Effects of crop rotation and tillage on blackleg disease of canola

X.W. Guo, W.G.D. Fernando, and M. Entz

Abstract: The effects of a 4-year crop rotation and two tillage systems on blackleg (*Leptosphaeria maculans*) disease of canola were determined in a field experiment carried out from 1999 through 2002 at Carman, Manitoba. Crops in rotation were canola (*Brassica napus*) (C), wheat (*Triticum aestivum*) (W), and flax (*Linum usitatissimum*) (F). Rotations were done under conventional tillage (T) or zero-till (Z) systems. Diseased stem incidence and severity were assessed. Diseased stem incidence and severity in 2001 and 2002 were significantly lower when canola was rotated with wheat, and rotated with wheat and flax under both tillage systems. The CCCCT rotation had a higher disease incidence than CWCCT, CWFCT, and CCWCT rotations, which were not significantly different from each other. Zero-till plots had significantly greater diseased stem incidence and severity than tilled plots, with or without rotation. Tillage reduced the disease when it was performed with a simple-crop rotation; however, the effect was reduced with a two-crop rotation. The results suggest that an appropriate rotation and tillage strategy could be effective in reducing the severity of blackleg of canola in farmers' fields.

Key words: crop rotation, tillage, canola, wheat, flax, *Leptosphaeria maculans*, blackleg disease.

Résumé : De 1999 à 2002 à Carman (Manitoba) les effets d'une rotation des cultures de 4 ans et de deux systèmes de travail du sol sur la maladie de la jambe noire (*Leptosphaeria maculans*) du canola furent déterminés dans une expérience au champ. Les cultures dans la rotation étaient le canola (*Brassica napus*) (C), le blé (*Triticum aestivum*) (W) et le lin (*Linum usitatissimum*) (F). Les rotations furent effectuées selon les systèmes de travail du sol classique (T) ou de semis direct (Z). La fréquence de tiges malades et la gravité de la maladie sur ces tiges furent évaluées. La fréquence de tiges malades et la gravité en 2001 et 2002 furent significativement plus faibles lorsque le canola fut inclus dans la rotation avec le blé et lorsqu'il fut inclus dans la rotation avec le blé et le lin pour les deux systèmes de travail du sol. La rotation CCCCT avait une fréquence de maladie plus élevée que les rotations CWCCT, CWFCT et CCWCT qui n'étaient pas significativement différentes l'une de l'autre. Les parcelles sans travail du sol avaient une fréquence de tiges malades et une gravité significativement plus élevées que les parcelles travaillées, avec ou sans rotation. Le travail du sol diminua la maladie lorsqu'il fut effectué avec une monoculture; cependant, son effet fut moins grand avec une rotation à deux cultures. Les résultats suggèrent qu'une stratégie appropriée de rotation et de travail du sol pourrait être efficace à réduire la gravité de la jambe noire du canola dans les champs des producteurs.

Mots clés : rotation des cultures, façons culturales, canola, blé, lin, *Leptosphaeria maculans*, maladie de la jambe noire.

Introduction

Canola (*Brassica napus* L., and *Brassica rapa* L.) is one of the major oilseed crops grown worldwide. With the significant expansion of acreage sown to canola during the past 2 decades (Howlett et al. 2001), blackleg caused by *Leptosphaeria maculans* (Desm.) Ces. et de Not. (anamorph: *Phoma lingam* (Tode ex Fr.) Desm.), one of the most serious diseases of canola, has started to appear in new areas (Fernando and Chen 2003; Fernando et al. 2003).

The most important source of inoculum of *L. maculans* is previously infected canola stubble, bearing pseudothecia from which ascospores can be released (Sawatsky 1989). Crop rotation and tillage are two methods of reducing the association between crop and pathogen and are relatively easy for farmers to employ. Theoretically, appropriate crop rotation lengthens the time between similar host plants, so pathogen populations will decline. Crop rotation takes advantage of the fact that plant pathogens important on one crop may not cause problems on another crop (Kharbanda 1999). Gracia-Garza et al. (2002) reported that 2-year crop rotations of corn (*Zea maize* L.) or winter wheat (*Triticum aestivum* L.) with soybean (*Glycine soja* Sieb. et Zucc.) had a significant effect on reducing production of apothecia by

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sclerotia of *Sclerotinia sclerotiorum* in soybean fields. Germination of sclerotia was decreased in crop rotations compared with continuous soybean. Dill-Macky and Jones (2000) found that fusarium head blight (FHB) of wheat could be significantly reduced with a wheat–soybean rotation. Bockus and Claassen (1992) showed that wheat–sorghum (*Sorghum bicolor* L.) rotation decreased severity of tan spot on winter wheat.

Tillage can affect survival of *L. maculans* in three ways: (1) breaking up infected stubble to promote its decomposition, (2) improving the environment for stubble-decomposing microorganisms to survive in the soil, e.g., by burying them to protect them from drying out at the soil surface, and (3) changing (for better or worse) the conditions in which the fungus exists in the soil (Kharbanda 1999). Bailey et al. (1992) reported that common root rot symptoms on wheat decreased in 3 of 4 years under minimum-till or zero-till compared with conventional tillage. Tinline and Spurr (1991) showed that common root rot was reduced significantly in 3 to 6 years under reduced tillage. Dill-Macky and Jones (2000) reported that moldboard plowing favors the decline of *Fusarium graminearum* Schwabe (Gibberellazeae (Schwein.: Fr.) Petch) on wheat residue, and prevents the development of the pathogen by burying residue. Wrather and Kendig (1998) found disk-tillage or moldboard plowing significantly reduced the soil population density of *Macrophomina phaseolina* (Tassi) Goid in a soybean field.

From the literature cited above, it is apparent that different pathogens react differently to conditions induced by rotation and tillage systems. Although survey reports are available, no systematic study has been published on the effects of rotation and tillage on blackleg disease of canola. Therefore the objective of this research was to investigate the effects of a 4-year study on crop rotation and two tillage systems on the incidence and severity of blackleg disease of canola.

Materials and methods

Inoculum preparation

Leptosphaeria maculans pathogenicity group 2 (PG-2) isolate 86-12 was collected from a field at Swan Lake, Manitoba, Canada, in 1986. The isolate was stored on potato dextrose agar (PDA) medium (Becton, Dickinson and Company, Sparks, Maryland, USA) under sterilized mineral oil at 20 °C in the dark and annually inoculated to and isolated from the canola cultivar 'Westar' to maintain pathogen virulence. Mycelia of the fungus were transferred from the PDA storage medium onto V-8 juice (Campbell Soup Company, Toronto, Ontario, Canada) agar media amended with 0.1g/L streptomycin sulfate (Sigma-Aldrich Co., St. Louis, Missouri, USA) and 0.75g/L $CaCO_3$ (Fisher Scientific, Fair Lawn, New Jersey, USA) and incubated under light at 24 °C for 15 days before use. Pycnidiospores produced on the V-8 agar were harvested in distilled sterilized water, filtered into 50-mL disposable sterile centrifuge tubes (Fisher Scientific, Pittsburgh, PA, USA), and counted using a haemocytometer (Hausser Scientific Company, Horsham, Pennsylvania). These tubes were stored on ice during transport to the field site (approximately one hour). Immediately before inoculation, the spores were diluted to a concentration of 1×10^7 spores/mL in distilled water. Tween 20 (Sigma Chemical Co., St. Louis, Missouri, USA) was added into the spore suspension at a ratio of l mL of Tween 20 to 1000 mL of spore suspension as a surfactant.

Experimental design and measurements

The study was conducted at the University of Manitoba's Carman Research Station, Carman, Manitoba, Canada (CRS), from 1999 to 2002. The soil type was a Rignold loam (moderately well-drained; particles are 0.002–0.005 mm in diameter). A split-plot design with three replications was used, with conventional tillage (T) and zero tillage (Z) as main plots and canola (C) in rotation with wheat (W) and flax (F) as subplots. There were four rotations within each tillage treatment: CCCCT (canola was grown for 4 years under conventional tillage in each year), CWCCT, CCWCT, CWFCT, CCCCZ, CWCCZ, CCWCZ, and CWFCZ. The experimental field size was 155 m × 81 m. Each plot was 10 m × 4 m. There was a distance of 15 m between plots and 10 m between a plot and border of the field. The canola cultivar used was 'Westar' (susceptible to *L. maculans* (Anonymous 1999)), the wheat cultivar was 'AC-Barrie', and the flax cultivar was 'Norlin'. Fall rye was sown as the border crop. In 1999, all plots were seeded to canola. A pycnidiospore suspension of *L. maculans* PG-2 isolate 86-12 at 1×10^7 spores/mL was inoculated onto the canola at the three-leaf stage, using a backpack sprayer, from 1900 to 2000 on June 10. One litre of spore suspension was used in each plot. Conventional tillage was conducted in the 12 plots marked as conventional tillage plots using a deep tiller with tine harrow after the crop was harvested each fall, followed by cultivation using a cultivator with tine harrow and coil packer each spring. No tillage was carried out in the 12 zero-till plots for the duration of the experiment. A canola rotation with one nonhost crop was defined as a simple rotation, and a canola rotation with two nonhost crops was defined as a diverse rotation.

Diseased stem incidence and severity were assessed in ten 3-m lines across the width of the plot in 2001 and 2002. The lines were 1 m apart and started 0.5 m away from the edge of the plot. Diseased stem incidence and severity were evaluated using 10 canola plants at the filling stage (seeds in pods turn yellow) along each line, with approximately 0.3 m between each sample. A total of 100 plants were sampled in each plot.

Data analysis

Diseased stem incidence (%) was expressed as number of infected plants per total number of plants sampled × 100, where assessment of infected plants was based on the presence or absence of lesions on a cross section of the crown. Diseased stem severity was assessed using the standard 0–5 scale: 0 = no infection, 1 = lesion area is less than 25% of a cross-sectional area of crown, 2 = 25%–50%, 3 = 51%–75%, 4 = 76%–100%, 5 = plant dead. Diseased stem severity was evaluated as average diseased stem severity over all the infected plants sampled in each plot.

Statistical analyses of diseased stem incidence and diseased stem severity measurements were conducted using ANOVA. For comparisons among the means of different treatments, differences among main-plot treatments (tillage) were tested against the main-plot error mean square, and subplot treatment (rotations) comparisons were tested against the subplot error. For the difference between two rotation systems within same tillage system, standard error of the mean difference was assessed as the square root of [2(error mean square of subplot) / number of replication of main treatments]. For the difference between the means of two tillage systems at single level of rotation system, standard error of the mean difference was assessed as the square root of 2{[(number of subplots per main plot – 1) × error mean square of subplot + error mean square of main plot] / (number of replication of main treatments × number of subplots per main plot)}. The Fisher's protected least significant difference test ($P \leq 0.05$) was performed to assess the significant differences in disease incidence among treatments using normalized data $\arcsin(\bar{x}^{1/2})$, and the differences in diseased stem severity using normalized data $\ln(\bar{x})$.

Mean daily temperature, relative humidity and precipitation, and mean monthly temperature and precipitation at Carman were provided by the CRS. There were no long-term (>10 years) records of weather conditions at CRS; thus mean monthly temperature and precipitation from 1971 through 2000 were recorded at Morden, Manitoba, approximately 30 km away from Carman, and were obtained from the Internet (Canadian Climate Normals 1971–2000, 2003. Website: http://www.climate.weatheroffice.ec.gc.ca/climate_normals).

Results

Tillage and rotation had significant effects on reducing the disease in 2001 (Tables 1 and 2). Growing canola in rotation with wheat (CWC) significantly reduced diseased stem incidence by 18% in the zero-till plots and by 8% in the tilled plots, compared with continuous canola (CCC). The least diseased stem incidence and severity were observed in tilled plots, when canola was rotated with wheat (CWCT). Zero-till plots had significantly greater diseased stem incidence and severity compared with tilled plots, with or without rotation.

The effects of rotation and tillage on diseased stem incidence were significant in 2002 (Table 1). Diseased stem incidence was greater in the CCCCZ rotation than in the CWCCZ, CCWCZ, and CWFCZ rotations (Table 1), greater in the CWCCZ rotation than in the CCWCZ rotation, greater in the CCWCZ rotation than in the CWFCZ rotation, and greater in the CCCCT rotation than in the CWCCT, CWFCT, and CCWCT rotations. The latter three rotations were not significantly different from each other. When canola was grown in rotation with wheat and flax (CWFC) in the tilled and zero-till plots, blackleg incidence was decreased by 28% and 61%, respectively. Tillage reduced diseased stem incidence in all rotations except in the CWFC rotation ($P \leq 0.05$).

Growing a crop other than canola for 1 year (CWCCZ and CCWCZ) resulted in a reduction in diseased stem severity, and not growing canola for 2 years (rotation CWFCZ) reduced diseased stem severity further in zero-till plots (Table 2). There was no difference between CWCCZ and CCWCZ. Diseased stem severity in plants from the CCCCT rotation was greater than that in plants from the CWCCT, CWFCT and CCWCT rotations. There were no significant differences among CWCCT, CWFCT, and CCWCT. Tillage reduced diseased stem severity of all rotations except in the CWFC rotation ($P \leq 0.05$).

Table 1. Blackleg incidence (%) on canola under different rotation and tillage systems at maturity in 2001 and 2002.

	Tillage	
Rotation	Conventional till (T)	Zero till (Z)
2001		
CCC	57.0 aA	81.0 aB
CWC	49.0 bA	63.0 bB
2002		
CCCC	49.3 aA	79.3 aB
CWCC	21.5 bA	61.0 bB
CCWC	16.3 bA	56.7 cB
CWFC	21.0 bA	18.7 dA

Note: C, canola; W, wheat; F, flax. Values represent means calculated from untransformed data; mean difference was calculated from transformed data $\arcsin(\bar{x}^{1/2})$. Different lowercase letters within a column for each year indicate significant difference between different rotation systems; different capital letters within a row indicate significant difference between different tillage systems according to the Fisher's protected least significant difference (LSD) test ($P \geq 0.05$).

Table 2. Blackleg disease severity on canola stems under different rotation and tillage systems at maturity in 2001 and 2002.

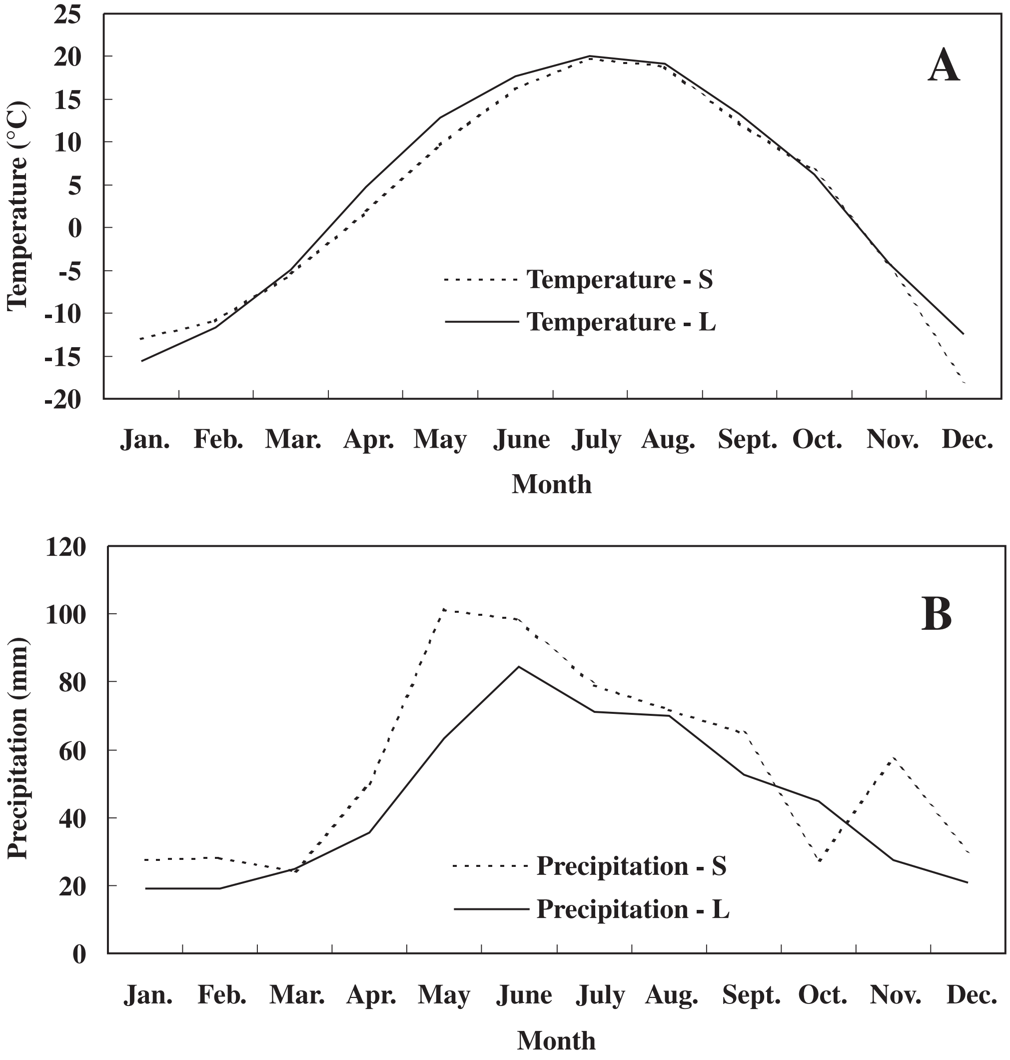
	Tillage	
Rotation	Conventional till (T)	Zero till (Z)
2001		
CCC	3.2 aA	3.6 aB
CWC	2.9 bA	3.4 bB
2002		
CCCC	1.3 aA	3.1 aB
CWCC	1.2 bA	2.0 bB
CCWC	1.0 bA	1.9 bB
CWFC	1.1 bA	1.1 cA

Note: C, canola; W, wheat; F, flax. Values represent means calculated from untransformed data; mean difference was calculated from transformed data $\ln(\bar{x})$. Different lowercase letters within a column for each year indicate significant difference between different rotation systems; different capital letters within a row indicate significant difference between different tillage systems according to the Fisher's protected least significant difference (LSD) test ($P \geq 0.05$).

Discussion

Rotation and tillage had significant effects on reducing blackleg disease at Carman, Manitoba. Canola in rotation with wheat, and in rotation with wheat and flax, can reduce the incidence and severity of blackleg disease in both tilled and zero-till plots. Tillage resulted in a decrease in the disease when a simple rotation was used; however, the effect

Fig. 1. Mean monthly temperature and precipitation from 1999 through 2002 at Carman, Manitoba, and long-term temperature and precipitation patterns from 1971 through 2000 at Morden, Manitoba, approximately 30 km from Carman. S, short term; L, long term.



of tillage on disease was reduced when a diverse rotation was used. This may be partly due to the rotations masking the effects of tillage with a diverse (two-crop) rotation. Therefore, zero tillage appears more feasible where diverse rotations are used, in terms of controlling blackleg disease on canola. This suggests that rotation to nonhost crops with tillage could significantly reduce blackleg disease of canola in farmers' fields.

Although rotation showed a significant decrease in disease in our 4-year study, other studies or surveys have shown crop rotations to have different effects on blackleg of canola. Petrie (1986) showed that a 3-year rotation between canola crops reduced the severity of blackleg disease. Turkington and Clayton (2000) reported that under conventional-tillage conditions, a 4-year rotation helped to limit the level of blackleg inoculum. Morrall et al. (1999) found that when canola was rotated to nonhost crops for more than 5 years, blackleg disease showed a decreasing trend, but contrary to our findings, not in rotations of 1, 2, or 3 years. In our study, the year of nonhost crops in rotation affected the reduction of blackleg incidence and severity. Gossen et al. (2003), in a study on integrated crop management systems, showed no significant differences in blackleg disease associated with a 5-year rotation. These differences may arise from different environmental factors in the study area, sampling design, or prevailing soil conditions that may or may not help the pathogen survival. Effects of crop rotation and tillage on blackleg disease could be different with changes of weather conditions in different years, which impact decomposition of canola stubble (Petrie 1986), formation of pseudothecia (Pérès et al. 1999), and spore dispersal (Guo and Fernando 2005). The numbers of spores trapped from tilled plots was less than than that from zero-till plots in a 1-year study (Guo 2004). Temperature, relative humidity, and precipitation during the period of this study showed little changes in comparison with the long-term weather pattern at Carman (Figs. 1A and 1B), suggesting that interpretation of results from this study should have little impact from the environmental conditions that prevailed from 1999 to 2002 in Carman, Manitoba.

Tillage can play an important role in controlling blackleg disease. Survival of stubble-borne pathogens has been closely correlated with stubble decomposition (Blenis et al. 1999; Kharbanda 1999; Wrather and Kending 1998).

Turkington and Clayton (2002) showed that canola stubble decomposition was more rapid in the soil than on the surface. However, the problem could become complicated with tillage over several years. Infected stubble already in the soil could be brought to the soil surface by the tillage and allow the pathogen to infect and spread within the current host crops. The present study showed that tillage had significant effects on reducing blackleg disease.

Differences in geographical location and cropping systems may be factors limiting our ability to make general conclusions about the effects of tillage and crop rotation on blackleg of canola. In dry regions, it may take longer periods for canola stubble to decompose than in dry sub humid regions such as Carman, Manitoba. The use of resistant cultivars could prove useful in shortening the periods of crop rotations. The effects of tillage on blackleg disease could be clarified with a further understanding of the effect of tillage on distribution of canola residue in the soil, and spore dispersal from crop residues.

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