Leptosphaeria maculans avirulence management for durable control of phoma stem canker on oilseed rape

J. S. West, A. O. Latunde-Dada, S. L. Rogers, Y-J. Huang, N. Evans, B. D. L. Fitt

Rothamsted Research, Harpenden, AL5 2JQ, UK Email: neal.evans@bbsrc.ac.uk

Abstract

The stability and durability of resistance genes in cultivars of arable crops grown commercially over several seasons depends upon the biology of the pathogen and its propensity to undergo mutation and recombination, particularly at avirulence loci. Pathogen avirulence management has been used for disease control in various crops, particularly against powdery mildew and rust diseases of cereals. New research is providing information on the population structure of *Leptosphaeria maculans* (cause of phoma stem canker of oilseed rape) in different regions and the resistance genes present in the host crop. This information could enable diversification in oilseed rape planting to decrease the risk of severe phoma stem canker epidemics and delay the changes in the *L. maculans* populations that lead to single gene resistance becoming ineffective. Integration of avirulence management with cultural practices that decrease disease severity can reduce inoculum production for the following season. This integration of control strategies can increase the durability of resistance. This review summarises recent research relating to this approach and draws extensively on papers that were recently published in a special edition of the European Journal of Plant Pathology; "Sustainable strategies for managing *Brassica napus* (oilseed rape) resistance to *Leptosphaeria maculans* (phoma stem canker) and as a book (Springer publisher; ISBN: 1402045247).

Key words: Integrated Avirulence Management, Phoma Stem Canker, *Leptosphaeria maculans*, Diversification

Introduction

Leptosphaeria maculans (phoma stem canker or blackleg of oilseed rape) has a high evolutionary potential and therefore a high ability to overcome certain types of host resistance due to its reproductive strategy, effective spore dispersal and large population reservoir (McDonald & Linde, 2002). As oilseed rape (*Brassica napus*) is increasingly regarded as a cash crop rather than as simply a break in cereal production, it is important to minimise yield loss to stem canker. The use of resistant cultivars is often the most cost effective control method against stem canker (reviewed by Delourme *et al*., 2006). Cultivar resistance scores used to advise growers each season are normally calculated from the results of trials in different locations within a particular country to account for local differences in the pathogen population. These trials produce a measurement of 'field resistance', comprising the effects of race-specific qualitative or major gene resistance, quantitative resistance, disease escape and tolerance. The potential for breakdown of resistance is affected by the types of resistance genes present (qualitative or quantitative), their deployment (through pyramiding in one host, use of cultivar mixtures, or spatio-temporal rotation), pathogen reproduction (sexual or asexual), pathogen dispersal (airborne spores, rain-splashed spores, seed movement etc.), environmental conditions and the size of the pathogen population as affected by control methods or the presence of alternative hosts (Fitt *et al*., 2006). Research is currently testing a set of pathogen isolates to find the number of different qualitative resistance host genes present. New genes may be introgressed from related species. Unfortunately, the qualitative resistance can be rendered ineffective by selection of a virulent pathogen population in only a few seasons, particularly if the qualitative resistance is set in a host without a good background of quantitative (polygenic, partial or horizontal) resistance (Rouxel *et al*., 2003; Li *et al*., 2003; Gladders *et al*., 2006; Sprague *et al*., 2006). Almost all qualitative resistance currently used in the UK has been rendered ineffective in terms of preventing infection but there may still be strong positive effects of such qualitative resistance in reducing the severity of leaf spotting compared to universally susceptible cvs and even altering the timing of successful leaf infection if spores of virulent strains occur over a shorter time-period than those of the entire pathogen population. Furthermore, anecdotal evidence suggests that some qualitative resistance genes, if overcome by a virulent pathogen strain, may still act as a component of quantitative resistance, e.g. reducing the growth rate of the pathogen *in planta*. Quantitative resistance occurs in many cultivars and is thought to be race non-specific. Possible phenotypic indicators of quantitative resistance include ascospore germination dynamics, the latent period, sporulation intensity, lesion size, growth rate down the petiole, time from leaf spotting to canker appearance and the rate of canker development. Some of the qualitative resistance genes and quantitative trait loci associated with quantitative resistance have been mapped.

L. maculans populations and host resistance

Populations of *L. maculans* may vary regionally in response to different selection pressures, particularly to resistance present in local wild or cultivated host plants. New races may occur by mutation or be introduced to a region by airborne spores or in contaminated seed, although this is relatively rare. In France, a recent survey of the *L. maculans* population was made to identify the predominant races present and their relative proportions, in order to improve the development and deployment of effective resistance genes. A total of 1797 isolates were taken from plots of plants lacking any major resistance

gene (cv. Drakkar) at 20 locations in France and these were genotyped at nine Avr loci (Fig 1; Balesdent *et al*., 2006).

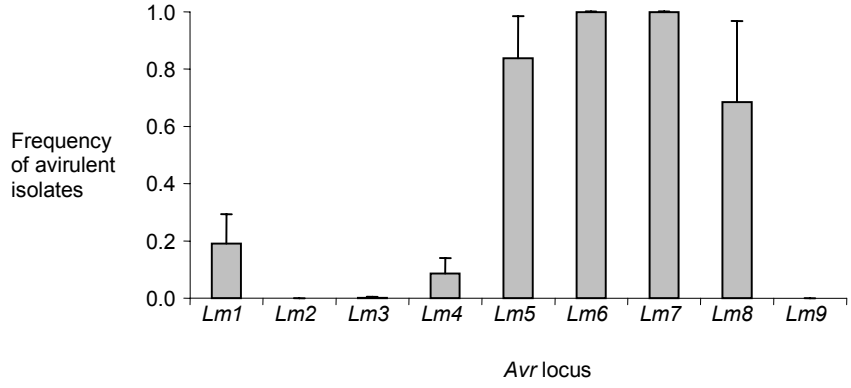

Fig. 1. Frequency of avirulence (AvrLm) alleles for nine loci in 1787 *L. maculans* isolates sampled in France. Bars represent standard deviation. Taken from Balesdent *et al*. (2006).

Balesdent *et al*. (2006) found that virulence alleles *avrLm*2 and *avrLm*9 were present in every isolate, while conversely the frequencies of the avirulence alleles *AvrLm*6 and *AvrLm*7 were over 99%. *AvrLm*1, *AvrLm*4, *AvrLm*5 and *AvrLm*8 were polymorphic, while *AvrLm*3 isolates were detected very rarely (<1% of all isolates). Only 11 races were found, with Av5-6-7-(8) (virulent on *Rlm*1, *Rlm*2, *Rlm*3, *Rlm*4 and *Rlm*9) present in 65% of isolates. They noted that virulent races, for example those with *avrLm5*, were present at some sites although the corresponding resistance gene had not been used in commercial oilseed rape crops there. Similar results were found in a Europe-wide survey (Stachowiak *et al*., 2006). The low number of races can be explained by the low number of resistance sources used in most oilseed rape in Europe. In western Europe, cultivars grown extensively in recent years include Capitol (*Rlm*1), Bristol (*Rlm*2, *Rlm*9), Express (*Rlm*2), Mendel (*Rlm*3), Falcon (*Rlm*4), Synergy (*Rlm*4) and Apex (*Rlm*9) (Gladders *et al*., 2006).

Diversification schemes and integrated avirulence management

CETIOM (Centre Technique Interprofessionnel des Oléagineux Métropolitains) has suggested the use of different diversification groups with respect to *L. maculans* on oilseed rape in France. Group 1 comprises cvs that have low susceptibility that is thought to be quite stable due to quantitative resistance; group 2 is cvs with a similar low susceptibility, based on both qualitative and quantitative resistance and this is divided into three sub-groups according to the major genes present (alone or in combination) with the recommendation that cvs with the same major genes are not grown in successive years or in nearby fields; group 3 is cvs with major gene resistance that has been overcome and cultivation is not advised in phoma stem canker affected areas; while group 4 is cvs that have effective major genes but that have no identified quantitative resistance and so in the short term can be used in rotation with cvs from groups 1 and 2 (Gladders *et al*., 2006). Clearly good farm cultivation records, control of volunteers and stubble management (conflicting with a possible trend towards minimal tillage) will help such diversification schemes.

Aubertot *et al*. (2006) introduced the term Integrated Avirulence Management (IAM) to describe a scheme to reduce the selection pressure placed on pathogens and simultaneously decrease the general level of disease using cultural, biological or chemical methods to reduce the potential for resistance to be overcome. The idea of integrated management of diseases using different cultivar resistance groups, fungicides and other factors has been described previously by Crute (1984), Wolfe (1984) and Mundt *et al.* (2002). However, Aubertot *et al*. (2006) illustrate the idea using a model to describe the effects of management practices on the control of *L. maculans*. By decreasing disease severity or increasing 'field resistance', IAM could reduce selection of a virulent pathogen population and therefore increase the durability of the resistance used for their control (McDonald & Linde, 2002; Mundt *et al*., 2002; Parlevliet, 2002). IAM not only varies the deployment of resistance genes but also advocates a combination of cultural, physical, biological or chemical control methods to reduce the pathogen population. Cultural practices that promote disease escape include an altered sowing date to avoid infections at the most susceptible growth stages. This can be complemented by manipulating plant density and fertiliser regime to increase leaf turn-over in the plant so that leaves may be shed before the fungus has grown down the petiole to reach the stem. New research using isolates genetically modified with reporter genes such as green fluorescent protein (GFP) is enabling the dynamics of pathogen growth in various host tissues to be studied (Eckert *et al*., 2005). Since a dense stand of plants produces leaves with longer petioles, there is at least a delay in the pathogen reaching the stem and possibly complete escape if the leaf is shed before stem infection occurs. However, too dense a canopy can lead to tall plants prone to lodging. High autumn nitrogen availability (> 250 kg ha^{-1}) was found to increase canker severity in some studies (Aubertot *et al*., 2004).

Foliar fungicide application can reduce final canker severity substantially if timed to protect the earliest leaf layers at risk of infection, since these lead to the most severe cankers. West *et al*. (2002) showed that canker severity could be reduced below yield-damaging levels in England using foliar fungicides soon after the onset of significant leaf spotting (*i.e.* > 10 to 20% plants affected) as this not only prevented new leaf infections but also slowed pathogen growth in the existing infections and reduced the risk of stem infection. A number of schemes have been researched to forecast the release of ascospores or the onset of leaf infections (Penaud *et al*., 1999; Salam *et al*., 2003).

Discussion

The deployment of resistance sources and the use of diversification schemes in Europe can be directed by knowledge of the population structure of *L. maculans*. The integration of molecular diagnostics with air sampling methods could be used in future research to facilitate more widespread sampling of the pathogen population from spores present in the air (Lacey & West, 2006). Aubertot *et al*. (2006) analyse the methods (cultural, physical, and chemical) that can limit the size of *L. maculans* populations to improve the efficacy of diversification schemes under Integrated Avirulence Management and the durability of specific resistance to *L. maculans*. Recent advances in mapping genes or QTLs associated with qualitative and quantitative resistance will help with future breeding research and can be coupled with knowledge from recent pathogen surveys to enhance the deployment of resistance genes. Resistance screening may be assisted by new methods under development to relate infection of plant tissues at particular growth stages to final canker severity. Further research is currently attempting to separate components of field resistance, especially to identify types of quantitative resistance and disease escape (e.g. the Defra LINK CORDISOR project, West *et al*., 2004). It is important that results are passed on to farmers and extension consultants (Gladders *et al*., 2006). The Internet is now being used more widely to disseminate information, e.g. in the UK a scheme to forecast the onset of phoma leaf spotting is under development (Gladders *et al*., 2006). These methods will be of increasing importance as oilseed rape production in Europe is set to expand, particularly for biofuel production under low input systems.

Acknowledgements

This work was funded by the European Commission under Framework Programme 5, Quality of Life (SECURE project, contract number QLK5-CT-2002-01813) and the UK Department for Environment, Food and Rural Affairs (Defra)(OREGIN project, ARO703). The CORDISOR project (LK 0956) is funded by the Defra Sustainable Arable LINK initiative. Rothamsted Research receives funding through the UK Biotechnology and Biological Sciences Research Council.

References

Aubertot J.-N., Pinochet X., Doré T. (2004). The effects of sowing date and nitrogen availability during vegetative stages *on Leptosphaeria maculans* development on winter oilseed rape. Crop Protection **23**, 635-645.

Aubertot, J.-N., West J.S., Bousset-Vaslin L., Salam M.U., Barbetti M.J., Diggle A.J. (2006). Modelling for Integrated Avirulence Management: case study of phoma stem canker of oilseed rape (*Brassica napus*). European Journal of Plant Pathology 114, 91-106.

Balesdent M.-H., Louvard K., Pinochet X., Rouxel T. (2006). A large-scale survey of races of *Leptosphaeria maculans* occurring on oilseed rape in France. *European Journal of Plant Pathology* **114**, 53-65.

Crute I.R. (1984). The Integrated Use of Genetic and Chemical Methods for Control of Lettuce Downy Mildew (*Bremia-lactucae* Regel). Crop Protection **3**, 223-241.

Delourme R., Chèvre A.-M., Brun H., Rouxel T., Balesdent M.-H., Dias J.S., Salisbury P., Renard M., Rimmer S.R. (2006). Major gene and polygenic resistance to *L. maculans* in oilseed rape (*Brassica napus*). European Journal of Plant Pathology **114**, 41-52.

Eckert M, Maguire K, Urban M, Foster S J, Fitt B D L, Lucas J A, Hammond-Kosack K. (2005). *Agrobacterium tumefaciens*-mediated transformation of *Leptosphaeria spp.* and *Oculimacula spp*. with the reef coral gene *DsRed* and the jellyfish gene *gfp* FEMS Microbiology Letters **253**, 67-74

Fitt B.D.L., Brun H., Barbetti M.J., Rimmer S.R. (2006). World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). European Journal of Plant Pathology **114**, 3-15.

Gladders P., Evans N., Marcroft S.J., Pinochet X. (2006). Dissemination of information about management strategies and changes in farming practices for the exploitation of resistance to *Leptosphaeria maculans* (phoma stem canker/blackleg) in oilseed rape cultivars. European Journal of Plant Pathology **114**, 117-126.

Lacey M.E., West J.S. (2006). *The Air Spora*. Pp. 156. Dordrecht, Springer.

Li H., Sivasithamparam K., Barbetti M.J. (2003). Breakdown of a *Brassica rapa* subsp *sylvestris* single dominant blackleg resistance gene in *B-napus* rapeseed by *Leptosphaeria maculans* field isolates in Australia. Plant Disease **87**, 752-752.

McDonald B.A., Linde C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology **40**, 349- 379.

Mundt C.C., Cowger C., Garrett K.A. (2002). Relevance of integrated disease management to resistance durability. Euphytica **124**, 245-252.

Parlevliet J.E. (2002). Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica **124**, 147-156.

Penaud A., Bernard C., Maisonneuve C., Pérès A., Pilorgé E. (1999). Decision rules for a chemical control of *Leptosphaeria maculans.* Proceedings of the 10th International Rapeseed Congress, Canberra, Australia. http://www.regional.org.au/au/gcirc/

Rouxel T., Penaud A., Pinochet X., Brun H., Gout L., Delourme R., Schmit J., Balesdent M.-H. (2003). A 10-year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the Rlm1 resistance gene of oilseed rape. European Journal of Plant Pathology **109**, 871-881.

Salam M.U., Khangura R.K., Diggle A.J., Barbetti M.J. (2003). Blackleg sporacle: A model for predicting onset of pseudothecia maturity and seasonal ascospore showers in relation to blackleg of canola. Phytopathology **93**, 1073-1081.

Sprague S.J., Balesdent M.-H., Brun H., Hayden H.L., Marcroft S.J., Pinochet X., Rouxel T., Howlett B.J. (2006). Major gene resistance in Brassica napus (oilseed rape) is overcome by changes in virulence of populations of Leptosphaeria maculans in France and Australia. European Journal of Plant Pathology **114**, 33-40.

Stachowiak A., Olechnowicz J., Jedryczka M., Rouxel T., Balesdent M.-H., Happstadius I., Gladders P., Latunde-Dada A.O., Evans N. (2006). Frequency of avirulence alleles in field populations of *Leptosphaeria maculans* in Europe. European Journal of Plant Pathology 114**, 67-75.**

West J.S., Fitt B.D.L., Leech P.K., Biddulph J.E., Huang Y.-J., Balesdent M.-H. (2002). Effects of timing of *Leptosphaeria maculans* ascospore release and fungicide regime on phoma leaf spot and phoma stem canker development on winter oilseed rape (*Brassica napus*) in southern England. Plant Pathology **51**, 454-463.

West J.S., Thomas J., Gladders P., Booth E., Latunde-Dada A.O., Evans N., Fitt B.D.L. (2004). Components of Resistance to Diseases in Winter Oilseed Rape: CORDISOR. GCIRC Bulletin **21**: XII 2004, pp.79-81.

Wolfe M.S. (1984). Trying to understand and control powdery mildew. Plant Pathology **33**, 451-466.