rate. In general, binucleate strains in combination with AG-2-1 or AG-4 only enhanced survival when the binucleate was applied at the high ratio. Seedling survival in the presence of AG-11 was high, and inoculation with binucleate AG did not significantly enhance survival (Fig. 6a).

When applied at the high rate, binucleate AGs resulted in significantly lower incidences of hypocotyl/root rot caused by AG-2-2. The low rate of AG-A, Bo and I combined with AG-2-2 and low rate of AG-Bo, I and K combined with AG-4 also resulted in low incidences of hypocotyl/root rot, while the high rate of each binucleate combined with AG-4 resulted in a significantly lower percent of seedlings with hypocotyl/root rot. Combining AG-2-1 or AG-11 with the binucleate AGs did not result in a significantly lower incidence of hypocotyl/root rot caused by AG-2-1 or AG-11 (Fig. 6b).

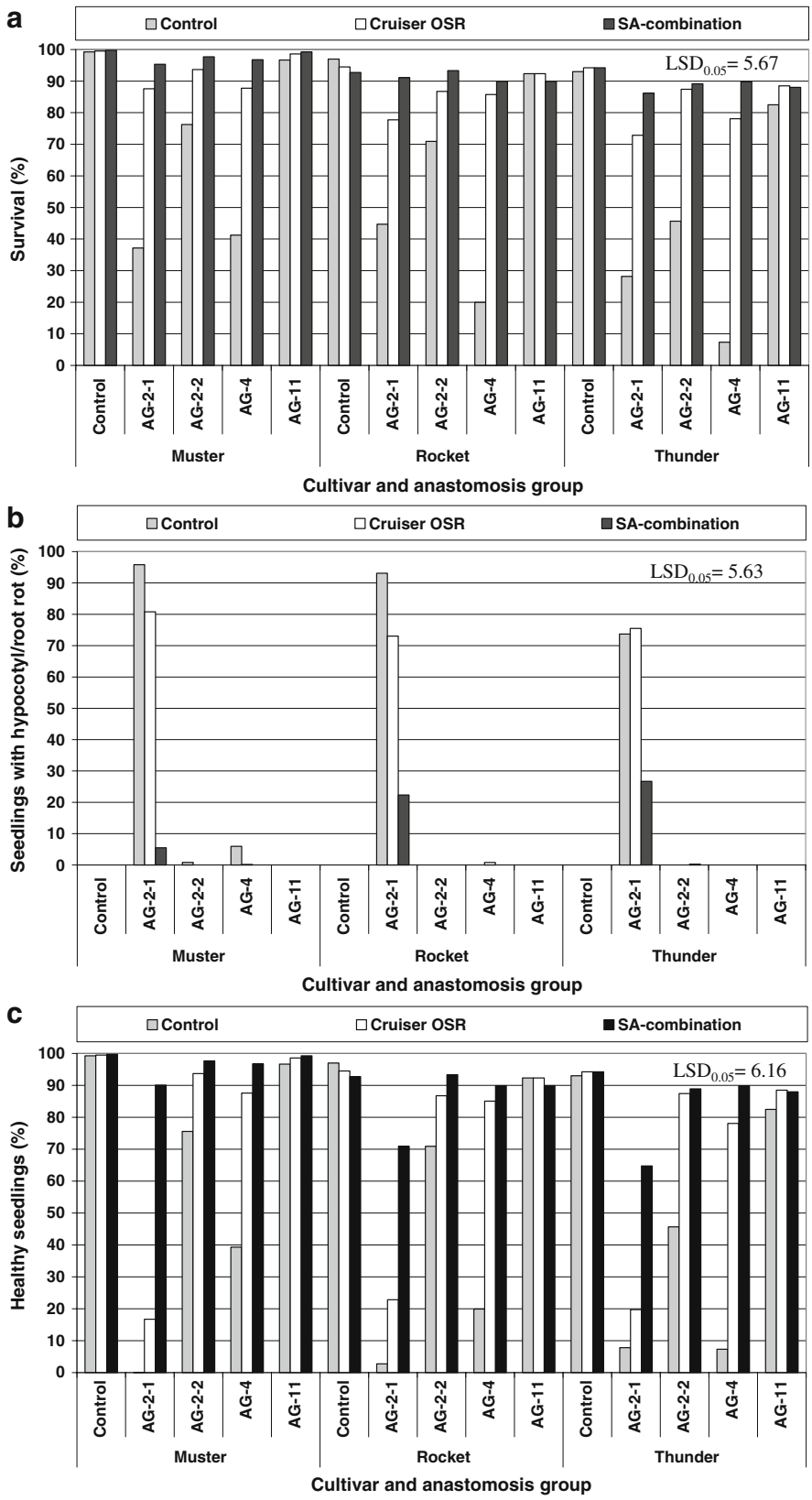

Fig. 3 Effect of seed treatments with Cruiser OSR and SA-combination on canola seedling survival, hypocotyl/root rot and percent healthy seedlings at 21 days after planting in soil inoculated with *Rhizoctonia solani* isolates of different anastomosis groups. a) % Survival = (Number of seedlings that survived/50)×100; b) % Seedlings with hypocotyl/root rot = (Number of seedlings with hypocotyl/root rot out of the number of seedlings that survived)×100; c) % Healthy seedlings = (Number of seedlings that survived minus number of seedlings with hypocotyl/root rot/50)×100

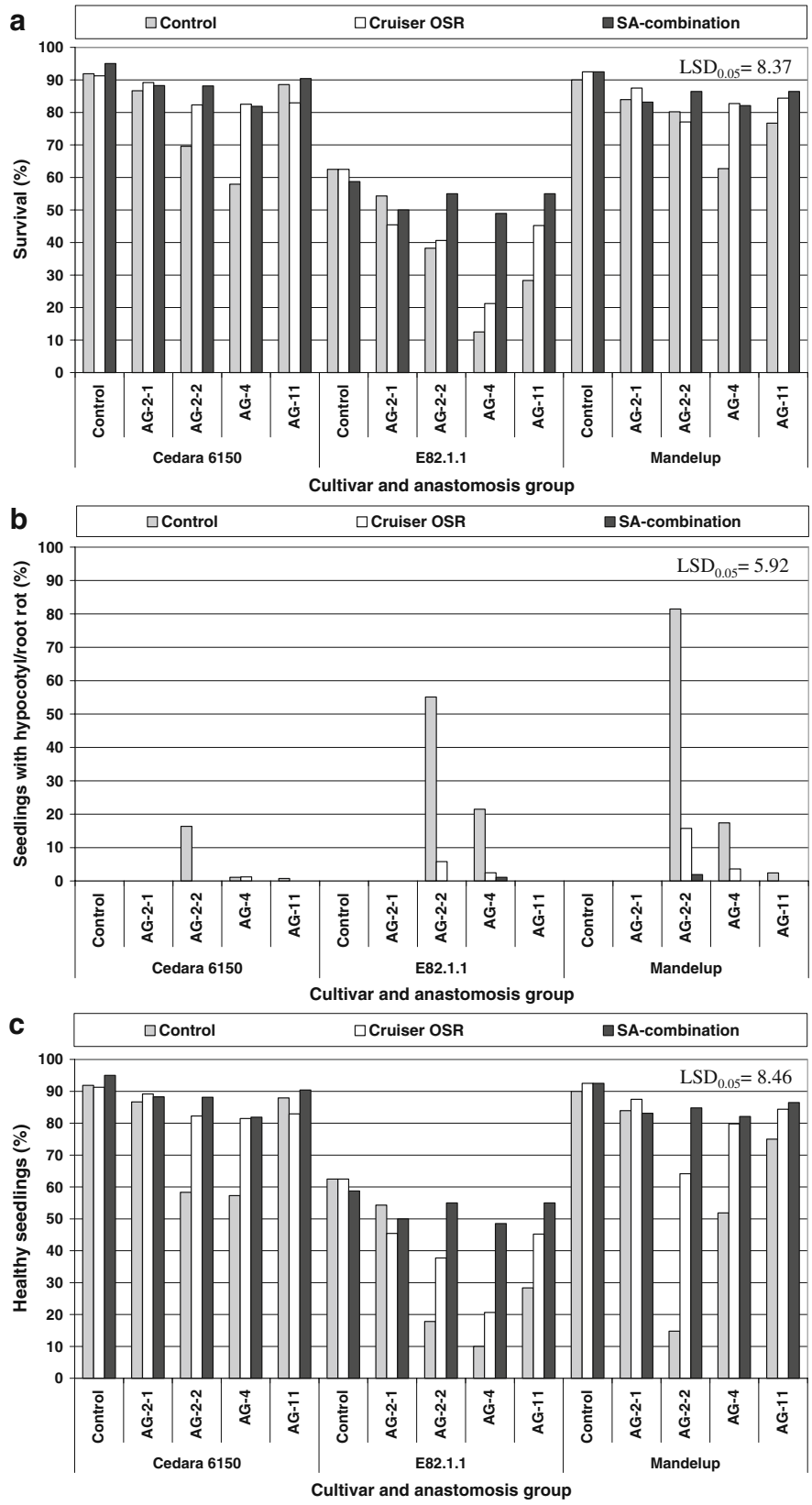

Fig. 4 Effect of seed treatments with Cruiser OSR and SA-combination on lupin seedling survival, hypocotyl/root rot and percent healthy seedlings at 21 days after planting in soil inoculated with *Rhizoctonia solani* isolates of different anastomosis groups. a) % Survival = (Number of seedlings that survived/20)×100; b) % Seedlings with hypocotyl/root rot = (Number of seedlings with hypocotyl/root rot out of the number of seedlings that survived)×100; c) % Healthy seedlings = (Number of seedlings that survived minus number of seedlings with hypocotyl/root rot/20)×100

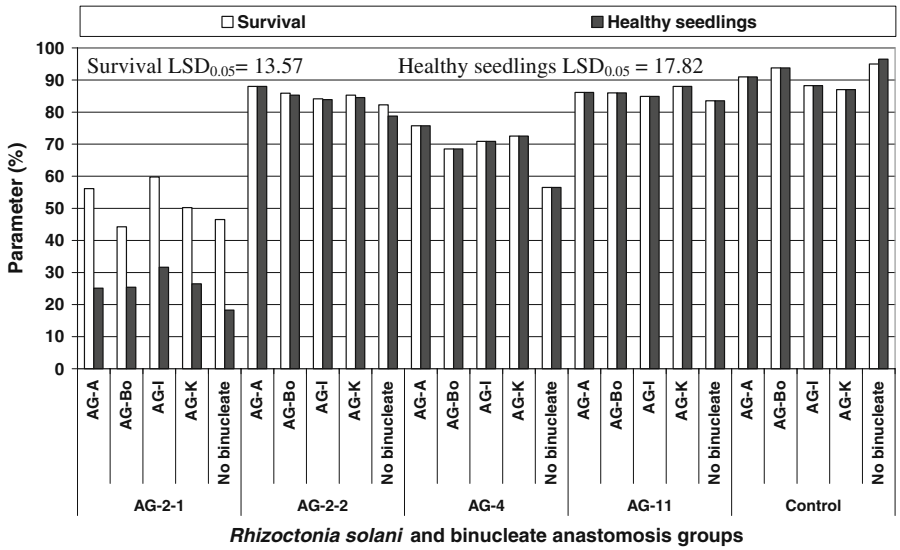

Fig. 5 Effect of binucleate *Rhizoctonia* (AG-A, AG-Bo, AG-I and AG-K) on percentage canola seedling survival and healthy seedlings in soils infested with multinucleate *R. solani* isolates (AG-2-1, AG-2-2, AG-4 and AG-11).% Survival = (Number of seedlings that survived/50)×100;% Healthy seedlings = (Number of seedlings that survived minus number of seedlings with hypocotyl/root rot/50)×100

When applied at a low rate, AG-I in the presence of AG-2-2 significantly improved the percentage of healthy seedlings from 0 (AG-2-2 + without binucleate) to 33.8%, whereas the combination of AG-2-2 with high concentrations of any of the binucleate AGs resulted in a significantly greater percentage of healthy seedlings. Combination of AG-4 with low and high concentrations of AG-Bo, I and K, low concentrations of AG-I or high concentration of AG-A also increased the percentage healthy seedlings. Combining AG-2-1 or AG-11 with low and high concentrations of the binucleate AGs did not significantly affect the percentage healthy seedlings except for the combination of AG-11 with the high concentration of AG-Bo that resulted in a significant increase in the number of healthy seedlings (Fig. 6c).

Discussion

In this study the reaction of canola and lupin cultivars/selections to *Rhizoctonia* diseases of seedlings (damping-off and hypocotyl/root rot), the efficacy of fungicide seed treatments against these diseases, and the protective effect of binucleate *Rhizoctonia* AGs against multinucleate *Rhizoctonia* AGs were investigated. Four multinucleate AGs viz. AG-2-1, 2–2, 4 and 11 and four binucleate AGs viz. AG-A, Bo, I and K isolates were included in the study. These AGs are common to field soils in the southern Cape (Tewoldemedhin et al. 2006) and recently were recovered in surveys conducted in rotation trials in the southern and western Cape in 2006 and 2007 (Lamprecht et al. unpublished). Although AG-4 was not obtained in 2006 and 2007, it was previously reported to occur in the southern Cape (Tewoldemedhin et al. 2006), and has been reported in other locations as an important pathogen on canola (Hwang et al. 1986; Gugel et al. 1987; Baird 1996). The current study confirmed that *R. solani* AG-2-1 and AG-4, and AG-2-2 and AG-4 are important seedling pathogens of canola and lupin, respectively (Tewoldemedhin et al. 2006).

The population of *R. solani* primarily responsible for damage to canola most commonly has been reported to be AG-2-1 and AG-4 (Kaminski and Verma 1985; Gugel et al. 1987; Yitbarek et al. 1987). However, a diversity of *Rhizoctonia* AG have been documented in association with canola and were able to elicit various levels of disease (Tewoldemedhin et al. 2006). Previous studies evaluating resistance of canola to *R. solani* have primarily focused on host susceptibility to isolates of AG 2–1 (Yang 1989; Yang and Verma 1992). An evaluation of *Brassica rapa* and *B. napus* cultivars found that none tested was immune to infection by the individual isolate of *R. solani* AG-2-1 employed, but significant differences in susceptibility among and within species was observed and plants became less susceptible with age (Yang and Verma 1992). Progeny from disease-free plants of *B. napus* cv. Midas were more resistant than the original parental strains, indicating that resistance could be improved through selection (Yang and Verma 1992).

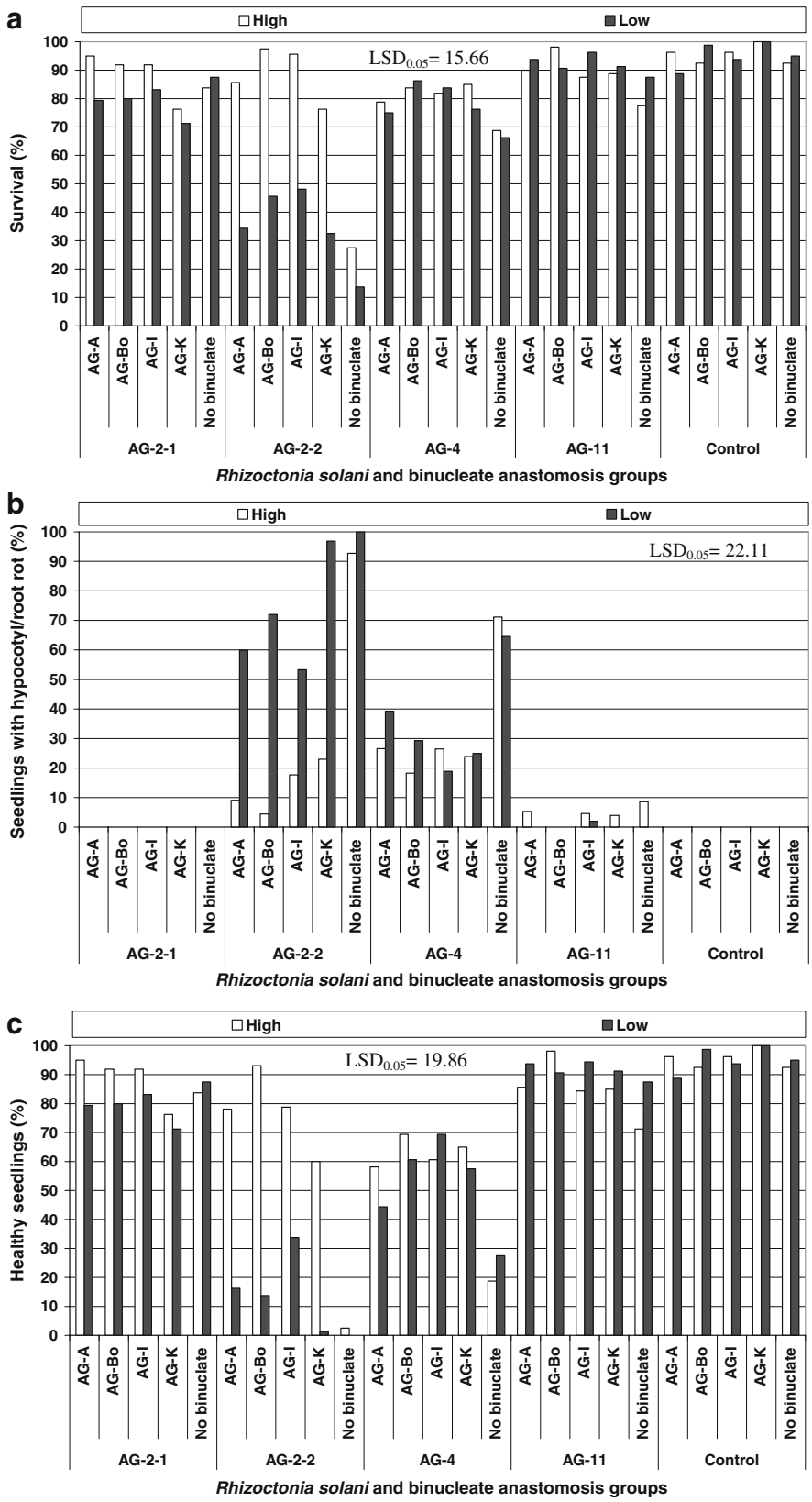

Fig. 6 The effect of combining multinucleate (AG-2-1, AG-2-2, AG-4 and AG-11) and binucleate (AG-A, AG-Bo, AG-I and AG-K) *Rhizoctonia* anastomosis groups in low (1:1) or high (1:10) ratios in soil on the percent survival of lupin seedlings, seedlings with hypocotyl/root rot and healthy seedlings. a) % Survival = (Number of seedlings that survived/20)×100; b) % Seedlings with hypocotyl/root rot = (Number of seedlings with hypocotyl/root rot out of the number of seedlings that survived)×100; c) % Healthy seedlings = (Number of seedlings that survived minus number of seedlings with hypocotyl/root rot/20)×100

In the current study, although the cultivars, Rocket, Spectrum and 44C11 were less affected, all eight canola cultivars evaluated in this study were highly susceptible to the three test isolates of AG-2-1.

To our knowledge there are no previous reports concerning the general screening of canola cultivars for resistance to isolates of *R. solani* belonging to AG other than AG-2-1. Although cultivars exhibited uniform levels of hypocotyl rot induced by AG-4, there were significant differences in seedling survival and percent healthy seedlings among the canola cultivars examined. Based upon these parameters, Spectrum and 44C73 appeared to possess lower levels of susceptibility to infection by AG-4. Simlarly, differential resistance to AG-2-2 was detected with cultivars Tornado and Rocket more susceptible to this AG based upon seedling survival and percent healthy seedlings. In contrast, *R. solani* AG-11 isolates were uniformly non-pathogenic towards all canola cultivars tested.

Few studies have examined resistance of pasture legumes to *R. solani*. You et al. (2008) reported useful levels of resistance to *R. solani* among cultivars of common birds-foot (*Ornithopus sativus*), with resistance to AG-1-1B more prevalent than resistance to AG-2-1 or AG-2-2 among the test cultivars. In the current study, lupin cultivars were shown to differ significantly in their reaction to *R. solani* AG-2-2, AG-4 and AG-11, with *L. albus* cultivar Cedara 6150 and selection E16 being less affected by isolates of *R. solani* belonging to multiple AGs (2–2, 4 and 11) and the *L. luteus* selections being most susceptible to these same isolates. *L. angustifolius* cultivars/selections were all quite susceptible to AG-2-2, Mandelup and Quilinock were moderately resistant to AG-4 and resistant to AG-11. This appears to be the first report on the reaction of lupin cultivars to AG-2-1, 2–2, 4 and 11.

As multiple *Rhizoctonia* AGs are well-known pathogens of canola and lupin seedlings in the Western Cape Province (Tewoldemedhin et al. 2006; Lamprecht et al. unpublished data) it is important to investigate the effect of seed treatments against the diversity of entities that comprise this pathogen population. Multiple studies have examined fungicide seed treatments for the control of seedling damping-off and root rot of canola incited by *R. solani* (Kataria and Verma 1990; Katatria et al. 1991; Kataria and Verma 1993). Studies were primarily limited to examination of isolates belonging to *R. solani* AG-2-1, and effective fungicide seed treatments were identified. The seed treatments examined in our study effectively controlled seedling disease of canola and lupin incited by multiple isolates representing different AGs of *R. solani*. Cruiser OSR and SA-combination seed treatments significantly improved seedling survival and reduced hypocotyl/root rot of canola. However, SA-combination was more effective than Cruiser OSR in protecting canola seedlings against AG-2-1 and AG-4. Researchers in Canada and Australia have reported iprodione (included in SA-combination) to be effective for control of *R. solani* in canola (Kataria and Verma 1993; Khangura, et al. 1999). De Villiers et al. (2006) evaluated Cruiser OSR and SA-combination [with imidachloprid (Gaucho) added] against damping-off of canola caused by uncharacterized *Rhizoctonia* isolates. Using seedling survival as evaluation criterion, they determined these treatments to be equally effective. Since Cruiser OSR is already registered as a seed treatment for canola in Europe and Brazil (also known as Helix; Paulsrud et al. 2001) and elsewhere (Doyle et al. 2002), further trials were conducted in South Africa to obtain the necessary information for registration locally (currently pending). Results of our current study show that SA-combination is a better seed treatment option with regard to the management of seedling diseases of canola caused by *Rhizoctonia* AG-2-1 and AG-4.

At present, there is no fungicide seed treatment registered in South Africa for control of damping-off of lupin (Nel et al. 2003). The efficacy of the two seed treatments in protecting lupin seedlings against *Rhizoctonia* was similar to that recorded for canola. SA-combination again appeared to be more effective against damping-off and hypocotyl/root rot caused by AGs 2–2, 4 and 11 than Cruiser OSR. Although *R. solani* is an important pathogen of lupin worldwide (Sweetingham 1989; Leach and Clapham 1992) the effect of seed treatments on *Rhizoctonia* diseases has not been studied widely and there is no report on the effect of fungicide seed treatments on specific AG associated with lupin.

Numerous studies have investigated the protective effect of binucleate *Rhizoctonia* AG against disease incited by *R. solani* AG-4 and AG-2-2 on various crop plants (Bell et al. 1984; Burpee and Goulty 1984; Masuhara et al. 1993; Schisler et al. 1993; Ross

et al. 1998; Hwang and Benson 2002). The study conducted by Tewoldemedhin et al. (2006) and the surveys conducted in 2006 and 2007 showed that the relative recovery of binucleate *Rhizoctonia* AG was significantly higher than the recovery of *R. solani* AG in the trials where canola and lupin were rotated with barley and wheat. Results also indicated a higher incidence of binucleate AG associated with lupin compared to canola. This prompted us to investigate the protective effect of binucleate *Rhizoctonia* AGs against *R. solani* AG on canola and lupin. Results obtained thus far indicate a clear reduction in disease caused by AG-2-2 and AG-4 and to a lesser extent AG-11 on lupin when inoculated into soil in combination with binucleate *Rhizoctonia* isolates from AG, A, Bo, I and K. AG-A, I and K also significantly improved survival of canola in the presence of AG-4. For lupin, a higher ratio (1:10) of the binucleate to multinucleate AG was significantly more effective in providing protection and it appears that AG-Bo and AG-I are more effective than AG-A and AG-K. Although the potential use of binucleate *Rhizoctonia* AGs as biocontrol agents against multinucleate AGs has been investigated on other crops (Herr 1995), this appears to be the first report demonstrating a reduction in Rhizoctonia diseases of canola and lupin seedlings in response to soil inoculation with binucleate *Rhizoctonia* AG.

The results of this study demonstrate that canola cultivars are highly susceptible to *R. solani* AG-2-1, but that the cultivars Spectrum and 44C73 are relatively less affected by AG-4 than the other cultivars examined. The reaction of lupin cultivars/selections were more clearly defined and cultivars/selections differed significantly in their reaction to AG-2-2, AG-4 and AG-11. It was also shown that fungicide seed treatments can be highly effective in protecting canola and lupin seedlings against the diversity of *R. solani* AGs that can incite diseases on these crop plants. Likewise, binucleate *Rhizoctonia* AGs were shown to protect canola and lupin seedlings against infection by multinucleate *R. solani* AGs. However, *R. solani* AG are only one element of a pathogen complex, which includes *Fusarium* and *Pythium* spp., contributing to the poor seedling establishment of canola and lupin in the Western Cape Province. In order to effectively manage seedling diseases of canola and lupin, it will also be important to characterize, in addition to *Rhizoctonia* spp., the role of *Fusarium* and *Pythium* spp. If the involvement of these two genera are found to be significant, then there will be a need to develop and incorporate aspects of crop rotation, cultivar resistance and seed treatments across all three genera into an integrated disease management strategy for controlling the full spectrum of damping-off and hypocotyl/root rot disorders of canola and lupin seedlings.

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