Eur J Plant Pathol (2011) 131:305–316
DOI 10.1007/s10658-011-9809-z

Effect of cropping system on composition of the *Rhizoctonia* populations recovered from canola and lupin in a winter rainfall region of South Africa

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Accepted: 12 May 2011 / Published online: 27 May 2011

Abstract *Rhizoctonia* spp. anastomosis groups (AGs) associated with canola and lupin in the southern and western production areas of the Western Cape province of South Africa were recovered during the 2006 and 2007 growing seasons and identified using sequence analyses of the rDNA internal transcribed spacer regions. The effect of crop rotation systems and tillage practices on the recovery of *Rhizoctonia* spp. was evaluated at Tygerhoek (southern Cape, Riviersonderend) and Langgewens (western Cape, Moorreesburg) experimental farms. Isolations were conducted from canola planted after barley, medic/clover mixture and wheat, and lupin planted after barley and wheat, with sampling at the seedling, mid-season and seedpod growth stages. In the 2006 study, 93.5% of the *Rhizoctonia* isolates recovered were binucleate and 6.5% multinucleate; in 2007, 72.8% were binucleate and 27.2% were multinucleate. The most abundant AGs within the population recovered included A, Bo, I and K, among binucleate isolates and 2-1, 2-2 and 11 among multinucleate isolates. Crop rotation sequence, tillage and plant growth stage at sampling all affected the incidence of recovery of *Rhizoctonia*, but certain effects were site-specific. The binucleate group was more frequently isolated from lupin and the multinucleate group from canola. AG-2-1 was only isolated from canola and AG-11 only from lupin. This study showed that important *Rhizoctonia* AGs such as AG-2-1, 2-2 and 11 occur in both the southern and the western production areas of the Western Cape province and that crop rotation consistently influences the incidence and composition of the *Rhizoctonia* community recovered from the cropping system.

Keywords Binucleate · Crop sequence · Multinucleate · Sampling time · Tillage practice

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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; Khanghura et al. 1999; Chang et al. 2005). *Rhizoctonia* spp. cause damping-off, as well as hypocotyl, root, and crown rot, and *R. solani* has been reported to cause significant yield losses (Davidson 1977; Sippell et al. 1985; Sweetingham et al. 1986; Huber et al. 1992; Kataria and Verma 1992; Verma 1996; Khanghura et al. 1999; Klein-Gebbinck and Woods 2002; Paulitz and Okubara 2006).

Canola and lupin are important crops in the Western Cape province of South Africa, and are grown mostly in rotations that include barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*). There are many problems with seedling establishment of canola and lupin in this region and expansion of production and ultimate viability of these crops are threatened by soilborne diseases. A complex of organisms, viz. *Fusarium*, *Pythium* and *Rhizoctonia* spp., were shown to contribute to poor establishment of these crops in the Western Cape region (De Villiers et al. 2006). However, the composition of the *Rhizoctonia* spp. population associated with these crops and the relative importance of specific AGs to disease development was not ascertained. Recently, Tewoldemedhin et al. (2006) characterized the *Rhizoctonia* AGs isolated from canola and lupin as well as certain crop species used in rotation systems with these crops in the southern production area of the Western Cape province. Several *Rhizoctonia* AGs were isolated from these production systems with AG-2-2 and AG-4 HG-II demonstrating the highest degree of virulence on all crops examined including canola and lupin. The Tewoldemedhin et al. (2006) study was conducted in the southern production area only of the Western Cape. At present, there is no information on the incidence of *Rhizoctonia* AGs on canola and lupin in the western production areas of the Western Cape, and there is no information on the effect of different rotation systems and tillage practices on the incidence of these AGs in either of the two production areas. Since the Western Cape normally has higher rainfall during the season than the Southern Cape, incidences of soilborne diseases (take-all and Fusarium crown rot) of wheat differ in the two production areas (Klaasen et al. 1991, 1992). It is therefore possible that there are also differences in *Rhizoctonia* spp. and diseases associated with canola and lupin in these two areas.

The western and southern production areas of the Western Cape are characterised by winter- and all-year rainfall respectively, with cool, wet winters and hot, dry summers. The western production area (Swartland) has a typically Mediterranean climate with more than 80% of annual rainfall occurring from mid-autumn to early-spring (April to September). Legume pastures that are used in crop–pasture rotations in the Swartland are restricted to annual species. Rainfall distribution in the southern production area varies from 75% between mid-autumn and early-spring (April to September) in the western parts to 55% in the eastern extremities. Lucerne pastures can be established and persist because a higher proportion of rain falls during the summer months and climate is milder and more temperate than in the western production area. Both production areas have rainfall of between 250 mm and 450 mm, but most of the area allocated to rain-fed crop and pasture production systems in the region receives less than 400 mm.

Canola, introduced into local cropping systems in the 1990s, has moderate yield potential but is limited by soil moisture availability. Despite research and on-farm management practice demonstrating the production potential and advantages of growing canola in the rotation, many producers find the crop difficult to manage and do not achieve its production potential. Lupins have been cultivated extensively, mainly as grazing for sheep, but also for grain production. Wheat, barley and oats are the most important cereal crops that are produced in the winter and all-year rainfall regions. Hard-seeded, self-regenerating annual (medics and clovers) and perennial (lucerne) legumes are the most important pastures used in short- and long-rotation systems with cereal, oil and protein crops such as canola and lupin.

The objectives of this study were to characterize the *Rhizoctonia* spp. population from canola and lupin in terms of anastomosis group identity and relative frequency of recovery during the growing season in both the southern and the western production areas in the Western Cape province, and to determine the effect of rotation systems and tillage practices on the incidences of these *Rhizoctonia* AGs.

Material and methods

Rhizoctonia spp. were isolated during the 2006 and 2007 growing seasons from plants in four on-going

crop rotation trials conducted by the Western Cape Department of Agriculture at the Tygerhoek (34° 10′ S and 19° 54′ E) and Langgewens (33° 17′ S and 18° 42′ E) experimental farms near the towns of Riviersonderend and Moorreesburg, respectively. Rainfall recorded during the 2006 and 2007 growing seasons was 346.9 and 585.5 mm, respectively at the Langgewens experimental farm (western production area) and 339.3 and 161.4 mm, respectively at the Tygerhoek experimental farm (southern production area). Long-term rainfall data for Langgewens and Tygerhoek experimental farms are 347.3 and 297.4 mm, respectively (ARC-ISCW, Agro-climatology Unit, Private Bag X5013, Stellenbosch 7599). Crops included in these trials were wheat (W) cv. SST 027, barley (B) cv. SSG 564, canola (C) cv. ATR Stubby, lupin (L) cv. Tanjil, a mixture of medic (*Medicago truncatula*) and clover (*Trifolium michelianum*), (M) cvs Sephi, Santiago, Paraggio, Balansa, Gosse and Nungarin.

Trials 1 and 2 were designed to determine the interaction between tillage practice [zero-till (soil left undisturbed and seed planted with a Star Wheel planter with minimal soil disturbance to a maximum depth of 5 mm) and minimum-till (soil scarified to a depth of 100–150 mm in late March/early April and then planted with a tined no-till planter)] and crop rotation on crop production and the physical and chemical properties of the soil. Trials 3 and 4 were designed to investigate the effects of crop rotation on crop production using no-till (Table 1) farming practises. Although the rotation trials included many crop sequences, only those containing canola and lupin were evaluated in this study. Two crop sequences were included from each of Trials 1 and 2, and six crop sequences from each of Trials 3 and 4 (Table 1). Trials 1 and 2 were established in 2000 and 2002 at Tygerhoek and Langgewens, respectively, and trials 3 and 4 in 2002 and 1996 at Tygerhoek and Langgewens, respectively.

The crop sequences evaluated in trials 1 and 2 were the same in 2006 and 2007; however, in 2007 the tillage treatments were altered as the zero-till plots had been re-designed to accommodate additional tillage treatments. The new design included zero-till, no-till (soil left undisturbed until planting then planted with a tined no-till planter that results in maximum of 20% soil disturbance to a depth of 100–150 mm in planting row), minimum-till and conventional tillage (soil scarified to a depth of 100–150 mm in late March/early April, then disked (Tygerhoek) or ploughed (Langgewens) to a depth of 150–200 mm just before planting and planted with a tined no-till planter). Sampling in 2007 was conducted on the four newly allocated tillage treatment plots. The difference between sampling years, for trials 1 and 2 therefore, is that the minimum-till plots had been tilled only once (in early 2007) since trials 1 and 2 were established, and the no-till and conventional tillage plots had also been disturbed only once (in 2007) since the trials were established.

The size of the experimental plots varied among and within trials. In trials 1 and 2 plot sizes were 0.12 ha in 2006 and 0.03 ha (after re-designing of tillage practices) in 2007. Plot size in trials 3 and 4 for both years were 0.25 ha and 0.5 ha respectively. Crop sequences in trials 1 and 2 were replicated four times, and in trials 3 and 4 there were two replicates of each crop sequence. All crop residues had been retained on all experimental plots since the start of trials 1, 2 and 3, and since 2002 in Trial 4.

Sampling and isolation

Canola and lupin plants were collected at the seedling (4 to 6 weeks after planting), mid-season (12 to

Table 1 Crop sequences[z] three years prior to sampling in the four trials that were included in this study

Trial 1 (Tygerhoek)	Trial 2 (Langgewens)	Trial 3 (Tygerhoek)	Trial 4 (Langgewens)
B-L-W-C	**W-L-W-C**	M-W-M-C	C-W-W-L
W-C-B-L	**W-C-W-L**	C-W-B-L	M-W-M-C
		L-W-B-C	**W-C-W-L**
		W-C-W-L	**W-L-W-C**
		W-L-W-C	W-W-L-C
		W-M-M-C	W-W-W-C

[z] B = Barley, C = Canola, L = Lupin, M = Mixture of medic and clover, W = Wheat; The crop that was evaluated in each sequence is underlined. Crop sequences where canola and lupin were compared within the same rotation sequence are in bold.

14 weeks after planting) and flowering/seedpod (20 to 22 weeks after planting) stages. Forty plants were collected from 10 sampling locations (four plants/location) along a zig-zag (W) pattern through each experimental plot at each sampling time. Plants were washed under running tap water to remove adhering soil, and rinsed twice in sterile distilled water. Small pieces of root and hypocotyl or crown tissue with lesions were excised and placed onto the following growth media: water agar (WA) (Agar Bacteriological; Biolab Diagnostics, Midrand, South Africa), WA amended with 200 μg ml^{-1} novostreptomycin, and potato dextrose agar (PDA; Biolab Diagnostics) amended with 200 μg ml^{-1} novostreptomycin. A total of 18 hypocotyl/coleoptile/crown and 18 root segments were plated for each experimental plot at each sampling time for each crop. The total number of tissue segments plated for each crop at each sampling time was 1152 (2006 season) and 1728 (2007 season) for canola and 864 (2006 season) and 1440 (2007 season) for lupin. The total number of tissue segments plated per crop depended upon the number of plots containing the specific crops within the rotation trial.

Rhizoctonia AG typing

Mycelia of *Rhizoctonia* cultures grown on PDA plates at 25°C with a 12 h photoperiod for 5 days were used for DNA extractions. DNA was extracted from mycelia according to the protocol of Lee and Taylor (1990). PCR amplification of *Rhizoctonia* DNA was conducted using the primer set ITS4 and ITS5 (White et al. 1990), and the reaction conditions as previously described (Tewoldemedhin et al. 2006). Amplification products were cloned into the vector pCR 4-TOPO® (Invitrogen, Carlsbad, CA, USA) and 2 μl of the cloning reaction was used to transform chemically competent *Eschericia coli* TOP10 (Invitrogen, Carlsbad, CA, USA). Plasmid DNA was isolated from transformed colonies using the S.N.A.P. miniprep plasmid purification kit (Invitrogen).

Sequencing of resulting clones was conducted in reactions using the CEQ™ DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA) and analyzed using a CEQ 8000 Genetic Analysis System capillary-based DNA sequencer (Beckman Coulter). Sequencing was conducted in one direction employing M13 reverse (5′-CAGGAAACAGCTATGAC-3′) as the primer, consistently yielding 600 to 650 bp reads. Identity of each sample was attained by comparing resulting DNA sequences to a local database collection and to GenBank (BLAST search).

Experimental design and statistical analysis

Trials 1 and 2 utilized a randomized block design with four replicates. The treatment design was a 2×2 and 2×4 factorial for 2006 and 2007, respectively, with factors of two crop sequences and two (2006 season) or four (2007 season) tillage practices. The repeated measurements (sampling times) were included as a subplot factor. Trials 3 and 4 also employed a randomized block design with six crop sequences replicated in two blocks. The repeated measurements (sampling times) were also included as a subplot factor.

Although isolations of *Rhizoctonia* spp. were conducted separately from crowns, hypocotyls and roots, these data were combined for analysis of variance. Percent incidence data were transformed, the Shapiro-Wilk test was performed to test for normality (Shapiro and Wilk 1965) and analysis of variance was conducted for each trial separately using SAS (SAS 1999). 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). The Student's t-least significant differences (LSD) were calculated to compare means at the 5% significance level.

Analyses of the data showed that there was not enough evidence for significant two and three factor interactions between crop sequence, tillage practice and sampling times, and main effects were therefore compared.

Results

Recovery and AG typing of *Rhizoctonia* spp

A total of 184 and 298 *Rhizoctonia* spp. isolates were obtained from the four trials during 2006 and 2007, resepctively. Of the 184 isolates obtained in 2006, 6.5% were multinucleate and 93.5% binucleate. In 2007, the *Rhizoctonia* population recovered consisted of 27.2% multinucleate and 72.8% binucleate isolates. Among the two crops, multinucleate *Rhizoctonia*

were more prevalent from canola than lupin, and vice versa with regard to the binucleate *Rhizoctonia* AGs.

Based on sequence analysis of the total *Rhizoctonia* population examined, *R. solani* contained isolates belonging to AG-2-1 [4.4% (2006), 15.4% (2007)], AG-2-2 [0.5% (2006), 7.7% (2007)] and AG-11 [1.6% (2006), 4.0% (2007)], whereas the binucleate *Rhizoctonia* spp. included isolates belonging to AG-A [3.3% (2006), 10.7%, (2007)], AG-Bo [7.6% (2006), 7.4% (2007)], AG-I [42.9% (2006), 30.9% (2007)] and AG-K [17.4% (2006), 23.8% (2007)]. Several (22.3%) of the binucleate isolates (unidentified group) did not exhibit a clear affinity with any binucleate *Rhizoctonia* spp. according to the ITS sequences contained in the Genbank library. Among the multinucleate *Rhizoctonia* groups, AG-2-1 and AG-2-2 were isolated from canola and AG-2-2 and AG-11 from lupin. *Rhizoctonia solani* AG-2-1, 2-2 and 11 were isolated from plants cultivated at both the Tygerhoek and Langgewens experimental farms. With the exception of AG-A, all binucleate *Rhizoctonia* AGs (including the unidentified group) were isolated from both canola and lupin at both locations.

Effect of crop sequences

In both 2006 and 2007, crop sequences had no significant effect on the frequency at which different AGs were recovered from canola and lupin in trial 1 (data not shown). In trial 2, AG-K and the binucleate *Rhizoctonia* group were recovered significantly ($P \leq 0.05$) more frequently from lupin than canola, in both years (Figs. 1 and 2). AG-I and AG-11 were also isolated more frequently from lupin than canola in 2007 (Fig. 1). *Rhizoctonia solani* AG-2-1 and the multinucleate *Rhizoctonia* group were isolated significantly more frequently from canola than lupin in 2007 (Figs. 1 and 2). In trial 3, the greatest incidence of binucleate *Rhizoctonia* was recorded on lupin in 2006 (Fig. 3). In 2007, AG-Bo and AG-11 were recorded significantly more frequently on lupin (2.3%) following wheat in the W-C-W-L system than lupin (0%) following barley in the C-W-B-L system and also more than on canola (0%) (data not shown). Incidence of the multinucleate *Rhizoctonia* group was not significantly affected by crop sequence in 2006, but in 2007 incidence of the multinucleate group was greater in canola following the medic/clover mixture in the M-W-M-C rotation system than canola following barley in the L-W-B-C system. Lupin following wheat (W-C-W-L) also had a higher incidence of multinucleate *Rhizoctonia* than lupin following barley (C-W-B-L) (Fig. 3). Crop sequences did not have a significant effect on individual AGs in 2006 in trial 4, but in 2007, recovery of AG-K was significantly greater on lupin (C-W-W-L=1.9% and W-C-W-L=0.9%) than canola (all crop sequences=0%) and recovery of AG-2-1 was significantly greater on canola in the W-L-W-C (1.4%) and W-W-W-C (0.5%) crop sequence than the other crop sequences (0%) (data not shown). Incidence of the binucleate group was also greater on lupin in crop sequence C-W-W-L and on lupin in crop sequence W-C-W-L than canola in crop sequences M-W-M-C and W-W-L-C in trial 4 in 2006. In 2007 incidences of the binucleate group was significantly greater on lupin than canola in all crop sequences (Fig. 4). Crop sequence did not affect the incidence of the multinucleate *Rhizoctonia* group in 2006, but in 2007 a significantly greater incidence of the multinucleate group was recorded on canola in the W-L-W-C crop sequence compared to canola in the M-W-M-C, W-W-L-C and W-W-W-C crop sequences and lupin in the C-W-W-L and W-C-W-L crop sequences (Fig. 4).

Tillage practices

In both years, tillage practices did not significantly affect the recovery of *Rhizoctonia* AGs in trial 1 at Tygerhoek (data not shown). Similar results were obtained for trial 2 in 2006; however in 2007, AG-I was recovered significantly more frequently from plants in the conventional tillage treatment (3.6%) than the other tillage treatments [zero-till (1.0%); no-till (1.0%) and minimum-till (0.7%)] (data not shown) and the incidence of binucleate *Rhizoctonia* group was significantly greater for the conventional than the zero-till and minimum-till treatments. Incidence of the multinucleate *Rhizoctonia* AGs was not affected by tillage practices (Fig. 5).

Sampling time

Sampling time did not significantly affect AG recovery in trial 1 in both years, except for a significantly higher recovery of AG-Bo at the first (0.5%) compared to the third sampling time (0%) in 2007 (data not shown). Sampling time had a

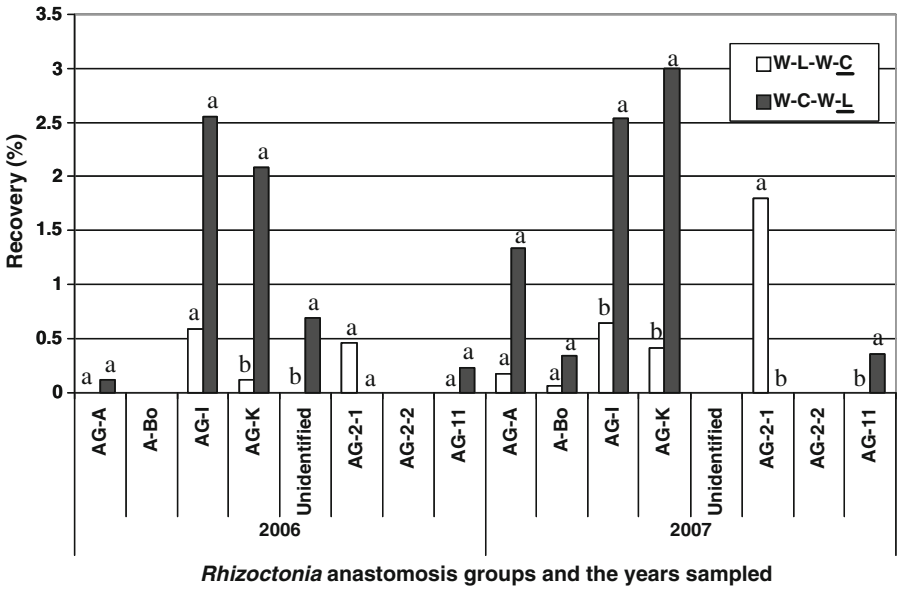

Fig. 1 Recovery of *Rhizoctonia* anastomosis groups (AG) from canola and lupin in trial 2 at the Langgewens experimental farm during 2006 and 2007. Crops that were sampled in this study are underlined in the crop sequence; C = Canola, L = Lupin, and W = Wheat. Values represent the mean percentage of plant tissue fragments out of 36 pieces per experimental unit (one plot) that yielded the AG. Values within an AG within a year followed by the same letter do not differ significantly at $P=0.05$ according to Student's t-LSD

significant effect on the recovery of AG-Bo in trials 2 [higher in the beginning (0.5%) and end (0.1%) of season] and 3 [higher mid-season (0.7%) than at the beginning (0.2%) and end (0.2%) of season] in 2007 (data not shown). In 2006, AG-I (Trial 4) and AG-K (Trails 2 and 4) were recorded at significantly higher rates at the end [AG-I (6.3%), AG-K (3.3%, Trial 2; 1.6%, Trial 4)] compared to the beginning [AG-I (0.9%), AG-K (0%, Trial 2; 0%, Trial 4)] of the season (data not shown). Recovery of AG-I in trial 2 was higher in the beginning (2.4%) and end (1.4%) than the middle of the growing season, and AG-K was recovered more frequently at the end (3.9%) than the beginning (0.7%) in 2007 (data not shown). In

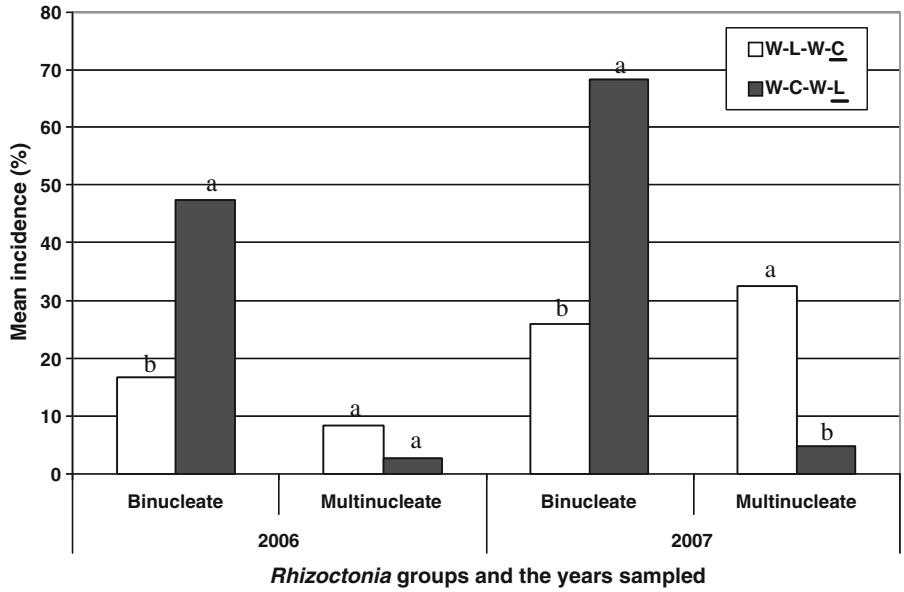

Fig. 2 Mean percentage incidence of bi- and multinucleate *Rhizoctonia* on canola and lupin in trial 2 at the Langgewens experimental farm during 2006 and 2007. Crops that were sampled in this study are underlined in the crop sequence; C = Canola, L = Lupin and W = Wheat. Values are mean percentage of binucleate or multinucleate isolates out of the total number of *Rhizoctonia* isolates obtained for all three sampling times for each rotation system for each year (season). Values within a *Rhizoctonia* group within a year followed by the same letter do not differ significantly at $P=0.05$ according to Student's t-LSD

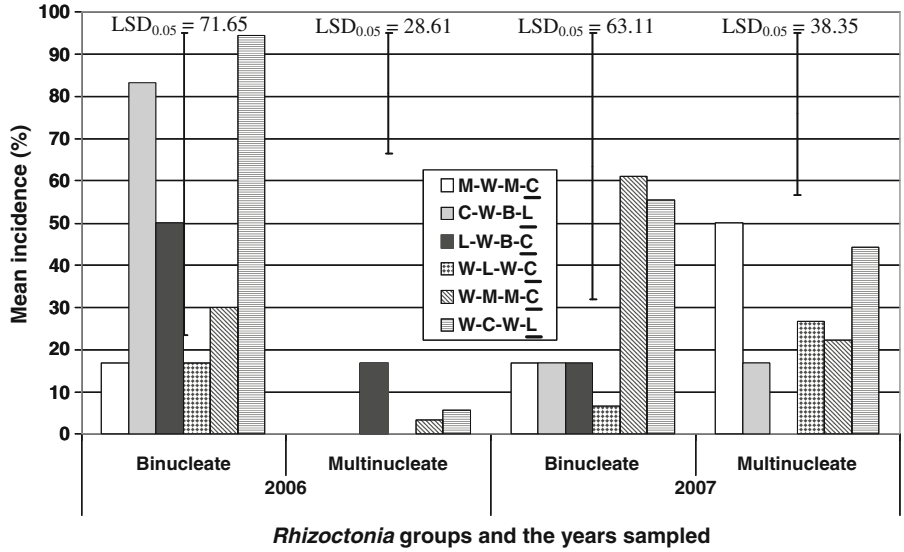

Fig. 3 Mean percentage of bi- and multinucleate *Rhizoctonia* on canola and lupin in crop sequences sampled in trial 3 at the Tygerhoek experimental farm during 2006 and 2007. Crops that were sampled in this study are underlined in the crop sequence; B = Barley, C = Canola, L = Lupin, M = medic/clover mix and W = Wheat. Values are mean percentage of binucleate or multinucleate isolates out of the total number of *Rhizoctonia* isolates obtained for all three sampling times for each rotation system for each year (season). Student's t-LSD at $P=0.05$ for each *Rhizoctonia* group for each year given at top of column

2007, the recovery of individual multinucleate AGs was often greater in the beginning and end than the middle of the season, but except for AG-2-1 (0.7, 0 and 0.2% respectively at the beginning, middle and end of season for Trial 4) and 11 (0.5, 0 and 0.7% respectively at the beginning, middle and end of the season for Trial 3) these differences were not statistically significant (data not shown). In 2006,

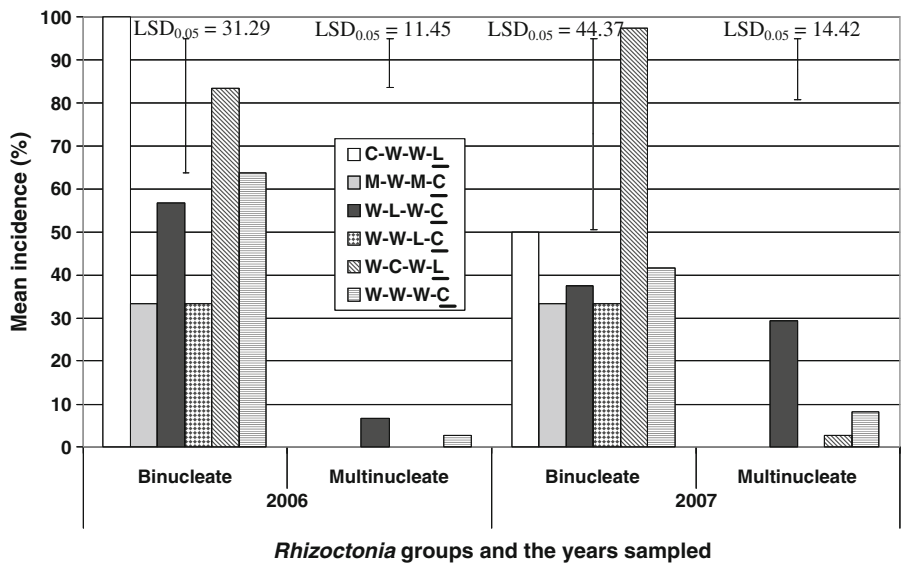

Fig. 4 Mean percentage of binucleate and multinucleate *Rhizoctonia* on canola and lupin in crop sequences sampled in trial 4 at the Langgewens experimental farm during 2006 and 2007. Crops that were sampled in this study are underlined in the crop sequence. C = Canola, L = Lupin, M = medic/clover mix and W = Wheat. Values are mean percentage of binucleate or multinucleate isolates out of the total number of *Rhizoctonia* isolates obtained for all three sampling times for each rotation system for each year (season). Student's t-LSD at $P=0.05$ for each *Rhizoctonia* group for each year given at top of column

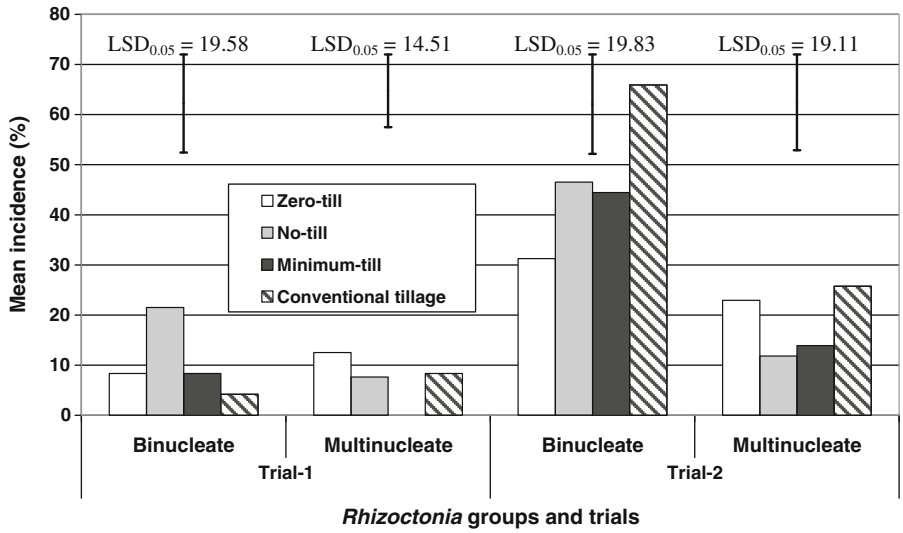

Fig. 5 Effect of tillage practice on mean percentage of bi- and multinucleate *Rhizoctonia* from canola and lupin sampled in trials 1 and 2 in 2007. Values are mean percentage of binucleate or multinucleate isolates out of the total number of *Rhizoctonia* isolates obtained for all three sampling times for each rotation system for each year (season). Student's t-LSD at $P=0.05$ for each *Rhizoctonia* group for each year given at top of each column

incidence of the binucleate and multinucleate *Rhizoctonia* groups was significantly greater in trial 2 at the end compared to the beginning of the season, and although not significant, the same trend was also recorded for trials 3 and 4 (Fig. 6a). In 2007, incidence of the binucleate group was high in the beginning and middle of the season and low at the end of the season at Tygerhoek (Trials 1 and 3), and at Langgewens (Trials 2 and 4), incidence of this group was high at the beginning and end of the season and low in mid-season (Fig. 6b). Recovery rate of the multinucleate group was high in the beginning and end of the season in all trials and significantly so in trials 2 and 3 (Fig. 6b).

Discussion

This study characterized composition of *Rhizoctonia* populations associated with canola and lupin grown in crop rotational trials conducted in the southern and western production areas of the Western Cape province of South Africa. A higher frequency of binucleate compared to multinucleate *Rhizoctonia* isolates was obtained. The results confirm those reported by Tewoldemedhin et al. (2006) for the southern production area, and by MacLeod and Sweetingham (1997) for lupin in Australia. In this study, among the two crops evaluated, a relatively higher incidence of binucleate AGs and lower incidences of multinucleate *Rhizoctonia* AGs were recorded on lupin and vice versa on canola. The greater overall incidence of binucleate compared to multinucleate isolates may reflect on the possible protection of plants against the more virulent multinucleate *Rhizoctonia* AGs by the less virulent binucleate AGs (Herr 1995), an aspect that will be evaluated later in this ongoing study.

In both the 2006 and 2007 growth seasons, four binucleate (A, Bo, I and K) and three multinucleate (2-1, 2-2 and 11) AGs were recovered from the four trials. All the binucleate AGs were obtained from both canola and lupin, but the multinucleate AG-2-1 was isolated from canola only and AG-11 only from lupin. In a previous study, Tewoldemedhin et al. (2006) recorded the same AGs obtained in the present study, with the exception of AG-Bo. They also isolated two AGs (AG-3 and 4 HG-II) from canola and lupin that were not recovered in our study, and AG-2-2 was not recoved from canola or lupin. Thus far in our studies in South Africa, we have not isolated *R. solani* AG-8 (MacNish 1983) from canola or lupin or thin binucleate *Rhizoctonia* (TBR) (MacLeod and Sweetingham 1997; MacLeod 2001; MacNish and O'Brien 2003) from lupin as reported in Australia.

All AGs identified in this study were obtained from both the western (Langgewens) and southern (Tygerhoek) production areas of the Western Cape province. The highly virulent AG-2-1 was commonly recovered from canola, an AG that is known to limit production in other parts of the world (Kaminski and Verma 1985; Hwang et al. 1986; Gugel et al. 1987;

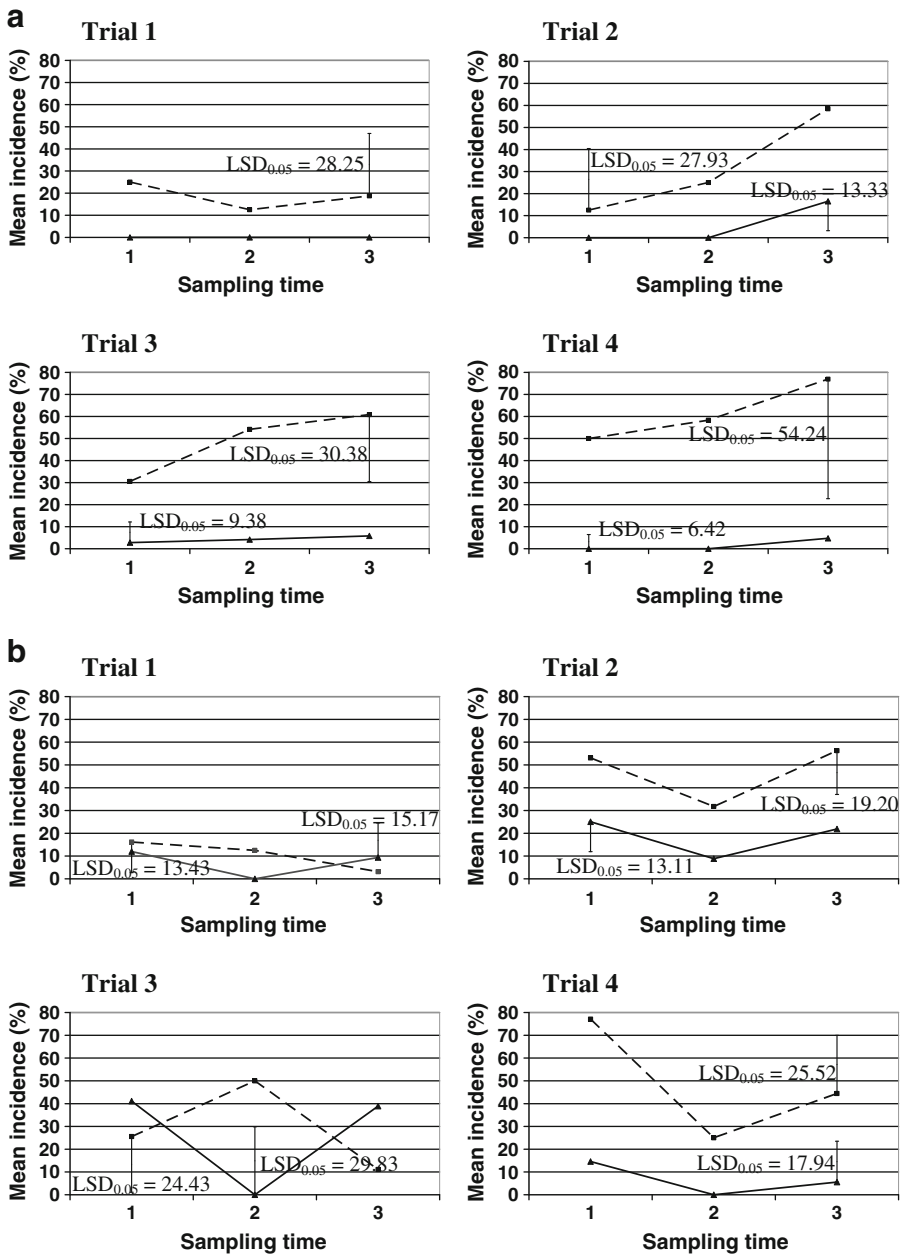

Fig. 6 Effect of sampling time on the mean percentage of binucleate (- - -) and multinucleate (▲) *Rhizoctonia* on canola and lupin sampled in the four trials during 2006 (**a**) and 2007 (**b**). Values are mean percentage of binucleate or multinucleate isolates out of the total number of *Rhizoctonia* isolates obtained for all three sampling times for each rotation system for each year (season). Student's t-LSD $P=0.05$ for each *Rhizoctonia* group for each trial and year given at each line

Khanghura et al. 1999; Klein-Gebbinck and Woods 2002; Paulitz and Okubara 2006). This AG was previously isolated from canola in studies restricted to the Tygerhoek experimental farm (Tewoldemedhin et al. 2006), which represents the southern production area in the Western Cape province. Our study therefore confirms that AG-2-1 is also present on canola in the western production area of this province. Although AG-4 HG-II was previously recorded on canola and lupin in the southern production area and proved to be highly virulent on these crops (Tewoldemedhin et al. 2006), the absence of this AG on these same crops in our study, even in rotations where canola was planted after crops such as medic, an important host of this pathogen (Kulik et al. 1995), suggests that this AG-4 HG-II is not an important pathogen of canola and lupin under field conditions in the Western Cape province.

Previously, AG-2-1 and 11 were shown to be highly virulent on lupin (Tewoldemedhin et al. 2006).

Although AG-11 was commonly isolated from this crop in the current study, AG-2-1 was not isolated from lupin, and there is only one other report of the pathogenicity of AG-2-1 on lupin (Sweetingham et al. 1986). These reports taken together indicate that AG-2-1 is not likely to function as a significant pathogen of lupin under field conditions. However, the recovery of AG-11 from both the southern and the western production areas indicates the importance of this AG on lupin in the Western Cape province.

Isolates of AG-2-2 were recovered less frequently than isolates of AG-2-1 in this study. Previously, Tewoldemedhin et al. (2006) also recorded a low incidence of AG-2-2 and showed that although it was isolated from medic only, it was highly virulent on canola, lupin and medic, and significantly more virulent on barley and wheat than AG-2-1. It is therefore possible that AG-2-2 can cause problems with establishment of canola and lupin planted after barley and wheat in rotation systems in the Western Cape province. However, isolations conducted in 2007 from canola exhibiting damping-off symptoms when planted after barley in the Western Cape province, have yielded only AG-2-1 (Lamprecht, unpublished).

The fact that canola following wheat, and lupin following wheat in the same crop sequences (W-L-W-C and W-C-W-L) had different multinucleate AGs associated with these crops emphasizes the selective effect of these crops for important Rhizoctonia pathogens. Furthermore, the high frequency of isolation of binucleate *Rhizoctonia* associated mostly with lupin compared with canola in these crop sequences also indicates a selective effect of lupin for binucleate *Rhizoctonia* AGs, especially AG-I and K.

Information on the effect of crop rotation on soilborne pathogens of canola and lupin is very limited. Yang et al. (1995) found that rotation of canola with barley for two or more years reduced the population of *R. solani* AG-2-1. There are no previous reports concerning the effect of crop rotation on the AGs of *Rhizoctonia* recovered from lupin in the Western Cape province. Among the rotation systems evaluated in this study, canola and lupin were included only every fourth year in the rotation sequence. In 2006, there were no significant differences in the incidences of AG-2-1 recorded on canola in the different systems evaluated. The same applied for AG-2-2 on canola and lupin and AG-11 on lupin. In 2007, however, canola planted after wheat (W-L-W-C and W-W-W-C) yielded a significantly higher incidence of AG-2-1 than canola planted after medic (M-W-M-C) and lupin (W-W-L-C) at Langgewens. Lupin planted after wheat (W-C-W-L) exhibited a significantly higher incidence of AG-11 than lupin planted after barley (C-W-B-L) at Tygerhoek. Since rainfall differed quite dramatically from 2006 to 2007 in the two areas, it seems possible that environmental conditions can influence the effect of rotation systems on the incidence of *Rhizoctonia* AGs on canola and lupin. Based on these findings, it is evident that utilization of barley, rather than wheat, as a pre-crop would be preferable.

In general, use of cropping sequences as a tool to manage soilborne diseases, including those incited by *R. solani* AGs, in lupin or canola production systems will likely benefit from incorporation of wheat or barley pre-crops rather than broadleaf crops such as medic. Canola and lupin are universal hosts for *Sclerotinia sclerotiorum* and canola, lupin and medic (annual *Medicago* spp.) are susceptible to *Pythium* spp. and *Fusarium avenaceum* (Lamprecht et al. 1988; Sweetingham 1989; Bateman et al. 1991; Simpfendorfer et al. 2004; De Villiers et al. 2006; Hemmati et al. 2009). It is therefore important that the profile of pathogens inciting soilborne diseases of canola and lupin in a specific area be taken into account when rotation systems for disease management are recommended.

Among the numerous trials evaluated in this study, tillage treatment was only observed to affect incidence of the binucleate *Rhizoctonia* group, with higher recovery from the conventional tillage treatment than minimum or no-till treatments, and only during the 2007 trial. In Australia, Brennan and Crabtree (1989) found that the incidence of *Rhizoctonia* bare patch disease of lupin (caused by *R. solani* AG-8) was decreased and yield increased by increasing depth of cultivation. In contrast with *Rhizoctonia* bare patch disease, Eradu-patch disease, caused by a binucleate *Rhizoctonia* (TBR), was not significantly reduced by conventional tillage practice (MacLeod and Sweetingham 1997). Limited research has been conducted on the effect of tillage on canola diseases (Kharbanda and Tewari 1996). Turkington et al. (1995) reported that tillage did not affect brown girdling root rot (caused by *R. solani* AG-2-1) of canola. However, Arshad et al. (1997) indicated that

no-till reduced this disease. Soon et al. (2005) also found that infection of canola with AG-2-1 increased with tillage intensity. Our results on the effect of tillage on the incidence of *Rhizoctonia* AGs in canola and lupin are not conclusive at this stage, and the conflicting information indicates that more targeted studies on the effect of tillage practices on *Rhizoctonia* diseases of canola and lupin is warranted.

In 2006, the recovery of *Rhizoctonia* isolates increased from the beginning to the end of the season, which corroborates the previous findings from studies conducted in the southern production area of the Western Cape province (Tewoldemedhin et al. 2006). MacLeod and Sweetingham (1997) also found an increase in the recovery of *Rhizoctonia* isolates on lupin from July to August. During 2007 the incidence of bi-and multinucleate *Rhizoctonia* AGs were high at the first and third sampling, but lower at the second sampling at Langgewens, whereas at Tygerhoek incidence of the binucleate group was high in the beginning and middle of the season and low at the end, but the multinucleate group at Tygerhoek followed the same trend as that observed for trials at Langgewens. The differences in rainfall patterns (abnormally high rainfall during mid-season at Langgewens) at the two locations may account for the differences in isolation patterns that were recorded.

The results of the present study showed that important *Rhizoctonia* AGs such as AG-2-1, AG-2-2 and AG-11 occur in both the southern and the western production areas in the Western Cape province and that crop rotation affects the incidence of *Rhizoctonia* AGs. Future research will focus on the development of integrated disease management strategies including seed treatment, cultivar resistance and the protective effect of binucleate AGs against disease inciting pathogenic multinucleate AGs on canola and lupin, to reduce losses caused by these pathogens on canola and lupin in the Western Cape province of South Africa.

Acknowledgements This research was financially supported by the Protein Research Trust (PRT), the Agricultural Research Council-Plant Protection Research Institute (ARC-PPRI), the Agricultural Research Services of the United States Department of Agriculture (USDA-ARS) and the Department of Agriculture of the Western Cape Province of South Africa. We also thank Alta Schoeman, Almarie Van den Heever, Sheryldene Williams, Gregory Anthony, John Deysel and Lizzie Hugo for technical assistance.

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