Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

The Race Structure of *Leptosphaeria maculans* in Western Canada

H.R. Kutcher[1], D.J. Cross[1], B. Marquer[2], C.L. Kirkham[1], A.-M. Chèvre[2], R. Delourme[2], M.-H. Balesdent[3], T. Rouxel[3] and H. Brun[2]

[1]Agriculture and Agri-Food Canada, Melfort, SK, Canada ; Institut National de la Recherche Agronomique, [2]Le Rheu and [3]Grignon, France

Introduction

Blackleg or Phoma stem canker of oilseed rape and canola (*Brassica napus* L.), caused by *Leptosphaeria maculans* (Desmaz.) Ces. & De Not. (Fig. 1), results in significant yield loss world wide. The pathogen also causes disease on many other *Brassica spp.* Resistant varieties and four-year crop rotations have controlled the disease very effectively in canola in western Canada over the past 15 years. However, changes in the virulence of populations of *L. maculans* have been reported recently. Changes in the pathogen population are likely to be a result of the introduction of resistance genes introduced into host varieties.

Figure 1. Blackleg infected canola plants from Saskatchewan.

Resistance to *L. maculans* in *B. napus* may be due to quantitative factors, or to specific resistance genes that interact with avirulence (*AvrLm*) genes of the pathogen in a gene-for-gene manner. Changes in the pathogen population were first observed when Canadian isolates were classified into pathogenicity groups or PGs (Kutcher et al. 1993, 2007), and more recently into races based on the avirulence genes these isolates carry (Kutcher et al. 2010). The latter research was based on a random collection of isolates of the pathogen isolated from unknown canola varieties. By determining the frequency of avirulence alleles in populations of *L. maculans,* collected from a single variety with no specific resistance genes at a number of locations across western Canada, an understanding of the race structure of the pathogen will be obtained.

Objective

To determine the race structure of *L. maculans* from isolates collected at nine locations in western Canada.

Materials and Methods

Variety 'Westar' canola, which carries no specific resistance genes, was seeded in blocks as a trap crop at nine locations in western Canada in 2007 or 2008. Leaves or basal stems exhibiting blackleg symptoms were collected from each location during the growing season or after harvest. From this material, isolations of *L. maculans* were made and isolates cultured from spores from a single pycnidia. Inoculum suspensions (1 x 10^7 spores mL^{-1}) of each isolate were prepared and used to inoculate the differential cultivars: Westar – no resistance genes, Quinta – *Rlm1,3*, Cooper – *Rlm 1,4*, MT 29 – *Rlm1,9*, Glacier – *Rlm 2,3*, Verona – *Rlm 2,4*, Samourai – *Rlm2,9*, Quantum – *Rlm3*, Falcon – *Rlm4*, Falcon MX – *Rlm4,6*, 01-23-2 – Rlm 7, Darmor – *Rlm9*, 1065 – *LepR1*, 1135 – *LepR2*, Surpass 400 – *Rlm1*, *LepR3*. Using the cotyledon inoculation test, cotyledons of four, seven day old plants of each differential were inoculated in two replicates. The plants were placed in a growth chamber at 22/16° C corresponding to a 16/8 hour photoperiod.

Disease symptoms, based on the scale of: 0 – no symptoms to 9 – severely diseased (Delwiche 1980), were assessed at 14 days after inoculation (Figure 2). Scores of 0 - 4.9 were considered avirulent reactions and 5.0 - 9.0 as virulent. Data was analyzed to determine the frequency of the avirulence allele of each gene at each location.

Figure 2. Cotyledon assessment scale (Delwiche 1980).

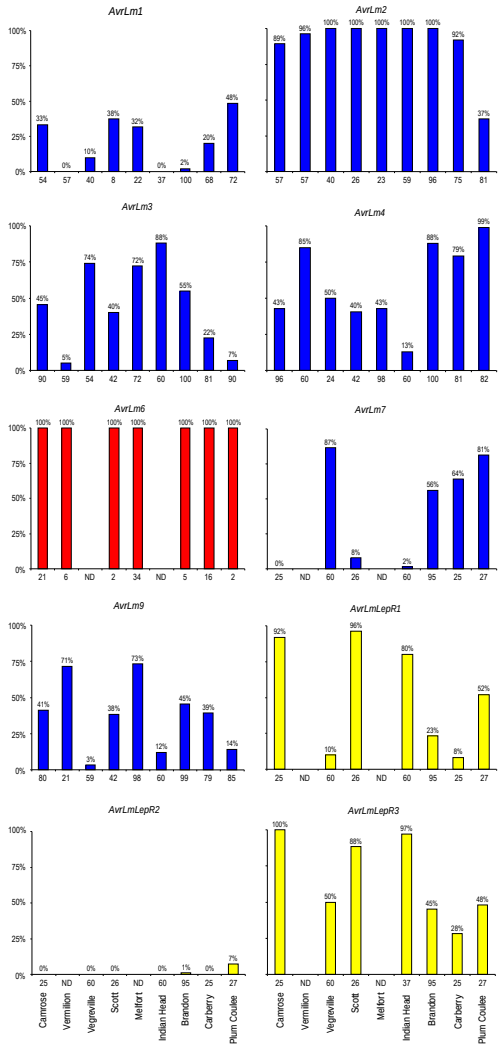

Figure 3. Percentage of *Leptosphaeria maculans* isolates carrying each avirulence allele. The number of isolates examined at each location is indicated below each column. Avirulence genes in blue correspond to resistance genes sourced from *B. napus*, red from *B. juncea* and yellow from *B. rapa* subsp. *sylvestris*. ND – no data.

Results to date

Considerable variation in the frequency of avirulence alleles was observed for seven of the 10 genes examined. Only three genes exhibited limited or no variation: *AvrLm2*, *AvrLm6* and *AvrLepR2* (Figure 3).

The frequency of *AvrLm1* among the isolates examined to date was ≤48% at all locations, and this allele was not detected at all at Vermillion, AB and Indian Head, SK.

The *AvrLm2* allele was present in all isolates at 5 of the 9 locations, present in 89 to 96% of isolates at three locations, two from western AB and one from eastern MB, but only detected in 36% of isolates from Plum Coulee, MB, the eastern most location in this study.

The presence of *AvrLm3* was highly variable among locations. It was lowest at Plum Coulee, MB (5%) and Vermilion, AB (7%) and highest at Indian Head, SK (88%). At other locations this allele was detected in 22 to 74% of isolates.

Most of the isolates from MB carried *AvrLm4* (≥79%). However, this allele was detected in only 13 to 43% of isolates from the SK locations, but was carried by 43 to 85% of isolates from AB.

Only a small sample of isolates from each location has been tested for *AvrLm6* to date. All isolates, regardless of location carried this allele.

AvrLm7 is highly variable among locations. It was not detected in isolates from Camrose, AB; was very low at Scott and Indian Head, SK (≤8%); and varied from 56 to 87% at Vermilion, AB, and the MB locations.

AvrLm9 was also quite variable among locations (3 to 73%), with no trend across the prairies. For example, the variation was 3 to 71% in AB, 12 to 73% in SK and 14 to 45% in MB.

The presence of *AvrLepR1* was detected in 8 to 96% of isolates among locations with no geographical trend.

AvrLepR2 was detected very infrequently, only in 1% of isolates at Brandon, MB and 6% at Plum Coulee, MB. This allele was not detected in isolates from the other five locations tested to date.

Detection of *AvrLepR3* varied from 28 to 100% with high rates of detection at Camrose, AB; Scott and Indian Head, SK (88 to 100%); and moderate rates (28 to 50%) at Vermilion, AB and the MB locations.

The results to date indicate that there is considerable variation in the frequency of avirulence alleles of the pathogen among these nine locations in western Canada.

An understanding of the race structure of *L. maculans* in western Canada will provide the knowledge required to improve management of this disease through cultural measures, such as crop rotation; resistance gene management, such as cultivar rotation; and breeding, through the development of cultivars with improved resistance.

References

Delwiche, P.A. 1980. Genetic aspects of blackleg (*Leptosphaeria maculans)* resistance in rapeseed (*Brassica napus*). PhD Thesis University of Wisconsin, Madison, WI 144 pp

Kutcher, H.R., van den Berg, C.G.J. and Rimmer, S.R. 1993. Variation in pathogenicity of *Leptosphaeria maculans* on *Brassica* spp. based on cotyledon and stem reactions. Can. J. Plant Pathol. 15:253-258.

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

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

Acknowledgements

The authors are grateful for the assistance provided by Coop students J. Fletcher and C. del Carman, casual employees, co-operators who facilitated isolate collection, and to Erin Cadieu for help with graphics. Financial support from the Alberta Canola Producers Association and SaskCanola through the CARP program of the Canola Council of Canada and the Matching Investment Initiative program of Agriculture and Agri-Food Canada is acknowledged.

Canada