Mitigating the risk of blackleg disease of canola using fungicide strategies.

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Executive summary

Blackleg, caused by *Leptosphaeria maculans* is the most widespread fungal disease of canola in western Canada. It has the potential to greatly reduce canola yields and decrease economic returns of growers. In the two decades before 2010, the disease has been managed primarily with the use of resistant cultivars and 4-year crop rotations. However, many growers are producing canola in 2-year rotations across the Prairies in response to market signals. Research has shown that there is variability for virulence in the pathogen population (Chen and Fernando 2006; Kutcher et al., 2007; Kutcher et al., 2010), which suggests that the pathogen may be able to overcome the resistance with some of the cultivars.

With the increasing risk of blackleg in many regions, especially southern Manitoba, east-central Alberta, northwestern and southeastern Saskatchewan, growers are asking for information on products available and expected benefits. Several fungicides are registered in Canada for control of blackleg on canola (*Brassica napus*), including the new and well-known product Headline® (pyraclostrobin). Tilt® (propiconazole) and Quadris® (azoxystrobin) are registered for many years, and a new product that combines the active ingredients in Tilt and Quadris has also become available under the name Quilt Xcel®. Often the questions from growers include is a fungicide treatment useful for control of blackleg as in the case of sclerotinia stem rot? When should I apply? Which product is most cost effective and will the potential plant growth benefit claimed for certain products such as Headline warrant regular preventative treatment of canola crops against blackleg, and more importantly, when should I spray?

Based on research trials in Saskatchewan, foliar fungicide treatment against blackleg often produces little yield benefit, especially on resistant cultivars (Kutcher et al., 2013). The blackleg level was relatively low for most of the years during this study. Therefore, it was not clear if fungicides should be recommended when cultivar resistance is overcome by the pathogen and the disease pressure is high. The objectives of this study were to assess the benefit of fungicide treatments in relation to application timing and host resistance based on multi-site and multi-year field trials across canola growing regions on the prairies.

Field plots were established at Vegreville, AB, Scott and Melfort, SK and Brandon and Carman, MB between 2011 and 2014, with the susceptible cv. Westar used to represent the worst-case scenario of resistance breakdown. Diseased canola residues from previous years were left in the plot area for pathogen inoculum. The fungicides Headline, Tilt, Quadris® and Quilt Xcel® were applied at the 2-4 leaf stage individually, in a split application (Headline then Tilt or vice versa) at the 2-4 leaf and prior to bolting, and Headline alone just prior to bolting. Unsprayed plots were used as a non-treated control. The resistant (R) cultivar 45H29 and moderately resistant (MR) cultivar 43E01 also were treated with Headline at the 2-4 leaf stage as additional checks. At crop maturity, blackleg incidence and severity were assessed on 50 plants by examining cross-sections of lower stems and tap roots in each plot. Seed yield was recorded after harvest.

Data from a total of 17 site-years showed varying levels of blackleg. When all site-years were analysed together, all treatments, except Tilt applied at the 2-4 leaf stage or Headline applied prior to bolting, reduced blackleg and increased seed yield of Westar. When data were analysed separately based on the disease severity (DS: 0-5), the trend was the same for trials with moderate to high levels (DS>1.0) of disease (8 site-years). However, no difference in disease incidence, severity or canola seed yield was observed with any of the treatments (9 site-years) when blackleg occurred at low levels (DS<1.0). The early application of Headline generally reduced the disease incidence and severity on MR and R canola cultivars but did not increase the yield significantly. Overall, the project was on time and on budget.

Introduction

Blackleg disease, caused by *Leptosphaeria maculans* (Desmaz.) Ces. & de Not, is a serious threat to canola production in western Canada, and an on-going issue in many canola/rapeseed production regions in Australia and Europe (Fitt et al. 2006). The disease can cause substantial yield losses if not managed carefully. Canola is grown traditionally in rotation once every 4 years in western Canada due mostly to the consideration of managing crop pests and diseases including blackleg. In recent years, however, growers have begun to produce canola more intensively due to market opportunities and cultivar improvements (Kutcher et al. 2013). Consequently the risk of blackleg is increasing, which can be caused by changes in the pathogen population and the emergence of virulent pathogen strains against the current cultivars. The breakdown of blackleg resistance has been reported in France and Australia (Rouxei et al. 2003; Li et al. 2005). Shorter rotations will likely exacerbate the situation by favoring rapid buildup of virulent pathogen inoculum (Petrie 1995; West et al. 2001; Sosnowski et al. 2006; Harker et al. 2015). New pathogen strains have been reported in western Canada (Chen and Fernando 2005, 2006; Fernando and Chen 2003) and this trend has been continuing in recent years, reflected by the diversity and changed race composition in the pathogen population (Kutcher et al., 2007; Kutcher et al., 2010). Field surveys in recent years generally indicated that blackleg continues to be one of the most common diseases of canola (Dokken-Bouchard *et al.*, 2011; Lange *et al.*, 2011; McLaren *et al.* 2011), and this poses a threat to canola production.

In general, only blackleg resistant canola cultivars are recommended in Canada (Saskatchewan Ministry of Agriculture. 2010) but this has not prevented the disease from occurring at severe levels in several regions, especially southern Manitoba and east central Alberta. Adjacent southeast and northwest regions in Saskatchewan also reported blackleg disease damage more frequently than other regions of the province. There has generally been an increase in blackleg prevalence and severity in western Canada (The Western Producer 2012), highlighting the threat to canola production. This may be related to shortened crop rotation in these regions because blackleg generally increases in rotations comprising more than one canola crop every four years (Kutcher et al. 2013), although resistant cultivars may be much less affected than susceptible cultivars under these conditions. It is also possible that the increased blackleg incidence and severity reflects a breakdown in cultivar resistance or at least a gradual erosion of resistance with time (Harker et al. 2015) due to adaptation of the pathogen population to current canola cultivars.

The value of using fungicides in blackleg management varies, depending on disease situations and cultivar resistance. In Western Australia, the triazole fungicide fluquinconazole used as a seed treatment on a susceptible cultivar significantly decreased blackleg severity and increased canola yields in most of the field trials conducted (Khangura and Barbetti 2004). In southern Australia, however, the similar treatment reduced the disease severity on resistant or moderately resistant canola cultivars but the yield was rarely improved (Marcroft and Potter

2008). In western Canada, foliar application of the fungicide azoxystrobin reduced blackleg incidence but canola grain yields were generally not affected in a 1998-2001 study in Saskatchewan (Kutcher et al. 2011). The blackleg levels were generally low in those trial years. It appears that a fungicide treatment may be of value to blackleg management only under high disease pressure or when the canola cultivar has lost resistance.

A strobilurin fungicide pyraclostrobin (Headline®) was registered recently in Canada for control of blackleg on canola. Products registered previously included propiconazole (Tilt®) and azoxystrobin (Quadris®). The latter is also a strobilurin fungicide. A combination of propiconazole and azoxystrobin has been developed by Syngenta under the name Quilt Xcel®, and this product received the Canadian approval for label expansion against blackleg on canola in 2014. All of the products above are foliar fungicides, and early application can be an efficient timing due to the opportunity of tank mixing with a post-emergent herbicide. On susceptible canola cultivars, often a single fungicide application at the 2-4 leaf stage may be sufficiently effective (Kutcher et al. 2003). However, it was not clear if the fungicide application can be delayed until bolting or a second application at the later stage will boost the control of blackleg, especially under high disease pressure and reduced cultivar resistance. The objectives of this study were to assess the efficacy of foliar fungicides in mitigating the risk of severe canola yield losses caused by blackleg when cultivar resistance is overcome by the pathogen population. Additionally, treatment timing and multiple applications were also assessed to determine the optimal efficacy of fungicide against blackleg.

Materials and Methods

The study consisted of five field sites located in Vegreville, AB, Scott and Melfort, SK, Brandon and Carman, MB between 2011 and 2014. Prior studies in Saskatchewan showed little benefit of fungicide treatments for blackleg on resistant canola cultivars (Kutcher et al. 2003, 2013). Therefore the susceptible (S) cultivar Westar was used to represent the worst case scenario of cultivar resistance erosion. The resistant (R) cultivar 45H29 and moderately resistant (MR) cultivar 43E01 were used but treated only with pyraclostrobin at 2-4 leaf stage for comparisons.

The plot size was 9.6 m^2 to 32 m^2 depending on the location, with seeding rates at 150 seed /m row for R and MR cultivars, and 175 seed/m row for the S cultivar Westar. The row spacing was about 20 cm. Because both Roundup-Ready (43E01 and 45H29) and conventional (Westar) cultivars were present at each location, the weed management used herbicides which were generally suitable for conventional canola. Due to generally high weed pressure at the Melfort site, Edge™ was broadcasted prior to seeding. At other sites, however, a tank mix of Poast®, Muster® and Lontrel™ was applied for post-emergent weed control (Saskatchewan Ministry of Agriculture, 2010).

In most sites/years, infection relied primarily on natural pathogen inoculum from stubbles in adjacent plots where a susceptible canola cultivar was seeded in the previous year. At the Carman site, a conidial suspension of *L. maculans* (PG2) was sprayed throughout the plot area on June 23, 2011, five days prior to the first fungicide application (at the 2-4 leaf stage) to enhance the infection because no prior canola crop was close to the plot area for several years. The fungicide rate followed label recommendations. The experiment used a randomized block design with 4 replicates.

Treatments:

***On the S cultivar Westar*:**

non-sprayed (control)
Headline® @ 2-4 leaf stage
Quadris® @ 2-4 leaf stage
Tilt® @ 2-4 leaf stage
Quilt Xcel® @ 2-4 leaf stage
Headline® @ just prior to bolting
Tilt® @ 2-4 leaf stage, Headline® @ just prior to bolting
Headline® @ 2-4 leaf stage, Tilt® @ just prior to bolting

On the MS cultivar 43E01

Non-sprayed (check 1)
Headline® @ 2-4 leaf stage

On the R cultivar 45H29

Non-sprayed (check 2)

Headline® @ 2-4 leaf stage

These treatments were applied at all site-years. At the growth stage of 5.1 to 5.3 (Harper and Berkenkamp 1975), fifty plants were uprooted from each replicate, cut through hypocotyls and/or tap roots, examined for presence/absence of blackleg symptoms and rated for disease severity using a 0-5 scale (**Table 1, Fig 1**) based on the % area of diseased tissue in the cross-section. After harvest, canola seeds were cleaned and yields taken from each plot.

Initially data from a total of 17 site-years were pooled for analysis due to the homogeneity of variance. The Logarithm transformation was used to improve the normality of disease incidence data prior to the analysis of variance (ANOVA). The treatment effect on yield was analyzed for different cultivars due to inherently different yield potential. Treatment means were separated using Dunnetts' Test ($P < 0.05$) which allowed the comparison of each treatment with non-treated controls. Data from site-years with light average blackleg severity (<1.0) were separated from those of moderate to high levels of disease severity (>1.0) during further analysis.

Table 1 Description of blackleg rating scale [a]

Rating	Description
0	No disease visible in the cross section
1	Diseased tissue occupies up to 25% of cross-section
2	Diseased tissue occupies 26 to 50% of cross-section
3	Diseased tissue occupies 51 to 75% of cross-section
4	Diseased tissue occupies more than 75% of cross-section with little or no constriction of affected tissues
5	Diseased tissue occupies 100% of cross-section with significant constriction of affected tissues; tissue dry and brittle; plant dead

[a] Based on the recommendation by the Western Canada Canola/Rapeseed Recommending Committee, 2009.

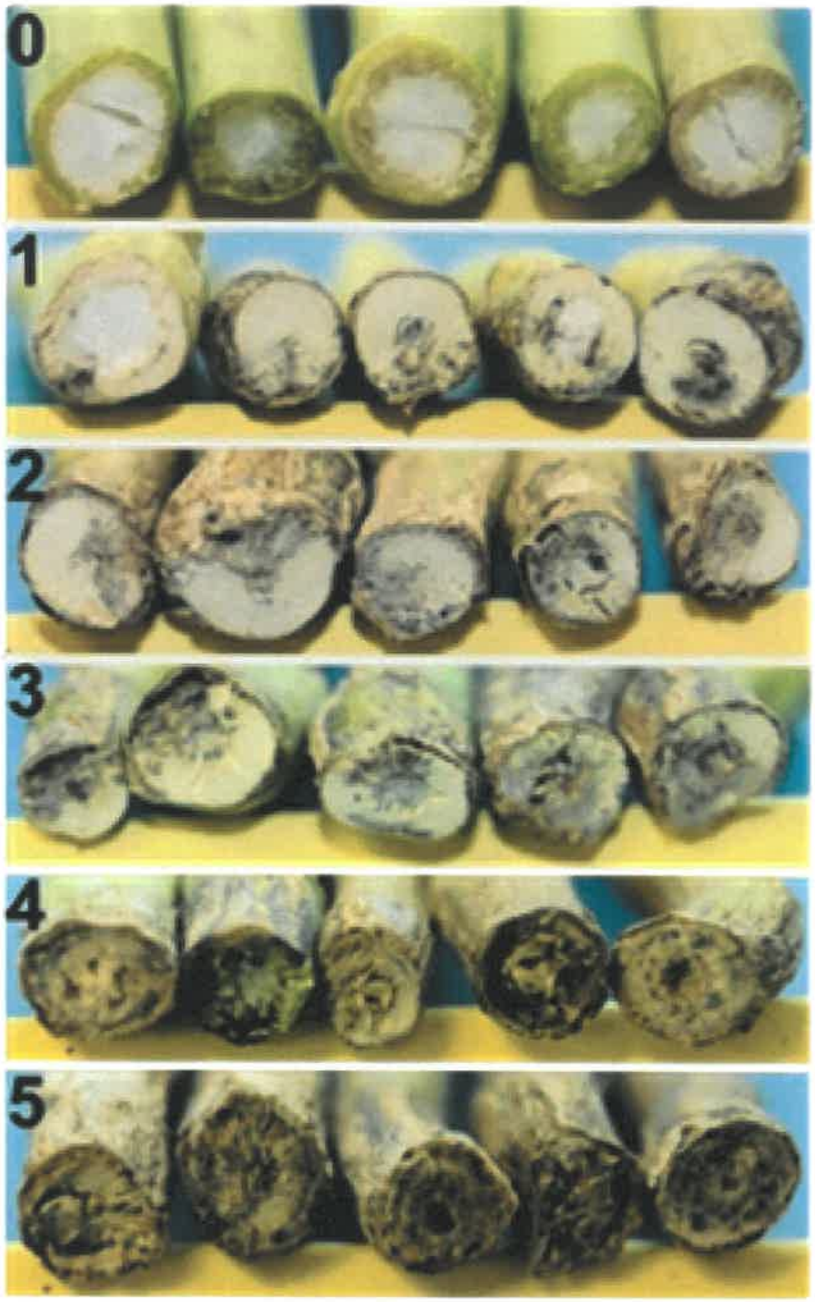

Figure 1 A pictorial range of blackleg disease severity (0-5).

Results

On the susceptible cultivar Westar which was used to simulate resistance breakdown, blackleg incidence and severity varied substantially depending on the location and year, with the disease levels ranging from light to moderately high in non-treated controls. This variation may be due to different pathogen inoculum and/or weather conditions. When data from all 17 site-years were analyzed together, the early application (2-4 leaf stage) of Headline, Quadris or Quilt Xcel reduced blackleg and increased canola grain yield significantly on Westar (**Table 2**). Tilt or late application of Headline (prior to bolting) did not reduce the disease or increase the grain yield, relative to the non-treated control. Two treatments using fungicides of different action modes did not achieve better efficacy relative to a single application of Headline or Quadris at the 2-4 leaf stage. The average yield benefit was 3-4 bushels/acre for the early fungicide treatment. On MR or R cultivars, however, an early application of Headline generally reduced disease incidence and severity relative to non-treated control but the effect was insignificant to canola yield (**Table 2**).

When data were separated into low (<1.0) and moderately high (>1.0) disease site-years for further analysis, several new trends emerged. Under the low disease severity, none of the fungicide treatments reduced the blackleg further or increased the yield substantially on Westar (**Table 3**). Similar phenomena were observed also on MR and R cultivars, except that the disease incidence on the MR was reduced slightly while the impact on yield was not significant.

Under moderate to moderately high disease pressure, the blackleg incidence and severity were generally higher on all treatments relative to those under lower disease pressure (**Table 3, Table 4**). The early application of Headline, Quadris or Quilt Xcel reduced blackleg severity and increased canola grain yields significantly. Similar to the earlier results based on all site-years, Tilt or late application of Headline was ineffective. On MR or R cultivars, an early application of Headline generally reduced the disease but failed to increase canola yield (**Table 4**) over non-treated controls. Fungicide treatments, however, did not increase the yield of Westar (30 bu/ac) to the level of MR (38 bu/ac) or R (50 bu/ac) cultivars (**Table 2**), and this is likely due to factors beyond the disease impact; some of the newer cultivars may have greater yield potential.

Table 2 Effect of fungicide treatment on blackleg and grain yield of canola with varying levels of disease resistance over 17 site-years between 2011 and 2014.

Cultivar	Treatment	Dis incidence (%)	Dis severity (0-5)	Grain yield (bu/ac)
Westar (S)	Non-treated control	54.1	1.5	26.4
	Headline (2-4 leaf)	42.8 *	0.9 *	30.4 *
	Quadris (2-4 leaf)	41.8 *	0.8 *	30.2 *
	Tilt (2-4 leaf)	57.0	1.5	27.1
	Quilt (2-4 leaf)	47.2	1.1 *	30.5 *
	Headline (rosette)	49.4	1.3	28.1
	Tilt + Headline #	46.8 *	1.2 *	29.4 *
	Headline + Tilt #	41.6 *	0.9 *	30.5 *
43E01 (MR)	Non-treated control	53.2	1.3	37.3
	Headline (2-4 leaf)	40.3 *	0.8 *	37.5
45H29 (R)	Non-treated control	44.1	0.9	49.3
	Headline (2-4 leaf)	35.9 *	0.6 *	50.2

* Means are significantly different from the non-treated control of the same cultivar ($P \leq 0.05$, Dunnett's Test).

Split applications at the 2-4 leaf and prior to bolting stages, respectively.

Table 3 Effect of fungicide treatment on blackleg and grain yield of canola over selected site-years (9) with low disease severity (<1.5) between 2011 and 2014.

Cultivar	Treatment	Dis incidence. (%)	Dis severity (0-5)	Grain yield (bu/ac)
Westar (S)	Non-treated control	29.7	0.5	31.8
	Headline (2-4 leaf)	29.3	0.5	33.5
	Quadris (2-4 leaf)	25.8	0.4	33.4
	Tilt (2-4 leaf)	33.2	0.5	32.8
	Quilt (2-4 leaf)	27.7	0.4	33.3
	Headline (rosette)	27.1	0.4	32.3
	Tilt + Headline [#]	26.5	0.4	34.2
	Headline + Tilt [#]	26.5	0.4	33.6
43E01 (MR)	Non-treated control	28.7	0.3	40.8
	Headline (2-4 leaf)	23.4 *	0.4	43.6
45H29 (R)	Non-treated control	20.1	0.2	50.5
	Headline (2-4 leaf)	20.3	0.2	51.1

* Means are significantly different from the non-treated control of the same cultivar ($P \leq 0.05$, Dunnett's Test).

[#] Split applications at the 2-4 leaf and prior to bolting stages, respectively.

Table 4 Effect of fungicide treatment on blackleg and grain yield of canola over selected site-years (8) with moderate to moderately high disease severity (>1.5) between 2011and 2014.

Cultivar	Treatment	Dis incidence (%)	Dis severity (0-5)	Grain yield (bu/ac)
Westar (S)	Non-treated control	80.8	2.5	20.5
	Headline (2-4 leaf)	57.4 *	1.5 *	27.0 *
	Quadris (2-4 leaf)	59.2 *	1.4 *	26.8 *
	Tilt (2-4 leaf)	83.0	2.6	20.8
	Quilt (2-4 leaf)	68.6 *	1.8 *	27.4 *
	Headline (rosette)	75.9	2.2	23.4
	Tilt + Headline #	69.1 *	2.1 *	24.2
	Headline + Tilt #	58.2 *	1.4 *	27.1 *
43E01 (MR)	Non-treated control	79.8	2.3	33.4
	Headline (2-4 leaf)	58.7 *	1.4 *	30.8
45H29 (R)	Non-treated control	70.4	1.6	48.1
	Headline (2-4 leaf)	53.0 *	0.9 *	49.2

* Means are significantly different from the non-treated control of the same cultivar ($P \leq 0.05$, Dunnett's Test).

Split applications at the 2-4 leaf and prior to bolting stages, respectively.

Discussion

Canola production is a multi-billion dollar industry in western Canada, with the annual crop volume surpassing 15 M tonnes. Negative impact by blackleg, even at a moderate scale may translate into a large amount of lost income for growers. This project takes a proactive approach by examining the efficacy and yield benefit of fungicide strategies in blackleg management in case the varietal resistance erodes rapidly. Although the genetic resistance and crop rotation will continue to be the mainstay for blackleg management in western Canada, increasingly tighter crop rotations can result in the build-up of pathogen inoculum and exacerbate the selection for pathogen strains capable of overcoming the current sources of resistance. Therefore, a second line of defense should be designed and assessed in case of rapid blackleg resistance breakdown.

Use of fungicides may be an option, but when and how to apply these fungicides need to be better tuned. Based on prior work in Saskatchewan (Kutcher et al., 2003; 2013), fungicides generally did not increase canola yield on resistant canola cultivars, but often did on susceptible cultivars. This study provided important information for assessment of fungicide strategies based on field trials in multiple locations across major canola crop regions in western Canada. In general, there is little benefit to apply a fungicide targeting blackleg if the cultivar resistance still holds. Even on a moderately resistant cultivar, the fungicide generally does not pay economically. This was demonstrated by the lack of fungicide effect on R and MR cultivars throughout the study. If the cultivar resistance fails and blackleg disease increases rapidly, application of a strobilurin fungicide, including Headline and Quadris, can effectively reduce the disease and alleviate canola yield losses. However, the old and well-known fungicide Tilt appears ineffective and the reason for this is yet to be understood. Since there has been strong evidence for fungal pathogens to develop insensitivity to strobilurin fungicides, caution should be exercised in considering fungicides for blackleg management; a fungicide treatment should only be recommended when there is a high risk for disease (high blackleg incidence in the previous crop, short rotation). Otherwise, frequent use of strobilurin fungicides may select fungicide tolerance/resistance in the pathogen population. There is currently no alternative chemistry registered for blackleg control.

To assess the risk of blackleg in a specific field, producers should check the disease incidence and severity shortly after swathing. The scouting will give a picture of pathogen inoculum pressure and cultivar resistance. If blackleg is found on a high number of plants (say >30%) and the average severity is greater than 1 (**Figure 1**), then steps need to be taken to mitigate the risk by changing canola cultivar, extending crop rotation and considering a fungicide treatment if rotation is shorter than 3 years.

The timing of fungicide at 2-4 leaf stage was intended to protect cotyledons and lower true leaves from infection, which tends to be most relevant to development of blackleg or basal stem canker later on. This timing efficacy was highlighted during this study in which the application of Headline at prior to bolting appeared ineffective. For efficiency and cost consideration, the early fungicide application may be tank mixed with post-emergent herbicides. For the purpose of seedling protection, seed dressing with a fungicide (Marcroft and Potter 2008) may also be an option. These additional fungicide timing/application options need to be assessed.

When compared to one application only at the 2-4 leaf stage, the second treatment prior to bolting did not increase the disease-control efficacy or canola yield substantially. It was hypothesized initially that the second treatment might reinforce the efficacy by protecting upper leaves from late-released ascospores or even by pycnidial spores (Ghanbarnia et al. 2009, 2011), hence reducing stem infection. Data from the current study, however, strongly emphasized the importance of targeting the early growth stage for the best result and economic return. It was therefore concluded that in most cases the second treatment is not necessary.

Despite the relatively high levels of disease observed at harvest on R and MR cultivars in some of the trials (**Table 4**), the impact on canola yield appears to be limited and the fungicide treatment would provide little economic benefit. For example, the disease incidence and severity on the MR cultivar were fairly similar to those on Westar, with or without the fungicide treatment, but the yield benefit from the fungicide was more pronounced on Westar. It appears that the yield of these newer MR or R cultivars is affected less by the blackleg severity at the harvest time as opposed to that of Westar; it is possible that the stem infection originated from cotyledon or lower-leaf infection progresses more slowly on MR or R cultivars relative to that on

Westar, but this aspect requires further study. Additionally, these MR or R cultivars certainly showed higher yield potential, producing more seed than Westar under similar blackleg disease levels **(Table 4)**.

Findings out of this study support the following extension messaging: Fungicides may be considered for blackleg management only when disease pressure is high, which is often related to the erosion of cultivar resistance, high blackleg incidence/severity in the previous crop and short crop rotations. Scouting after swathing/harvest is important to understanding the risk potential and making fungicide decisions. Strobilurin fungicides are effective against blackleg, and the early application (2-4 leaf stage) is more effective than a late treatment (rosette, prior to bolting). Multiple applications generally are not required for maximum efficacy.

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Additional professionals involved in the project

Chang Liu. M.Sc. student (U of M) who worked on the project at Carman, MB site between 2011 and 2013. Chang graduated in 2014 under the supervision of Dr. Fernando.

Additional productivity

Publications

1. Peng G, Fernando WGD, Kirkham CL, Lange R, Kutcher HR, McLaren D, Johnson E, Turkington KT. 2012. Mitigating the risk of blackleg disease of canola using fungicide strategies. In *Proc. Soil and Crops 2012*. University of Saskatchewan Press (CD), 7 pp.
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