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Crop Residue Affects Rhizoctonia solani Population Dynamics and Seedling Blight of Canola

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Abstract: Seedling blight caused by *Rhizoctonia solani* Kühn substantially reduces stand establishment and seed yield of canola (*Brassica napus* L.) in western Canada. The effect of crop residue on soil populations of *R. solani* and canola seedling blight was examined under field, greenhouse and laboratory conditions. Field plots were established with inoculation or noninoculation with *R. solani* as the main plot and barley, canola, oat and field pea residues as the sub-plots. Soil samples were collected from each subplot for analysis in a greenhouse bioassay and laboratory assay of *R. solani* population before seeding canola. The crop residue effect was not significant. Under inoculation with *R. solani*, the yield was consistently greater when canola was grown on barley residue compared to the canola residue over two-year trials, although oat and pea residue contributed to greater yield. Without inoculation, canola yield was greatest when grown on barley residue, intermediate on oat and pea and the least on canola in the first trial and in the second trial greater yield was obtained on barley and oat residues compared to other residues. In the greenhouse bioassay, canola seedling emergence was greater, while damping off and root rot were less severe, following barley or oat compared to canola or field pea in both inoculated and non-inoculated treatments. Populations of *Rhizoctonia* were lower following barley or oat relative to canola or field pea. Crop rotation and incorporation of barley or oat residue between canola crops may be a useful strategy to reduce seedling blight of canola.

Key words: Barley, Brassica napus, crop residue, crop rotation, cultural management, field pea, oat

INTRODUCTION

Seedling blight, also known as damping-off of canola (Brassica napus L., B. rapa L.) caused solani Kühn Rhizoctonia [teleomorph: Thanatephorus cucumeris (Frank) Donk], has been a serious problem in the Canadian prairies for many years (Petrie and Vanterpool, 1970; Rimmer and Platford, 1982; Sippell et al., 1985). Rhizoctonia solani can cause seed decay or seedlings may shrivel and die shortly after emergence, resulting in pre- and post-emergent damping off, respectively. The symptoms appear as constrictions at or below the soil line. Severe seedling blight may reduce stand establishment and seed yield substantially. For example, in central Alberta, Canada, seedling establishment of canola was reduced in over 20% of fields in 2000-2002 (Hwang et al., 2009). Early reports also indicated that in the Peace River and central regions of Alberta, 80-100% of plants were observed to be infected

in some canola fields and estimated yield losses were in excess of 20% (Sippell *et al.*, 1985; Gugel *et al.*, 1987). *Rhizoctonia solani* can cause root rot or foot rot in adult plants which reduces plant vigour and subsequent yield potential by reducing the number of roots available for nutrient and water uptake (Xi *et al.*, 1995).

Crop rotation has been one of the key components of the disease management of field crops in western Canada (Christen and Sieling, 1995; Howard, 1996; Kharbanda and Tewari, 1996). However, in the recent times canola rotations have been shortened in Alberta (Hartman, 2012; Kutcher *et al.*, 2013) and crops are now seeded directly into standing stubble, into a cooler and wetter soil compared to traditional cultivation practices (Kutcher and Brandt, 2006). A study by Yitbarek *et al.* (1988) has shown that short rotations increase the populations of soil-borne pathogens.

Genetic resistance to seedling blight and foot rot of canola is currently not available (Kataria and Verma, 1992)

and fungicide seed treatments are not highly effective (Soon et al., 2005). Manipulation of cultural practices or inclusion of suitable crops in the rotation system may change the soil environment so that it becomes unfavorable for disease development (Kharbanda and Tewari, 1996; Krupinsky et al., 2002). Therefore, effective management of seedling blight should combine the use of available control options, including cultural practices, for an integrated management system.

There is little quantitative data on the effect of crop residues on *R. solani* populations causing seedling blight of canola or on their impact on canola stand establishment and seed yield. However, under reduced tillage, cultivation of a non-host crop such as barley (Hordeum vulgare L.) for two or more years between canola crops may reduce seedling blight and root rot of canola (Yang et al., 1995). While a higher incidence root rot on canola due to *R. solani* was found when canola was grown after fescue (Festuca L.) (Evans,1994). Therefore, the objective of the study was to examine the dynamics of *R. solani* populations on seedling blight and yield of canola after incorporation of barley, canola, oat (Avena sativa L.) or field pea (Pisum sativum L.) residues into the soil.

MATERIALS AND METHODS

Inoculum preparation: Canola seedlings with symptoms of seedling blight or root rot were collected from commercial fields near Edmonton, AB, in 2009. Diseased sections of the stems were plated on water agar and purified by removing the hyphal tips and transferring them to fresh Potato Dextrose Agar (PDA) growth aggressive isolate of R. solani medium. An (isolate CA7-9, AG 2) was selected for use in the study based on previous testing (data not shown). The isolate was maintained at 4°C until needed on Potato Dextrose Agar (PDA) medium. Inoculum was produced using the method described by Hwang (1988). Briefly, the isolate was cultured on sterilized grain (rye-oat grains; 1:1) until they were covered with fungal mycelium. The colonized grains were air-dried, ground and sifted using a 2 mm screen and then were stored at 4°C until used as inoculum. This process yielded a population density of 2×10⁷ CFU g⁻¹ of inoculum.

Field experiment: Field plots were established at Crop Development Centre North, Edmonton (53°39' N, 113°21' W) on a black Chernozemic loam soil in 2010. The study was arranged in a split-plot design with four replications, with inoculation (*Rhizoctonia* and a non-inoculated control) as the main plot treatments and

crop residue type (barley, canola, oat or pea) as the sub-plot treatments. Prior to seeding in the spring, the site was cultivated and then rototilled on May 31. Each plot was 6×1 m, with 1 m between plots and 2 m between blocks. Four rows of barley cv. (CDC) Coalition, Canola cv. 45H29 (treated with thiamethoxam (Cruiser 350 FS at 800 mL/100 kg seed) to discourage flea beetles (Pyllotreta spp.)), oat cv. Derby or field pea cv. Midas were seeded with a mechanical seeder in each sub-plot on May 31, 2010. The seeding rate for canola was 1.25 kg ha⁻¹ (0.75 g row⁻¹), for barley and oat it was 25 kg ha⁻¹ and for pea it was 33.3 kg ha⁻¹. Inoculum of R. solani was placed in the seed row at a rate of 25 L ha⁻¹ (15 mL row⁻¹) during seeding. Routine crop management practices, such as fertilization and weed control were carried out as for commercial crops in the region. At maturity, the crops were harvested and the residues were ploughed and incorporated into the soil with a rototiller on October 18, 2010. The working surfaces of the rototiller were cleaned with a pressure washer; all non-inoculated plots were cultivated first, followed by the inoculated plots.

Each plot in the trial was seeded to canola cv. 45H29 at 1.25 kg ha⁻¹ on May 31, 2011 as described previously, except that no pre-seeding tillage was applied and no inoculum was added. Seedling emergence was assessed (every plant in each plot) at 3 weeks after seeding and the crop was harvested on September 14, 2011 with a small-plot combine. Seed yield was determined after the samples were dried and weighed.

Prior to seeding in the spring of 2011, five soil samples (one from each of five locations) of about 5 kg for each plot were collected (from the top 10 cm of soil) and mixed thoroughly for use in the greenhouse study (described below).

A repetition of the experiment was initiated in 2011 using the same design, methods and agronomic practices as described above. The site was disked and rototilled on April 24, 2011, the four crops were seeded on May 31, crop residue was incorporated with a rototiller on October 18, soil samples were collected the following spring in 2012, canola was seeded on May 31, 2012 and the plots were harvested on September 19, 2012.

Greenhouse bioassay: Twenty replicate cups (9 cm dia) for each field treatment (five from each plot) were filled with the bulked soil sample that had been collected in the spring of 2011. The cups were placed on the greenhouse bench in a split plot design with four replicates, with inoculated and non-inoculated soil as main plots and cropping treatments from the field as sub-plots. The greenhouse temperature was maintained at 20±2°C, with

a 16 h photoperiod at an intensity of 140 µmol m⁻² sec⁻¹. Ten seeds of canola cv. 45H29 were sown into each cup and grown for 3 weeks. Seedling emergence and post-emergence damping-off were counted at 8 days after seeding and plant height was measured 10 days after seeding. Root rot severity at the base of the seedlings was rated two weeks after seeding on a 0-4 scale, where: (0) Healthy seedling, (1) Discoloration at the base, (2) Small lesions, (3) Sunken lesions but stem not girdled and (4) Stem girdled, seedling dead or dying (Hwang *et al.*, 2007). After the disease data were recorded, the plants were air-dried at 37°C and dry weight was measured. The experiment was repeated in 2012 with soil from the repetition of the field trial.

Quantification of Rhizoctonia: Rhizoctonia populations were estimated via soil dilution plating onto a Rhizoctonia-selective medium (Ko and Hora, 1971). A 15-20 g subsample of each soil sample collected for the greenhouse bioassay was ground with a mortar and pestle and sifted through a 30 mesh sieve. Ten grams of the resulting sample were added to 100 mL of 0.1% sterile water agar medium to obtain a 10x dilution. A 1 mL aliquot of the suspension of each sample was transferred onto each of six replicate 10 cm diameter Petri dishes containing the selective medium (Ko and Hora, 1971). The dishes were incubated at room temperature on a laboratory bench. Colony counts were performed at 5 days after inoculation.

Data analysis: The data analyses were conducted using SAS software (SAS, 2008). Prior to analysis, each data set was tested for homogeneity of variance using a normal probability plot and any outliers in the data sets were eliminated using the Univariate Procedure in SAS. Analysis of variance revealed significant effects of repetition and a repetition x treatment interaction for several response variables, so tests from each experiment were analyzed separately using the GLM procedure of SAS. Means were compared using the LSMEAN T-test at p≤0.05 unless otherwise specified.

RESULTS

Field experiment: Under field conditions, inoculation with *R. solani* reduced seedling emergence but there was no effect of crop residue or an inoculation×residue interaction in either year (Table 1). In the 2010-2011 trial, seed yield in the inoculated plots was lower when canola was grown on canola residue compared to canola grown on barley, oat or field pea residues but there was no difference in yield among the barley, oat or field pea

Table 1: Effect of the residues of barley, oat, field pea and canola under inoculation or non-inoculation with *Rhizoctonia solani* on canola stand establishment and seed yield in field trials at Edmonton, AB, in 2011- 2012

		2011		
			2012	
Inoculation	Residue	Plants/row	Yield (t ha ⁻¹)	Yield (t ha ⁻¹)
R. solani	Barley	49.4ª	2.85ª	1.99⁴
	Oat	63.1ª	2.92ª	1.43^{b}
	Field pea	47.1ª	2.69a	1.30°
	Canola	48.1ª	2.25^{b}	$1.12^{\rm b}$
	Mean	51.9 ^B	2.68^{A}	1.46^{A}
Non-inoculated	Barley	67.4ª	3.47ª	1.91⁴
	Oat	64.6ª	3.37^{b}	1.80^{a}
	Field pea	65.1ª	3.08^{b}	$1.24^{\rm b}$
	Canola	58.3ª	2.60°	$0.91^{\rm b}$
	Mean	63.9 ^A	3.13 ^A	1.47 ^A

Data are the mean of four replications. Means followed by the same lowercase letter within R. solani inoculated or non-inoculated treatments are not significantly different at $p \le 0.05$ according to the LS Means T-test. Overall means (boldface font) for inoculated and non-inoculated treatments (plants/row and yield) that are followed by the same uppercase letter are not significantly different at $p \le 0.05$ according to the LS Means T-test

residue treatments. A similar pattern of results was observed in the non-inoculated plots, except that canola yield was higher on barley residue than on oat and field pea residue. In the 2011-2012 trial, seed yield was generally lower than in 2010-2011. In the inoculated plots, seed yield was higher on barley residue than on oat, pea and canola residues but the difference among the oat, pea and canola residues was not significant. In the non-inoculated plots, seed yield was higher on barley and oat residue than on canola and pea residue.

Greenhouse bioassay: In both tests of the greenhouse trial, canola seedling emergence was higher and root rot severity was lower in soil from the barley and oat residue treatments compared to soil from the canola and pea residue (Table 2). In soil collected in 2011 from the field experiment in 2010, the number of plants with symptoms of damping-off was higher in canola on field pea residue than on any of the other residues in non-inoculated plots.

In 2012, damping off was greater in canola and pea residue compared to barley and oat residue (Table 2). In 2011, damping-off was equally severe when canola was grown after incorporation of canola or pea residues in inoculated plots and was more severe in canola and pea residue compared to either barley or oat residue (Table 2). Canola seedlings were shortest on field pea residue in the inoculated treatments and shortest on canola residue in the non-inoculated treatments but there were no differences among the other crop residue treatments. In 2012, damping-off was greater in canola residue compared to all other crops in soil from the inoculated plot (Table 2). In 2011, plant height was greater for all residue types compared to the pea residue in the inoculated plots and was greater for all residue types compared to the canola

Table 2: Effect of residue from the preceding crop and inoculation with Rhizoctonia solani on seedling emergence, seedling blight and damping-off and growth of canola plants in soils collected from field trials at Edmonton, AB, in the spring of 2010-2011 under greenhouse conditions

	8	ase contains	Damping	Root rot		Plant dry
Inoculation	Crop	Emergence	e-off	severity	height	weight
treatment	residue	(%)	(%)	(0-4)	(cm)	(g)
2010						
Rhizoctonia	Barley	72ª	8.5 ^b	1.67^{b}	2.75ª	0.16^{a}
	Oat	66ª	3.3°	1.65^{b}	2.68^{a}	0.15^{a}
	Pea	45 ^b	13.5a	2.59a	2.61^{b}	0.15^{a}
	Canola	44 ^b	13.3ª	2.60^{a}	2.91ª	0.16^{a}
	Mean	57 ^B	9.7 ^A	2.13^{A}	2.74 ^A	0.16^{A}
Noninoculate d	Barley	77ª	$3.3^{\rm b}$	1.32^{b}	3.03ª	0.17^{b}
	Oat	74ª	4.8°	1.37^{b}	3.06^{a}	0.19⁴
	Pea	75ª	7.0°	2.00^{a}	2.84^{a}	0.17^{b}
	Canola	74ª	4.5^{b}	2.03ª	2.53^{b}	0.15°
	Mean	75 ^A	4.9^{A}	1.68^{B}	2.87 ^A	0.17^{A}
2011						
Rhizoctonia	Barley	73ª	5.4 ^b	0.58^{b}	2.36^{a}	0.21ª
	Oat	80^{a}	$3.3^{\rm b}$	0.69^{b}	2.61ª	0.20^{a}
	Pea	48 ^b	6.3^{f}	1.16ª	2.56^{a}	0.24^{a}
	Canola	45^{b}	10.4^{a}	1.12ª	2.62^{a}	0.23^{a}
	Mean	62 ^A	6.4 ^A	0.89^{A}	2.54^{A}	0.22^{A}
Non inoculated	Barley	73ª	2.9°	0.69^{b}	2.62^{a}	0.25a
	Oat	74ª	$3.3^{\rm b}$	0.64^{b}	2.47 ^b	0.22^{a}
	Pea	46⁰	7.9ª	1.17^{a}	2.81ª	0.24^{a}
	Canola	50°	7.9ª	1.03ª	2.41^{b}	0.22^a
	Mean	61 ^A	5.5 ^A	0.88^{A}	2.58 ^A	0.23^{A}

Data are the mean of two repetitions x 20 replicate cups (four composite soil samples representing four field replication of a treatment×five cups soil⁻¹ sample). Means followed by the same lowercase letter within inoculation treatment and year do not differ at p≤0.05 according to the LS Means T-test. Overall means for inoculated and non-inoculated treatments (plants row⁻¹ and yield) that are followed by the same uppercase letter are not significantly different at p≤0.05 according to the LS Means T-test

residue in the non-inoculated plots. In 2012, plant height was similar for all residue types in the inoculated plots but was greater in the plants grown in soil with barley or pea residue compared to the canola or oat residue in soil from the non-inoculated plots (Table 2). Crop residue treatment only affected plant weight of plants sown on canola residue in the non-inoculated plots, where weight was highest on oat residue and lowest on canola residue in 2011. There was no effect of treatment on plant dry weight in 2012 (Table 2).

Quantification of *Rhizoctonia*: In the isolation study, populations of *R. solani* were generally higher in the inoculated versus non inoculated treatments, irrespective of crop residue treatment, although the differences were not always significant (Fig. 1a-b). Populations in the inoculated plots were lower on oat residue than on barley, canola or pea residues from 2011 (Fig. 1a). In the non-inoculated plots, populations were lower on barley and oat residue than on canola or field pea residue. In 2012, populations of *R. solani* were lower on barley and oat residue than on canola and field pea residue in both the inoculated and non-inoculated treatments (Fig. 1b).

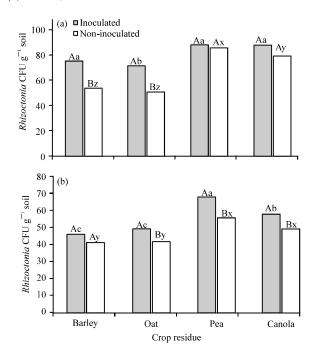


Fig. 1(a-b): Effect of crop residues on the population of *Rhizoctonia solani* in soil in two trials of field experiments conducted during, (a) 2010-2011 and (b) 2011-2012, respectively. Each bar represents the mean CFU of 24 plates (four replicate soil samples representing four field replications of a treatment x six replicate plates for each soil sample). Uppercase letters (A and B) indicate differences within residues (inoculated vs. non-inoculated), while lower case letters indicate differences among residues in inoculated treatments (a, b, c) or non-inoculated treatments (x, y, z) (p≤0.05). CFU: Colony forming unit

DISCUSSION

Cultural practices including the application of organic soil amendments and management of the type and quantity of crop residue have a direct impact on plant health and crop productivity and have been shown to influence the intensity of a range of plant diseases (Abawi and Widmer, 2000; Bailey and Lazarovits, 2003). In the present study, seedling emergence was improved and damping-off and root rot severity were reduced, in canola following crops of barley or oat relative to canola following canola or field pea in field trials in 2011 and 2012. A similar pattern of response was observed in a greenhouse bioassay of soil collected from each study site in the early spring of the assessment year. In the field study, the seed yield of canola was higher when grown on

barley, oat or field pea residues compared to canola residue. Similar results were reported where the yields of canola increased when grown following wheat, barley or pea compared to consecutive crops of *B. napus* (Christen and Sieling, 1995). Moreover, they reported that continuous cropping of any crop result in increased pathogens or insect pests specific to that crop. These phenomena may have contributed the reduction of canola yield when canola was grown on canola residue relative to the other crops in the rotation.

In the current study, populations of R. solani were generally higher in inoculated than non-inoculated treatments, irrespective of the crop residue treatment, although the differences were not always significant. Rhizoctonia solani populations were reduced, however, when oat or barley residue was incorporated into the soil compared with canola or field pea residue. This is consistent with the results of a previous study that reported that Rhizoctonia populations were reduced when barley was grown for two to three years after canola in zero tillage (Yang et al., 1995). Studies have shown that crop residue incorporation in the soil creates soil suppression by enhancing antagonistic microorganism (Sturz et al., 1997; Peters et al., 2003) resulting reduction in the soil pathogen. In this study, barley and oat residues may have been the preferred substrate of growing antagonistic micro-organism that reduces R. solani population in this study.

Crop rotation with barley or oat between canola crops may be a useful strategy to manage seedling blight of canola. Rotation with field pea was generally less effective at increasing seedling emergence and reducing damping-off and root rot severity. It is possible that decomposition of the nitrogen-rich pea residue encouraged a build-up of *Rhizoctonia* populations. However, it is also possible that populations of *R. solani* had increased on the preceding crop of canola and field pea via infection of the roots. Whatever the underlying reason, these data indicate that populations of *R. solani* were reduced, damping-off was reduced and seedling emergence and seed yield of canola was increased by inclusion of barley or oat in rotation with canola.

CONCLUSION

This study revealed that barley and oat residues caused a greater reduction in canola damping off, disease severity and *Rhizoctonia* populations than pea or canola residues. Since seedling blight of canola is a serious concern in stand establishment and no resistance cultivar is available crop rotation and incorporation with barley or oat residues between canola crops may be a useful strategy to manage seedling blight of canola.

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