

The Changing Phase of Canola-Blackleg Populations in Western Canada: Is it Cause for Alarm or Time for Sound Management Practices to be Adopted?

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Blackleg caused by the fungus *Leptosphaeria maculans* is an economically important disease of canola and rapeseed cultivars grown worldwide. It is by far the most destructive pathogen of canola in North America; however, there has been good resistance in commercially available cultivars for almost 20 years against the predominant race - PG2, which has been invaluable to the farmers. New and virulent isolates belonging to PG3, PGT and PG4 races caused significant disease in locations where they appeared recently in Western Canada and North Dakota, USA. Greenhouse experiments have confirmed that there is no seedling resistance to these new strains in the commercially available cultivars. We are using marker assisted selection methods to pyramid (stack) resistance genes into cultivars, for it to withstand attack from all virulent races. To understand whether these isolates have evolved through sexual recombination or have migrated/introduced to western Canada and north central plains of the US, a study was conducted using blackleg populations collected from a single field in La Riviere, Manitoba, North Dakota, Brazil, Australia and United Kingdom using molecular markers. Results have shown that the blackleg pathogen is extremely diverse in its population. Each population consisted of isolates with high number of unique genotypes. All populations were clustered together indicating a high rate of gene flow existed among all populations, with the greatest rate being between isolates from La Riviere, Manitoba and North Dakota. Extensive cultivation of cultivars with single gene resistance helped the emergence of new and virulent strains in France and Australia in the recent past, and caused significant economic losses to farmers. In areas where the new races have been isolated, rotation away from canola for a minimum of 3-4 years, and use of conventional tillage may help reduce the spread of these new strains further.

Introduction

Leptosphaeria maculans (Desmaz.) Ces. & De Not. (anamorph *Phoma lingam* Tode ex Fr.), causes blackleg disease of rapeseed/canola crops (*Brassica napus* L. and *B. rapa* L.) worldwide. The disease is particularly important and causes significant yield losses in Europe, Australia and Canada (West et al., 2001). Pathogenicity test based on an interaction phenotype (IP) is useful in determining the pathogenicity groups of the isolates based on a limited number of differential cultivars (Mengistu et al., 1991). To date, four PGs have been recognized using *B. napus* cultivars Westar, Glacier and Quinta (Mengistu et al., 1991). The PG-1 (B-group) isolates are non-aggressive on Westar, Glacier and Quinta, and have been classified as a separate species *L. biglobosa* (Shoemaker and Brun, 2001). A-group isolates are quite diverse in aggressiveness and can be named as PG-2, PG-3 and PG-4 in response to their virulent spectra on the differentials. PGT, a new aggressive type, was found in the North American population of *L. maculans* (Chen and Fernando, 2005).

Breakdown of rapeseed/canola resistance to blackleg disease, resulting from the shift in *L. maculans* population, has been reported in Europe (Rouxel et al., 2003) and Australia (Li et al., 2003). Cultivars with moderate to high levels of resistance to *L. maculans* were developed in Canada (Rimmer, 2006). It is unknown whether these cultivars will provide long lasting protection against the natural population of *L. maculans*. In 2002 and 2003, a resistant cultivar (to PG-2) Q₂ which is generally used as the resistant-check cultivar in canola/rapeseed co-op trials by breeding programs in Western Canada was found severely infected in Roland and Morden, Manitoba, Canada. If there is a pathogen population change and breakdown of resistance, the canola industry could be affected. The working hypothesis for this study was that there has been a shift in the strain structure of *L. maculans* population in Western Canada and North Dakota. In this study we describe the application of the pathogenicity test to evaluate variability for virulence in *L. maculans* population using isolates collected from North America especially Western Canada and North Dakota from 2002 through 2004, which was then compared to a collection from 1984

through 2001. We were also interested in doing an extensive study on the *L. maculans* population in a single field. We are using marker assisted selection methods to pyramid (stack) resistance genes into cultivars, for it to withstand attack from all virulent races.

Materials and methods

Origin of isolates

The isolates of *L. maculans* used for test of pathogenicity groups mainly came from two sources: 1) a total of 39 isolates retrieved from *L. maculans* collection at the University of Manitoba (storage from 1984 to 2001); 2) isolates obtained either from different locations across Western Canada and North Dakota from 2002 to 2004, or from a single field (La Riviere, Manitoba) in 2004. The pathogen was isolated and single spored using standard methods (Chen and Fernando, 2006).

Host cultivar, inoculation and evaluation

Cultivars Westar, Glacier, Quinta were seeded and seven days after seeding, cotyledons of each differential cultivar were inoculated with each isolate. Interaction phenotype (IP) on cotyledons was assessed 12 days post-inoculation by using a 0-9 scale (Williams, 1985). Sources of resistance against PG3 and PG4 were searched from the university *Brassica* germplasm. Resistant parents were selected and used in cross with Westar as a susceptible parent. F2 and F3 progeny were developed for resistance against PG3 and PG4 respectively and were used for resistance segregation assay at the cotyledon stage.

Results

A total of 28 Manitoba isolates and 11 North Dakota isolates were retrieved from *L. maculans* collection of 1984-2001 at the University of Manitoba (Table 1). These isolates were either in weakly virulent PG-1 (15% of isolates) or in virulent PG-2 (85% of isolates), no PG-3 or PG-4 isolates were detected (Table 2, Fig.1).

In 2002, a total of 44 isolates were obtained from three locations in Manitoba, three locations in Saskatchewan and six locations in North Dakota, U.S.A (Table 1). Similar to the 1984-2001 collection ($\chi^2=0.558 < 3.84, p > 0.05$), 84% of the isolates were PG-2 and 14% were PG-1 (Fig. 1). One isolate, PL02-02, from Selkirk, Manitoba was identified as PG-3 in 2002.

In 2003, 22 isolates from 8 locations in Manitoba, 17 isolates from 3 locations in Saskatchewan, 12 isolates from 5 locations in Alberta and 107 isolates from at least 13 locations in North Dakota (U.S.A.) were evaluated for pathogenicity on the canola differential set (Table 1). The surveys in Manitoba yielded eight PG-3 isolates in total. PG-3 was not isolated from Saskatchewan samples but PGT, a new pathogenicity group, was recognized to be present in Saskatchewan's collection (65% of isolates, Fig.1). In North Dakota six PG-3 isolates from at least two locations (Cavalier and Ward) were isolated and confirmed through the differential test. In addition, isolates PL03-54-01 from Camrose, Alberta and PL03-53-31 from Cavalier, North Dakota were classified as PG-4 for the first time.

In order to investigate the pathogenicity profile of *L. maculans* population from a single field in 2004, 108 isolates were isolated from a canola field in La Riviere, Manitoba (Table 2), the same field where PG-3 isolates were found in 2003. All five pathogenicity groups were identified within this *L. maculans* population. One percent of isolates were PG-1, 75% of isolates PG-2, 9.3 % PG-3, 7.4 % PGT and 7.4 % PG-4. This is the first identification of PG-4 isolates in Manitoba. Pathogenicity profile of *L. maculans* isolates obtained from 2002 to 2004 (Table 1) indicated that PG-3 isolates were found at least in four locations in Manitoba (Selkirk, Roland, Carman and La Riviere) and two locations in North Dakota (Cavalier, Ward); PGT isolates were detected at four locations in Manitoba (Selkirk, Plum Coulee, Brandon and La Riviere), four locations in Saskatchewan (Antler, Kenaston, Tisdale and Arboorfield), two locations in Alberta (Viking and Camrose) and five locations in North Dakota (Ramsey, Towner,

Cavalier, Ward and Pierce); PG-4 isolates were identified in Manitoba (La Riviere), Alberta (Camrose) and North Dakota (Cavalier).

Resistance against PG3 was traced in the Australian spring *Brassica napus* variety Dunkeld. The Winter Variety Quinta used as differential is also known to carry resistance against PG3. Cotyledon stage pathogenicity assays conducted with F2 progeny from crosses between Westar and Dunkeld as well as Westar and Quinta revealed that the resistance segregation ratio was 3:1. Resistance against PG4 was found in the *Brassica juncea* variety Cutlass. Progeny from crosses between Westar and Cutlass displayed a poor seed set and a F3 progeny was required for resistance segregation analysis. The segregation ratio of resistance against PG4 was 8:1. These sources of resistance are being used for resistance pyramiding in order to develop lines carrying resistance against PG2, PG3 and PG4 together. For this purpose, *Brassica napus* varieties Q2 and Crésor that are PG2 resistant were used to add resistance against PG3 and PG4.

Discussion

Our results show that weakly virulent isolates PG-1, now classified into a separate species *L. biglobosa* (Shoemaker and Brun, 2001), and highly virulent pathogenicity complex PG-2, PG-3, PGT and PG-4 in the species *L. maculans*, are present in canola fields of Western Canada and North Dakota. No obvious relationship was observed between pathogenicity group and geographic location. PG-1 occurred at all sites surveyed but at low levels. Prior to 2001, only 15.3% of isolates were found to be PG-1. The largest % proportion of PG-1 (21.3 %) was observed in 2002 (Fig. 1).

PG-2 isolates are prevalent in all locations of Western Canada. They were the predominant population each year in all locations. For instance, population size of PG-2 was always over 70% of isolates during four years of investigation (Fig. 1). PG-2 has been reported in Western Canada since 1975 (Mengistu et al., 1991). However, similarity of pathogenicity between isolates collected in 1980's and in the early 21st century, grouping either in PG-1 or PG-2, revealed that *L. maculans* populations in Western Canada and North Dakota have remained relatively unchanged during that period. PG-3 and PG-4 isolates have been detected previously in Ontario, Canada (Mahuku et al., 1997) and Georgia, USA (Phillips et al., 1999). The samples we analyzed in 2002, 2003 and 2004 presented the occurrence of PG-3, PGT and PG-4 isolates to a great extent across the western prairies of Canada and North Dakota U.S.A. Pongam (1999) indicated that isolates of *L. maculans* in Ontario were genetically linked to Australia, France and Germany, whereas isolates of *L. maculans* in Western Canada, North Dakota and Georgia were genetically similar to UK. If that is correct, the origin of virulent isolates found in Ontario and Western Canada could be different. PG-2 isolates are rare in Western Europe (Penaud et al., 1999) and Australia (Keri, 1999), whereas PG-3 and PG-4 are most abundant (Williams, 1992). Why new strains such as PG-3 and PG-4 are arising in Western Canada is unknown. One scenario is introduction of new strains from other regions or continents via seed (Purwantara et al., 2000). Canadian Seed Growers Association (CSGA) has strict regulations of crop seed trades. *Leptosphaeria maculans* is a quarantined pathogen/disease in the importing/exporting of canola seeds. In addition, seed treatment with fungicides against blackleg pathogen is required in Canada. However, the contribution of seed-lot to variation among the isolates was still noticed in Canada (Mahuku et al., 1997). Secondly population shifts associated with the sexual recombination of this pathogen are well known across the world. Pathogenicity group test for the isolates from a single field at La Riviere in 2004 showed the co-existence of all PG types, perhaps suggesting that sexual recombination occurs in the environment. The detection of PGT isolates, which are found only in Western Canada and North Dakota, also suggests sexual recombination may be occurring. The study of Mahuku et al. (1997) indicated the variation among isolates of *L. maculans* caused by sexual reproduction in Canadian canola fields. Finally, monoculture of similar host resistance may exert selection pressure on *L. maculans* populations (Balesdent et al., 2005) and cause the variation of the population structure. In France, the large-scale use of single *Rlm1* gene within ten years (1990 to 2000) shifted the population of isolates from *Avlml1* to others (Rouxel et al., 2003). Similarly in Australia, a

single dominant resistance gene derived from *B. rapa* subsp. *sylvestris* collapsed within a period of 3 years of commercial use due to the change of population structure (Li et al., 2003).

Although PG-3, PGT, and PG-4 are present in Western Canada and North Dakota, the frequency of new strains within this region is still low (Fig. 1). The new strains first identified within this region were either from commercial canola fields or from canola co-op blackleg nursery fields. For instance, the PG-3 isolate found in 2002 came from a canola farm located in Selkirk, Manitoba, which was grown with canola (cv. Hyola 401) in 2000 and then planted with barley (2001) and flax (2002). In 2003, PG-3 isolates were obtained from co-op blackleg nursery fields in Roland and Carman, Manitoba where blackleg-resistant canola cv Q₂ was severely infected. Also PG-3 isolates were detected in a farmer's field at La Riviere, 200 km southwest of Winnipeg, where severe blackleg was reported during the growing season of 2003. The PG-4 isolates were first detected in 2003 at Camrose, Alberta in a co-op blackleg nursery field and a commercial field in Cavalier, North Dakota. PGT isolates were identified in 2003 and 2004 from many canola farms. In 2005, the canola industry reported fields with moderately resistant or resistant canola cultivars had significant blackleg. We were able to isolate PG-3 and PGT strains from all diseased canola stem samples sent to us by these individual growers or seed companies (data not presented).

Because the new PG-groups have established in canola growing regions of Western Canada and North Dakota, USA, the growers, in the immediate future, will need to deploy cultural management strategies such as crop isolation, and longer rotations between canola crops to minimize the impact of these new strains. Ultimately the canola industry will have to develop new resistant cultivars against all PG isolates.

The first generation of canola lines carrying resistance genes against *L. maculans* PG2, PG3, and P4 found in Western Canada are being developed. However reports from Australia and Europe have revealed that changes within the pathogen populations quickly break down resistance of newly introduced varieties (Rouxel et al., 2003, Li et al., 2003). Pathogen populations changes are attributed to sexual reproduction of *L. maculans* which is common in the Canadian prairies. It is then expected that due to the coexistence of all pathogenicity groups in Western Canada and North Dakota, effective blackleg management will require continuous resistance breeding efforts.

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Table 1. Pathogenicity groups of *Leptosphaeria maculans* / *L. biglobosa* (reproduced from Chen and Fernando, CJPP 2006)

Year ^a	Origin ^b	Location	# of Isolates	PG-				
				1	PG-2	PG-3	PGT	PG-4
1984-	Canada	Manitoba	28	6	22	0	0	0
2001	USA	North Dakota	11	0	11	0	0	0
2002	MB	Selkirk	13	6	6	1	0	0
		Grosse isle	3	0	3	0	0	0
		Winnipeg	1	0	1	0	0	0
	SK	Yorktown	1	0	1	0	0	0
		Melfort	1	0	1	0	0	0
		Tadmore	1	0	1	0	0	0
	ND	Cavalier	13	2	11	0	0	0
		Bottineau	1	0	1	0	0	0
		Mclean	2	0	2	0	0	0
		Ward	4	2	2	0	0	0
		Benson	2	0	2	0	0	0
		Towner	5	0	5	0	0	0
	2003	MB	Roland	4	0	0	4	0
Darlingford			3	0	3	0	0	0
La Riviere			3	0	1	2	0	0
Plum Coulee			5	0	1	0	4	0
Carman			2	0	0	2	0	0
Gretna			2	0	2	0	0	0
Dauphin			1	1	0	0	0	0
Brandon			2	0	1	0	1	0
SK		Kenaston	5	0	1	0	4	0
		Tisdale	9	0	4	0	5	0
		Arboorfield	3	0	1	0	2	0
AB		Viking	3	0	1	0	2	0
		Vermilion	3	3	0	0	0	0
		Camrose	2	0	0	0	1	1
		Killam	1	1	0	0	0	0
		Westlock	3	3	0	0	0	0

Table 2 (Cont.). Pathogenicity groups of *Leptosphaeria maculans* / *L. biglobosa*

Year	Origin	Location	Isolate No.	PG-1	PG-2	PG-3	PGT	PG-4
2003	ND	Ramsey	2	0	2	0	0	0
		Towner	8	0	8	0	0	0
		Foster	5	0	5	0	0	0
		Cavalier	6	0	4	1	0	1
		Ward	24	1	20	3	0	0
		Eddy	1	0	1	0	0	0
		Mclean	7	0	7	0	0	0
		Mountrail	4	1	3	0	0	0
		Bottineau	10	0	10	0	0	0
		Renville	5	0	5	0	0	0
		Ward	2	0	2	0	0	0
		Osnabrock	1	0	1	0	0	0
		n/a ^c	32	0	28	2	2	0
2004	MB	Roland	3	0	2	1	0	0
		Darlingford	4	0	4	0	0	0
		La Riviere	108	1	81	10	8	8
		Selkirk	37	0	36	0	1	0
		Morden	7	0	5	2	0	0
	SK	N. Battlefield	1	0	1	0	0	0
		Antler	5	0	4	0	1	0
		n/a	3	0	3	0	0	0
	AB	Camrose	8	0	0	0	7	1
		n/a	3	0	2	1	0	0
	ND	Ramsey	4	0	3	0	1	0
		Towner	9	0	6	1	2	0
		Burke	2	0	2	0	0	0
		Cavalier	12	2	8	0	2	0
		Ward	7	0	6	0	1	0
		Nelson	4	0	4	0	0	0
		Mclean	4	0	4	0	0	0
		Mountrail	3	0	3	0	0	0
		Bottineau	4	0	4	0	0	0
		Renville	5	1	4	0	0	0
Pierce		6	0	5	0	1	0	
Benson		3	0	3	0	0	0	
Divide		1	0	1	0	0	0	
Williams		1	0	1	0	0	0	
McHenry		1	0	1	0	0	0	

^aThe time when the isolate was collected from field.

^bAB – Alberta, MB – Manitoba, SK – Saskatchewan, ND – North Dakota.

^cn/a – not available.

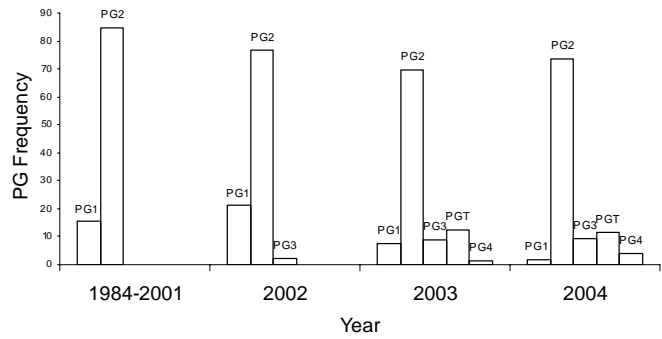


Figure 1. Frequency of pathogenicity groups of *Leptosphaeria maculans* / *L. biglobosa* detected in Western Canada and North Dakota from 2001 – 2004(reproduced from Chen and Fernando, CJPP 2006).

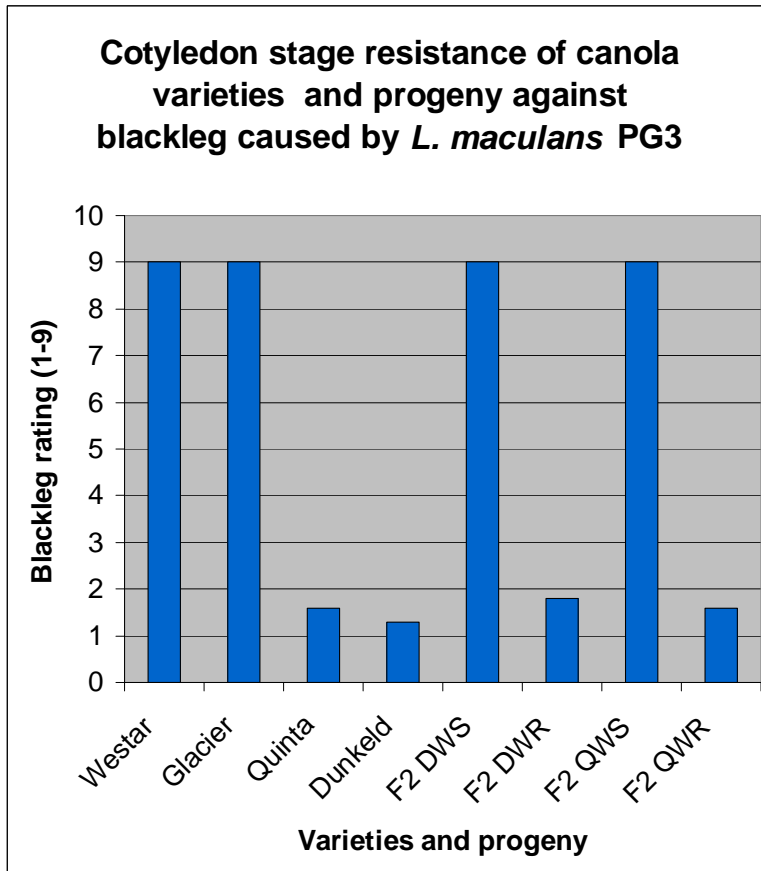


Figure 2. Segregation of resistance against PG3 in F2 progeny from Westar x Dunkeld and Westar x Quinta crosses

DWS: susceptible progeny from Westar x Dunkeld; DWR: resistant progeny from Westar x Dunkeld; QWS: susceptible progeny from Westar x Quinta; QWR: resistant progeny from Westar x Quinta

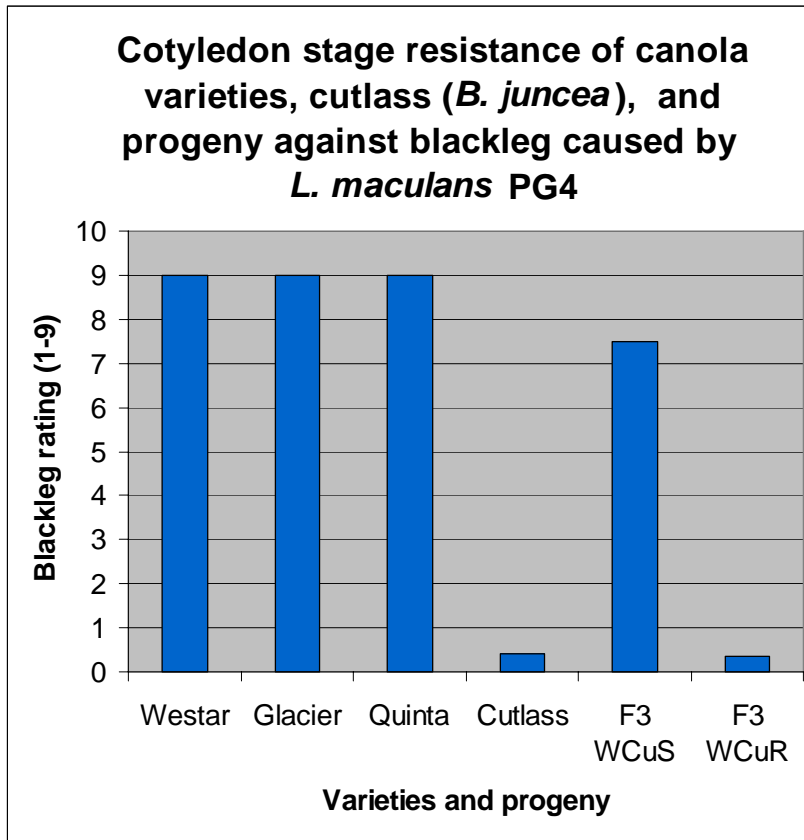


Figure 3: Segregation of resistance against PG4 in F3 progeny from a Westar x Cutlass cross
 WCuS: Susceptible Westar x Cutlass progeny; WCuR: Resistant Westar x Cutlass progeny