A large-scale survey of races of *L. maculans* occurring on canola in western Canada

H.R. Kutcher, D. Cross, K. Nabetani and C.L. Kirkham

Agriculture and Agri-Food Canada,
Melfort Research Farm
Box 1240, Highway 6 South
Melfort, SK S0E 1A0

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Canola Agronomic Research Program
c/o Gail Hoskins
400-167 Lombard Avenue, Winnipeg, MB, R3B 0T6
hoskinsg@canola-council.org

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ABSTRACT

*Specific resistance genes are an effective means of disease control when the pathogen population is mainly avirulent on the cultivated varieties carrying the corresponding resistance gene. Host genetic resistance may be rapidly overcome however, with the development of new races of the pathogen. Genetic resistance has been used effectively to control blackleg of oilseed rape or canola (*Brassica napus *L.) in western Canada. This study determined the race structure of* Leptosphaeria maculans*, the species responsible for the most damaging symptoms of blackleg. The frequency of avirulence alleles at ten avirulence loci in over 800 isolates of the pathogen were determined from material collected from nine locations, three in each of Alberta, Saskatchewan and Manitoba. The frequency of avirulence alleles* AvrLm1*,* AvrLm3*,* AvrLm4*,* AvrLm7*,* AvrLm9*,* AvrLmLepR1*,* AvrLmLepR2 *and* AvrLmLepR3 *varied from 0 to 97% depending on the loci and the geographic location. At eight locations ≥ 80% of the isolates carried the avirulence allele* AvrLm2*, but only 34% did so at a ninth location. The avirulence allele at* AvrLm6 *was present in all isolates tested from eight locations, but only in 48% of isolates at the ninth location. Knowledge of avirulence allele frequency and the race structure of* L. maculans *in canola producing regions of western Canada will be crucial to develop strategies to maintain the efficacy of resistance genes.*

INTRODUCTION

Development of effective plant disease resistance management strategies depend on an understanding of the genetics of the interaction between the host and pathogen (Kutcher et al. 2010a). Blackleg disease of canola (*Brassica napus* L.), caused by *Leptosphaeria maculans* (Desmaz.) Ces. & de Not., has been effectively controlled through use of resistant canola varieties and rotation of the crop with several other crop species. However, variation

for virulence of *L. maculans* has been observed in western Canada (Chen and Fernando 2006, Kutcher et al. 2007, Kutcher et al. 2010b). The cause of this variation is likely due to the adaptation of the pathogen to varieties of canola with high levels of genetic resistance to the disease, as has been found in other countries (Rouxel et al. 2003, Sprague et al. 2006).

Resistance to the pathogen is described as being of two types: race-specific (qualitative) or race-nonspecific (quantitative), and at present most *B. napus* cultivars in Canada are classed as resistant to *L. maculans,* although the basis for this resistance is generally unknown (Rimmer 2006). Quantitative resistance is believed to be inherited polygenically and expressed at the adult plant stage. Race-specific resistance is controlled by Mendelian inherited resistance (R) genes and a resistant response occurs as a result of recognition by the plant of corresponding pathogen avirulence (Avr) gene products in a gene-for-gene manner (Flor 1942). In the *L. maculans* - *Brassica* interaction, race-specific resistance is usually effective at the leaf level, as early as the cotyledon stage.

Race-specific R genes were initially observed to be very effective in controlling blackleg of oilseed rape in Europe and Australia (Howlett 2004). However, populations of *L. maculans* were observed to change rapidly as a result of selection pressure arising from wide-spread production of varieties carrying specific R genes. For example, *Rlm1* in France (Rouxel et al. 2003) and *LepR3* in Australia (Sprague et al. 2006) both broke down after approximately 3 years of widespread use. The development of new pathogen races, coupled with more intensive production of canola, or perhaps as a result of intensive production, increases the risk that the current genetic resistance in canola may be overcome by the pathogen. In western Canada, characterization of isolates of *L. maculans* using the pathogenicity group (PG) system (Mengistu et al. 1991) indicated that the pathogenic variability of the isolate population changed over the last 15 years from predominately PG2 to include PG3, PGT and PG4. Since this system includes R genes: *Rlm1*, *Rlm2* and *Rlm3*, this indicates that canola genotypes depending solely on these genes will be susceptible to races of *L. maculans* carrying virulence genes that correspond to these R genes. However, there have been many other R genes identified that condition resistance to *L. maculans* in various *Brassica* spp. (Balesdent et al. 2005, Rimmer 2007).

Recently, isolates of *L. maculans* from western Canada have been classified by the avirulence genes they carry, which correspond to specific R genes in *B. napus* (Kutcher et al. 2010b). In that study a random collection of *L. maculans* from western Canada identified 16 races based on the analysis of 10 avirulence genes in the pathogen, with considerable

variation in the frequency of avirulence alleles among these 10 genes. The isolates examined were collected from unknown varieties of canola, many of which likely carried specific R genes. Collection of isolates from a variety of *B. napus* with no specific R genes will result in a sample unbiased by the presence of specific R genes of the canola variety. Collection of isolates at different locations will provide insight into the population structure of the pathogen, i.e. the races of the pathogen that exist and the relative proportions of each race at each collection site.

The specific objective of this project was to conduct a large-scale survey of isolates of *L. maculans* in western Canada to determine the pathogen's population structure. Knowledge of the structure of the pathogen population will provide the information needed to develop blackleg resistance management strategies for varieties dependent on specific R genes. These strategies include adhering to crop rotations long enough to allow breakdown of the previous crop residue that harbours the pathogen, and therefore the pathogen itself, rotation of varieties with different specific R genes, or combining race-specific resistance with race non-specific resistance. This work is important to inform canola breeders of the existence of races of the pathogen with the ability to overcome sources of resistance currently used in their breeding programs and provide lead time for breeders to incorporate new sources of resistance or at least strategically manage current sources of resistance.

MATERIALS & METHODS

1) Collection of infected material and preparation of *L. maculans* isolates

Canola (cv. Westar) leaves and stem bases infected with *L. maculans* were collected from nine sites across the prairies (Camrose, Vegreville and Vermillion, AB; Scott, Melfort, and Indian Head, SK; and Brandon, Carberry and Plum Coulee, MB) with the support of co-operators (Figure 1). Approximately 100 isolates of *L. maculans* were cultured from this material from each location except Vegreville where only 47 isolates were obtained.

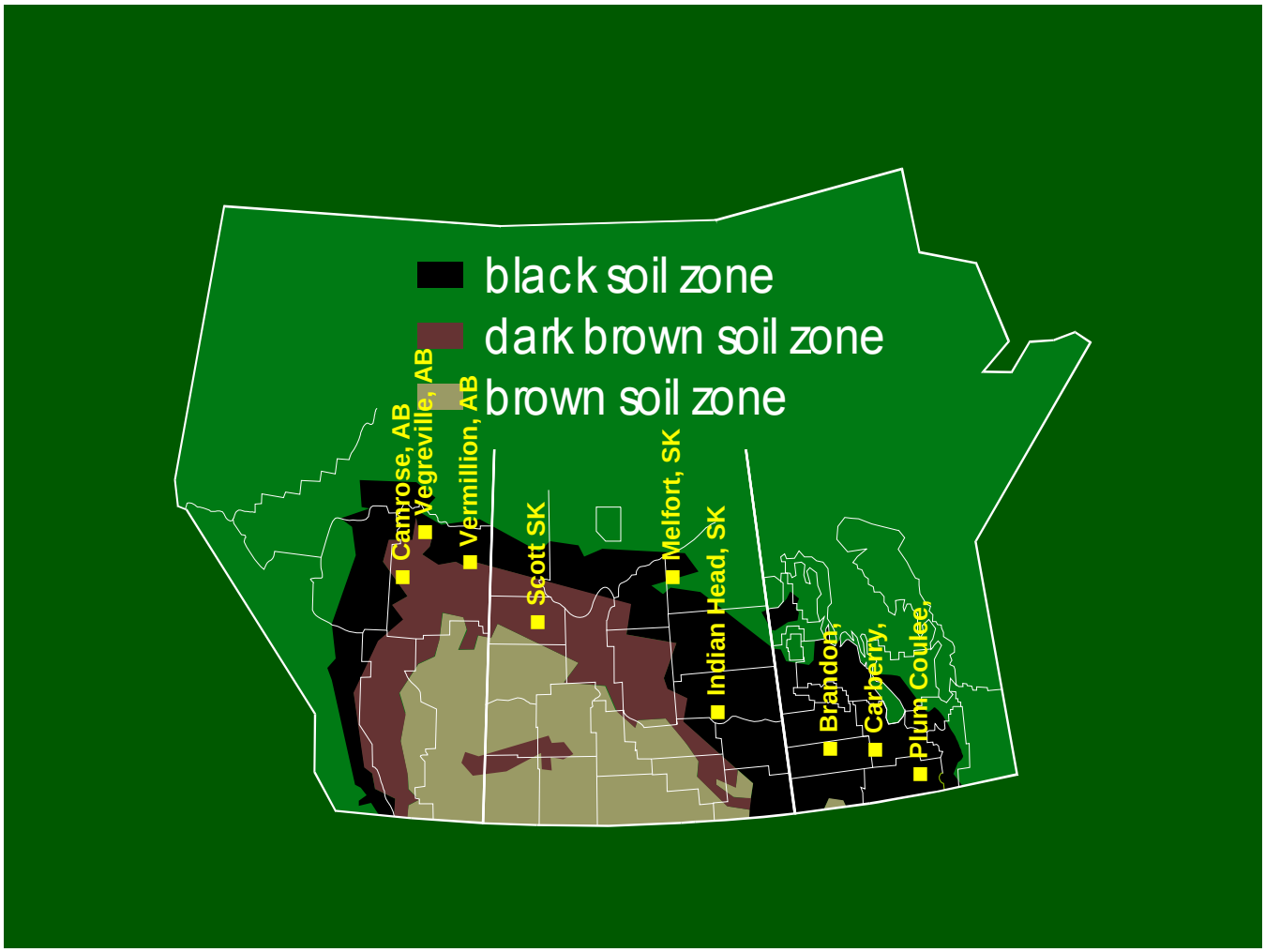

Figure 1. Locations in western Canada from which isolates of *L. maculans* were collected.

Infected plant material was plated on V8-juice (Campbell Soup Company Ltd, Toronto, Canada) agar to which 1% streptomycin sulphate and 40 mg l^{-1} Rose Bengal were added to restrict bacterial growth. From single pycnidia, a minute amount of exudate was re-plated on V8-juice agar and the culture grown for approximately 10 days at 20ºC under 12 hours of cool white fluorescent light. These plates were then harvested by flooding with 7 mL of sterile distilled water and the surface of the plates scraped gently with a stain-less steel rod to dislodge pycnidiospores. The pycnidiospore mixture was filtered through sterile 'Cheese cloth' or a 70 µm mesh cell strainer (Fisher Scientific Canada, Edmonton, Canada) into sterile 8 mL tubes. To prepare inoculum for host plant inoculation, the frozen concentrated spore solution was thawed on ice or in the refrigerator and the spore concentration was determined by counting spores in a 10% diluted solution with

haemocytometer. Based on the determined concentration, sterile distilled water was added to arrive at a concentration of 1 x 10^7 pycnidiospores mL^{-1}**.**

2) Host differentials and evaluation

Seed of varieties and lines of *Brassica* spp. were chosen to form a differential set. Seed of a number of these varieties, many of which are winter-types, were grown under controlled conditions for 4-6 weeks, vernalized for 6-8 weeks, and planted at a field site on the Melfort Research Farm to increase seed. The plants were grown individually or in groups of 2 or 3 under a 'cage' to prevent cross-pollination, thereby ensuring homozygous seed lots for each variety.

Varieties or lines of *Brassica* spp. used in the characterization of the isolates, and the R-genes carried by each are listed in Table 1. These varieties allow evaluation of the isolates for the presence of the avirulence genes (Avr-genes) corresponding to the R gene: *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4, Rlm6, Rlm7, Rlm9, LepR1, LepR2 and LepR3*. We used the *B. juncea* variety Cutlass to characterize the isolates, which may carry *Rlm5* and *Rlm6*.

3) Cotyledon inoculation and evaluation

Seeds of each variety were planted directly into soil, Sunshine Mix #3 (Sun Gro Horticulture Canada Ltd., Vancouver, Canada) in flats in the growth chamber. One hundred twenty seeds per flat were planted, and then the emerged plants thinned to 64 per flat for inoculation. Leaves other than the cotyledons were removed weekly after inoculation in order to keep the cotyledons from senescing. All plants were watered with 7 L of tap water per 3 flats as required and kept at 22/16°C day/night temperature and a 16 h photoperiod. Nutrients (20-20-20 fertilizer, 4 grams per liter, 4 liters per flat of 64 plants) were added at planting.

Isolates were evaluated on 2 plants of each variety in 2 replicates (4 plants in total). Each plant was inoculated at 4 sites (2 on each cotyledon) for a total of 16 inoculation sites. The cotyledons of the differential lines were inoculated with the pycnidiospore suspensions of the *L. maculans* isolates 7 days after seeding. Both lobes of each cotyledon received a 0.5 mm diameter wound using a modified tweezers (Figure 2a) and a 10 µL droplet of inoculum placed onto each wound (Figure 2b). Plants were evaluated for symptom

development and appearance at 14 days after inoculation using a scale that considers lesion size, tissue collapse, necrosis or chlorosis and pycnidia formation (Figure 3, Table 2). The average score for each isolate-host genotype interaction was classified as avirulent (score = 0 – 4.9, Avr) or virulent (score = 5.0 – 9.0, avr).

Table 1. Varieties or lines of *Brassica napus* and *B. juncea* and their R genes used to evaluate isolates of *L. maculans* for the presence of avirulence genes.

Variety or line of *Brassica* spp.	R-gene(s)	Source of R gene
Westar	none	
MT29	*Rlm1* and *Rlm9*	*B. napus*
Quinta	*Rlm1* and *Rlm3*	*B. napus*
Cooper	*Rlm1* and *Rlm4*	*B. napus*
Samouraï	*Rlm2* and *Rlm9*	*B. napus*
Glacier	*Rlm2* and *Rlm3*	*B. napus*
Verona	*Rlm2* and *Rlm4*	*B. napus*
Quantum	*Rlm3*	*B. napus*
Falcon	*Rlm4*	*B. napus*
Falcon MX	*Rlm4* and *Rlm6*	*B. napus,B.juncea*
Line 01-23-02	*Rlm7*	*B. napus,B.juncea*
Darmor	*Rlm9*	*B. napus*
Darmor MX	*Rlm9 and Rlm6*	*B. napus, B.juncea*
1065	*LepR1*	*B. rapa* subsp. *sylvestris*
1135	*LepR2*	*B. rapa* subsp. *sylvestris*
Surpass 400	*LepR3 and Rlm1*	*B. rapa* subsp. *sylvestris*
Cutlass	*Rlm5 and Rlm6?*	*B. juncea*

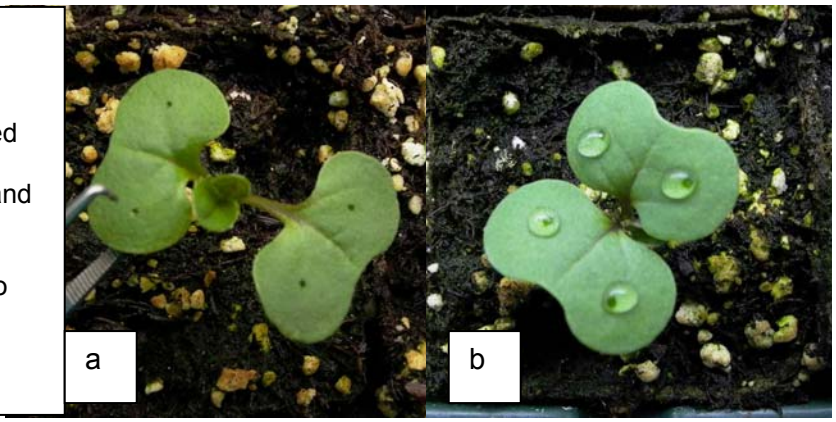

Figure 2.

a) modified tweezers used to wound cotyledons, and

b) inoculated plants prior to return to the growth chamber.

Figure 3. Cotyledon inoculation assessment scale modified from Newman, 1980.

Table 2. Cotyledon rating scale for the evaluation of symptoms on *Brassica* ssp. caused by *Leptosphaeria maculans*, modified from Delwiche (1980).

Disease Reaction	Description
0	No darkening of tissue around the wound. Typical response of non-inoculated cotyledon
1	Limited blackening around wound; lesion diameter 0.5-1.5 mm. Faint chlorotic halo may be present.
3	Dark necrotic lesion, 1.5-3.0 mm. Chlorotic halo may be present. Sporulation absent.
5	3.0-6.0 mm lesion, may be delimited by darkened necrotic tissue or may show grayish-green tissue collapse characteristic of reactions 7 & 9. Sporulation absent.
7	Greyish-green tissue collapse. Lesion 3.0-6.0 mm, with sharply delimited, non-darkened margins, limited number of pycnidia may be present.
9	Rapid tissue collapse at about 10 days accompanied by presence of many pycnidia in large lesions (>than 5 mm) with diffuse, non-darkened margins.

RESULTS

From the trap crops of cv. 'Westar' at the nine locations, approximately 850 isolates (~100 from each location) were derived from single pycnidia. From the Vegreville location where only 47 isolates were obtained because the population was composed of a high proportion of *L. biglobosa*. This work was conducted at the Melfort Research Farm except for data on *AvrLm6*, which was obtained in collaboration with Dr. H. Brun and Mr. B. Marquer at INRA, Rennes, France. A subset of approximately 50 isolates from each of the nine locations was sent to Rennes for characterization of *AvrLm6*.

The data is presented in Figures 4-15. The combined data of all isolates regardless of location indicated that the alleles *AvrLm1* and *AvrLepR2* were observed to be at a very low frequency, 8 and 1%, respectively (Figure 4). Avirulence genes: *AvrLm3*, *AvrLm4*, *AvrLm7*, *AvrLm9* and *AvrLepR1* varied in frequency between 25 and 53%. Only three avirulence genes were observed to occur at a high frequency: *AvrLm2* (86%), *AvrLm6* (94%) and *AvrLepR3* (77%).

There was significant variation for each avirulence allele among the nine locations included in this study. The percentage of isolates carrying *AvrLm1* (Figure 5) was relatively low (≤ 9%) at all locations except Plum Coulee, MB (33%). The *AvrLm2* avirulence allele (Figure 6) was carried by 90% or more of the isolates at each location except Carberry (83%) and Plum Coulee, MB (34%).

The *AvrLm3* allele was highly variable among the *L. maculans* populations from the various locations (Figure 7). Its frequency ranged from only 2% at Plum Coulee, MB to 93% at Indian Head, SK. In general, *AvrLm3* was relatively high in SK (≥ 80%), variable in AB (10 – 79%) and low in MB (2 – 47%). The situation was the reverse for *AvrLm4* allele (Figure 8). *AvrLm4* was present at low to moderate frequency (13 – 39%) in five of the six AB and SK locations (except at Vermillion, AB - 86%) but relatively high at all MB locations (60 – 97%).

Data for *AvrLm6* were obtained from our collaboration with colleagues at INRA, Rennes, France. All isolates examined at all locations except Plum Coulee, MB carry this avirulence allele (Figure 9). However, only 48% of isolates carry *AvrLm6* at Plum Coulee. The avirulence alleles carried by *B. juncea* variety 'Cutlass' has not been determined, but may carry *Rlm5* and *Rlm6*. A resistant or avirulent reaction was observed for all isolates

from all locations on 'Cutlass', except for two isolates (2%) at Plum Coulee, MB (Figure 10). *Brassica juncea* may carry *Rlm5*, *Rlm6* and possibly other unknown resistance genes.

Similar to *AvrLm4*, the frequency of *AvrLm7* is greater at the MB locations than the SK locations and variable at the AB locations (Figure 11). However for specific locations, such as Brandon, MB, the frequency of *AvrLm7* is only 53%, while that of *AvrLm4* is 85%. Another example where results differ is at Vegreville, AB where the frequency of *AvrLm7* was 87%, but *AvrLm4* only 19%. The frequency of AvrLm9 was generally low (4- 45%) at all locations (Figure 12), except Melfort, SK (78%).

The proportion of isolates carrying *AvrLmLepR1* varied from a low of 8% at Vermillion, AB to 53% at Indian Head, SK (Figure 13). Avirulence allele *AvrLmLepR2* was either not present or present in very few isolates (≤5%) of the populations tested from any location (Figure 14). Avirulence allele *AvrLmLepR3* was present at a high frequency at Vegreville, AB; all SK locations and Plum Coulee, MB, but a lower frequency (48 – 61%) at Vermillion, AB, and Brandon and Carberry, MB (Figure 15).

DISCUSSION

Characterization of isolates for avirulence genes corresponding to the ten resistance genes examined in this study indicated that the frequency of each avirulence allele in the *L. maculans* populations varied significantly in the western Canadian pathogen population. The frequency of most of the avirulence alleles, seven of the ten, was low to moderate (≤ 53%). However, some of these alleles were at high frequency at particular locations. For example, over all locations the frequencies of *AvrLm3* and *AvrLm4* were moderate, but *AvrLm3* was high (≥ 80%) in SK, low in MB and variable in AB. The opposite occurred for *AvrLm4*, which was high in MB, low in SK and again variable in AB. This indicates that the corresponding R genes in the host might be deployed in varieties specific to regions of the prairies where the frequency of these avirulence alleles are high. Canola varieties carrying *Rlm3* might do well to combat *L. maculans* in SK, but not MB and similarly, varieties carrying *Rlm4* may be effective in MB, but likely would not be useful in SK.

Other R genes such as *Rlm1*, *Rlm9*, *LepR1* and *LepR2* do not appear to be useful against *L. maculans* at any location in western Canada due to the low frequency of the corresponding avirulence genes in the pathogen populations at these locations. Therefore

these R genes would not be good candidates for breeding programs, based on this data. However, it is possible that there is a fitness cost to the pathogen to carry the virulence alleles and that the frequency of the avirulence alleles could change over time. This would result in the corresponding R genes becoming effective against the pathogen at some future time. For example, the virulence allele *avrLm4* has been shown to have a fitness cost in Europe and the pathogen population change over time to carry a high frequency of *AvrLm4* (Huang et al. 2006). The frequency of *AvrLm1* was also reported to be low (<20%) in Europe and France and *AvrLm9* not present in the pathogen population at all (Stachowiak et al. 2006, Balesdent et al. 2006). There appears to have been a reduction in the frequency of *AvrLm1* (~45%) from isolates collected in western Canada between 1997 and 2005 (Kutcher et al. 2010b) and those collected in 2007 and 2008 (8%) for this study. This is a similar trend to that observed by the Europeans.

The resistance gene *Rlm7* is common in recent European varieties. However, the small proportion of isolates carrying *AvrLm7* at the SK locations (≤ 7%) and moderate at Camrose, AB (42%), Brandon, MB (53%) and Carberry, MB (64%) indicate it is not a good candidate for these western Canadian locations. This result is supported by a previous finding, which indicated a population of western Canadian isolates of *L. maculans* did not carry a high proportion (25%) of *AvrLm7* (Kutcher et al. 2010b). However, at a few locations, such as Vegreville and Vermillion, AB and Plum Coulee, MB, the frequency of *AvrLm7* was fairly high (≥ 87%) suggesting that there are locations in western Canada where varieties carrying *Rlm7* could be effective against *L. maculans. AvrLm7* is reported to be the same allele as *AvrLm4*, as this allele recognizes both *Rlm4* and *Rlm7* (Parlange et al. 2009). However, data from this study suggests that while there are similarities, there are also differences between the results for *AvrLm4* and *AvrLm7* (Figures 8 and 11).

Three avirulence alleles were observed to be at high frequency in this study: *AvrLm2*, *AvrLm6* and *AvrLepR3*. The corresponding R genes could therefore be good candidates for use in variety development to condition resistance to *L. maculans* in western Canada. These results are similar to that found in the previous study by Kutcher et al. (2010b). These results differ from Europe, where *AvrLm2* is not found in the pathogen population (Stachowiak et al. 2006). In Europe, the corresponding R gene, *Rlm2*, is believed to have been one of the first R genes used in European varieties and is no longer effective. The fact that the frequency of *AvrLm2* in this study of western Canadian isolates is 86% indicates there are 14% of isolates in this study that can overcome the resistance of

the corresponding R gene, *Rlm2*. These isolates were obtained from Camrose and Vermillion, AB, and all three MB locations. The frequency of AvrLm2 was particularly low at Plum Coulee, MB. Therefore reliance of any variety or varieties only on *Rlm2* would likely be short-lived. This suggests that this R gene should be used in a strategic way to maintain its usefulness in regions of western Canada where it is effective. This may be as part of a gene pyramid or in combination with quantitative sources of resistance. The same applies to *AvrLm6* and *AvrLepR3*, which were observed at high frequencies, but less than 100%. Under experimental conditions in France, a variety carrying *Rlm6*, which was grown repeatedly for 3 years on its own stubble resulted in the detection of isolates of *L. maculans* that could overcome *Rlm6* (Brun et al. 2000). Similarly in Australia, varieties dependent on *LepR3* broke down very quickly (Li et al. 2003).

The R gene, *Rlm6* originally was detected in *B. juncea* mustard and was introgressed into *B. napus*. The fact that *AvrLm6* was carried by all isolates tested at eight of the nine locations was not surprising because the corresponding R gene, *Rlm6* is not believe to be in use in Canadian canola varieties. However, what was very surprising is that the frequency of *AvrLm6* was only 48% at Plum Coulee, MB. We have no logical explanation for this observation. Because varieties carrying *Rlm6* are not grown in western Canada to our knowledge, there should be no selection for virulence allele *avrLm6*. *Brassica juncea* mustard is grown in western Canada and it is possible the selection for *avrLm6* at Plum Coulee could have resulted from this. However, mustard is not usually grown at Plum Coulee, MB. Characterization of isolates on the *B. juncea* mustard variety 'Cutlass' indicated that two isolates (2%) overcame the resistance of this variety at Plum Coulee, MB. At all other locations this variety was resistant (avirulent reaction) to the isolates examined. The R genes carried by 'Cutlass' are not known, but it may contain *Rlm6* and possibly others such as *Rlm5*.

The frequency of *AvrLepR3* was generally high, but not 100% at any location. The corresponding R gene has likely been used by Advanta (now Monsanto) in some varieties over 10 years ago in western Canada. This gene still appears to be useful to combat *L. maculans*, but again intensive use of the gene in many varieties grown under short canola rotations would likely result in its breakdown.

The results indicate considerable variation in the frequency of avirulence alleles of *L. maculans* among locations in western Canada. This knowledge, coupled with knowledge of R genes present in current canola varieties, should be useful to provide recommendations

on variety selection to canola growers based on frequencies of avirulence gene in the pathogen populations (races) that exist in their area. The information will also be very useful in the development of improved varietal resistance and the strategic use of these resistance resources by the industry. This improved understanding of the race structure of *L. maculans* in western Canada will improve management of this disease through other means as well, such as reinforcing to producers and the industry, the importance of crop rotation.

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Van de Wouw, A.p.**,** Marcroft, S.J., Barbetti, M.J., Li, H., Salisbury, P.A., Gout, L., Rouxel, T., Howlett, B.J., and Balesdent, M.H. 2008. Dual control of avirulence in *Leptosphaeria maculans* towards a *Brassica napus* cultivar with 'sylvestris-derived' resistance suggests involvement of two resistance genes. Plant Pathol. 58: 305-313.

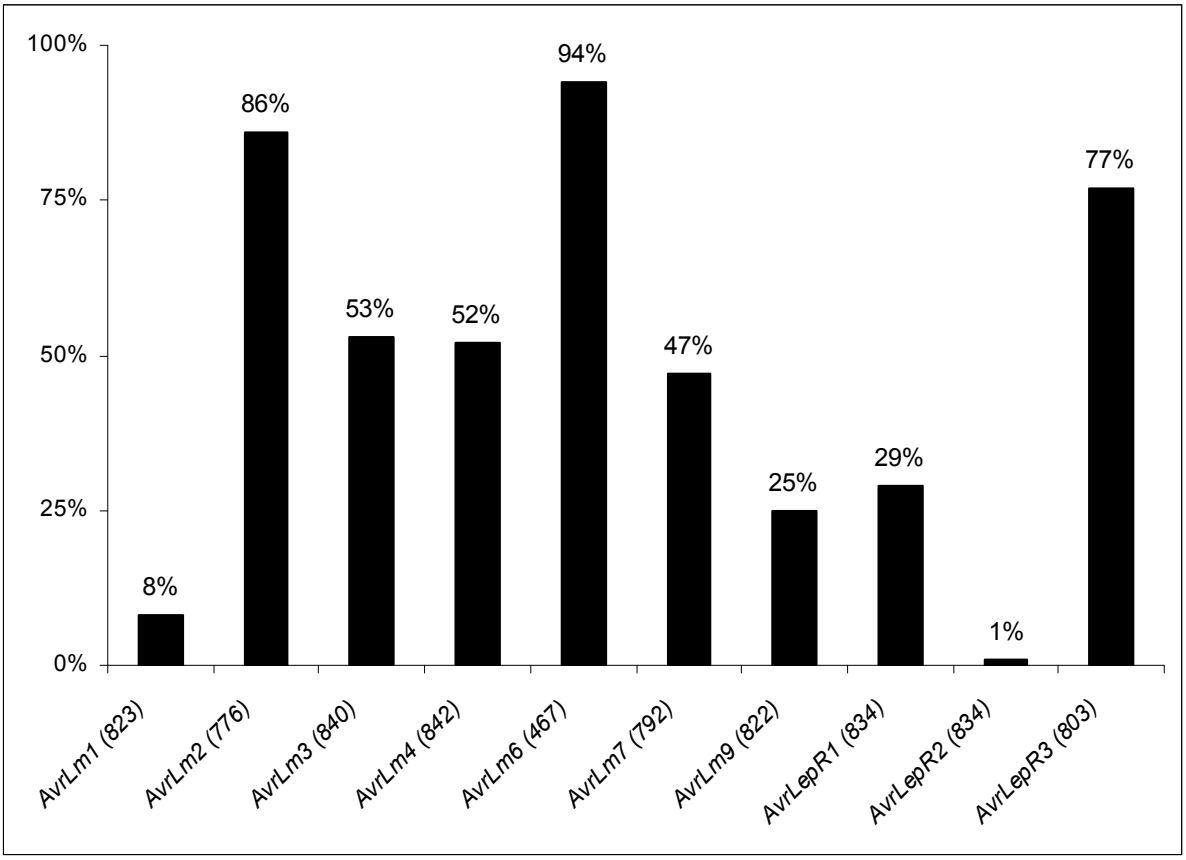

Figure 4. Frequency (%) of avirulence alleles in the *L. maculans* population of western Canada (all locations combined, number of isolates determined for each Avr gene is indicated in brackets).

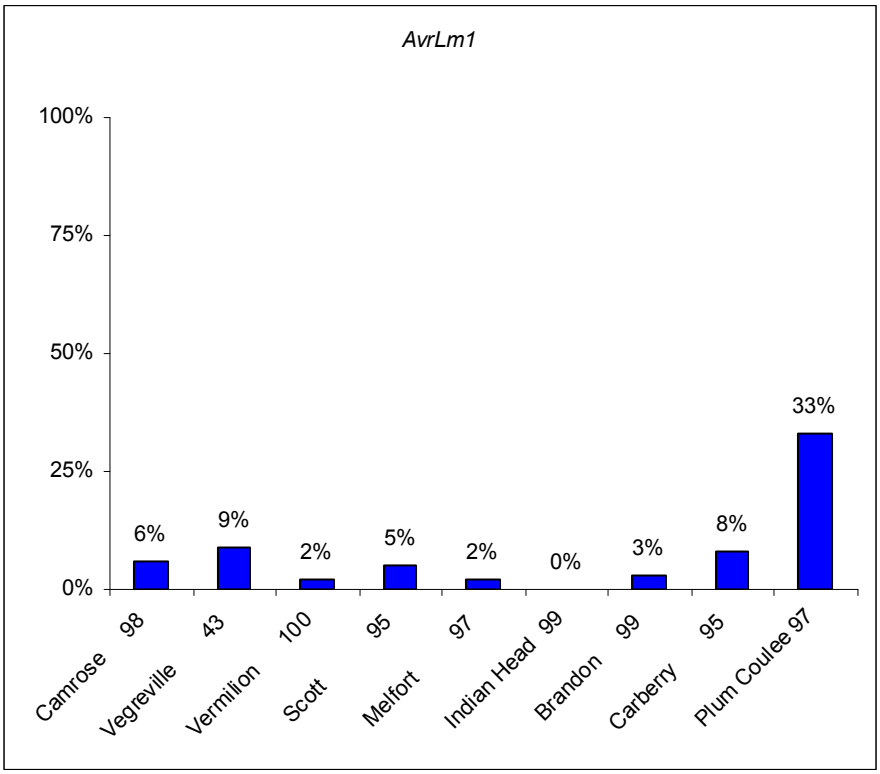

Figure 5. Frequency (%) of avirulence allele *AvrLm1* in the *L. maculans* population of western Canada at each of nine locations (numbers following each location indicates the number of isolates that were characterized for the presence or absence of *AvrLm1*).

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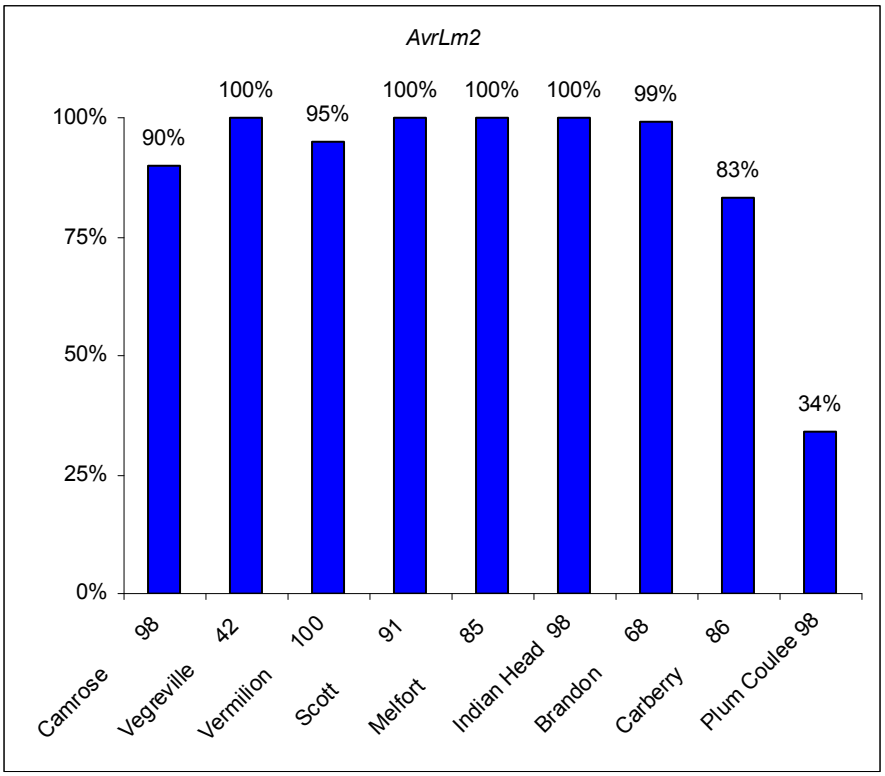

Figure 6. Frequency (%) of avirulence allele *AvrLm2* in the *L. maculans* population of western Canada at each of nine locations (numbers following each location indicates the number of isolates that were characterized for the presence or absence of *AvrLm2*).

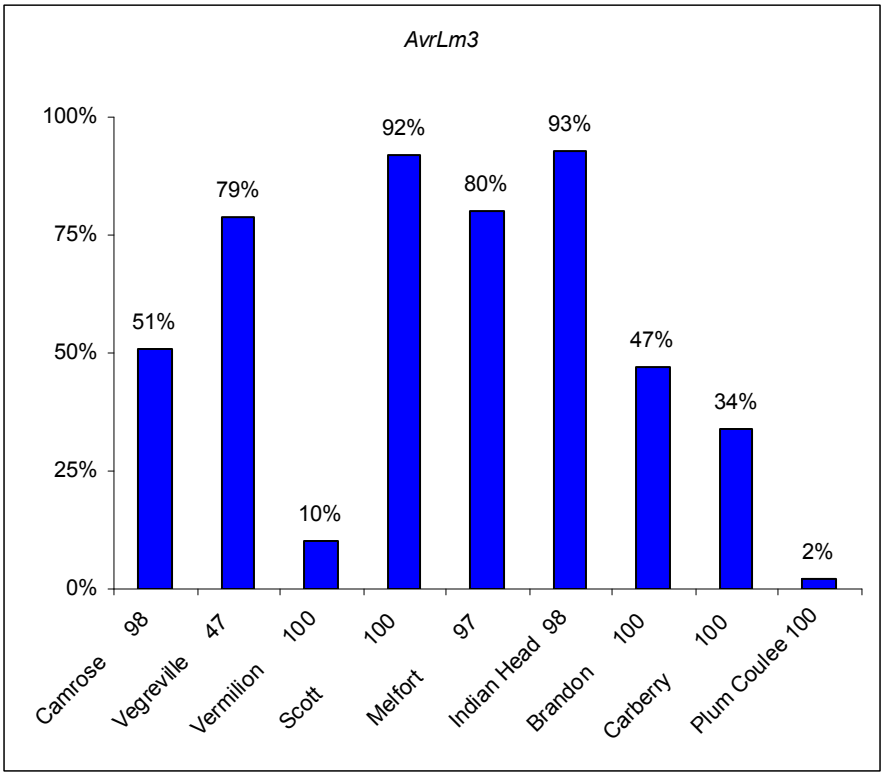

Figure 7. Frequency (%) of avirulence allele *AvrLm3* in the *L. maculans* population of western Canada at each of nine locations (numbers following each location indicates the number of isolates that were characterized for the presence or absence of *AvrLm3*).

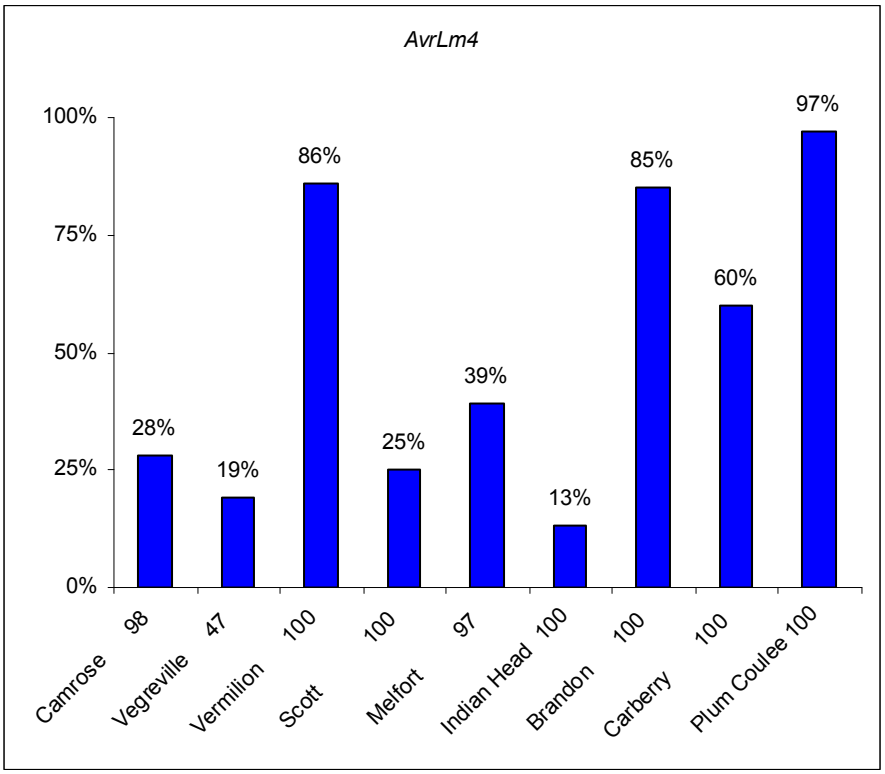

Figure 8. Frequency (%) of avirulence allele *AvrLm4* in the *L. maculans* population of western Canada at each of nine locations (numbers following each location indicates the number of isolates that were characterized for the presence or absence of *AvrLm4*).

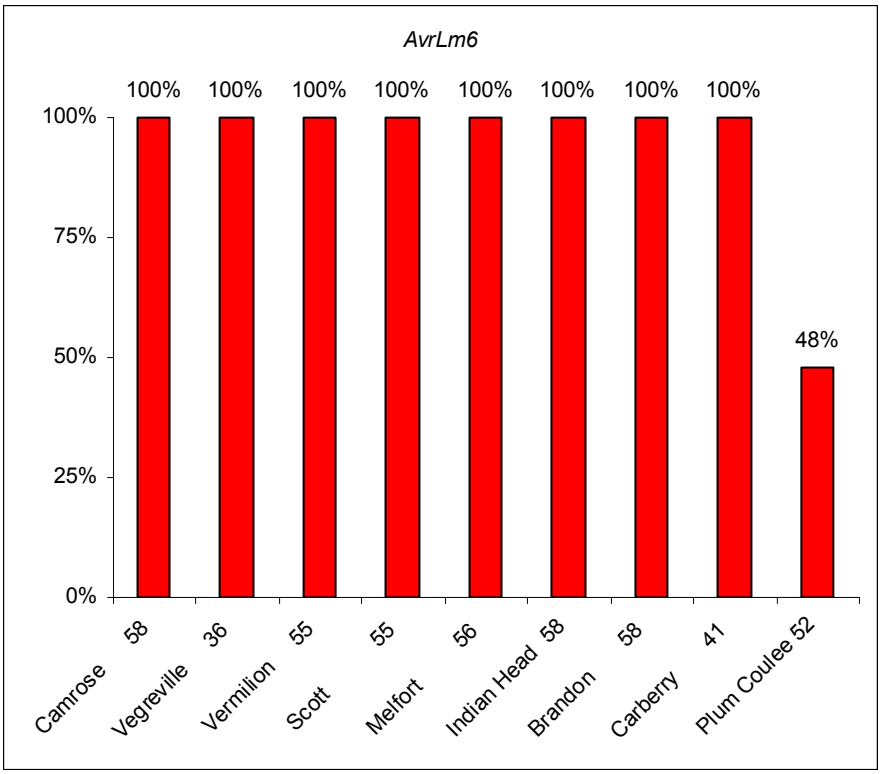

Figure 9. Frequency (%) of avirulence allele *AvrLm6* in the *L. maculans* population of western Canada at each of nine locations (numbers following each location indicates the number of isolates that were characterized for the presence or absence of *AvrLm6*).

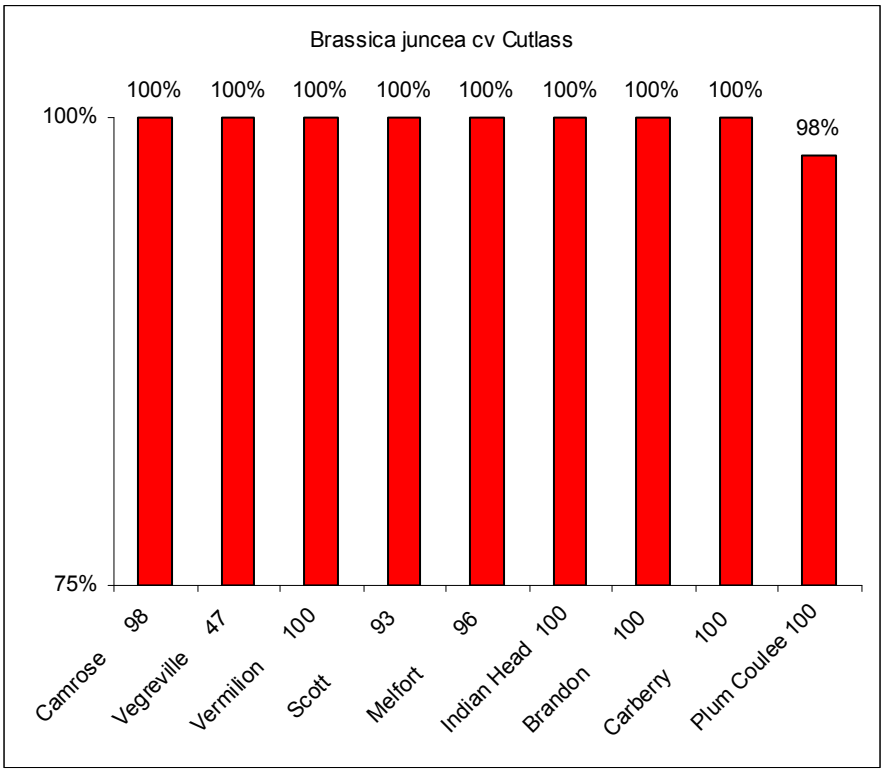

Figure 10. Frequency (%) of avirulent (resistant) reactions on variety 'Cutlass' in the *L. maculans* population of western Canada at each of nine locations (numbers following each location indicates the number of isolates that were characterized for the virulent or avirulent reaction).

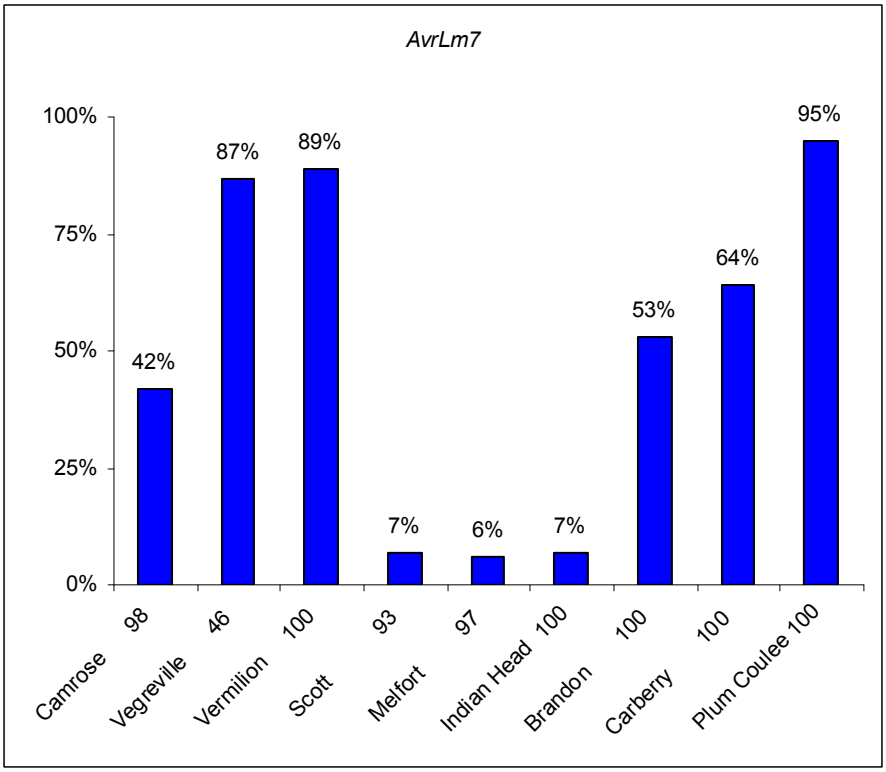

Figure 11. Frequency (%) of avirulence allele *AvrLm7* in the *L. maculans* population of western Canada at each of nine locations (numbers following each location indicates the number of isolates that were characterized for the presence or absence of *AvrLm7*).

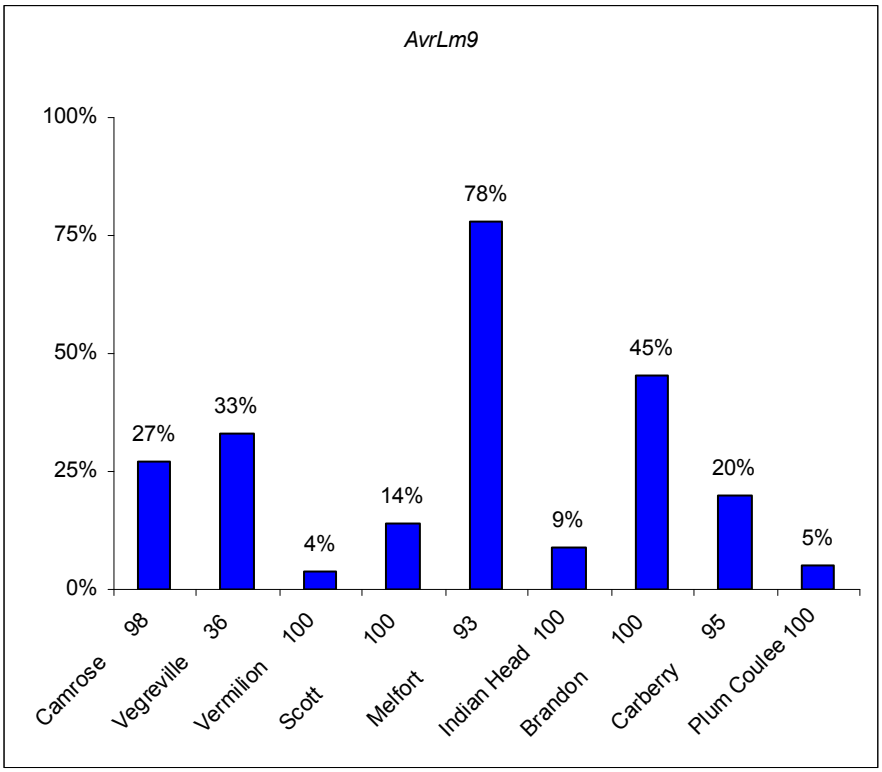

Figure 12. Frequency (%) of avirulence allele *AvrLm9* in the *L. maculans* population of western Canada at each of nine locations (numbers following each location indicates the number of isolates that were characterized for the presence or absence of *AvrLm9*).

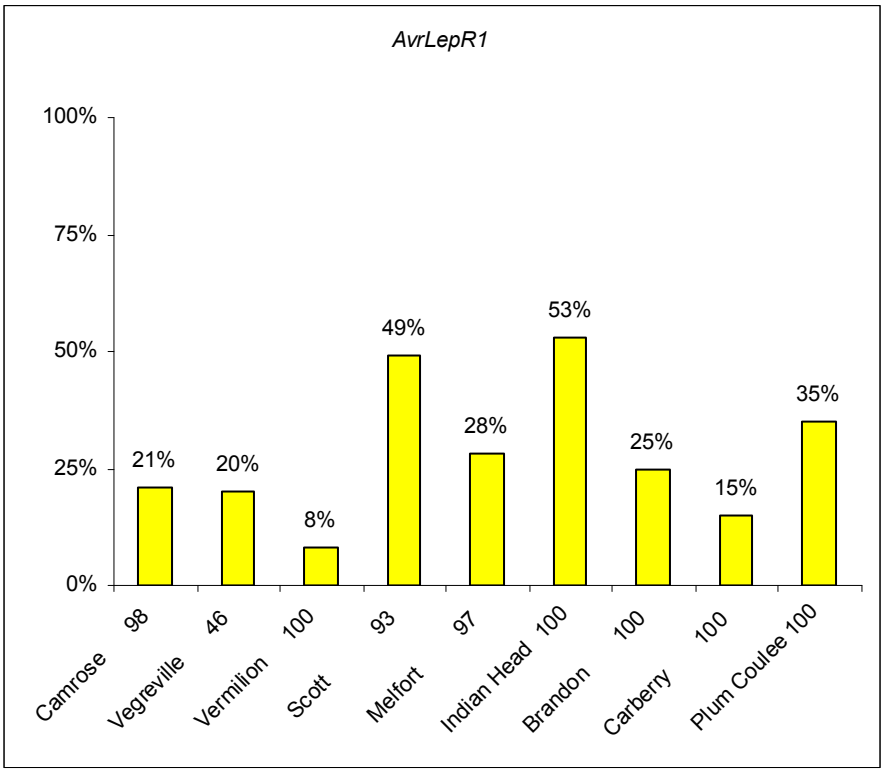

Figure 13. Frequency (%) of avirulence allele *AvrLepR1* in the *L. maculans* population of western Canada at each of nine locations (numbers following each location indicates the number of isolates that were characterized for the presence or absence of *AvrLepR1*).

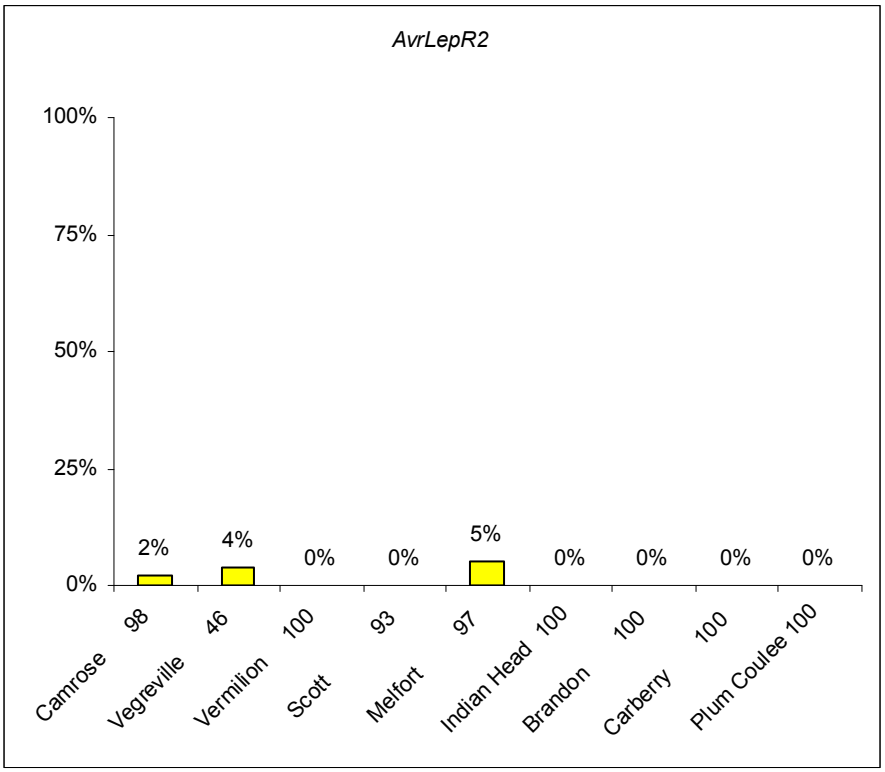

Figure 14. Frequency (%) of avirulence allele *AvrLepR2* in the *L. maculans* population of western Canada at each of nine locations (numbers following each location indicates the number of isolates that were characterized for the presence or absence of *AvrLepR2*).

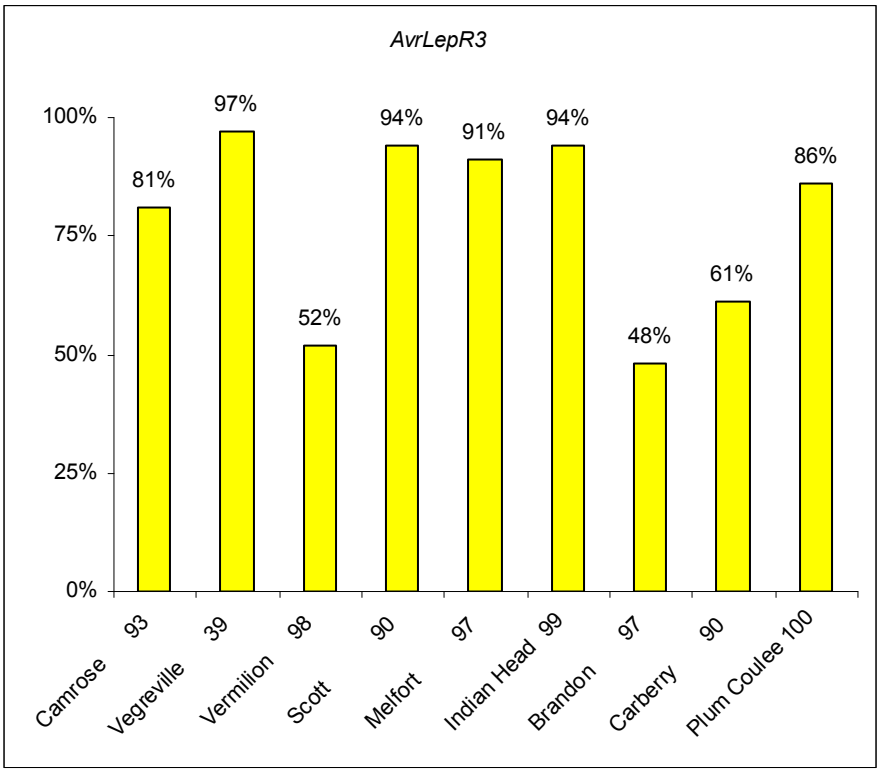

Figure 15. Frequency (%) of avirulence allele *AvrLepR3* in the *L. maculans* population of western Canada at each of nine locations (numbers following each location indicates the number of isolates that were characterized for the presence or absence of *AvrLepR3*).

Technology Transfer Activities (Cumulative):

Contributed talk at the joint meeting of the Saskatchewan Phytopathological Society and the Plant Pathology Society of Alberta, 'Understanding the population structure of *Leptosphaeria maculans* in western Canada'. October 20-22, 2008, Lloydminster, AB.

Poster entitled: Determining the race structure of *Leptosphaeria maculans* in western Canada. Presented at the Soils & Crops Workshop, February 25-26, 2009, University of Saskatchewan, Saskatoon, SK

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

Invited symposium speaker: 'Signalling in Plant Pathogen Interactions', at the Canadian Phytopathological Society Annual Meeting in Winnipeg, June 22 to 25, 2009.

Interviewed by CJVR radio, Melfort on the Alice McFarlane Agriculture Program that is heard province-wide. Discussed sclerotinia and blackleg disease management in canola. June 29, 2009.

Invited talk on canola disease management - clubroot, blackleg and sclerotinia at the Scott Field Day, July 15th, 2009.

Presentations at the Melfort Field Day, July 17th, 2009. Topics were: the biodiesel study of increased canola production, Northeast Agricultural Research Foundation cereal fungicide trials, Pea-canola intercropping study, sclerotinia resistant variety study, crop recovery study and race identification of blackleg diseases. Also discussed clubroot issue.

In person interview at the Melfort Field Day followed up with a telephone interview for an Agricultural edition of the Melfort Journal at Melfort. Discussed the blackleg study and the significance of the project to combat blackleg disease of canola. July 17th and 27th, 2009.

Local researcher combats blackleg' article in the Harvest 2009 issue of the Parkland Review, Tisdale, August 14, 2009.

Blackleg study with Melfort roots moves to western Canada' article in the Harvest 2009, Special supplement to the Northeast Sun, Page A2.

Poster entitled: 'Determining the race structure of *Leptosphaeria maculans* in western Canada', Fletcher, J., Cross, D., Kirkham, C. and Kutcher, H.R. presented at the SK Canadian Phytopathological Society meeting, December 3rd, 2009, Saskatoon, SK

Blackleg revisited – races and resistance. Oral presentation at the Manitoba Agronomists Conference. December 14-16, 2009.

"Rotate for Results". Canola Digest article, February 2010, Pages 18-20.

Determining the race structure of *Leptosphaeria maculans* in western Canada, Soils and Crops Research Workshop, Saskatoon, SK, February 24-25, 2010.

Management of canola and pulse diseases. Oral presentation at the Crop Opportunity and Scott Research Update, North Battleford, SK, March 2, 2010 (Organized by the SK Ministry of Agriculture, the Western Applied Research Corporation and AAFC, Scott.

Management of field pea and canola diseases. Oral presentation at the Grain Market and Production Meeting, Melfort, SK, March 4, 2010 (Organized by the SK Ministry of Agriculture and Viterra)

Blackleg lurking, article in Canola, Pulse and Special Crops Guide (magazine). February, 2010 edition, page 32.

Canola Disease Update. 2010 Spring Canola Grower Meeting, Hosted by The Saskatchewan Canola Growers and SaskCanola, Evergreen Centre, Nipawin, Wednesday, March 24, 2010.

Interviewed by Karen Briere for the Western Producer on blackleg disease of canola and the implications of our research on races of the disease. April 14th. Article in Western Producer May 6, Page 69.

Interviewed on management of blackleg disease in canola. What can producers do to reduce the risk. May 3rd, 2010 for CJVR and CJWW radio in NE SK

Ghanbarnia, K., Larkan, N., Rimmer, S.R., Li, G., Kutcher, H..R., McVetty, P.B.E., Fernando, W.G.D., Lydiate, D. 2010. Towards map-based cloning of AvrLepR1, a novel avirulence gene in *Leptosphaeria maculans* corresponding to LepR1 resistance gene in *Brassica napus*. Poster at the Canadian Phytopathology conference, June 20-23. Vancouver, B.C.

Kutcher, H.R., Cross, D., Marquer, B., Kirkham, C.L., Chèvre, A.-M., Delourme, R., Balesdent, M.-H., Rouxel, T., Brun, H. 2010. The race structure of *Leptosphaeria maculans* in western Canada. Poster at the Canadian Phytopathology conference, June 20-23. Vancouver, B.C.

Field day presentations at the PCARP tour in Lacombe June 29th (CCC) and again on June 30th (general grower tour) regarding disease management in various canola projects (including the biodiesel study), discussing blackleg, sclerotinia and clubroot.

Western Producer article intitled: 'Canola diseases lurk in weather' includes quotes from myself and Kelly Turkington on blackleg and sclerotinia research and disease management. Page 62, July 15th issue.

Melfort Field Day. Four presentations on: intensive production of canola (blackleg, sclerotinia stem rot and clubroot) and fungicide use in cereals (barley and wheat).

Top Crop Manger, Article on blackleg genetics of resistance". Volume 36 (16), Pages 6-8, December, 2010.

Invited speaker to the Canadian Weed Science Society annual conference to give a talk, 'Pest implications of intensive canola rotations', during the follow up workshop to the Plenary Session 'New crops and crops with second-generation traits'. Approximately 200 partipants at the conference from across Canada. November 17th, 2010.

Canola Watch, web-based and email publication of the Canola Council of Canada, 'Look for blackleg in tight rotations'. Email article to subscribers and posted to Canola Council web-site as of Nov 29, 2010.

Poster 'The race structure of Leptosphaeria maculans in western Canada' displayed at the CPS meeting Saskatoon, December 9, 2010.

Invited speaker: 'Pest implications of intensive canola rotations', to the SK Plant Disease Committee meeting, December, 2010, Saskatoon, SK.

Invited speaker to 2 sessions for the Viterra crop agonomist training 'Canola disease management and at bit of barley work', Dec 14 & 15, 2010.

Invited speaker: 'Pest implications of intensive canola rotations' at the U of S update, an information update for Ministry of Agriculture crop specialists, Dec 16, 2010.

Presentation at the Crop Production Show on blackleg work at Melfort. Work presented at the AAFC booth during Trade Show, January 10, 2011.

Canola Disease Resistance Management, Invited talk at the Top Notch Farming 2011 meeting sponsored by SaskCanola, SK Ministry of Agriculture and Viterra, Melfort SK, Travelodge Hotel, February 9, 2011

"Agriculture Forum held last week at the Kerry Vickar Centre - Rotate for Results". Melfort Journal, February 15, 2011, Page 12.

Canola Disease Resistance Management, Invited talk to 2 sessions of the Viterra , Strategies for Successful Farming Meeting, Saskatoon, SK, Travelodge Hotel, March 1, 2011 (~125 producers and Viterra staff).

Canola Disease Resistance Management, Invited talk to 2 sessions of the Viterra , Strategies for Successful Farming Meeting, Regina, SK, Travelodge Hotel, March 2, 2011 (~125 producers and Viterra staff).

Canola Disease Resistance Management, Invited talk to the SK Ministry of Agriculture and Pineland Coop 'Crop Talk 2011' meeting at Prince Albert, SK, March 8, 2011 (~50 producers and industry in attendance).

Canola Disease Resistance Management, Invited talk to the BASF grower meeting at Yorkton, SK, March 17, 2011 (~50 producers and industry in attendance).

Canola Disease Resistance Management, Invited talk to the Viterra, Strategies for Successful Farming Meeting, Wadena, SK, Legion Hall, March 23, 2011 (~200 farmers and 50 industry).

Canola Disease Resistance Management, Invited talk to the Northeast Terminal Association grower meeting, Wadena, SK, March 31, 2011 (~45 farmers and 10 industry).

Canola Disease Resistance Management, Invited talk to the Cargill sponsored producer meeting, Canwood, SK, April 5, 2011 (12 producers and industry).

Invited talk 'Blackleg disease survey and pathogen variability in Canada' given at the Blackleg Workshop, Winnipeg, MB, April 14, 2011 (~120 industry).

Kutcher, H.R., D.J. Cross, B. Marquer, C.L. Kirkham, A.-M. Chèvre, R. Delourme, M.-H. Balesdent, T. Rouxel and H. Brun. The race structure of *Leptosphaeria maculans* in western Canada. Poster to be presented at the International Rapeseed Congress, Prague, Czech Republic, June 5-9, 2011.

Scientific Publications from project or related to project (Cumulative):

Cross, D.J., Kirkham, C.L. and Kutcher, H.R. 2009. Determining the race structure of *Leptosphaeria maculans* in western Canada. Proceedings of the Soils & Crops Workshop, February 25-26, 2009, University of Saskatchewan, Saskatoon, SK [CD_ROM]

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.

Cross, D.J., Kirkham, C.L. and Kutcher, H.R. 2009. Determining the race structure of *Leptosphaeria maculans* in western Canada. Proceedings of the Soils & Crops Workshop, February 25-26, 2009, University of Saskatchewan, Saskatoon, SK [CD_ROM].

Kutcher, H.R., and Yu, F. 2009. Blackleg revisited – races and resistance. Proceedings of the Manitoba Agronomists Conference. December 14-16. Published on-line at http://www.umanitoba.ca/afs/agronomists_conf/2009/proceedings2009.html

Kutcher, H.R., Yu, F., Brun, H. 2009. Using knowledge of genetic interactions between *Brassica napus* and *Leptosphaeria maculans* to manage specific resistance of blackleg of oilseed rape. Can. J. Plant Pathol. 31: 502 (abstract).

Dokken-Bouchard, F.L., Bassendowski, K.A., Boyle, T., Cowell, L.E., Gugel, R.K., Ippolito, J., Kirkham, C.L., Kutcher, H.R., Lewchuk, Z., Miller, S.G., Morrall, R.A.A., Phelps, S., Schemenauer, I., Sommerfeld, S., and Vakulabharanam, V. 2010. Survey of canola diseases in Saskatchewan, 2009. Canadian Plant Disease Survey 90: 127-129.

Kutcher, H.R., Balesdent, M.H., Rimmer, Rouxel, T., S.R., Delourme, R., Chèvre, A.M., and Brun, H. 2010. Frequency of avirulence genes among isolates of *Leptosphaeria maculans* in western Canada. Can. J. Plant Pathol. 32 (1): 77-85.

Kutcher, H.R., Yu, F.Q., and Brun, H. 2010. Improving blackleg disease management of *Brassica napus* from knowledge of genetic interactions with *Leptosphaeria maculans*. Can. J. Plant Pathol. 32 (1): 29-34.

Kutcher, H.R., W.G.D. Fernando, T.K. Turkington and D.L. McLaren. 2011. Best Management Practices for Blackleg Disease of Canola. Prairie Soils and Crops eJournal. In press.

Kutcher, H.R., C. Eynck, B.D. Gossen, T.K. Turkington, S.A. Brandt and K. Xi. 2011. Disease implications of canola-intensive crop rotations. Book Chapter in Canadian Weed Science Society. In press.

Kutcher, H.R., Cross, D., Marquer, B., Kirkham, C.L., Chèvre, A.-M., Delourme, R., Balesdent, M.-H., Rouxel, T., Brun, H. 2010. The race structure of *Leptosphaeria maculans* in western Canada. Can. J. Plant Pathol. Abstract. In press.

Kutcher, H.R., D.J. Cross, B. Marquer, C.L. Kirkham, A.-M. Chèvre, R. Delourme, M.-H. Balesdent, T. Rouxel and H. Brun. The race structure of *Leptosphaeria maculans* in western Canada. Proceedings of the International Rapeseed Congress, Prague, Czech Republic, June 5-9, 2011, in press.