Epidemiology / Épidémiologie

Prevalence of pathogenicity groups of *Leptosphaeria maculans* in western Canada and North Dakota, USA

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Abstract: Blackleg, caused by *Leptosphaeria maculans*, is an economically important disease of canola (*Brassica napus*). Little is known about the current distribution of pathogenicity groups (PGs) of *L. maculans* in Canada and the United States. Four hundred and eighty-nine isolates of *L. maculans* or *Leptosphaeria biglobosa* from western Canada and North Dakota, United States, were placed in five PGs (PG-1 recognized as *L. biglobosa* since 2001, PG-2, PG-3, PG-4, and PGT) on the basis of a series of inoculations on canola cultivars ('Westar', 'Glacier', and 'Quinta'). Only PG-1 and PG-2 were observed in populations from a 1984 to 2001 collection. All five PGs were observed in populations collected between 2002 and 2004. New, more aggressive strains of *L. maculans* may pose a significant threat to the canola industry in Canada and the United States.

Key words: canola, blackleg, *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, pathogenicity group, prevalence.

Résumé : La jambe noire du canola (*Brassica napus*), causée par le *Leptosphaeria maculans*, est une maladie économiquement importante. La distribution actuelle des groupes de pathogénicité (GP) du *L. maculans* au Canada et aux États-Unis est mal connue. Quatre cent quatre-vingt-neuf isolats de *L. maculans* ou *Leptosphaeria biglobosa* de l'Ouest du Canada et du Dakota du Nord, États-Unis, furent classés en cinq GP (PG-1 reconnu comme *L. biglobosa* depuis 2001, GP-2, GP-3, GP-4 et GPT) d'après une série d'inoculations sur des cultivars de canola ('Westar', 'Glacier' et 'Quinta'). Seuls GP-1 et GP-2 furent observés dans les populations d'une collection couvrant les années 1984 à 2001. Tous les cinq GP furent observés dans des populations obtenues de 2002 à 2004. De nouvelles souches plus agressives de *L. maculans* peuvent menacer l'industrie du canola au Canada et aux États-Unis.

Mots clés : canola, jambe noire, *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, groupe de pathogénicité, prévalence.

Introduction

Leptosphaeria maculans (Desm.) Ces. & De Not. (anamorph *Phoma lingam* (Tode ex Fr.) Desm.), causes blackleg of rapeseed/canola crops (*Brassica napus* L. and *Brassica rapa* L.) worldwide. The disease is particularly important and causes significant yield losses in Europe, Australia, and Canada (West et al. 2001). The occurrence and severity of the disease vary among growing seasons (Howlett 2004), cultivars (Aubertot et al. 2006), agricultural practices (Aubertot et al. 2004), and environmental conditions (Sosnowski et al. 2004). *Leptosphaeria maculans* is known to have been present in Europe for over 60 years (Aubertot et al. 2004), in Australia for at least 80 years (Sivasithamparam et al. 2005), and in Canada for 30 years (Petrie 1978). Variation in the pathogen populations has been reported, and efforts have been made to characterize different isolates through morphological (Petrie 1988), physiological (McGee and Petrie 1978), biochemical (Soledade et al. 2000), genetic (Gall et al. 1995), and molecular markers (Goodwin and Annis 1991; Pongam et al. 1999; Purwantara et al. 2000). Among these, virulence markers of pathogenicity groups (PGs) are most widely used for characterizing the pathogen populations (Pongam et al. 1999).

Pathogenicity testing based on an interaction phenotype is useful in determining the PGs of the isolates on a limited number of differential cultivars (Keri 1999; Koch et al. 1991; Kutcher et al. 1993; Mengistu et al. 1991). Variation in virulence of the isolates is displayed on differential cultivars in the form of resistant or susceptible reactions. Usually, isolates of *L. maculans* are primarily grouped as either nonaggressive (group B) or aggressive (group A) types, on the basis of reactions on the cotyledons of susceptible cultivars such as 'Westar'. To date, four PGs have been recognized using *B. napus* 'Westar', 'Glacier', and 'Quinta' (Keri 1999; Koch et al. 1991; Mengistu et al. 1991). The PG-1 (group B) isolates are nonaggressive on 'Westar',

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'Glacier', and 'Quinta' and have been classified as a separate species, *Leptosphaeria biglobosa* R.A. Shoemaker & H. Brun (Shoemaker and Brun 2001). Isolates in group A are quite diverse in aggressiveness and can be named 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 populations of *L. maculans* (Chen and Fernando 2005; Rimmer 2006).

A breakdown of rapeseed/canola resistance to blackleg, resulting from a shift in the *L. maculans* populations, 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* have been developed in Canada (Rimmer 2006). It is not known whether these cultivars will provide long-lasting protection against the natural populations of *L. maculans*. In 2002 and 2003, 'Q_2', a cultivar resistant to PG-2 that is generally used as the resistant control in canola/rapeseed cooperative trials in breeding programs in western Canada, was found to be severely infected in Roland and Morden, Manitoba. If there has been a change in the pathogen populations and a resulting breakdown in canola 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 the *L. maculans* populations in western Canada and North Dakota, United States. In this study we describe the application of pathogenicity testing to evaluate the variability of virulence in the *L. maculans* populations, using isolates collected from North America, especially western Canada and North Dakota, from 2002 through 2004, which were then compared with a collection made from 1984 through 2001. We were also interested in carrying out an extensive study on the *L. maculans* population in a single field.

Studies on population biology and structure are relevant to the development of rational control strategies to manage the entire pathogen population rather than a single isolate or a race. An epidemic of plant disease that causes significant crop loss involves thousands if not millions of infection events. For management strategies to be effective, it is important to recognize and understand the population at large. This is even more imperative in the case of the blackleg pathogen complex. It is vital for us to understand the population structure and evolution of *L. maculans*, with emphasis on pathogenicity analysis, so that we can answer the following questions: why and how are the *L. maculans* populations in western Canada and North Dakota changing?

Materials and methods

Origin of isolates

The isolates of *L. maculans* used for testing PGs came mainly from two sources: (1) a total of 39 isolates retrieved from the *L. maculans* collection at the University of Manitoba, Winnipeg (stored 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 Rivière, Manitoba, 200 km southwest of Winnipeg) in 2004. The mycelia or pycnidia of *L. maculans* from the collection at the University of Manitoba are grown on a slant of potato dextrose agar and completely covered with a thick layer of sterile mineral oil for long-term storage. To reculture an isolate, a small piece of agar was cut from the slant of specimen bottle and transferred onto an autoclaved filter paper. The oil on the piece of agar was gently blotted onto the sterile filter paper. The piece of agar was then placed on V8 agar (200 mL of V8 juice (Campbell Soup Company Ltd., Toronto, Ont., Canada), 0.75 g of $CaCO_3$, 15 g of agar in 800 mL of distilled water) and incubated under continuous cool-white fluorescent light at room temperature (20 °C).

Sporulating cultures (pycnidia) that formed on the V8-agar plates were harvested and kept in a highly concentrated state in a microcentrifuge tube (2.0 mL) at –20 °C for short-term storage (viable for at least 1 year). All retrieved isolates were inoculated to *B. napus* 'Westar' (very susceptible to blackleg) once to revive and maintain virulence, following which each isolate was recovered and single-spored.

Isolation of the blackleg pathogen from leaf lesions or stubble collected in Canada and the United States from 2002 through 2004

Small pieces of stubble bearing pseudothecia or small pieces of infected leaf tissue were surface-sterilized in 1% sodium hypochlorite (NaOCl) solution (3–5 min for stubble and 30 s for leaf tissue). Stubble and leaf pieces were then rinsed three times in sterile distilled water, dried on autoclaved filter paper, placed on V8 agar, and incubated under continuous cool-white fluorescent light for 5–7 days. The V8 agar was amended with 0.03% chloramphenicol (EC200-2874, Sigma, St. Louis, Mo., USA) to inhibit bacterial growth. After subculturing, isolates were characterized as *L. maculans* by means of colony and pycnidia morphology. All isolates were inoculated onto 'Westar' to bioassay virulence and then stored at –20 °C.

Single-spore isolation and inoculum preparation

One microlitre of concentrated pycnidiospore suspension was mixed in 10–15 μL of sterile distilled water and spread on 2% water agar with a flamed L-shaped glass rod. The plates were wrapped in aluminum foil to block the light and incubated at room temperature for 36–48 h. Single germinating pycnidiospores were transferred to V8 agar under the microscope (magnification, 100×), using a sterile needle. After they had grown on V8 agar for 14 days at room temperature, sporulating cultures were flooded with 10 mL of sterile distilled water, and the surface of the plates was scraped gently with a flamed glass rod to release pycnidiospores from the pycnidia. The pycnidiospores were harvested by filtering through sterilized Miracloth® (Calbiochem, La Jolla, Calif., USA) and centrifuged at 9000 r/min (1 r = 2π rad) for 20 min. The supernatant was decanted and the spore pellet was resuspended in approximately 1 mL of sterile distilled water. The concentrated pycnidiospore suspension was transferred to a microcentrifuge tube (2.0 mL) and stored at –20 °C. Prior to inoculation, a few drops of concentrated spore suspension were mixed in a sterile tube containing about 10 mL of sterile distilled water. The concentration of the inoculum was adjusted to 1×10^7 spores/mL with the aid of a hemacytometer (Hausser Scientific Company, Horsham, Penn., USA).

Host cultivar, inoculation and evaluation

'Westar', 'Glacier', and 'Quinta' were seeded and grown in flats with soilless mix (MetroMix®, W.R. Grace and Co. Ltd., Ajax, Ont.). All seedlings were watered daily and kept in a growth chamber (16 °C (night) : 21 °C (day); photoperiod, 16 h (light) : 8 h (dark)). Seven days after seeding, 24 fully expanded cotyledons of each differential cultivar were inoculated with each isolate. Each cotyledon was wounded once with a sterile needle, and a droplet (10 μL) of pycnidiospore suspension was pipetted onto the wound. The inoculated cotyledons were allowed to dry at room temperature for at least 12 h before the next watering. After inoculation, cotyledons were moved back to the same growth chamber and incubated. The inoculation experiment was repeated at least once for each isolate. The interaction phenotype on the cotyledons was assessed 12 days after inoculation, using a scale of 0–9 (Williams 1985): 0, no darkening around the wounds; 1, limited blackening around the wound, lesion diameter 0.5–1.5 mm, a faint chlorotic halo may be present, sporulation absent; 3, dark necrotic lesions, a chlorotic halo 1.5–3.0 mm in diameter may be present, sporulation absent; 5, nonsporulating, but lesions 3–5 mm in diameter, sharply delimited by a dark necrotic margin, may show gray-green tissue collapse; 7, gray-green tissue collapse, lesions 3–5 mm in diameter, sharply delimited by a nondarkened margin; 9, rapid tissue collapse at about 10 days, accompanied by profuse sporulation in large lesions, more than 5 mm in diameter, with a diffuse margin. On this scale, 0–2 was classified as resistant, 3–6 as intermediate, and 7–9 as susceptible. PG-1, PG-2, PG-3, PGT, and PG-4 were assigned to each isolate, according to the interaction phenotype on 'Westar', 'Glacier', and 'Quinta' (Table 1).

Results

A total of 28 Manitoba isolates and 11 North Dakota isolates were retrieved from the *L. maculans* collection from 1984–2001 at the University of Manitoba (Table 2). 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 47 isolates were obtained from three locations in Manitoba, three locations in Saskatchewan, and six locations in North Dakota (Table 2). The frequencies of PG-2 and PG-1 isolates in 2002 were 76.6% and 21.3%, respectively, and remained similar to those observed in the 1984–2001 collection ($\chi^2 = 0.558$, df = 1, $p > 0.05$) (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 were evaluated for pathogenicity on the canola differential set (Table 2). The surveys in Manitoba yielded eight PG-3 isolates in total. PG-3 was not isolated from the Saskatchewan samples, but the presence of PGT, a new PG, was recognized in the Saskatchewan 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.

Table 1. Pathogenicity groups (PGs) of *Leptosphaeria maculans* and (or) *Leptosphaeria biglobosa** and their interaction phenotypes on three canola cultivars in western Canada and North Dakota, United States, from 1984 to 2004.

	Interaction phenotype		
	'Westar'	'Glacier'	'Quinta'
PG-1*	0 (R)	0 (R)	0 (R)
PG-2	7–9 (S)	0–2 (R)	3–6 (I)
PG-3	7–9 (S)	7–9 (S)	3–6 (I)
PGT	7–9 (S)	3–6 (I)	7–9 (S)
PG-4	7–9 (S)	7–9 (S)	7–9 (S)

Note: Interaction phenotypes were rated on a scale of 0–9 (Williams 1985). See Materials and methods for a description of the scale. I, intermediate; R, resistant; S, susceptible. Table modified after Mengistu et al. (1991).

*PG-1 has been recognized as *L. biglobosa* by Shoemaker and Brun (2001).

To investigate the pathogenicity profile of the *L. maculans* population from a single field in 2004, 108 isolates were isolated from a canola field in La Rivière, Manitoba (Table 2), the same field from which PG-3 isolates were found in 2003. All five PGs were identified within this *L. maculans* population in 2004. One percent of isolates were PG-1, 75% were PG-2, 9.3% were PG-3, 7.4% were PGT, and 7.4% were PG-4. This was the first identification of PG-4 isolates in Manitoba. Several other locations were found to have PG-3 or PGT, such as Roland, Selkirk, and Morden in Manitoba, Antler in Saskatchewan, Camrose in Alberta, and Ramsey, Towner, Cavalier, Ward, and Pierce in North Dakota (Table 2). Again, one isolate, PL04-25-01 from Camrose, Alberta, was confirmed to be in PG-4 in the 2004 test.

The pathogenicity profile of *L. maculans* isolates obtained between 2002 and 2004 (Table 2) indicated that PG-3 isolates were found at least in five locations in Manitoba (Selkirk, Roland, La Rivière, Carman, and Morden) and three locations in North Dakota (Cavalier, Ward, and Towner); PGT isolates were detected at four locations in Manitoba (Plum Coulee, Brandon, La Rivière, and Selkirk), four locations in Saskatchewan (Antler, Kenaston, Tisdale, and Arborfield), 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 Rivière), Alberta (Camrose), and North Dakota (Cavalier).

Discussion

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

Table 2. Pathogenicity groups (PGs) of *Leptosphaeria maculans* and (or) *Leptosphaeria biglobosa** from canola in western Canada and North Dakota, United States, from 1984 to 2004.

	Number of isolates in pathogenicity group					
Year† and origin	Total	PG-1*	PG-2	PG-3	PGT	PG-4
1984–2001						
Manitoba, Canada	28	6	22	0	0	0
North Dakota, United States	11	0	11	0	0	0
2002						
Manitoba						
Selkirk	13	6	6	1	0	0
Grosse Isle	3	0	3	0	0	0
Winnipeg	1	0	1	0	0	0
Saskatchewan						
Melfort	1	0	1	0	0	0
Tadmore	1	0	1	0	0	0
Yorktown	1	0	1	0	0	0
North Dakota						
Cavalier	13	2	11	0	0	0
Towner	5	0	5	0	0	0
Ward	4	2	2	0	0	0
Benson	2	0	2	0	0	0
McLean	2	0	2	0	0	0
Bottineau	1	0	1	0	0	0
2003						
Alberta						
Vermilion	3	3	0	0	0	0
Westlock	3	3	0	0	0	0
Viking	3	0	1	0	2	0
Camrose	2	0	0	0	1	1
Killam	1	1	0	0	0	0
Manitoba						
Plum Coulee	5	0	1	0	4	0
Roland	4	0	0	4	0	0
Darlingford	3	0	3	0	0	0
La Rivière	3	0	1	2	0	0
Carman	2	0	0	2	0	0
Gretna	2	0	2	0	0	0
Brandon	2	0	1	0	1	0
Dauphin	1	1	0	0	0	0
Saskatchewan						
Tisdale	9	0	4	0	5	0
Kenaston	5	0	1	0	4	0
Arborfield	3	0	1	0	2	0
North Dakota						
Ward	24	1	20	3	0	0
Bottineau	10	0	10	0	0	0
Towner	8	0	8	0	0	0
McLean	7	0	7	0	0	0
Cavalier	6	0	4	1	0	1
Foster	5	0	5	0	0	0
Renville	5	0	5	0	0	0
Mountrail	4	1	3	0	0	0
Ramsey	2	0	2	0	0	0
Ward	2	0	2	0	0	0
Eddy	1	0	1	0	0	0
Osnabrock	1	0	1	0	0	0
Origin not available	32	0	28	2	2	0

Table 2. (*concluded*).

Year† and origin	Number of isolates in pathogenicity group					
	Total	PG-1*	PG-2	PG-3	PGT	PG-4
2004						
Alberta						
Camrose	8	0	0	0	7	1
Origin not available	3	0	2	1	0	0
Manitoba						
La Rivière	108	1	81	10	8	8
Selkirk	37	0	36	0	1	0
Morden	7	0	5	2	0	0
Darlingford	4	0	4	0	0	0
Roland	3	0	2	1	0	0
Saskatchewan						
Antler	5	0	4	0	1	0
North Battleford	1	0	1	0	0	0
Origin not available	3	0	3	0	0	0
North Dakota						
Cavalier	12	2	8	0	2	0
Towner	9	0	6	1	2	0
Ward	7	0	6	0	1	0
Pierce	6	0	5	0	1	0
Renville	5	1	4	0	0	0
Bottineau	4	0	4	0	0	0
McLean	4	0	4	0	0	0
Nelson	4	0	4	0	0	0
Ramsey	4	0	3	0	1	0
Benson	3	0	3	0	0	0
Mountrail	3	0	3	0	0	0
Burke	2	0	2	0	0	0
Divide	1	0	1	0	0	0
McHenry	1	0	1	0	0	0
Williams	1	0	1	0	0	0

Note: For 1984 to 2001, *L. maculans* were retrieved from a collection at the University of Manitoba, Winnipeg. For 2002 to 2004, isolates were obtained either from different locations across western Canada and North Dakota or from a single field (La Rivière, Manitoba) in 2004.

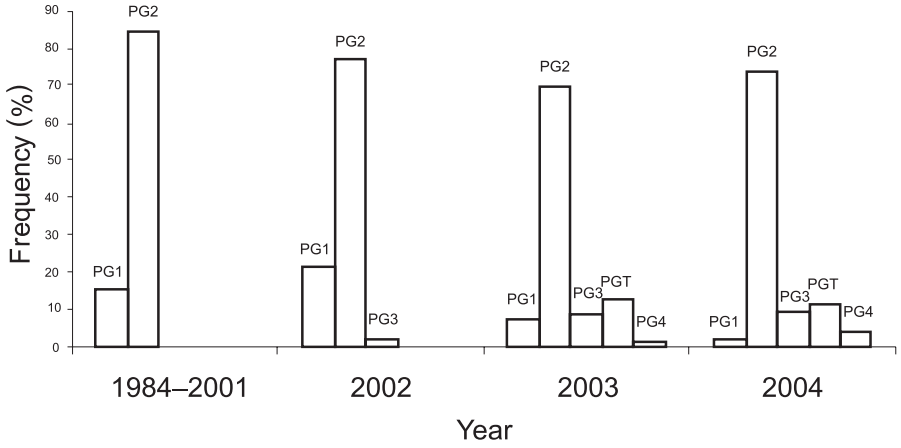
*PG-1 has been recognized as *L. biglobosa* by Shoemaker and Brun (2001).

†When the isolate was collected from the field.

et al. 2002) and Australia (Plummer et al. 1994), although the origin of PG-1 isolates is unknown. According to amplified fragment length polymorphism analysis, PG-1 isolates from Europe and North America show little phylogenic similarity (Purwantara et al. 2000). On the basis of this analysis, three genetically distinct subgroups have been recognized in group B (PG-1). The distinction between these PGs and subgroups is supported by a variety of isozyme and protein studies (Gall et al. 1995), analysis of internal transcribed spacers (Balesdent et al. 1998), and amplified fragment length polymorphism (Purwantara et al. 2000). Because PG-1 isolates are weakly virulent, they are not the cause of the blackleg epidemic in North America.

PG-2 isolates were predominant each year from 1984 to 2004 in all locations from the western Canada and North Dakota (Fig. 1). For instance, PG-2 always represented over 70% of the isolates within the overall *L. maculans* populations during the period of our investigation (Fig. 1). PG-2 has been reported in western Canada since 1975 (Kutcher et al. 1993; Mahuku et al. 1997; Mengistu et al. 1991). The similarity of pathogenicity between isolates collected in the 1980s and the early 21st century, which were grouped in either PG-1 or PG-2, revealed that *L. maculans* populations in western Canada and North Dakota 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, United States (Phillips et al. 1999). The samples we analyzed in 2002, 2003, and 2004, revealed that PG-3, PGT, and PG-4 isolates occurred across most of the western prairies of Canada and North Dakota. Pongam et al. (1999) indicated that isolates of *L. maculans* in Ontario were genetically linked to those in Australia, France, and Germany, whereas isolates of *L. maculans* in western Canada, North Dakota, and Georgia were genetically similar to those in the United Kingdom. If this is correct, the origins of the 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), while PG-3 and PG-4 are the most abundant (Williams 1992). Why new strains such as PG-3

Fig. 1. Frequencies of pathogenicity groups of *Leptosphaeria maculans* and (or) *Leptosphaeria biglobosa* (PG-1 has been recognized as *L. biglobosa* by Shoemaker and Brun (2001)) detected in western Canada and North Dakota from 1984 to 2004.



and PG-4 are arising in western Canada is not known. One scenario is that new strains are introduced from other regions or continents via seed (McGee and Petrie 1978; Purwantara et al. 2000). The Canadian Seed Growers Association has strict regulations concerning crop-seed trade. *Leptosphaeria maculans* is a quarantined pathogen in the importing and exporting of canola seeds. In addition, seed treatment with fungicides against the blackleg pathogen is required in Canada. However, the contribution of seed lots to variation among the isolates was still noted in Canada according to Mahuku et al. (1997). Secondly, population shifts associated with sexual recombination in this pathogen are well known across the world. Pathogenicity testing of the isolates from a single field at La Rivière in 2004 showed the coexistence of all virulent PG types, which perhaps suggests that sexual recombination occurs in the environment. The detection of PGT isolates, which are found only in western Canada and North Dakota, also suggests that sexual recombination may be occurring. The study by Mahuku et al. (1997) indicated variation among isolates of *L. maculans* caused by sexual reproduction in Canadian canola fields. Finally, monocultures with similar host resistance may exert selection pressure on *L. maculans* populations (Balesdent et al. 2005) and cause variation in the population structure. In France, the large-scale use of a single *Rlm1* gene shifted the population of isolates from *Avlm1* to others within 10 years (1990–2000) (Rouxel et al. 2003). Similarly, in Australia, a change in population structure of the pathogen, causing the host resistance to collapse, was observed within a period of 3 years of commercial use of all cultivars in which resistance was associated with the incorporation of a single dominant gene derived from *B. rapa* L. subsp. *sylvestris* (L.) Janch. (Li et al. 2003).

Although PG-3, PGT, and PG-4 are present in western Canada and North Dakota, the frequencies of these new strains within this region are still low (Fig. 1). The first reports of these strains within this region were either from commercial canola fields or from canola cooperative blackleg nursery fields. For instance, the PG-3 isolate found in 2002 came from a canola farm in Selkirk, Manitoba, on which canola was grown ('Hyola 401') in 2000, followed by barley in 2001 and flax in 2002. In 2003, PG-3 isolates were obtained from cooperative blackleg nursery fields in Roland and Carman, Manitoba, where blackleg-resistant canola 'Q_2' was severely infected. Also, PG-3 isolates were detected in a farmer's field at La Rivière, where severe blackleg was reported during the growing season of 2003. The PG-4 isolates were first detected in 2003 in a cooperative blackleg nursery field in Camrose, Alberta, 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 that fields seeded with canola cultivars previously rated as moderately resistant or resistant to *L. maculans* had significant levels of 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 PGs have established in canola-growing regions of western Canada and North Dakota, in the immediate future, the growers 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.

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