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Dynamics of infection by **Leptosphaeria maculans** on canola (**Brassica napus**) as influenced by crop rotation and tillage

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Dynamics of infection by *Leptosphaeria maculans* on canola (*Brassica napus*) as influenced by crop rotation and tillage

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Abstract
This study, intending to understand the effects of crop rotation and tillage on blackleg disease, was conducted in a field at Carman, Manitoba, Canada, from 1999 – 2002. Canola, wheat and flax were among the rotated crops. Rotations were performed under conventional or zero-till conditions. The number of infected plants, infected leaves per plant, lesions per plant, and percentage of leaf coverage with lesions decreased when canola was rotated with wheat and flax under zero till. The number of lesions per plant and percentage of leaf coverage with lesions were strongly correlated with stem disease severity, and the number of infected plants with stem disease incidence. Ascospores and pycnidiospores of *Leptosphaeria maculans* were reduced by crop rotation and tillage. This study suggests that the appropriate combination of rotation and tillage may lower airborne inoculum and reduce infection of canola plants by *L. maculans*.

Keywords: Leptosphaeria maculans, Brassica napus, *cropping practice, development of infection, spore dispersal*

Introduction

Blackleg disease, caused by *Leptosphaeria maculans* (Desm.) Ces. et de Not. (anamorph: *Phoma lingam* (Tode ex Fr.) Desm.) is a major disease throughout the world, which causes significant yield losses of canola (*Brassica napus* L.) under favorable conditions, especially high moisture and precipitation.

The disease can attack cotyledons, true leaves, pods and stems of the host plant. Lesions on leaves are white to grey in color, round to irregular in shape, and dotted with numerous black pycnidia. The infection on leaves prior to 3-leaf stage is the major cause of stem canker, which may penetrate and girdle the crown, causing plant lodging and resulting in yield loss (Sawatsky 1989). Ascospores of *L. maculans*, released from pseudothecia under high moisture, are the primary inoculum (Sawatsky 1989). Pycnidiospores, discharged from pycnidia by rain splash, contribute to secondary spread of the disease (Salam et al. 2003).

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Crop rotation and tillage – two methods of breaking the association between a host and a pathogen – are important in infection by fusarium head blight (*Gibberella zeae* [Schwein.] Petch) disease (Dill-Macky & Jones 2000), tan spot (*Pyrenophora tritici-repenstis* [Died.] Drechs.) (Bockus & Claassen 1992), and common root rot (*Cochliobolus sativus* [Ito & Kuribayashi] Drechs. Ex Dast.) (Bailey et al. 1992) in wheat (*Triticum aestivum* L.), and charcoal rot (*Macrophomina phaseolina* [Tassi] Goid) in soybean (Wrather & Kending 1998). Some surveys reported that a long rotation (>5 years) reduced incidence of blackleg disease on canola crops (Kaminski et al. 1996; Morrall et al. 1999; Khangura & Barbetti 2001). However, a controlled field experiment showed that a three-year rotation and tillage had little effect on the disease (Martin et al. 2001). Guo et al. (2005) reported that crop rotation and tillage significantly reduced blackleg stem disease incidence and severity in canola in rotation with wheat and flax in both conventional tillage and zero tillage. Conventional tillage with a one-non-host-crop rotation reduced the disease. There have been few studies on the impacts of crop rotation and tillage on inoculum levels of *L. maculans* in the field and infection of canola leaves and plants. Hammond et al. (1985) reported that stem canker lesions of canola were attributed to the lesions on leaves at the rosette stage, whereas it is not well understood whether stem canker and leaf lesions consistently indicate the degree of infection of canola. In this paper we report (i) development of infection by *L. maculans* on canola plants during growth stages; (ii) dispersal patterns of ascospores and pycnidiospores of *L. maculans*; and (iii) the relationship between the levels of infection on canola plants during primary growth stages and disease at ripening under different crop rotation and tillage systems.

Materials and methods

Experimental design

This study was conducted from 1999 – 2002 at the University of Manitoba's Carman Research Station, Carman, Manitoba, Canada. A split-plot design was deployed. The main plots were tillage (conventional tillage and zero tillage), and minor plots were rotation, in which were canola (*Brassica napus* L.) (C), wheat (*Triticum aestivum* L.) (W), and flax (*Linum usitatissimum*) (F). There were three replications. Four rotations were under conventional tillage: CCCCT (canola was grown for four years under conventional tillage in each year), CWCCT, CCWCT, CWFCT, and the other four rotations were under zero tillage: CCCCZ (canola was grown for four years under zero tillage in each year), CWCCZ, CCWCZ, and CWFCZ. The experiment was carried out in a field of 155 m × 81 m, including 24 10-m × 4-m plots with 15 m between plots and 10 m between a plot and the border of the field.

Preparation of the isolate

Leptosphaeria maculans isolate 86-12 was collected at Swan Lake, Manitoba, Canada, and identified as pathogenicity group 2 (PG-2) in 1986. The isolate was stored on the potato dextrose agar (PDA; 39 g of potato dextrose agar (Becton, Dickinson and Company, Sparks, MD, USA) per liter sterilized distilled water) under sterilized mineral oil at 20°C until use. Pycnidiospores were harvested 15 days after the isolate was transferred to V-8 juice agar (200 ml of V-8 juice (Campbell Soup Company, Toronto, ON, Canada) per liter, 15 g technical agar, and 0.75 $g l^{-1}$ $CaCO_3$). A concentration of 1×10^7 spores ml^{-1} was made using a haemocytometer (Hausser Scientific Company, Horsham, PA) before inoculation was carried out.

Wheat cultivars, tillage and inoculation

Canola cultivar 'Westar' (susceptible to *L. maculans* [Blackleg of Canola. Available at: http://www.agf.gov.bc.ca/cropprot/blackleg.htm.]), wheat cultivar 'AC-Barrie' and the flax cultivar 'Norlin' were used in this study. Fall rye was sown in the buffer area. 'Westar' was seeded in all plots in 1999. One liter of *L. maculans* spore suspension of 1×10^7 spores ml^{-1} was sprayed, using a backpack sprayer, on canola at the 3-leaf stage in each plot on June 10. Disease rating was performed in 2002. In the conventional-tillage plots, a deep tiller with tine harrow was used for each fall and a cultivator with tine harrow and coil packer for each spring after the crops were harvested.

Measurements

The number of infected plants, number of infected leaves per plant and number of lesions per plant were assessed on 1, 5, 9, 13 and 17 July 2002, by walking through the plots in a 17.5 m zigzag line of five equidistant segments. One canola plant was collected approximately every 0.6 m on the zigzag line. There were in total 29 plants for the measurement in each plot. Percentage of leaf coverage with lesions was recorded in a $1\,m \times 1\,m$ square in the center of each canola plot on 7, 11, 15, 19 and 23 July in the same year. The fourth true leaf of each plant infected by *L. maculans* was tagged on 7 July, and was photographed with a digital camera (Olympus D-620L; Olympus Optical Co. Ltd., London, UK). Percentage of leaf coverage with lesions was assessed on a computer using the ASSESS program (Assess 2002). The number of *L. maculans* spores dispersed was assessed in one-replicate plot of each treatment, in which was a rotorod impaction spore samplers (Aerobiology Company, Nepean, Ontario, Canada), from 21 June – 26 July 2002. Spore samplers were connected to a CR 10 datalogger (Campbell Scientific, Logan, Utah, America), which was programmed to run the rotorods for 5 min out of each hour. The rotorods were replaced every 24 h and the spores on the rotorod were counted under a microscope (400×).

Data analysis

The number of infected plants was expressed as the number of infected plants among the total plants sampled at each sampling time. The number of infected leaves per plant was assessed as the total number of infected leaves divided by the number of all plants. The number of lesions per plant was evaluated as the total number of lesions divided by the number of all plants. Percentage of leaf coverage with lesions was defined as the lesion size divided by the leaf area multiplied by 100. Spore concentration (spores m^{-3}) was assessed as $S/(RPM \times K \times T)$, where S was the total number of spores on a spore trap, RPM was rotations per minute, K was rod constant 0.0197, T was sampling period (min).

The ANOVA was used for statistical analysis of all parameters in this study. The data of the number of infected plants, number of infected leaves per plant, and percentage of leaf coverage with lesions were normalized with ln(x). Standard error of the mean difference between rotations in the same tillage system was expressed as $\sqrt{}$ [2(error mean square of subplot)/number of replications of main treatments]. Standard error of the mean difference between tillage systems in the same rotation system was assessed as the square root of $2\{[(\text{number of subplots per main plot} - 1) \times \text{error mean square of subplot} + \text{error mean square of main plot}]/(\text{number of replication of main treatments} \times \text{number of subplots per main plot})\}$. The significant differences among treatments were assessed using the Fisher's protected least significant difference test ($p < 0.05$).

Results

In 2002 there were no visible symptoms on leaves when canola was rotated with wheat and flax whether they were on zero-till or tilled plots. Also, no leaf symptoms were visible when canola was rotated only with wheat in the second or third year and were on tilled plots. Therefore, only those rotations in which disease was visible (CCCCZ, CWCCZ, CCWCZ and CCCCT) will be discussed.

The number of infected plants

The effects of rotation and tillage on the number of infected plants were significant from 1 – 17 July (Figure 1A). The number of infected plants increased over the growing season in all treatments in which disease occurred (Figure 1A). The increase was more evident after 9 July. Among the different treatments, the number of infected plants in rotation CCCCZ was significantly higher than in rotations CWCCZ, CCWCZ and CCCCT from 1 – 17 July. The numbers of infected plants in rotation CWCCZ was higher than in rotation CCWCZ on 9 and 17 July, and there was no difference between these two rotations on other dates (Figure 1A).

The number of infected leaves per plant

The effects of rotation and tillage on the number of infected leaves per plant were significant from 1 – 17 July (Figure 1B). There were no significant differences among CCCCZ, CWCCZ and CCWCZ on 1 July, but CCCCZ had significantly higher number of infected leaves than other rotations from 1 – 17 July except 13 July (Figure 1B). There were no significant differences between CWCCZ and CCWCZ during the growing season.

The number of lesions per plant

The effects of rotation, tillage and their interaction on the number of lesions per plant were significant (Figure 1C). The number of lesions per plant in the CCCCZ rotation was significantly higher than in the CWCCZ and CCWCZ rotations during the growing season (Figure 1C). There were no significant differences between CWCCZ and CCWCZ throughout the growing season.

Percentage of leaf coverage with lesions

The effects of rotation and tillage on the percentage of leaf coverage with lesions were significant (Figure 1D). CCCCZ rotation had significantly higher percentage of leaf coverage with lesions than CWCCZ, CCWCZ and CCCCT rotations from 7 – 23 July; and the percentage of leaf coverage with lesions in CWCCZ was significantly higher than CCWCZ except on 23 July (Figure 1D).

Dispersal of ascospores and pycnidiospores

Numbers of ascospores and pycnidiospores trapped were higher in CCCCZ, CWCCZ and CCWCZ rotations than in CWFCZ rotation (Figure 2A – D). Ascospores were trapped from the middle of June to the beginning of July, approximately eight days earlier than when the first pycnidiospores were trapped. The number of ascospores trapped was much lower than

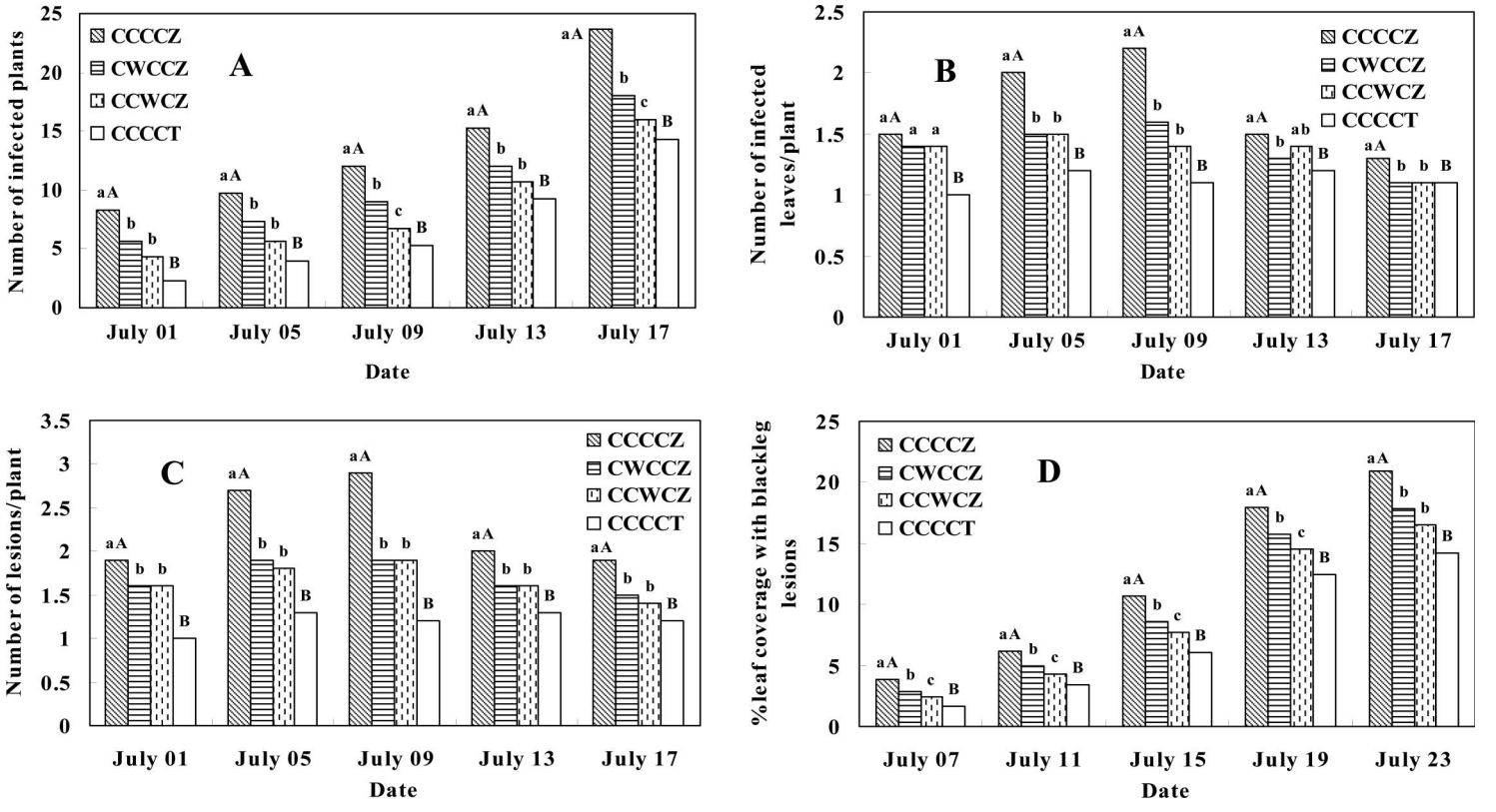

Figure 1. Number of infected plants (A), number of infected leaves per plant (B), number of lesions per plant (C), and percentage of leaf coverage with blackleg lesions (D) in different crop rotation and tillage systems from 1–17 July. C, canola; W, wheat; Z, zero-tilled; T, tilled. Values of the bars represent means calculated from untransformed data; mean difference was calculated from transformed data ln(x). Different lowercase letters on the bars for each date indicate significant difference between different rotation systems; different capital letters indicate significant difference between CCCCZ and CCCCT according to the Fisher's protected least significant difference (LSD) ($p < 0.05$).

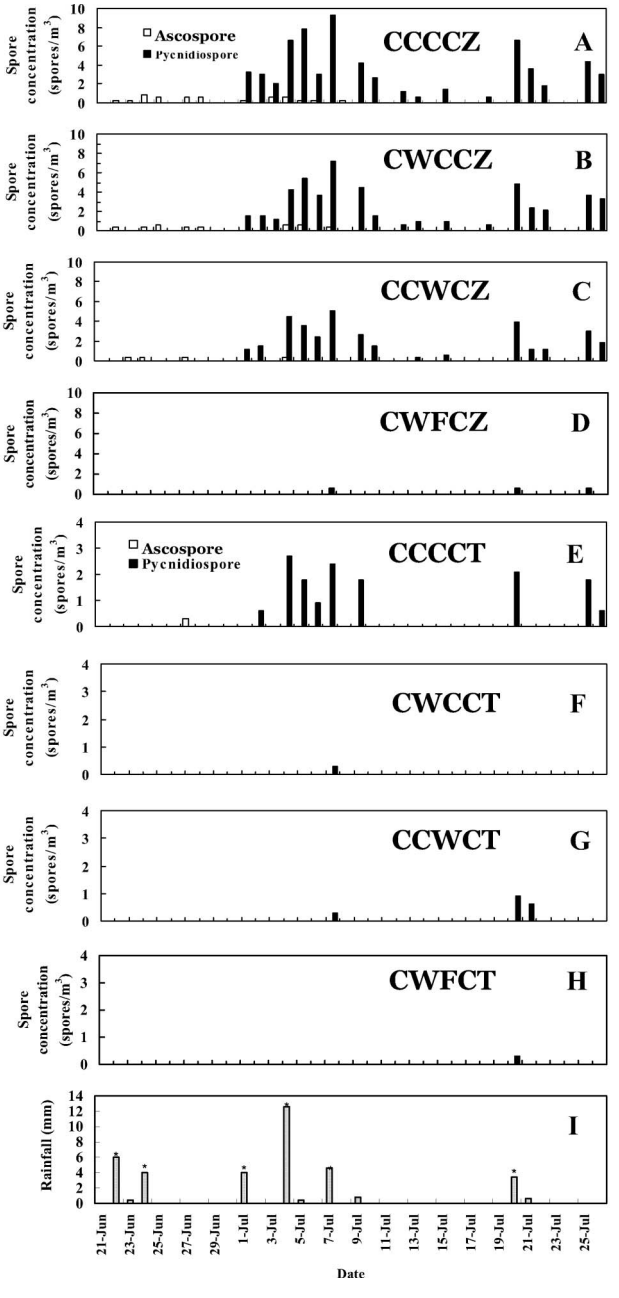

Figure 2. Daily spore count of ascospores of *L. maculans* and pycnidiospores of *P. lingam* trapped from plots under different rotations with zero tillage (Z). Pycnidiospores and ascospores were calculated as spores/m^3. C, canola; W, wheat; F, flax; Z, zero-tilled; T, tilled. Graph A: CCCCZ rotation; B: CWCCZ rotation; C: CCWCZ rotation; D: CWFCZ rotation; E: CCCCT rotation; F: CWFCT rotation; G: CCWCT rotation; H: CWCCT rotation.

pycnidiospores. The total number of ascospores and pycnidiospores trapped from all the plots during the whole assessing period were 38 and 584 per m^3, respectively.

Spore dispersal was associated with rainfall (Figure 2A–I). Ascospore dispersal on 22–25 June in plot CCCCZ, on 22, 24 and 25 June in plot CWCCZ and on 23 and 24 June in plot

CCWCZ occurred on similar days to the rainfall events on 22–24 June. When rainfall events occurred on 1, 4, 5, and 7 July, ascospores were trapped on 1, and 3–7 July in plot CCCCZ, on 4, 5, and 7 July in plot CWCCZ, and on 4 July in plot CCWCZ. During the same rainfall period, there was a mass of pycnidiospores dispersed in plots CCCCZ, CWCCZ, CCWCZ, and CCCCT (Figures 2A–C, E, and I). When rainfall events occurred on 20 and 21 July, a number of pycnidiospores were trapped around this period in plots CCCCZ, CWWCZ, CCWCZ, CCCCT and CCWCT (Figures 2A–C, E, G, and I).

The numbers of spores trapped were lower in plots with rotation and tillage than with no rotation and no tillage (Figure 2A–H). In plots under crop rotations, ascospores and pycnidiospores trapped in the rotation CWFCZ plot were much lower than in plots with rotations CWCCZ and CCWCZ, which had lower spore amounts than CCCCZ rotation (Figure 2A–D). The number of spores trapped in plots with rotations CWFCT, CWCCT and CCWCT was less than in the plot with rotation CCCCT. Spore release in rotations under conventional tillage was less than in rotations under zero tillage. There were ascospores trapped in plots with rotations under zero tillage except where the rotation had a 2-year break from canola, CWFCZ (Figure 2A–D). Low numbers of ascospores were also observed in tilled plots under continuous canola. However, pycnidiospores were trapped from plots with all rotations (Figure 2A–H).

Discussion

In the present study, the number of infected plants and the percentage of leaf coverage with lesions increased during the growing period of canola, and infected leaves and lesions per plant increased prior to early flowering stage and decreased after because the lower leaves declined and fell. Rotation and tillage significantly reduced blackleg disease in terms of the number of infected plants, infected leaves and lesions per plant, and percentage of leaf coverage with lesions. The number of infected plants, infected leaves and lesions per plant, and percentage of leaf coverage with lesions were lower when canola was rotated with wheat. Guo et al. (2005), in the same experiment, reported that the diseased stem incidence and severity were lower with wheat and flax as rotation crops in both tilled and zero-tilled plots. Tillage reduced stem disease incidence and severity when a simple rotation was used, however, the effects of tillage was reduced when a diverse rotation was used.

Crop rotation and tillage showed a similar effect on the number of infected plants, infected leaves and lesions per plant, and the percentage of leaf coverage with lesions to stem disease incidence and severity, therefore it is hypothesized that stem disease incidence and severity result from development and accumulation of infection on the plants; the latter is dependent on the dispersal time and number of *L. maculans* spores from pseudothecia on canola stubble; and the amount of canola stubble is affected by crop rotation and tillage systems.

McGee (1977), Blenis et al. (1999), and Turkington and Clayton (2000) reported that canola stubble decomposed quickly and the volume of stubble from the previous crops was more than ten times that of older stubble on the soil surface, resulting from crop rotation. Guo (2004) described the persistence of *L. maculans* on canola stubble at the surface and at depths of 5 and 10 cm in the soil. Eight, nine and ten months after stubble was buried, pathogen survival at a depth of 10 cm in the soil was significantly lower than pathogen viability at a depth of 5 cm, and the latter was significantly lower than on the soil surface. Therefore, inoculum levels of *L. maculans* on canola stubble may be reduced when the stubble is buried into soil by tillage.

Turkington et al. (2000) reduced *L. maculans* pycnidiospores by incorporating canola stubble into soil, which reduced the amount of stubble by 40%. Guo and Fernando (2005)

showed that a greater number of ascospores and pycnidiospores trapped from the zero-tilled canola-planted area than the tilled area. Hershman and Perkins (1995) found that the number of ascospores dispersed from canola stubble in fall and winter after the crop was harvested was significantly greater than infested stubble from the previous year because of stubble decomposition. Thus, the number of spore dispersal is largely associated with the amount of infected canola stubble on the soil surface.

There have been no studies on the effects of crop rotation and tillage on spore dispersal of *L. maculans* from canola stubble, and the relationship between *L. maculans* spore dispersal and infection of canola plant. The present study showed that the numbers of ascospores and pycnidiospores dispersed were less from tilled plots than from no-tilled plots in the same rotations, and were less in crop-rotation plots than non-rotation plots in the same tillages. Appropriate crop rotation lengthens the time between similar host plants, so pathogen populations have time to decline (Kharbanda 1999).

Guo and Fernando (2005) described the association of *L. maculans* ascospore and pycnidiospore dispersal with rainfall, and showed that ascospores were dispersed during the periods of rainfall and five days after rain. In the 4-year canola practice under conventional tillage and zero tillage, and rotations with one-year break of four years by wheat under zero-tillage, the dispersal of ascospores in late June and pycnidiospores in early July might have been triggered by rainfall events from 22 – 24 June and in early July, because there were no rainfall events within the seven days before June 21. The ascospores dispersed from the middle to end of June and in the beginning of July could contribute to the infection of canola plants observed in early and middle July, respectively. This study also showed that ascospores were trapped approx. eight days earlier than pycnidiospores, which were not long enough for lesion and pycnidial formation (Guo 2004), indicating pycnidiospores play an equally important role in the primary infection. A similar result was reported by Biddulph et al. (1999).

There have been no studies addressing the relationships between the number and the size of lesions on the growing plants and stem disease severity, or studies on the relationships between the number of infected plants with blackleg and stem disease incidence in the crop rotation and tillage systems. The present paper correlated the stem disease incidence and severity with the number of lesions, percentage of leaf coverage with lesions per plant, and showed the stem disease severity was highly correlated with the number of lesions ($R^2 = 0.97$) and percentage of leaf coverage with lesions per plant ($R^2 = 0.97$) at the flowering stage, respectively (Figures 3A and B). Stem disease incidence was highly correlated with the number of infected plants ($R^2 = 0.99$) (Figure 3C). This suggests that the number and size of lesions can be utilized in the early prediction of the blackleg disease as influenced by crop rotation and tillage. This observation is supported by Hammond et al. (1985) who reported that when colonizing the lamina and petiole, the pathogen hyphae were predominant to intercellular spaces in the parenchyma and the adjacent cell appeared obviously necrotic, and stem cankers were produced through a systemic pathway of infection.

This study suggests that appropriate crop rotation and tillage systems have positive effects on reducing blackleg disease in canola, though some studies showed no significant effects of similar cropping practices on this disease, which were conducted under different geographical and weather conditions, and with the tillage systems different from this study. Ascospore dispersal of *L. maculans* from canola stubble is associated with rainfall, thus, airborne inoculum levels, the primary source for the infection of plants, at different growing stages of the crop can be predicted based on stubble-borne inoculum levels and weather conditions. Furthermore, assessment of leaf infection at the early stage of canola can be used for predicting stem disease incidence and severity at maturity, making the disease control facilitated by timing the application of fungicides in the fields.

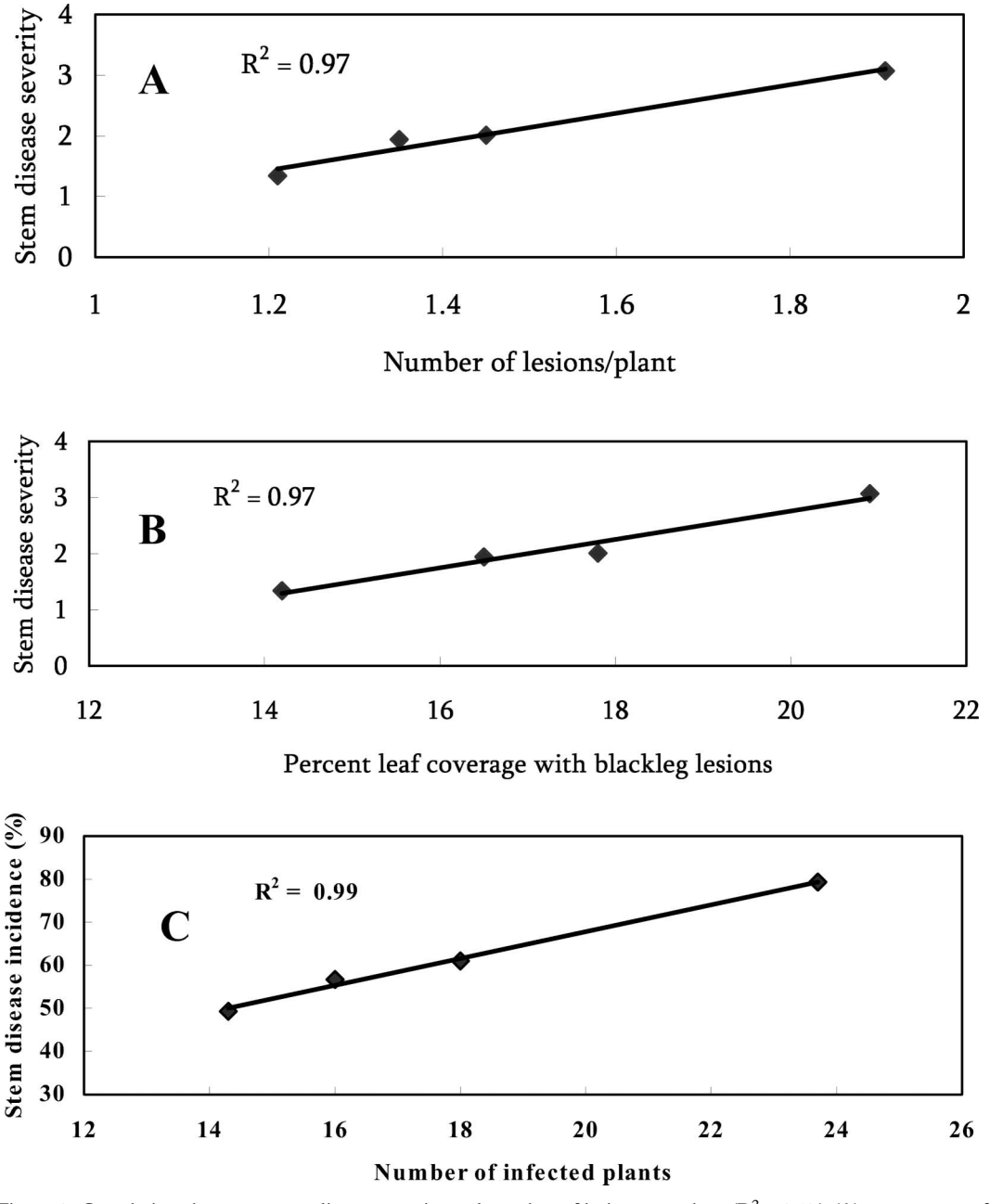

Figure 3. Correlations between stem disease severity and number of lesions per plant ($R^2 = 0.97$) (A); percentage of leaf coverage with blackleg lesions ($R^2 = 0.97$) (B); between stem disease incidence and number of infected plants ($R^2 = 0.99$) (C). R^2 = efficient of determination. The data of stem disease severity and disease incidence were derived from author's another study with the same experimental design (Guo et al. 2005).

Acknowledgements

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