Determination of pathogenic variability of *Leptosphaeria maculans* in western Canada and resistance in Canadian *Brassica napus* cultivars

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SUMMARY

Blackleg of canola and oilseed rape, caused by *Leptosphaeria maculans* (Desmaz.) Ces. & De Not. is the most damaging disease of these crops in Canada and world-wide. Since the introduction of cultivars with improved resistance to blackleg, the threat to the canola industry has been reduced, but not eliminated. Blackleg continues to be found throughout the canola growing area and recent changes in the pathogenic variability of *L. maculans* (new strains) in western Canada have been observed. New strains, coupled with more intensive production of canola increases the risk that the current genetic resistance in canola may be overcome by the pathogen. The specific resistance (R) genes in Canadian canola cultivars and the corresponding avirulence (*AvrLm*) genes carried in isolates of *L. maculans* collected from western Canada are unknown. Our objectives were to determine: 1) the avirulence genes in the pathogen population, and 2) the specific resistance genes in a selection of Canadian varieties and other lines.

Objective 1. Changes in pathogenicity of populations of *Leptosphaeria maculans* in western Canada are believed to have resulted from the use of specific resistance genes in *Brassica* spp., many of which have only recently been identified. Under controlled conditions, analysis was conducted for 103 isolates of *Leptosphaeria* spp. collected between 1997 and 2005 in western Canada. Ninety-six were determined to be *L. maculans* and seven *L. biglobosa*. The *L. maculans* isolates were characterized for avirulence genes (AvrLm) corresponding to resistance genes present in a differential set of host varieties or lines: *LepR3* and *Rlm1* to *Rlm10* (except for *Rlm8*). Due to the presence of confounding resistance genes in the differential set, *AvrLm1* and *AvrLm2* could not be determined in 9 of the isolates, *AvrLm6* in 2 and *AvrLepR3* in 49. *AvrLm1* was determined to be present in 46% of the isolates in this collection. The proportion of isolates carrying *AvrLm2* and *LepR3* was 97% and 98%, respectively, and all isolates carried *AvrLm6* and *AvrLm10*. The proportion of *L. maculans* isolates that carried *AvrLm3*, *AvrLm4*, AvrLm5, and *AvrLm7* was much lower and varied between 10% and 29%, and 60% of the isolates carried *AvrLm9*. Characterization of the isolates for these avirulence genes identified 16

races of *L. maculans*, with seven races accounting for 90% of the isolates. This Information will be useful for development of resistance management strategies to control blackleg disease in canola (*B. napus*) in western Canada.

Objective 2. A selection of open-pollinated, non-herbicide-tolerant *Brassica napus* L., *B. rapa* L. and *B. juncea* L. varieties or lines was evaluated for the presence of ten specific resistance genes to *L. maculans* (*Rlm1* to *Rlm10*). The specific resistance genes carried by each variety or line were deduced from reactions observed under controlled conditions on cotyledons of 14 plants challenged with a differential set of ten *L. maculans* isolates previously characterized for avirulence genes. Many varieties or lines (Cyclone, Dac1, Dac2, Dac3, Excel, Hyola 401, Hylite 201, Karat, Radikal, Sentry, Sibniik, Topas and Westar) did not appear to carry any of the specific resistance genes. *Rlm1* was detected in only a few varieties (71-20 CF from Monsanto, Surpass 400, Hyola 60, Hyola 440 and Quinta), and *Rlm2* was not detected, except in Glacier. *Rlm3* was the most commonly detected resistance gene in Canadian varieties (Conquest, Hi-Q, Quantum, Q2, Sprint, LBD449RR, SP Banner and possibly 45H72). This gene was also detected in the Australian variety AG-Castle and in Quinta and Glacier. A number of Australian varieties carried *Rlm4* (AV-Sapphire, Lantern and Rainbow) as well as the French cultivar Jet Neuf and the line Val1, but it was not detected in the Canadian varieties examined. The resistance genes *Rlm5, Rlm6, Rlm7, Rlm8* and *Rlm10* were not detected in any of the varieties. *Rlm9* was not detected in any of the varieties, but this may have been due to difficulties with the differential isolate used to detect this resistance gene. Reactions of the differential isolate on check varieties carrying *Rlm9* were not as expected and therefore conclusions could not be drawn for the presence of *Rlm9* in the varieties examined. Most importantly, specific resistance genes were not detected in many currently grown Canadian varieties, most of which are herbicide tolerant, hybrids. These varieties generally have a high resistance rating in the field evaluation, which suggests that resistance to *L. maculans* in these varieties may depend on quantitative resistance rather than specific gene resistance.

Determination of pathogenic variability of *Leptosphaeria maculans* and resistance in *Brassica* spp.

INTRODUCTION

Blackleg disease of canola, caused by the fungal pathogen *Leptosphaeria maculans* (Desmaz.) Ces. & De Not. is the most damaging disease of this crop worldwide (Howlett 2004, Fitt et al. 2006). The disease was a serious impediment to canola production throughout western Canada in the 1980s. In the late 1980s and early 1990s cultivars of canola with high levels of genetic resistance to the disease were introduced and cultural controls such as rotation were adopted by producers. These measures have reduced the yield and quality losses associated with the disease but have not eliminated the pathogen.

Recently the frequency of canola in the rotation has been increased by many producers and perhaps coincidentally changes in virulence (an indication of the potential differentiation of strains) of *L. maculans* has been reported (Keri et al. 2001, Chen and Fernando 2006, Kutcher et al. 2007). The changes in virulence were recognized through the use of the pathogenicity group (PG) system, which classifies isolates based on the specific interactions of *L. maculans* and the *B. napus* varieties 'Quinta' and 'Glacier' (Mengistu et al. 1991). The variety 'Quinta' was shown to carry the dominant resistance gene (R-gene), *Rlm1* and 'Glacier' to carry *Rlm2* and *Rlm3* (Ansan-Melayah et al. 1995, Ansan-Melayah et al. 1998, Balesdent et al. 2002). The variety 'Quinta' has been reported to be heterogeneous for *Rlm4* (Balesdent et al. 2001), although *Rlm3* has been detected in other seed lots (Kutcher et al., this study). *Rlm3* has been identified in the cv. Columbus, *Rlm4* from Jet Neuf, and *Rlm7* and *Rlm9* from *B. napus* accessions (Delourme et al. 2006). The genes *Rlm5* and *Rlm6* are found naturally in *B. juncea* and *Rlm6* has been introgressed into *B. napus* (Chèvre et al. 1997). *Rlm8* was identified in *B. rapa* and *Rlm10* from *B. nigra* (Chèvre et al. 1996) and both were introgressed into *B. napus*. Another four R-genes designated *LepR1* to *LepR4* have reported (Rimmer 2007) to bring the total to 14. Were all 14 R-genes in use in commercial cultivars, the number of possible races of the pathogen would be 2^{14} or over 16,000.

The specific R-genes on which current canola varieties depend could be overcome if new strains of the pathogen develop. Therefore understanding the genetic control of the host-pathogen interaction between *L. maculans* and *B. napus* is necessary to develop integrated management strategies and durable resistance to the disease. The objectives of this project are to characterize *L. maculans* isolates from the Canadian prairies against differential genotypes of *Brassica* spp. to identify avirulence genes (Avr-genes) carried by each isolate, and to characterize a selection of canola cultivars for R-genes using a differential set of *L. maculans* isolates carrying known Avr-genes. This research was conducted in collaboration with scientists at the Institut National de la Recherche Agronomique (INRA), France.

MATERIALS & METHODS

1) Characterization of Canadian *L. maculans* isolates for Avr-genes

This part of the project was conducted at the Institut National de la Recherche Agronomique, Rennes, France, except for the initial fungal isolations and single sporing of isolates.

Fungal material

One hundred and three isolates of *L. maculans* and *L. biglobosa* were isolated from mature canola plants collected at random from farmers' fields in western Canada between 1997 and 2005. *Leptosphaeria* spp. were isolated from canola residues on V8-juice (Campbell Soup Company Ltd, Toronto, Canada) agar to which 1% streptomycin sulphate was added to restrict bacterial growth. Test cultures were prepared from single pycnidiospores.

Inoculum preparation

Isolates derived from single pycnidiospores were cultured on V8-juice agar plates under black light at 20°C. After 10 days, sporulating cultures were flooded with 5 mL of sterile distilled water and the surface of the plates scraped gently with a flamed glass rod to dislodge pycnidiospores (Figure 1).

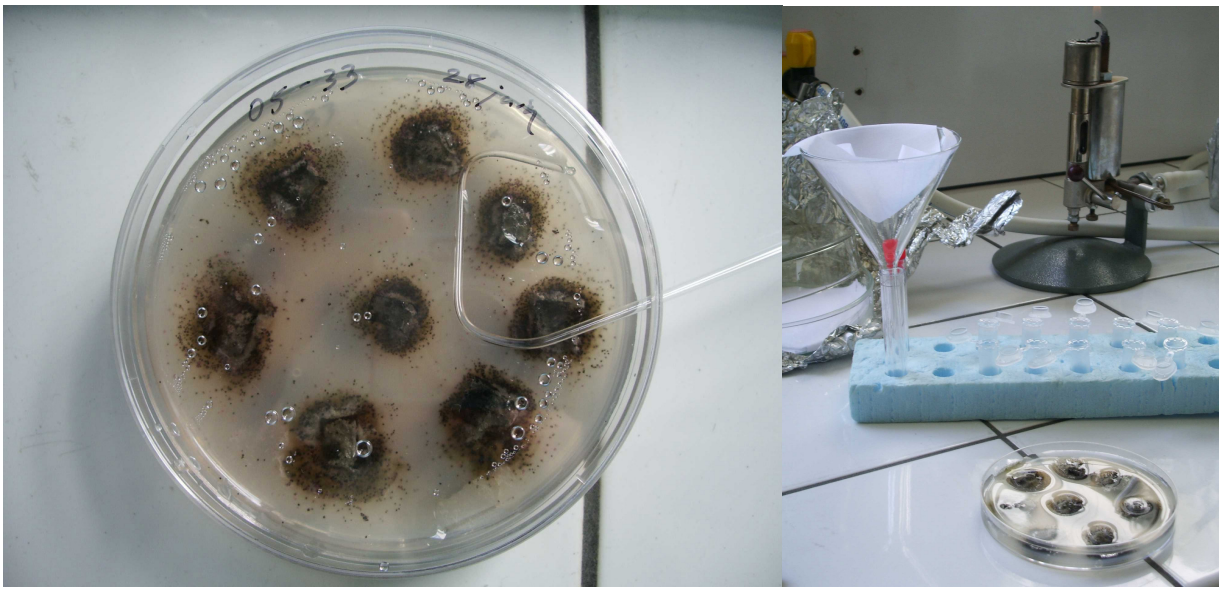

Figure 1. Inoculum production of an isolate of *Leptosphaeria spp*.

The pycnidiospore mixture was filtered through sterile 'Cheese cloth' (Fisher Scientific Canada, Edmonton, Canada) into sterile 5 mL tubes, and the concentration of the spore suspension determined using a haemocytometer. To prepare spore inoculum for host plant inoculation, the frozen concentrated spore solution was thawed on ice or in the refrigerator and aliquots added to sterile distilled water to arrive at a concentration of 1×10^7 pycnidiospores mL^{-1}.

Host material

A differential set of lines carrying previously characterized R-genes in *B. napus* or *B. juncea* genetic backgrounds were chosen for the study: Westar (no R-genes), MT29 (*Rlm1,9*), Samouraï (*Rlm 2,9*), 22-1-1 (*Rlm 3*), Falcon (*Rlm 4*), 150-2-1, (*Rlm 5*), 23-2-1 (*Rlm 7*), Darmor (*Rlm 9*), Westar 74 (*Rlm 10*) and Surpass 400 (*LepR3*). To determine the presence of *AvrLm6* isolates were tested on Falcon MX (*Rlm4,6*) and or Darmor MX (*Rlm6,9*). Use of these two genotypes detected the presence of *AvrLm6* for isolates that did not carry either *AvrLm4* or *AvrLm9*. Since MT29 and Samouraï each contain *Rlm9* as well as *Rlm1* and *Rlm2*, respectively, these lines could not be used to discriminate the presence of *AvrLm1* and *AvrLm2* when the isolates carried *AvrLm9*. In these cases the isolates were tested again using *B. napus* varieties characterized for *Rlm1* and *Rlm2* but which also contained other R-genes: Cooper (*Rlm1,4*), Grizzly (*Rlm1,3*), Glacier (*Rlm2,3*) and Verona (*Rlm2,4*). These additional differentials allowed discrimination of *AvrLm1* and *AvrLm2* in isolates that carried *Avrlm9* but not *AvrLm3* and or *AvrLm4*.

Isolates were evaluated on 2 plants of each *Brassica* genotype in 2 replicates for a total of 4 plants, each with 4 inoculation sites. Seeds of each genotype were pre-germinated on moistened filter paper for 2 days and then seeded into flats containing soil and incubated in the greenhouse for 10 days. Flats were then placed in the growth chamber. Leaves other than the cotyledons were removed. All plants were watered with tap water as required and kept at 22/16°C day/night temperature and a 14 h photoperiod.

Cotyledon inoculation and evaluation

Cotyledons of the differential lines were inoculated with pycnidiospore suspensions of the different *Leptosphaeria spp.* isolates, 13 days after beginning the pre-germination of seed. Both lobes of each cotyledon received a 1 mm diameter wound using a modified tweezers (Figure 2a) and a ten µL droplet of inoculum placed onto each wound (Figure 2b). The inoculated plants were returned to the growth chamber and flats covered with a plastic top and a plastic sheet to retain humidity for 24 hours in the dark (Figure 2c). Emerging leaves were removed from all seedling plants weekly to ensure that the cotyledons remained green. Both cotyledons of each plant, for a total of 4 lesions

on each plant, were evaluated for interaction phenotypes (IP) 14 and 21 days after inoculation on a scale of 0 to 11 according to lesion size, tissue collapse and necrosis or chlorosis (Figure 3). Isolate reactions on each cotyledon were classified as avirulent (Avr) or virulent (avr). The classification was based on the mean score with consideration of the standard deviation: ≤ 5.0 (resistant) or > 5.0 at 14 days, and or ≤ 7.0 (resistant) or > 7.0 at 21 days. For categories of 7 to 11 consideration was given to the appearance of the lesion (symptom of susceptibility or resistance) when determining the virulence classification.

Figure 2. a) modified tweezers used to wound cotyledons, b) inoculated plants prior to return to the growth chamber, and c) flats covered with plastic tops and a plastic sheet for 24 hours.

2) Characterization of Canadian canola cultivars for R-genes

Most of this part of the trial was conducted at Agriculture and Agri-Food Canada, Melfort, SK, but in addition a selection of non-genetically modified varieties was tested at Rennes.

Inoculum preparation and cotyledon evaluation

Inoculum of ten isolates of *Leptosphaeria maculans*, characterized for Avr-genes (Table 1), was prepared following the same protocol as described above. This differential collection of isolates allowed discrimination of R-genes *Rlm1* to *Rlm10* in the Brassica varieties or lines examined. Disease assessment was made on the 0-11 rating scale for the test conducted at Rennes (Figure 3) as explained above, and using the 0-9 scale for all tests conducted at Melfort (Figure 4). For the latter rating scale, a score ≥ 5.0 was accepted as a virulent reaction in the analysis. This indicated that there were no R-genes in the differential variety, which corresponded to the Avr-genes carried by the pathogen isolate.

Host material

Varieties or lines of canola that have been characterized are listed in Table 3. Seed germination and plant production was conducted as described in Part 1. Fourteen plants of each variety were tested in 2 replicates by inoculating each lobe of one cotyledon on each plant with each isolate. Therefore a total of 28 lesions were scored for each isolate-variety combination. A selection of non-genetically modified varieties was examined at Rennes and all varieties or lines in Table 3 were assessed at least once at Melfort. From the results of the initial testing at Melfort, varieties that appeared to carry a specific R-gene(s) were retested twice (Re-test A and Re-test B) and for some varieties, certain isolate-variety combinations were tested a third time (Re-test C).

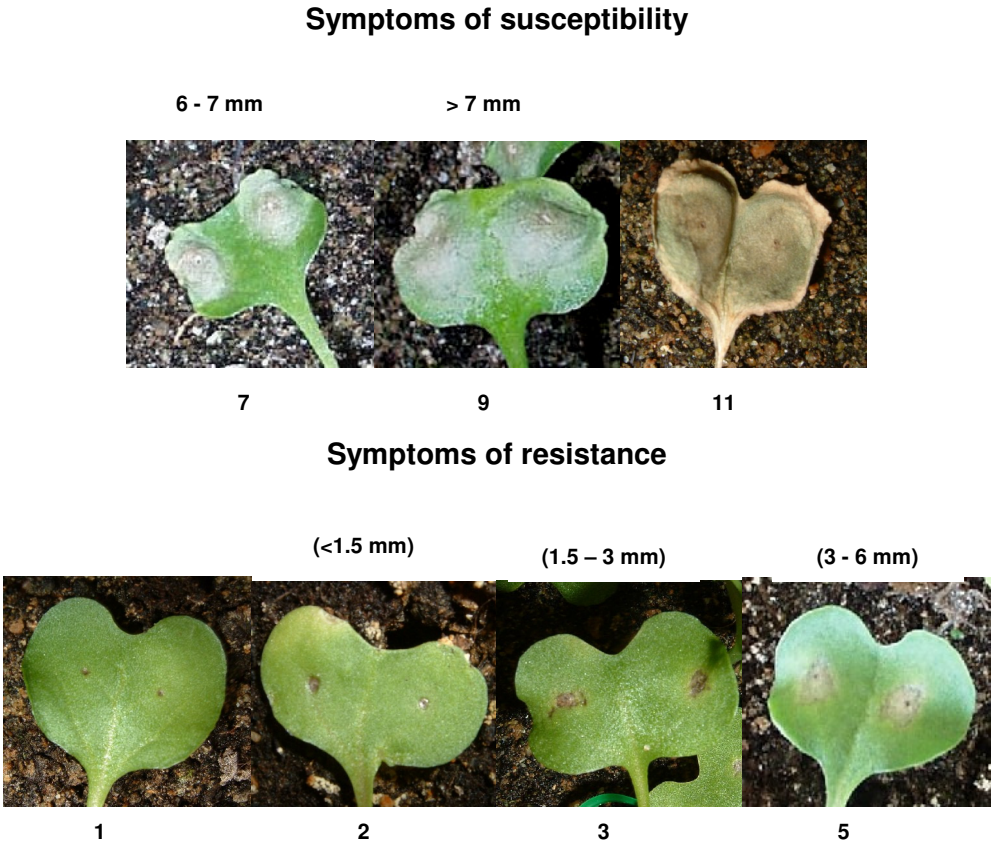

Figure 3. Cotyledon evaluation scale (0 - no symptoms to 11) in evaluation of symptoms resulting from inoculation of *Leptosphaeria maculans* isolates on *Brassica* cotyledons for characterization of pathogen isolates. Photos courtesy of H. Brun.

1 3 5 7 9

Figure 4. Cotyledon evaluation scale (0 - no symptoms to 9) in evaluation of symptoms resulting from inoculation of *Leptosphaeria maculans* isolates on *Brassica* cotyledons for evaluation of varieties or genotypes of *Brassica* spp., modified from Newman (1981).

RESULTS

1) Characterization of Canadian *L. maculans* isolates for Avr-genes

One hundred and three *Leptosphaeria spp*. isolates were evaluated. Seven isolates were determined to be *L. biglobosa*, as identified by the particular symptoms caused on the differential lines, although the isolates could not be differentiated from each other by the lines. The symptoms resulting from the inoculation were similar among canola varieties, and consisted of small, necrotic flecks resulting in a mottled appearance (Figure 5).

Figure 5. Symptoms of isolate 99-29 (*Leptosphaeria biglobosa*) on *Brassica napus* cotyledons at 14 (left) and 21 days (right).

Ninety-six isolates were identified as *L. maculans* and each isolate was characterized for Avr-genes by scoring the symptoms for each isolate-variety combination as in Figure 6.

Figure 6. Variation in the reaction of isolate 05-08 among differential Brassica varieties or lines.

The Avr-genes, *AvrLm1* and *AvrLm2* could not be determined for nine of the isolates due to the presence of other R-genes in the host differentials. The *AvrLm6* allele could not be determined in two isolates, and because Surpass 400 is believed to carry *Rlm1* as well as *LepR3*, *AvrLepR3* could not be determined in 49 isolates. Of the 87 isolates for which *AvrLm1* and *AvrLm2* could be determined, 16 races of the pathogen were identified based on the Avr-genes each isolate carried (Table 2). The fact that only 16 races were detected indicates that variation on many R-genes is low. Ninety percent of the isolates belonged to one of seven races, each of which was composed of between three and 20 isolates. The remaining nine races were represented by single isolates. Three races, Av-1-2-6-(8)-9-10-(LepR3), Av1-2-6-(8)-10-(LepR3) and Av2-6-(8)-9-10-(LepR3) represented 52 isolates (60%) and differed from each other only due to variation for *AvrLm1* or *AvrLm9*. Following the nomenclature of Balesdent et al. (2005), the avirulence alleles in parentheses were not or could not be determined in these races. One race, represented by a single isolate, carried the maximum number of avirulence alleles possible in this study, i.e. avirulence alleles corresponding to eight and possibly all ten R-genes. The races with the least number of avirulence alleles: Av1-2-6-(8)-10-(LepR3) and Av-2-6-(8)-10-LepR3, included 13 and three isolates, respectively. The other 14 races carried between five and seven avirulence alleles.

All isolates carried *AvrLm10* (96 isolates characterized) and *AvrLm6* (94 isolates characterized), 97% of the isolates carried *AvrLm2* (87 isolates characterized) and 98% (49 isolates characterized) carried *AvrLepR3* (Figure 7). Other Avr alleles were carried by many

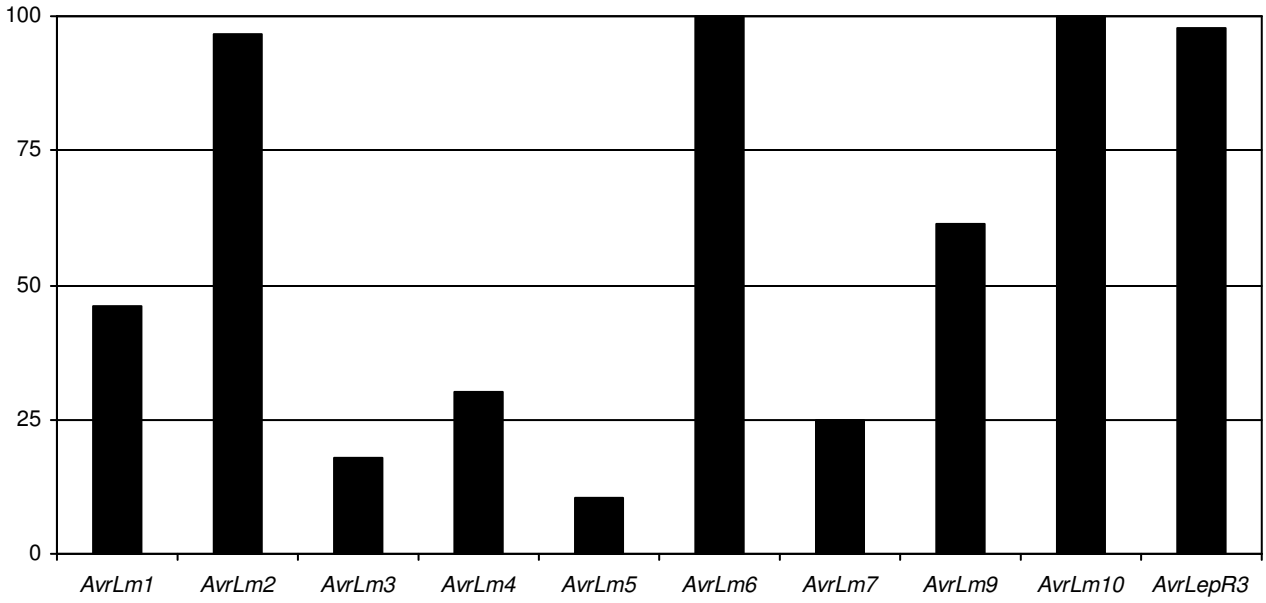

Figure 7. Percentage of *Leptosphaeria maculans* isolates carrying each Avr-gene. Data represent 47 isolates for *AvrLepR3*, 87 isolates for *AvrLm1* and *AvrLm2*, 94 isolates for *AvrLm6* and 96 isolates for all other avirulence alleles. *AvrLm8* was not assessed.

fewer isolates: *AvrLm3* – 18%, *AvrLm4* – 29%, *AvrLm5* – 10%, and *AvrLm7* – 25% (each characterized for all 96 isolates). *AvrLm1* was carried by 46% of the 87 isolates characterized and *AvrLm9* by 60% of the 96 isolates characterized. The fact that all isolates carried *AvrLm6* and *AvrLm10* means that the corresponding R-genes, *Rlm6* and *Rlm10*, effectively conditioned resistance against all the isolates that could be characterized in this collection. Both of these specific R-genes have been introgressed into *B. napus* from other *Brassica* spp., *Rlm6* from *B. juncea* (Chèvre et al. 1997) and *Rlm10* from *B. nigra* (Chèvre et al. 1996; Chèvre, personal communication). Therefore the fact that all isolates were avirulent on these R-genes is not surprising since these genes have been used only experimentally and are not believed to be present in commercial varieties of canola. However, in France under experimental field conditions, Brun et al. (2000) have shown that resistance conditioned by *Rlm6* in a susceptible spring-type *B. napus* background broke down over a three year period of cultivation. In addition, a survey of *L. maculans* races in France indicated that virulent alleles (*avrLm6*) were present in the pathogen

population, although at a very low level (1 of 1787 isolates analysed), even though this source of resistance has not been exploited commercially in France (Balesdent et al. 2006). In Australia, resistance to *L. maculans* has been introgressed from R-genes derived from *B. rapa* subsp. *sylvestris* into *B. napus* oilseed rape varieties. The variety Surpass 400, which carries *LepR3* (Yu et al. 2008), was overcome within three years of introduction in some regions of Australia (Li et al. 2003; Sprague et al. 2006).

The avirulence allele *AvrLm2* was present in all but three of the 96 isolates examined and *AvrLepR3* was present in all isolates but one (Figure 7). This is somewhat surprising because the corresponding R-genes (*Rlm2* and *LepR3*) have been available to Canadian plant breeders for some time and therefore might be expected to be carried by some current Canadian *B. napus* varieties. If this were the case a greater degree of adaptation of the pathogen to these genes would have been expected. Contrary to the results of this study, current European populations of *L. maculans* do not carry *AvrLm2* as concluded from recent surveys (Balesdent et al. 2006; Stachowiak et al. 2006) and as mentioned above, in a number of regions in Australia, *L. maculans* has been documented to overcome *LepR3*, indicating that a high proportion of the pathogen population of these regions must carry *AvrLepR3*.

The *AvrLm1* allele was detected in 46% of the isolates characterized (Figure 7), indicating that the pathogen population has adapted to *Rlm1* and suggesting that this R-gene may be present in some Canadian *B. napus* varieties. Rouxel et al. (2003) reported that *Rlm1* was common in French varieties of oilseed rape in the 1990s. However, the resistance imparted by this gene was overcome within three years in the late 1990's due to selection pressure on the pathogen population by the extensive use of the gene in widely grown varieties in France.

The avirulence alleles *AvrLm3* and *AvrLm4* were observed at relatively low frequency in this study, 18 and 29%, respectively (Figure 7). This suggests the pathogen population has adapted to the corresponding R-gene, *Rlm3*, rendering it relatively ineffective. The R-gene, *Rlm3* was present in a number of Canadian varieties and *Rlm4* in some recent Australian varieties (Kutcher et al. 2008). Although not to the same degree, the proportions of *AvrLm3* and *AvrLm4* in these isolates characterized from western Canada show a similar trend to that observed in the pathogen population in France in 2000-2001 (Balesdent et al. 2006), where *AvrLm3* was reported at extremely low levels (3 of 1787 isolates) and in other oilseed rape growing countries of northern Europe, where *AvrLm3* was not observed in 2002 and 2003 (Stachowiak et al. 2006). In the French and European studies, *AvrLm4* was also reported to be in relatively low proportions: 9% in France and 2% in Europe as a whole.

The observation that only a minor proportion of the isolates carried *AvrLm5* (10%) and *AvrLm7* (26%) is surprising since the corresponding R-genes are not believed to be in use in Canadian canola varieties. The *Rlm5* gene is found in *B. juncea* and is not likely present in any *B. napus* lines. However, it is possible that virulent isolates of *L. maculans* were selected through the production of *B. juncea* condiment mustard crops or canola-quality *B. juncea* even though blackleg is generally not a problem for mustard growers and the production of canola-quality *B. juncea* is recent and very limited. The low proportion of isolates carrying *AvrLm7* is curious since there is no evidence that *Rlm7* is present in either older or current Canadian *B. napus* varieties. The proportions of both *AvrLm5* (~85%) and *AvrLm7* (>99%), are very high in both the French and European reports (Balesdent et al. 2006; Stachowiak et al. 2006). At present, the *Rlm7* gene is very effective in France and carried by a number of French oilseed rape varieties.

In this study *AvrLm9* was detected in 60% of the isolates (Figure 7), indicating that *Rlm9* may be beneficial as a source of resistance in Canadian canola varieties, depending on how and where this resistance is employed. European populations of *L. maculans* have been reported to lack *AvrLm9* (Balesdent et al. 2006; Stachowiak et al. 2006). *Rlm9* is common in European winter-type oilseed rape varieties and selection pressure to overcome this R-gene has been proposed as the reason why *AvrLm9* is not present in the European pathogen population (Balesdent et al. 2005).

Generally, the results of this study agree with those observed by Balesdent et al. (2005), who found the absence of *AvrLm7* (and *AvrLm8*), but the presence of *AvrLm1*, *AvrLm2*, *AvrLm3* and *AvrLm9* in isolates from Saskatchewan. They concluded that due to the relatively low frequency of *AvrLm7*, the corresponding R-gene (*Rlm7*), may be of limited value to combat blackleg in western Canada, even though it appears to be a very effective source of resistance in Europe. On the other hand, *AvrLm2* was found at a high level in the pathogen population of this study. This indicates that the corresponding R-gene (*Rlm2)*, could be a useful source of resistance for western Canadian canola varieties, even though it is not effective for control of blackleg in Europe. *AvrLm1* and *AvrLm9* were found at moderate frequencies in the pathogen population in this study, suggesting that *Rlm1* and *Rlm9* may still have some value to control the pathogen in western Canada, unlike in Europe where *AvrLm1* is at a low level and *AvrLm9* is not present. In western Canada, *AvrLm3* is found at a low frequency in the pathogen population, suggesting that *Rlm3* may be of limited use as is the case in Europe where *AvrLm3* is almost non-existent. Differences in frequencies of avirulence alleles observed between this study concerning spring type varieties and studies from Europe, where winter-type varieties are mainly cultivated, may be due to differences in the R-genes used between the continents and to the length of time the various R-

genes have been carried by *B. napus* varieties. The study indicates that some R-genes, including some not likely to be carried by Canadian canola varieties, are unlikely to be effective against the population of *L. maculans* in western Canada. However, other R-genes that do not appear to be present in current Canadian canola cultivars, may be useful sources of resistance.

2) Characterization of Canadian canola cultivars for R-genes

Evaluation of *Brassica napus, B. rapa* and B. juncea varieties and lines for specific R-genes are summarized in Table 3. For many of the varieties evaluated there was considerable variability in the results as measured by the standard deviation of the scores and differences among tests in the detection of specific R-genes in some varieties. This variation was likely due to a combination of factors. Firstly, some of the varieties are believed to be somewhat genetically heterogeneous, such as the Australian cultivars. Second, some of the hybrid varieties tested were produced a number of years ago, and the proportion of actual hybrid seed may have been significantly less than 100%. Third, the protocol used at Melfort was modified from that used in France, where similar work has been conducted. This may have contributed to the variability observed, particularly in the first test of all varieties conducted at Melfort, although even at Rennes, variability for some of the R-genes was observed. A resistant reaction on the variety Darmor was not observed when inoculated with isolate PHW1223 as was expected. A resistant reaction indicates the presence of *Rlm9* in Darmor and *AvrLm9* in the isolate. Therefore conclusions could not be drawn regarding the presence of *Rlm9* in the varieties.

Many of the *B. napus* varieties or lines tested did not carry any of the R-genes for which they were examined. This is not surprising for the older, open-pollinated varieties, many of which were developed before breeding for resistance to blackleg was a high priority. As examples, Westar and Hyola 401, the first hybrid variety released in the mid-1990s, were popular with growers for many years but known to be very susceptible to blackleg. Other varieties or lines that did not appear to carry R-genes in this study were: Radikal and Sibniik, landraces from Siberia; Topas developed by Svalof A. B., Sweden in the 1980s; and Karat, a European spring type variety. The Dac1, Dac2 and Dac3 lines are believed to be from China but do not appear to carry any of the R-genes tested. Other Canadian varieties released in the late 1990s, such as Cyclone, Excel and Sentry, were suggested to have improved blackleg resistance over Westar at the time of their release, but did not appear to carry any of the R-genes examined in this study.

The absence of specific genes for resistance was surprising in the more recently registered cultivars, most of which were herbicide tolerant, hybrids (Table 3). Specific R-genes were not detected in most of the cultivars from the companies DSV, Viterra, Bayer and Pioneer. The exceptions were LBD449RR (DSV) and SP Banner (Viterra), in which *Rlm3* was consistently detected. The data indicates this gene may also be present in 45H72 (Pioneer), although the results were not consistent. The cultivar 71-20CL (Monsanto) appears to carry the *Rlm1* R-gene. The fact that most of these cultivars were rated by the Western Canadian Canola and Rapeseed Recommending Committee as carrying a high (R) or at least moderate (MR) level of resistance to blackleg, and yet carry few or more often no specific R-genes, suggests these varieties may depend on quantitative or polygenic resistance to *L. maculans*.

The presense of *Rlm1* was suggested in the Australian cultivar Surpass 400 and others believed to carry resistance introgressed from *B. napus* subsp. *sylvestris* (Hyola 60 and Hyola 440). There was some inconsistency as Surpass 400 appeared to be resistant to all the differential isolates used in the initial test at Melfort and all isolates but V23-2.1 in the test at Rennes. However, Surpass 400 was susceptible to most isolates except S7 and p27D (which detect *Rlm1*) in Re-tests A and B. As mentioned above, variety 71-20 CF (Monsanto) also appeared to carry *Rlm1*, as did Quinta. In this study, Quinta also appeared to carry *Rlm3*, which has not reported previously, although it has been suggested to carry *Rlm4* (Balesdent et al. 2001). Many researchers have maintained their own seed stocks of Quinta over the many years of its use in blackleg testing. Differences among seed lots are a distinct possibility. Given that analysis of the pathogen population detected *AvrLm1* in only 46% of the isolates, it is surprising that *Rlm1* was detected in only one of the Canadian cultivars (71-20 CF). It was expected that *Rlm1* would have been detected in a number of cultivars in order to exert sufficient selection pressure on the pathogen to result in the moderate proportion of isolates carrying *AvrLm1*.

None of the varieties characterized in this study were confirmed to carry *Rlm2*, except Glacier. If few or no cultivars grown in western Canada carry *Rlm2*, this may explain the small proportion of isolates that do not carry *AvrLm2*, as was found in Part 1 of this study. There would have been no selection pressure on the pathogen exerted by this R-gene if not carried by the varieties grown. The fact that some isolates were found that do not carry *AvrLm2*, and that many PG3 and PG4 isolates (by definition these pathotypes do not carry *AvrLm2*), have been collected, suggests there should have been selection pressure on the pathogen population to result in the detection of these pathotypes. Perhaps there are other cultivars grown in western Canada that carry *Rlm2*, but were not tested in this study.

A number of varieties or lines appeared to carry *Rlm3* and or *Rlm4*. Hi-Q, Quantum, Q2, Conquest and Sprint all had improved resistance to blackleg when released, compared to the check variety, Westar. The varieties Conquest, Hi-Q, Quantum and Q2 were developed from the same breeding program at the University of Alberta. It is believed material from France, by way of Australia, was used in this program. It had been assumed that the specific R-gene in this material was likely *Rlm4* derived originally from Jet Neuf. However, in this study the data is consistent that all these cultivars carry *Rlm3*.

The Australian cultivar, AG-Castle also appears to carry *Rlm3*. Two other Australian cultivars, Lantern and Rainbow appeared to carry *Rlm3* and *Rlm4* in the test conducted at Rennes. However, further testing at Melfort did not detect *Rlm3*, but only *Rlm4*. The Australian cultivar AV-Sapphire appears to carry only *Rlm4*, as did Jet Neuf, as expect, and the unregistered line Val1. Reactions of some isolates on Jet Neuf were intermediate or had a high degree of variability associated with the mean. Jet Neuf is known to carry a high level of quantitative resistance to *L. maculans*, which possibly influenced the reactions observed.

None of the *B. napus* cultivars appeared to carry R-genes other than *Rlm1*, *Rlm2*, *Rlm3* and *Rlm4*. The R-genes *Rlm5* and *Rlm6* originate from *B. juncea* and have been used only experimentally. Therefore it was not expected that any of the cultivars would carry these genes. *Rlm7* is used in recent cultivars in France, but was not observed to be carried by the Canadian cultivars examined in this study. *Rlm8* and *Rlm10* are genes characterized by French scientists, derived from *B. rapa* and *B. nigra*, respectively and have not been used commercially. As expected they were not detected in the cultivars examined in this study. It is possible that some of the cultivars examined may carry *Rlm9*. However, since the isolate used to detect *Rlm9* failed to do so under the conditions of the study at Melfort we cannot confirm this.

There were no specific R-genes detected in the four *B. rapa* cultivars characterized in this study. All three *B. juncea* cultivars examined were resistant to all differential isolates. The genes *Rlm5* and *Rlm6* have been reported to occur in *B. juncea*. However, the data suggests these varieties likely carry other unidentified R-genes because resistant reactions were obtained on these cultivars when inoculated with isolate V45-30, which does not carry *AvrLm5* or *AvrLm6*.

CONCLUSIONS

This study provides the first analysis of the Avr-gene frequency among isolates from the population of *L. maculans* present in western Canada. A limited number of races were detected among the 96 isolates of *L. maculans* characterized. The pathogen population, as represented by the isolates characterized, has within it individual isolates that can overcome most of the host R-genes tested. Exceptions are *Rlm6* and *Rlm10* for which all of the isolates that were characterized carried Avr-genes *AvrLm6* and *AvrLm10*. Other R-genes that were highly effective against the majority of isolates were *Rlm2* and *LepR3*. The proportion of the pathogen population that carried *AvrLm1*, *AvrLm3* and *AvrLm4* varied between 18 and 46%, indicating the corresponding R-genes have limited ability to resist infection by *L. maculans*. Unexpectedly, R-genes that are not known to be present in Canadian cultivars such as *Rlm5* and *Rlm7* were not effective against a majority of the *L. maculans* isolates characterized, and therefore likely have limited value as new sources of resistance.

It must be remembered that this research was based on a rather limited number of isolates collected from unidentified varieties of *B. napus*, consequently a more precise study is being undertaken to obtain isolates from a variety of *B. napus* known to have no specific R-genes to be sown at various locations to obtain an unbiased and extensive characterization of the western Canadian *L. maculans* population. Nevertheless, this study provides significant knowledge of avirulence allele variation of the pathogen population of *L. maculans* in western Canada and therefore provides some of the information needed to develop effective, durable blackleg resistance management strategies.

Analysis of canola cultivars detected the presence of only 4 specific R-genes (*Rlm1*, *Rlm2*, *Rlm3* and *Rlm4*). R-gene *Rlm1* was detected in Quinta, 71-20 CL (Monsanto), Surpass 400, Hyola 60 and Hyola 440. However, the results for Surpass 400 were variable among the tests conducted. *Rlm2* could only be detected consistently in Glacier. *Rlm3* was observed in a number of open-pollinated Canadian cultivars, most developed in the 1990s, and a few more recently released cultivars, some of which are herbicide tolerant. The Australian cultivar AG-Castle also appeared to carry *Rlm3*. In the other Australian cultivars: AV-Sapphire, Lantern and Rainbow, *Rlm4* was detected. Specific R-genes were not detected in most of the most recent cultivars, which tend to be herbicide-tolerant and often hybrids, despite high resistance ratings from adult plant field evaluation. This suggests these cultivars may derive resistance to *L. maculans* from quantitative sources.

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Table 1. Isolates of *Leptosphaeria maculans* used to characterize varieties of canola for resistance genes. Avirulent (Avr) reactions are indicated by a resistance phenotype and virulent (avr) by a susceptible phenotype for each isolate and variety interaction.

Isolate identification	Avr-genes	Expected reaction on canola varieties or lines carrying R-genes									
		Rlm1	*Rlm2*	*Rlm3*	*Rlm4*	*Rlm5*	*Rlm6*	*Rlm7*	*Rlm8*	*Rlm9*	*Rlm10*
290	5, 6, 7, 8, 10	*avr*	*avr*	*avr*	*avr*	***Avr***	***Avr***	***Avr***	***Avr***	*avr*	***Avr***
P27D	1, 5, 6, 7, (8)*, 10	***Avr***	*avr*	*avr*	*avr*	***Avr***	***Avr***	***Avr***	-	*avr*	***Avr***
V45-30	2, 7, (10)	*avr*	***Avr***	*avr*	*avr*	*avr*	*avr*	***Avr***	*avr*	*avr*	-
19.4.24	3, 5, 6, 8, (10)	*avr*	*avr*	***Avr***	*avr*	***Avr***	***Avr***	*avr*	***Avr***	*avr*	-
V23-2.1	4, 5, 6, 7, 8, (10)	*avr*	*avr*	*avr*	***Avr***	***Avr***	***Avr***	***Avr***	***Avr***	*avr*	-
NZ-T4	5, 6, 8, (10)	*avr*	*avr*	*avr*	*avr*	***Avr***	***Avr***	*avr*	***Avr***	*avr*	-
R2[†]	5, 7, (8), 10	*avr*	*avr*	*avr*	*avr*	***Avr***	*avr*	***Avr***	-	*avr*	***Avr***
PHW1223	5, 6, 8, 9	*avr*	*avr*	*avr*	*avr*	***Avr***	***Avr***	*avr*	***Avr***	***Avr***	-
IBCN 14	5, 6	*avr*	*avr*	*avr*	*avr*	***Avr***	***Avr***	*avr*	*avr*	*avr*	***Avr***
S7[‡]	1, 5, 6, 7, (8)	***Avr***	*avr*	*avr*	*avr*	***Avr***	***Avr***	***Avr***	-	*avr*	-

* Avr-genes in brackets have not been determined in these isolates. [†] Somda et al. 1999, [‡]Brun et al. 2001.

Table 2. Race identification of 87 isolates of *Leptosphaeria maculans* collected in western Canada between 1997 and 2005.

Race designation[1]	Number of isolates of each race	Frequency (%)	Number of avirulence alleles
Av1-2-3-4-5-(6)-7-(8)-9-10-(LepR3)[2]	1	1.2	8
Av1-2-4-5-(6)-7-(8)-9-10-(LepR3)	1	1.2	7
Av1-2-3-6-(8)-9-10-(LepR3)	1	1.2	6
Av1-2-4-6-7-(8)-10-(LepR3)	1	1.2	6
Av1-4-5-6-7-(8)-10-(LepR3)	1	1.2	6
Av2-3-6-(8)-9-10-LepR3	7	8.1	6
Av2-4-6-7-(8)-10-LepR3	13	14.9	6
Av1-2-6-7-(8)-10-(LepR3)	1	1.2	5
Av1-2-6-(8)-9-10-(LepR3)	20	23.0	5
Av1-4-6-7-(8)-10-(LepR3)	1	1.2	5
Av2-4-6-7-(8)-10	1	1.2	5
Av2-6-7-(8)-10- LepR3	3	3.5	5
Av2-6-(8)-9-10- LepR3	19	21.8	5
Av4-6-7-(8)-10- LepR3	1	1.2	5
Av1-2-6-(8)-10-(LepR3)	13	14.9	4
Av2-6-(8)-10- LepR3	3	3.5	4

[1] race nomenclature indicates the avirulence loci (Av) for which the isolate is avirulent (Balesdent et al. 2005), *AvrLm8* was not assessed.
[2] *AvrLepR3* could not be determined for isolates carrying *AvrLm1*, nor could *AvrLm6* be determined in isolates carrying both *AvrLm4* and *AvrLm9*.

Table 3. Results of the tests conducted to identify specific resistance genes carried by the *Brassica napus*, *B. rapa* and *B. juncea* varieties characterized in this study.

Variety	Company	Herb. Tol.*	Type‡	Blackleg Rating (WCCRRC)●	Putative R-genes - † signifies high variability and/or intermediate reaction score				
					Test at Rennes	Initial test at Melfort	Re-Test A	Re-Test B	Re-Test C
B. napus									
LBD449RR	DSV	RR	OP	R	-	*Rlm3*	*Rlm3*	none	*Rlm3*
LBD612RR	DSV	RR	OP	MR	-	none	-	-	
624RR	DSV	RR	Hyb	R	-	none	none	none	
SP 451 RR	Viterra	RR	Hyb	MR	-	none	-	-	
Desirable RR	Viterra	RR	Synth	R	-	none	-	-	
SP Banner	Viterra	RR	OP	R	-	*Rlm1*†, *2*†, *3*	*Rlm3*	*Rlm3*†	*Rlm3*
Invigor 5020	Bayer	LL	Hyb	R	-	*Rlm1*†, *2*†, *3*	none	none	
Invigor 5030	Bayer	LL	Hyb	R	-	none	-	-	
Invigor 5070	Bayer	LL	Hyb	R	-	none	-	-	
Invigor 2573	Bayer	LL	Hyb	R (VG)	-	none	-	-	
Invigor 2733	Bayer	LL	Hyb	MR - R	-	none	-	-	
Invigor 2663	Bayer	LL	Hyb	R (VG)	-	*Rlm2*, *3*	none	none	
71-20 CL	Monsanto	CF	Hyb	R	*Rlm1*, *2*, *3, 9*†	*Rlm1*, *2*	*Rlm1*	*Rlm1*, *Rlm2*	no *Rlm2*
71-25 RR	Monsanto	RR	Hyb	R	-	*Rlm3*†, *9*†	*Rlm2*	-	no *Rlm2*
71-45 RR	Monsanto	RR	Hyb	MR	-	*Rlm2*†	-	-	no *Rlm2*
34-55	Monsanto	RR	OP	MR - R	-	none	-	-	no *Rlm2*
32-75	Monsanto	RR	OP	R	-	*Rlm2*, *3*	-	-	no *Rlm2*

Table 3. continued

Variety	Company	Herb. Tol.*	Type‡	Blackleg Rating (WCCRRC)®	Putative R-genes - † signifies high variability and/or intermediate reaction score				
					Test at Rennes	Initial test at Melfort	Re-Test A	Re-Test B	Re-Test C
45H21	Pioneer	RR	Hyb	R	-	*Rlm2*†	-	-	no *Rlm2*
46H23	Pioneer	RR	Hyb	R	-	none	-	-	
45H72	Pioneer	CF	Hyb	R	*Rlm3*	*Rlm3*	none	none	*Rlm3*
45A55	Pioneer	RR	Hyb	R (VG)	-	none	-	-	
45A71	Pioneer	CF	OP	MS (F)	none	*Rlm9*†	-	-	
Cyclone		CON	OP	MR (G)	none	none	-	-	
Dac1		CON	OP	-	none	none	-	-	
Dac2		CON	OP	-	none	none	-	-	
Dac3		CON	OP	-	none	none	-	-	
Excel		CON	OP	MS (F)	none	none	-	-	
Hyola 401		CON	Hyb	-	none	-	-	-	
Hylite 201		CON	OP?	-	-	none	-	-	
Karat		CON	OP	-	none	-	-	-	
Radikal		CON	OP	-	none	none	-	-	
Sentry		CON	OP	-	none	none	-	-	
Sibniik		CON	OP	-	none	none	-	-	
Topas		CON	OP	-	none	*Rlm2*†	-	-	no *Rlm2*
Westar		CON	OP	-	none	none	none	none	
Surpass 400		CON	OP	-	R to all ex V23-2.1	R to all isolates	*Rlm1*	*Rlm1*	
Hyola 60 (4-6 plants/isolates due to poor seed)		CON	Hyb		-	-	*Rlm1*	-	
Hyola 440		CON	Hyb	-	-	*Rlm1*, *2*†	*Rlm1, 2*†	-	no *Rlm2*
Quinta		CON	OP	-	*Rlm1*, *3*	*Rlm1*, *3*†	-	-	

Table 3. continued

Variety	Company Tol.*	Herb.	Type‡	Blackleg Rating (WCCRRC)●	Putative R-genes - † signifies high variability and/or intermediate reaction score				
					Test at Rennes	Initial test at Melfort	Re-Test A	Re-Test B	Re-Test C
AG-Castle		CON	OP	-	*Rlm3*	*Rlm3*	-	-	
Conquest		RR	OP	R (VG)	-	*Rlm3*	-	-	*Rlm3*
Glacier		CON	OP	-	*Rlm2*, *3*	*Rlm2*, *3*, *9*†	-	-	
Hi-Q		CON	OP	R (VG)	*Rlm3*	*Rlm2*, *3*	*Rlm3*	-	*Rlm3*
Quantum		CON	OP	R (VG)	*Rlm3*	*Rlm3*	-	*Rlm3*†	*Rlm3*
Q2		CON	OP	R (VG)	-	-	-	-	*Rlm3*
Sprint		CON	OP	-	*Rlm3*	*Rlm3*†	-	-	*Rlm3*
AV-Sapphire		CON	OP	-	*Rlm1*†, *4*	*Rlm2*†	*Rlm4*	-	*Rlm4* no *2*
Jet Neuf		CON	OP	-	*Rlm1*, *2*, *4*, *9*†	*Rlm2*, *4*	-	-	*Rlm4*
Lantern		CON	OP	-	*Rlm3*, *4*	-	*Rlm4*	-	*Rlm4*
Rainbow		CON	OP	-	*Rlm3*, *4*	*Rlm2*†, *3*†, *9*	*Rlm4*	*Rlm4*	*Rlm4*
Val1		CON	OP	-	*Rlm4*	*Rlm4*	*Rlm4*†	*Rlm4*	*Rlm4*
B. rapa									
Hysyn 110		CON	Synth	-	-	none	-	-	
Reward		CON	OP	-	-	none	-	-	
Valleyview		CON	OP	-	-	none	-	-	
Yantarnaya		CON	OP	-	-	none	-	-	
B. juncea									
Estlin (canola quality)	Viterra	CON	OP	MR-R	R to all isolates	R to all isolates	-	-	
Dahinda (canola quality)	Viterra	CON	OP	-	-	R to all isolates	-	-	
Cutlass (mustard)		CON	OP	-	R to all isolates	R to all isolates	-	-	

*** -** RR – roundup ready, LL – liberty link, CF – Clearfield, CON – conventional herb

‡ - cultivar type: OP – open pollinated, Hyb – hybrid, Synth - synthetic

●- blackleg field rating at time of release based on evaluation by the WCCRRC (Western Canadian Canola and Rapeseed Recommending Committee): R – resistant, MR – moderately resistant, MS – moderately susceptible, S – susceptible (VG – very good, G – good, F – fair, P – susceptible, terms used previously, which correspond in general to R, MR, MS and S)

Technology Transfer Activities

Discussed oilseed rape (canola) blackleg management with the plant pathology group at Rothamsted Research Station, Harpenden, U.K. Met with Dr. B. Fitt, J. West, O. Latunde-Dada, Dr. J. Lucas (Head of Division), and other researchers and students with programs on diseases of oilseed rape, toured research plots, including the long-term rotation experiments (>150 years), April 11, 2007.

Interviewed by Kevin Hursh for two radio spots that played across Saskatchewan in on various radio stations (i.e. 650 CJOM) on intensive canola rotations to manage diseases and the genetic characterization of the fungus that causes blackleg disease, May, 2007.

Interviewed by Nathalie Blanc for article: ' Français et Canadiens s'allient contre le parasite du colza', in Sciences ouest magazine (France), mai, 2007, page18.

Invited talk at the Institut National de la Recherche Agronomique (INRA), Lutte contre le Phoma du colza au Canada, Rennes France, May 24, 2007.

Scott Field Day, July 11th, 2007 - spoke to ~250 farmers and industry representatives on changes in the pathogenicity of *Leptosphaeria maculans*.

Melfort Field Day, Jul 18th, 2007 - spoke to ~150 farmers and industry representatives on changes in the pathogenicity of *Leptosphaeria maculans*.

Interviewed for an article: ' Four-year crop rotation plan effective in reducing blackleg' in the Northeast SUN, Harvest 2007 AGRIculture supplement, August 10, 2007, page 9.

Interviewed by Donna Fleury for the article: 'Can canola rotations be shortened?' in Top Crop Manager magazine, November, 2007, pages 66-70.

Presentation at the Saskatchewan Phytopathological Society Annual Meeting. Detection of genes for resistance against *Leptosphaeria maculans* in *Brassica napus*. December 3, 2007.

Invited talk to the blackleg sub-committee of the Western Canadian Canola/Rapeseed Recommending Committee. Analysis of *Leptosphaeria maculans* isolates and detection of *Brassica* R-genes. December 10, 2007

Invited talk at the Agriculture and Agri-Food Canada, Saskatoon Research Centre, Seminar Series. Understanding genetic interactions between *Leptosphaeria maculans* and *Brassica* spp. January 23, 2008

Identification of races of *Leptosphaeria maculans* in western Canada, contributed presentation at the Soils and Crops Workshop, February 28 and 29, 2008, Saskatoon, SK

Invited talk on management of blackleg disease of canola at the Nisku, AB, Brandon, MB and Saskatoon, SK Canola Colleges, organized by the Canola Council of Canada, February 26, 28 and March 4, 2008.

Invited by the Saskatchewan Canola Growers to give a research update on blackleg research and disease management, Humboldt, SK March 20, 2008.

GX94 Radio Yorkton, April 2, 2008. Interview discussing the consequences of intensive canola production in rotation

CJWW Radio, Saskatoon, April 2, 2008. Interview discussing the consequences of intensive canola production in rotation.

Technical Bulletin "Agronomic Spotlight: Canola in Rotation' published by Monsanto for canola growers, May 14, 2008.

Rotate canola to maintain blackleg-tolerant varieties. Manitoba Co-operator, page 17, April 10th, 2008. Interviewed April 2, 2008.

Bayer CropScience e-newsletter sent to hundreds of growers that included information on canola rotations and blackleg risk. Spring, 2008

Melfort Field Day, Jul 23rd, 2008 - spoke to ~100 farmers and industry representatives on blackleg disease management.

Country Guide article 'Blackleg the sequel', article on managing blackleg disease of canola (rotations and genetics of resistance). Pages 38-39, June/July, 2008 issue. Phone interview May 13, 2008.

Technical Bulletin "Agronomic Spotlight: Blackleg in Canola' published by Monsanto for canola growers, August 29, 2008; reprinted in the Western Producer September 18, 2008, Page 17.

Top Crop Manager article: "Rotation, resistance and fungicides to manage diseases of canola and field pea", Pages 45-46, November, 2008 issue.

Managing blackleg, sclerotinia stem rot and (hopefully not) clubroot. Invited talk to Cargill agronomists and canola growers in Battleford, SK, February 10, 2009.

Scientific Publications from project or related to project:

Kutcher, H.R., M. Keri, D.L. McLaren and S.R. Rimmer. 2007. Pathogenic variation of *Leptosphaeria maculans* in western Canada. Can. J. Plant Pathol. 29: 388-393.

Kutcher, H.R., Brun, H., Rimmer, S.R., Balesdent, M.H., and Rouxel, T. 2007. Variability of *Leptosphaeria maculans* in western Canada based on avirulence genes. Annual Meeting of CPS-SCP (with Plant Canada 2007), Saskatoon, SK, Canada, June 10-14, 2007, pp. C1-1.

Kutcher,H.R., S.R. Rimmer, M.H. Balesdent, T. Rouxel and H. Brun. 2008. Detection of specific resistance genes against *Leptosphaeria maculans* in *Brassica napus.* Can. J. Plant Pathol. 30: 386 (abstract).

Kutcher, H.R., Rimmer, S.R., Balesdent, M.H., Rouxel, T. and Brun, H. 2008. Identification of *Leptosphaeria maculans* races in western Canada. Proceedings of the Soils and Crops Workshop, February 28-29, Saskatoon, SK [CD_ROM]

Kutcher,H.R., M.H. Balesdent, S.R. Rimmer, T. Rouxel A.M. Chèvre, R. Delourme and H. Brun. 2009. Frequency of avirulence genes among isolates of *Leptosphaeria maculans* in western Canada. Can. J. Plant Pathol. Submitted January, 2009.

Kutcher, H.R., Balesdent, M.H., Rimmer, S.R., Rouxel, T., Chevre, A.M., Delourme, R. and Brun, H. 2009. Identifying races of *Leptosphaeria maculans*, cause of blackleg disease of canola in western Canada. Proceedings of FarmTech 2009, Edmonton, AB, January 28-30.