# Variation in frequency of avirulence genes in *Leptosphaeria maculans* in western Canada

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## Introduction

At present, most *Brassica napus* L. canola varieties in Canada are considered resistant or moderately resistant to *Leptosphaeria maculans* Ces. & de Not., cause of blackleg disease, although the basis for this resistance is generally unknown (Rimmer 2006). Interactions between canola varieties and pathogen isolates from western Canada, an indication of race-specificity, have been reported (Kutcher et al. 2007). In specific interactions, the resistance or susceptibility of a line of *B. napus* to isolates of *L. maculans* depends on the gene for gene interaction of the resistance (R) genes in the host and the corresponding avirulence (Avr) genes in the isolate of *L. maculans*.

Variation in the pathogenicity of *L. maculans* has been recognized in Canada by classifying isolates into pathogenicity groups (PGs) based on their reaction on the winter type *B. napus* varieties Quinta and Glacier. The PG classification system has been useful to reveal the variation in the pathogen population that has occurred over the last 20 years. However, the information obtained from the PG classification system is limited since it is based on 3 or at most 4 specific R-genes. Currently there are as many as 14 R-genes that have been identified in various *Brassica* spp. (Rimmer 2007).

Knowledge of the Avr-genes present in the pathogen population will help to develop durable resistance in canola varieties, as well as cultural controls. The objective of this study was to determine the presence or absence of 10 avirulence genes in a collection of *L. maculans* isolates from Alberta, Saskatchewan and Manitoba obtained between 1997 and 2005.

## Experimental Method

A differential host set of 15 varieties or lines of *Brassica* spp. (MT29, Samourai, Line 22-1-1, Falcon, Line 150-2-1, Westar MX, Line 23-2-1, Darmor, Surpass 400, Falcon MX, Darmor MX, Verona, Cooper, Grizzly and Glacier), each carrying one, or sometimes two, of the following ten R-genes: *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, *Rlm5*, *Rlm6*, *Rlm7*, *Rlm9*, *Rlm10* and *LepR3*, was challenged with 96 isolates of *L. maculans* obtained from western Canada and derived from single pycnidiospores. The cotyledon inoculation method (Kutcher et al. 2007) was used under controlled conditions and cotyledon reactions for each *L. maculans* isolate – *Brassica* genotype identified each isolate as avirulent (resistant phenotype) or virulent (susceptible phenotype).

## Results and discussion

Cotyledon reactions for all isolate – *Brassica* genotype combinations were obtained for 87 of the 96 isolates. Avirulence genes carried by 9 isolates could not be determined for *AvrLm1* and *AvrLm2* due to the presence of more than one R-gene in the differential host set. Analysis of Avr-genes in the isolates indicated that 45% and 62% of isolates carried *AvrLm1* and *AvrLm9*, respectively, indicating that the corresponding R-genes, *Rlm1* and *Rlm9* in the host effectively conditioned resistance against these proportions of isolates in the collection studied (Figure 1). Variation for these Avr-genes suggests that the R-genes may be in use in Canadian canola varieties.

A high percentage of isolates carried *AvrLm2* (97%), *AvrLm6* (100%), *AvrLm10* (100%) and *AvrLmLepR3* (99%). This indicates that the corresponding R-genes condition resistance to all, or nearly all, pathogen isolates in this collection. This is not surprising for *Rlm6* and *Rlm10*, which are not at present believed to be carried in commercial canola varieties. This is also logical for *LepR3*, which has been used in Canada only since about 2000, and may not be used extensively in Canadian canola varieties. The high proportion of isolates carrying *AvrLm2* in this study indicated that the corresponding R-gene was highly effective against this collection of isolates. However, this is opposite to the situation in Europe where isolates carrying *AvrLm2* are not found (Balesdent et al. 2006).

Other Avr-genes were carried by many fewer isolates: *AvrLm3* – 18%, *AvrLm4* – 30%, *AvrLm5* – 10%, and *AvrLm7* – 24%. The R-genes, *Rlm3* and *Rlm4*, are likely to have been used

as sources of resistance in Canadian varieties for some time, which may explain the low frequency of these Avr-genes in this study, i.e., the pathogen population in western Canada may have adapted to the presence of these sources of resistance. The observation that a low proportion of the isolates carried *AvrLm5* and *AvrLm7* is surprising since the corresponding resistance genes are not believed to be in use in Canadian canola varieties. The *Rlm5* gene is in *B. juncea* and is not likely present in any *B. napus* varieties. Similarly, there is no reason to suspect that *Rlm7* is carried in Canadian canola varieties, although this source of resistance originates from *B. napus*. *Rlm7* is currently very effective in France and carried by several new French oilseed rape varieties (Balesdent et al. 2006).

The observation that many isolates could overcome *Rlm1* and *Rlm4* agrees with previous reports of increasing frequencies of isolates identified as PGT, which results from a susceptible reaction on the variety Quinta (*Rlm1* and possibly *Rlm4*) and a resistant reaction on the variety Glacier (*Rlm2* and *Rlm3*) (Chen and Fernando 2006, Kutcher et al. 2007). However, most isolates carried *AvrLm2*, an indication that few of the isolates in this collection would be classified as PG3 or PG4.

**Conclusions**

An understanding of Avr-gene frequency in the *L. maculans* population of western Canada indicates that effective sources of major gene-mediated resistance exist, but in some cases isolates that can overcome that resistance are already present in the pathogen population. In the latter case, the risk of rapid pathogen adaptation to the R-gene, and consequently the possible loss of effectiveness of the R-gene, is high in absence of a resistance management strategy. In other cases, the proportion of isolates carrying Avr-genes corresponding to certain R-genes was low. This indicates that these sources of resistance are unlikely to provide adequate blackleg disease control in western Canada.

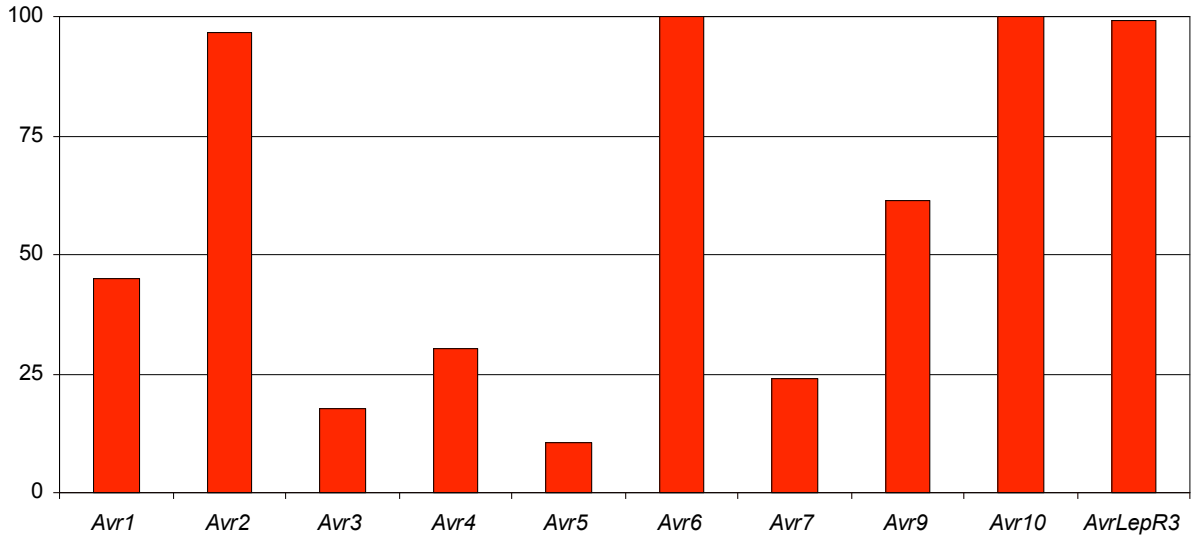


**Figure 1.** Percentage of *Leptosphaeria. maculans* isolates carrying each avirulence gene. Data based on 87 isolates for *Avr1* and *Avr2* and 96 isolates for all other avirulence genes.

## References

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