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Project: Effect of herbicide and disease resistance on survival and inoculum production of *Leptosphaeria maculans,* the causal agent of blackleg.

Introduction

Leptosphaeria maculans, the cause of blackleg, causes significant yield loss in canola crops. The pathogen survives on infested residues from previous years' crops. There is evidence that infection of resistant cultivars occurs similarly to infection in susceptible cultivars but symptom expression in resistant cultivars is inhibited or delayed. This suggests that, even on resistant cultivars, the fungus will colonize the crop residues, reproduce and provide inoculum for subsequent canola crops. This is an important concept because if the pathogen reproduces successfully even on resistant hosts it is to be expected that there will be little selection pressure for the pathogen to become more virulent on resistant cultivars. If this is true then resistance should be stable and durable. Presently, we do not know what the effect of planting resistant versus susceptible cultivars has either on survival of the pathogen on crop residues, or in terms of subsequent inoculum production from infested residues.

Previous studies have indicated that certain herbicides and fungicides are inhibitory to *L. maculans* and might reduce the survival of the pathogen on infected residues. Two of these herbicides are glufosinate-ammonium (Liberty) and glyphosate (RoundUp), which are now widely used in western Canada to control weeds in herbicide tolerant canola (HT canola) cultivars.

Thus, widespread planting of herbicide tolerant but blackleg susceptible cultivars, and the use of Liberty and RoundUp may have a significant effect on blackleg inoculum. This study reports on our investigations into the effects of two herbicides (Liberty and RoundUp) and fungicides (Tilt, Sportak and Quadris) on pathogen survival and inoculum production (and potential infectivity) of infested residues in both blackleg resistant cultivars and herbicide tolerant but blackleg susceptible cultivars. The study attempted to determine the optimal application of pesticides for control of blackleg on residues. It also assessed the risk of development of new and more pathogenic strains of the fungus. An understanding of the effects of these pesticides in canola cultivars with blackleg resistance or herbicide tolerance will provide

rationale for improved options in disease management.

Objectives

The objectives were to determine the ability of the blackleg fungus to survive and produce ascospore inoculum on different canola residues treated with a combination of herbicides and fungicides. These treatments were:

1. residues from herbicide tolerant cultivars treated with herbicide or fungicide compared to "normal" cultivars
2. residues treated with fall application of herbicide or fungicide
3. residues from blackleg resistant cultivars compared to susceptible cultivars

Experimental:

Collection of blackleg infested residues from established sites

Two previously established sites were used to obtain canola residues to investigate the effect of resistant (R) or susceptible (S) cultivars on ascospore liberation and quantification. The sites were at Rosthern, SK, (Dow Agro Sciences Site) planted with Excel (S) and Quantum (R) and at Viking, AB, Alberta Agriculture planted with Quantum R), Westar (Highly S) and Reward (S). The Dow experimental plots from 1998 were sampled in May 1999 for collection of blackleg infested residues. The Viking site was a four year rotation study designed to study the effect of crop rotation on blackleg severity. This site was established in 1995 and sampled in October 1999.

Results

Ascospores were obtained from these residues using a plastic wind tunnel. The data are shown in Table 1. In these samples there were high levels of ascospore release from residues of the susceptible cultivar Westar at Viking with continuous canola rotation, and Excel at Rosthern. Low levels of ascospores were obtained from the resistant cultivar Quantum from both Rosthern and Viking. Low numbers of spores were released from residues of Westar after 3 year rotation.

Generation of treated residues from field experiments

Three field experiments were established at two locations (Agriculture and Agri-Food Canada, Saskatoon Research Farm, Saskatoon and Melfort Research Farm) in 1999. The duration of these experiments was three years. Randomized complete block (RCBD) and split plot designs were used. All treatments were replicated at least four times within a site.

Experiment: 1. Effect of host plant resistance on blackleg sporulation

Objective. To determine the effect of plant host resistance on pathogen reproduction.

Experimental.

The experiment was planted at Melfort on May 26, 1999 and Saskatoon on June 2, 1999

on canola stubble. The experimental design was a RCBD with six replicates. The *Brassica napus* cultivars were Cresor (R), Quantum (R), Sentry (R) and Westar (HS). Disease ratings on all plots (0-5 scale) were made in July and August 1999. Collection of 200 infected stalks per plot was made after harvest for subsequent assessment of sporulation. All plant samples were sent to Saskatoon. Plant samples were placed in mesh bags and left on the soil surface at Saskatoon Research Farm to enable the fungus to develop naturally under field conditions. These residues produced mature fruiting bodies (ascocarps) for later release of ascospores. Spore production was measured in a small plastic wind tunnel and the first measurement of the spore production was made in the June/July 2000. The experiment was repeated in 2000 at Melfort. Disease assessment of the experiment at Melfort was made the last week of August. Ascospore numbers were recorded from samples of all treatments in July and October 2000 and May and October 2001 for plantings in 1999. Ascospore numbers were recorded in July and October 2001 from plantings in 2000.

Results

Disease ratings of four cultivars planted at Melfort and Saskatoon in1999 and at Melfort in 2000 are presented in Table 2 and Table 3 respectively. Moderate levels of disease occurred at Melfort in 1999 on cv. Westar but at Saskatoon severe disease levels for blackleg were obtained. Cultivars Cresor, Quantum and Sentry were highly resistant. High disease levels were observed at Melfort in 2000 on the susceptible cultivar Westar. The purpose of the disease rating was to provide a base line for symptom expression on the cultivars prior to ascospore release assessment from the residues obtained from each of the cultivars.

The first samples for ascospore release from 1999 plantings were made in June/July 2000, a second sample was taken in October 2000, a third sample was taken in May 2001 and the last sample was taken in October 2001. Spores numbers were high from samples from the Melfort site in the first sampling period and declined to low levels by the third and fourth sampling dates. (Table 2). A reverse trend occurred in samples from Saskatoon, where the highest spore release was observed in the third sampling period. These number declined in the fourth sample (Table 2). High numbers of spores were released from residues from the resistant cultivars, Cresor, Quantum and Sentry in the first two samplings from Melfort and these numbers were similar to those from Westar, the susceptible check. The highest numbers of spores were released from Westar at the third sampling from the Saskatoon site. Here, of the resistant cultivars, only Quantum released a significant number of spores.

No spores were obtained from residues from Melfort in 2000 at the two sampling dates in July and October 2001 (Table 3).

Experiment 2. Effect of herbicides/fungicides on blackleg sporulation

Objective. To determine the effect of fall application of herbicide or fungicide on pathogen reproduction. The experiment was continued for 3 years (1999, 2000, 2001).

Experimental.

The experiment was planted at Melfort on May 26,1999 and Saskatoon on June 2, 1999

on canola stubble. The experimental design was a RCBD with six replicates of seven treatments. The *B. napus* cultivar was Westar (HS). Disease ratings on all plots (0-5 scale) were made in July and August 1999. Treatments (glyphosate (RoundUp), glufosinate-ammonium (Liberty), prochloraz (Sportak), propiconazole (Tilt), azoxystrobin (Quadris), and two checks (untreated)) were applied after harvest in the fall. Plots were rated for disease severity at GS 5.0 (August 19, 1999 in Melfort and September 19, 1999 in Saskatoon). Residues were left *in situ* to enable the fungus to produce mature fruiting bodies to measure spore production. First assessment of the sporulation was made in the June/July 2000. The experiment was repeated in Melfort in 2000. Disease ratings of the treatments were made in August 2000. Sampling of spores from residues from Melfort and Saskatoon were made in July 2000, October 2000, and May 2001. Sampling from residues from Melfort 2000 was made in July 2001.

Results

The disease ratings and spore release for 1999 for Melfort and Saskatoon are presented in Table 4 A similar trend in relation to spore release was observed in this experiment as occurred in experiment 1, where high numbers of spores were liberated from the Melfort site at the first and second sampling periods. These numbers declined to virtually zero for the third and fourth assessments. The reverse occurred at the Saskatoon site where few spores were released from most treatments for the first sampling period. Maximum numbers of spores were released at the third sampling date with especially high numbers occurring in the Sportak fungicide treatment.. However, overall, no effect of herbicide or fungicide on total spore production was observed. Inexplicably, at the Saskatoon site the lowest numbers of spores were released from the check treatments. An attempt to repeat the experiment in 2000 at Melfort was unsuccessful because, despite the occurrence of high disease levels in the plots, no spores were released from the samples (Table 5).

Experiment: 3. Effect of herbicides on blackleg sporulation

Objective. To determine the effect of weed control applications (on HTC canola) during the growing season on pathogen reproduction.

Experimental.

This experiment was planted at Melfort on May 26,1999 and Saskatoon on June 2, 1999 on canola stubble. The experimental design was a nested-factorial with 6 cultivars and 3 times of herbicide application. The cultivars were 1. RoundUp Ready: Quest (S) & LG3235 (moderately resistant) - with treatments RoundUp applied at seedling, bolting and to residue after harvest, 2. Liberty Link: Invigor 2153 (S) & Invigor 2473 (R) - with Liberty applied at seedling, bolting and to residue after harvest, 3. Westar (HS) and Quantum (R): with water applied at seedling, bolting and to residue after harvest. The 18 treatment combinations were applied as a RCBD with four replicates. Random samples of 5 plants per plot were collected at three different growth stages (seedling, bolting-early bloom and late bloom early maturity). The stems of these plants were surface sterilized in 0.6 % Javex and plated onto V8 juice agar to determine the relative infection

levels of blackleg over the growing season. Disease severity using a standard 0-4 scale on 30 plants per plot was made at maturity. Spores were sampled from the 1999 plantings in July and October 2000 and May and October 2001. The experiment was repeated at Melfort in 2000. Disease severity data were obtained in August 2000 and spores were sampled in July and October 2001.

Results

Disease severity levels for the treatments are presented in Table 6. High levels of blackleg disease were obtained at Melfort (Westar average 4.9-5.0) and moderate to high levels of disease were obtained at Saskatoon (Westar average 4.3-4.4). Thus good infestation of residues by the pathogen was expected. Spore collection and determination of the treatment effects as specified in the objectives commenced in July 2000 and subsequent samples were made in October 2000, May 2001, and October 2001 (Table 7).

Again, the highest numbers of spores were released in this experiment at Melfort at the first and second sampling dates with virtually no spore release occurring in the third and last sampling dates. Unfortunately only residues from the post harvest treatment at Melfort was collected in 1999. It is apparent that high numbers of spores were obtained from Quantum (almost as many as from Westar) even though disease levels were much lower.

High numbers of spores were released for the first three sampling dates from Saskatoon and spores were still being produced by the fourth sampling date. However, no differences were observed due to the effects of resistance, herbicide treatment or timing of application for the numbers of spores released. No spores were obtained in the samples from the experiment planted in 2000 at Melfort (Table 8).

The RoundUp Ready HT lines (Quest, susceptible, and LG3235, moderately resistant to blackleg) showed the most difference in spore production (Table 7). Oddly, it was the susceptible cultivar Quest which resulted in the least number of spores produced. Both these lines were treated similarly with Roundup, so any differences would relate to cultivar differences not to herbicide treatment. In Saskatoon, over the three RoundUp treatments, the highest number of spores were produced on residues from LG3235 the more resistant cultivar.

Virtually no difference in the blackleg disease levels with the two Liberty link resistant lines, InVigor2153 and InVigor2473 were observed at Melfort, although at Saskatoon InVigor 2473 was more resistant and produced lower numbers of spores on residues over the four sampling dates (Table 7). Again any differences are more likely due to cultivars and not to application of Liberty.

Conclusions and Summary

The timing of ascospore release from infected canola stem residues varied significantly by location. From plantings at Melfort in 1999 spores were released in large numbers the following year (2000) whereas from plantings at Saskatoon spores were released in highest numbers in the second year after planting (2001). These data support the currently held view that short crop rotations are conducive to high blackleg disease levels.

With the cultivars used, ascospore release occurs from residues from susceptible and

resistant canola cultivars and is not highly associated with the disease ratings observed at harvest. Thus high numbers of ascospores can be produced on residues of resistant cultivars which at harvest have shown no disease symptoms. This implies that sexual reproduction of the pathogen may occur at similar frequencies on both resistant and susceptible cultivars and that the resistance employed in these cultivars probably does not result in significant selection pressure on the pathogen population to shift towards greater virulence on resistant cultivars. This could mean that the type of resistance in *Brassica napus* cultivars employed currently could be durable. This hypothesis could be tested by comparing the frequencies of different virulence types of isolates obtained from ascospores released from resistant cultivars compared to isolates released from susceptible cultivars. No research of this kind has been conducted in Canada, though some studies along these lines are in progress in Australia.

The use of RoundUp or Liberty herbicides on HT canola cultivars probably does not result in reduced numbers of spore released from stem residues when applied at recommended rates for weed control and under normal agronomic situations. These chemicals have shown inhibitory effects on the pathogen under laboratory conditions but this does not seem to occur under field conditions. Even fungicide applications to infected residues was not effective in subsequently reducing inoculum production.

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Table 1. Effective of cultivar as source of ascospore inoculum at two locations in 1998

Location	Cultivar	Pseudothecia rating (10-100)	No. ascospores released/cm2
Rosthern, SK	Excel	90	1400
	Quantum	25	90
Viking, AB	Westar - C3	40	360
	Westar - NT	65	2320
	Quantum - C3	35	270
	Quantum - NT	30	390

Table 2. Effect of plant host resistance on reproduction of *Leptosphaeria maculans* on stem residues from canola grown at two locations in 1999

Location	Cultivar	Final Disease Severity	Mean Spores July 2000	Mean Spores October 2000	Mean Spores May 2001	Mean Spores October 2001
Melfort						
	Cresor	0.3	3125	1340	15	0.5
	Quantum	0.2	2825	1070	2	8
	Sentry	0.4	4000	1010	25	0
	Westar	2.5	4170	315	0	0
			ns	ns	ns	ns
Saskatoon						
	Cresor	0.3	0	50	50	7
	Quantum	0.2	10	0	580	90
	Sentry	0.4	25	0	190	20
	Westar	4.6	15	200	2840	125
			ns	ns		ns

Table 3. Effect of plant host resistance on reproduction of *Leptosphaeria maculans* on stem residues from canola grown in Melfort in 2000

Location	Cultivar	Final Disease Severity	Mean Number Plants Infected	Mean Relative Infection %	Mean No. of spores July 2001	Mean No. of spores October 2001
Melfort						
	Cresor	1.0	2.3	47	0	0
	Quantum	0.7	1.5	30	0	0
	Sentry	1.4	2.2	43	0	0
	Westar	4.0	4.3	87	0	0

Note: The mean number of plants infected and the % mean relative infection are based on sampling at the early maturity stage of growth.

Table 4. The effect of fall application of herbicides (Roundup, Liberty) or fungicides (Tilt, Sportak, Quadris) on reproduction of *Leptosphaeria maculans* on canola residues in 1999 at two locations.

Location	Treatment	Disease Severity	Mean No. of spores July 2000	Mean No. of spores October 2000	Mean No. of spores May 2001	Mean No. of spores October 2001
Melfort						
	Check	3.4	3850	880	15	10
	Roundup	3.4	1900	1860	0	0
	Liberty	3.6	2410	1780	0	0
	Check	3.8	2950	1390	0	0
	Tilt	3.1	2560	2380	0	0
	Quadris	3.2	560	1160	0	0
	Sportak	3.1	390	1850	5	0
Saskatoon						
	Check	4.2	180	50	500	5
	Roundup	4.1	1	140	3180	20
	Liberty	4.1	0	990	760	240
	Check	4.2	1570	300	550	30
	Tilt	4.2	0	920	2230	340
	Quadris	4.2	590	0	1290	30
	Sportak	4.0	0	10	7950	75

Table 5. The effect of fall application of herbicides (Roundup, Liberty) or fungicides (Tilt, Sportak, Quadris) on reproduction of *Leptosphaeria maculans* on canola residues in 2000 at Melfort

Location	Treatment	Disease Severity	Mean No. of spores July 2001	Mean No. of spores Oct 01
Melfort				
	Check	4.5	0	0
	Roundup	4.6	0	0
	Liberty	4.5	0	0
	Check	4.7	0	0
	Tilt	4.6	0	0
	Quadris	4.5	0	0
	Sportak	4.6	0	0

Table 6. The effect of weed control applications during the growing season on HTC canola on disease severity blackleg caused by *Leptosphaeria maculans* in 1999

Location			Disease Severity	Mean Number Plants Infected	Mean Relative Infection %
Melfort	Cultivar	Treatment			
	Quest	RP sdlg	4.0	2.0	40
	Quest	RP bltg	3.9	1.75	35
	Quest	RP ph	3.8	1.75	35
	LG3235	RP sdlg	2.0	2.5	50
	LG3235	RP bltg	1.6	1.5	30
	LG3235	RP ph	1.7	1.25	25
	InVigor 2153	LL sdlg	2.6	3.0	60
	InVigor 2153	LL bltg	2.8	1.0	20
	InVigor 2153	LL ph	3.1	0.5	10
	InVigor 2473	LL sdlg	2.6	1.75	35
	InVigor 2473	LL bltg	2.3	3.0	60
	InVigor 2473	LL ph	3.0	1.5	30
	Quantum	H_2O sdlg	0.8	1.0	20
	Quantum	H_2O bltg	1.0	0.25	5
	Quantum	H_2O ph	0.7	0.0	0
	Westar	H_2O sdlg	5.0	1.25	25
	Westar	H_2O bltg	4.9	1.0	20
	Westar	H_2O ph	5.0	1.5	30
Saskatoon	Cultivar	Treatment			
	Quest	RP sdlg	2.9	3.5	70
	Quest	RP bltg	2.8	3	60
	Quest	RP ph	2.2	3.5	70
	LG3235	RP sdlg	1.4	3.5	70
	LG3235	RP bltg	1.0	3.75	75
	LG3235	RP ph	1.4	3.75	75
	InVigor 2153	LL sdlg	2.3	4.75	95
	InVigor 2153	LL bltg	2.5	4.25	85
	InVigor 2153	LL ph	2.9	4	80
	InVigor 2473	LL sdlg	1.5	4.25	85
	InVigor 2473	LL bltg	1.3	3.5	70
	InVigor 2473	LL ph	1.6	3.75	75
	Quantum	H_2O sdlg	0.4	4.25	85
	Quantum	H_2O bltg	0.5	4	80
	Quantum	H_2O ph	0.5	4.25	85
	Westar	H_2O sdlg	4.4	3.75	75
	Westar	H_2O bltg	4.3	4.25	85
	Westar	H_2O ph	4.3	4.75	95

Note: RP = Roundup; LL = Liberty;H_2O = water; sdlg = seedling application (4 leaf stage); bltg = bolting stage application; ph = post harvest application.

Table 7. The effect of weed control applications in 1999 during the growing season on HTC canola on reproduction of *Leptosphaeria maculans* on subsequent canola residues

Location				First Spore Evaluation	Second Spore Evaluation	Third Spore Evaluation	Fourth Spore Evaluation
Melfort	Cultivar	Treatment	Disease Severity	July 2000	October 2000	May 2001	October 2001
	Quest	RP sdlg	4.0	.	.	.	.
	Quest	RP bltg	3.9	.	.	.	.
	Quest	RP ph	3.8	0	480	70	0
	LG3235	RP sdlg	2.0	.	.	.	.
	LG3235	RP bltg	1.6	.	.	.	.
	LG3235	RP ph	1.7	1300	910	0	0
	InVigor 2153	LL sdlg	2.6	.	.	.	.
	InVigor 2153	LL bltg	2.8	.	.	.	.
	InVigor 2153	LL ph	3.1	1515	440	0	0
	InVigor 2473	LL sdlg	2.6	.	.	.	.
	InVigor 2473	LL bltg	2.3	.	.	.	.
	InVigor 2473	LL ph	3.0	35	1150	10	0
	Quantum	H_2O sdlg	0.8	.	.	.	.
	Quantum	H_2O bltg	1.0	.	.	.	.
	Quantum	H_2O ph	0.7	1005	260	0	0
	Westar	H_2O sdlg	5.0	.	.	.	.
	Westar	H_2O bltg	4.9	.	.	.	.
	Westar	H_2O ph	5.0	1600	520	0	0
Saskatoon	Cultivar	Treatment					
	Quest	RP sdlg	2.9	155	5	2570	10
	Quest	RP bltg	2.8	25	10	320	25
	Quest	RP ph	2.2	45	185	870	15
	LG3235	RP sdlg	1.4	195	145	2090	650
	LG3235	RP bltg	1.0	1725	690	2140	20
	LG3235	RP ph	1.4	40	40	1900	180
	InVigor 2153	LL sdlg	2.3	10	440	1790	170
	InVigor 2153	LL bltg	2.5	1745	45	1590	5
	InVigor 2153	LL ph	2.9	120	60	3750	65
	InVigor 2473	LL sdlg	1.5	0	10	440	135
	InVigor 2473	LL bltg	1.3	305	0	20	175
	InVigor 2473	LL ph	1.6	0	510	770	95
	Quantum	H_2O sdlg	0.4	55	170	10	5
	Quantum	H_2O bltg	0.5	255	20	40	5
	Quantum	H_2O ph	0.5	1265	0	30	0
	Westar	H_2O sdlg	4.4	1435	70	1650	125
	Westar	H_2O bltg	4.3	800	20	2370	35
	Westar	H_2O ph	4.3	20	190	910	90

Note: RP = Roundup; LL = Liberty;H_2O = water; sdlg = seedling application (4 leaf stage); bltg = bolting stage application; ph = post harvest application.

Table 8. The effect of weed control applications during the growing season on HTC canola on disease severity blackleg caused by *Leptosphaeria maculans* in 2000

Location			Disease	Mean Number	Mean Relative	Mean No. of spores	Mean No. of spores
Melfort	Cultivar	Treatment	Severity	Plants Infected	Infection %	July 2001	October 2001
	Quest	RP sdlg	2.1	2	40	0	0
	Quest	RP bltg	2.3	1.25	25	0	0
	Quest	RP ph	2.1	2.75	55	0	0
	LG3235	RP sdlg	1.7	1.5	30	0	0
	LG3235	RP bltg	1.6	2.25	45	0	0
	LG3235	RP ph	2.0	1.75	35	0	0
	InVigor 2153	LL sdlg	2.8	1.5	30	0	0
	InVigor 2153	LL bltg	2.8	1.75	35	0	0
	InVigor 2153	LL ph	2.9	2	40	0	0
	InVigor 2473	LL sdlg	1.1	1.25	25	0	0
	InVigor 2473	LL bltg	1.2	2.5	50	0	0
	InVigor 2473	LL ph	1.3	1.75	35	0	0
	Quantum	h2o sdlg	0.7	2.25	45	0	0
	Quantum	H_2O bltg	0.7	2	40	0	0
	Quantum	H_2O ph	0.7	1.5	30	0	0
	Westar	H_2O sdlg	4.3	0.75	15	0	0
	Westar	H_2O bltg	4.3	1.75	35	0	0
	Westar	H_2O ph	4.2	2.5	50	0	0

Note: RP = Roundup; LL = Liberty; H_2O = water; sdlg = seedling appliaction (4 leaf stage); bltg = bolting stage application; ph = post harvest application.