The SECURE project – Stem Canker of oilseed rape: Molecular methods and mathematical modeling to deploy durable resistance

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Abstract

Modelling done during the SECURE project has demonstrated the dynamic nature of the interaction between phoma stem canker (*Leptosphaeria maculans*), the oilseed rape host (*Brassica napus*) and the environment. Experiments done with near-isogenic lines of *L. maculans* to investigate pathogen fitness support field data that suggest a positive effect of the avirulence allele AvrLm4 on pathogen fitness, and that the loss of this allele renders isolates less competitive under field conditions on cultivars without the resistance gene *Rlm4*. The highlight of molecular work was the cloning of *AvrLm1* and *AvrLm6*. *L. maculans* is now one of the few fungal species for which two avirulence loci have been cloned. Subsequent research focused on understanding the function of *AvrLm1* and *AvrLm6* and on the analysis of sequences of virulent isolates to understand molecular evolution towards virulence. Isolates of *L. maculans* transformed with GFP and/or DsRed were used to follow growth of the fungus in *B. napus* near-isogenic-lines (NIL) with or without MX (*Rlm6*) resistance under different temperature and wetness conditions. The results greatly enhanced our knowledge of the infection process and the rate and extent of *in planta* growth on different cultivars. Conclusions from work to model durability of resistance have been tested under field conditions through a series of experiments to compare durability of resistance conferred by the major resistance gene *Rlm6* alone in a susceptible background (EurolMX) or in a resistant background (DarmorMX) under recurrent selection over 4 growing seasons. A major priority of the project was knowledge transfer of results and recommendations to target audiences such as plant breeding companies and extension services. CETIOM developed a "diversification scheme" that encourages French growers to make an informed choice about the cultivars that are grown within the rotation based on the resistance genes carried by the individual cultivars. Use of such schemes, in association with survey data on the population structure of *L. maculans* at both national and European scales will provide opportunities for breeders and the industry to manage available *B. napus* resistance more effectively.

Key words: avirulence, *Brassica napus*, cloning, durability, *Leptosphaeria maculans*, *Phoma lingam*, resistance.

Introduction

Phoma stem canker (blackleg), caused by *Leptosphaeria maculans*, is the most serious disease on winter oilseed rape (*Brassica napus*, canola) in Europe, North America and Australia (Fitt et al., 2006). Although sources of resistance against *L. maculans* are available, the narrow genetic base of the *B. napus* host means that major gene and polygenic resistance is finite (Delourme et al., 2006). In addition, when used in isolation in commercial cultivars, the resistance conferred by major genes is quickly rendered ineffective due to rapid selection for virulence in the *L. maculans* population (Balesdent et al., 2006; Sprague et al., 2006). Therefore there is a need for the use of currently available and future resistance to be managed in order that the durability of resistance is improved. The aim of SECURE was to investigate aspects of the interaction between *L. maculans*, the *B. napus* host and the environment to understand more fully the factors affecting the effectiveness of the resistance response and to make recommendations as to the best strategy for the deployment of resistance genes to ensure resistance is durable.

Materials and methods

Workpackages

The SECURE project consists of five inter-related workpackages (WP)

- WP1 - Modelling the life cycle of *L. maculans*.

A mechanistic model of the life cycle of *L. maculans* has been produced. This has been validated using existing data and new data from WP3.

- WP2 - Effects of pathogen variation at Avr loci on durability of resistance.

Two avirulence loci (*AvrLm1* and *AvrLm6*) were analysed. The analysis included cloning and functional characterisation of the genes. In addition, an analysis of the molecular events leading to virulence was done. Fitness of virulent isolates was also assessed in controlled environment and glasshouse experiments.

- WP3 - Effects of genotype/environment on durability of resistance.

L. maculans population race structure was quantified at sites in four countries extending data previously available only in France. The influence of plant genetic background and the environment on durability of resistance was analysed through a series of field experiments across the main oilseed rape growing regions of Europe.

- WP4 - Strategy for sustainable deployment of durable resistance

A model was developed to investigate the interactions between resistance and avirulent/virulent *L. maculans* isolates. This was used to develop new criteria to assess measures of durability of resistance (security, longevity, effectiveness) (van den Bosch and Gilligan, 2003) and to predict the effects of deployment strategy on durability of new resistance genes.

- WP5 - Diffusion of results

Results and recommendations were delivered to target groups/stakeholders through popular literature, meetings and peer reviewed publications. Results and recommendations were published on the SECURE website at www.secure.rothamsted.ac.uk.

Results

A prototype life-cycle model was developed during the first year of the SECURE project (WP1). Existing data and data generated during the first three seasons were used to validate the model. The model fit to data was generally very good, with fits of predicted to observed data having an $R^2 > 80\%$ for 50% of data sets (See Evans et al. this proceedings).

Experiments done in WP2 using near-isogenic lines of the *L. maculans* pathogen to investigate pathogen fitness have produced data which support evidence from field experiments (Huang et al., 2006b). The field data suggested a positive effect of the avirulence allele AvrLm4 on pathogenic fitness, and that the loss of this allele rendered isolates less competitive than avirulent isolates under field conditions on cultivars without *Rlm4*. The highlight from the molecular work under WP2 was the cloning of *AvrLm1* (Gout et al., 2006) and *AvrLm6* (Fudal et al., 2006). Subsequent research focused on understanding the function of the avirulence alleles, analysis of sequences of virulent isolates to understand molecular evolution towards virulence and other work on the genomic organisation of *L. maculans* (Kuhn et al., 2006). Other research done during the SECURE project was the transformation of *L. maculans* with GFP and/or DsRed (Eckert et al., 2005). The transformed isolates were used to follow growth of the fungus in *B. napus* near-isogenic-lines (NIL) with or without MX (*Rlm6*) resistance under different temperature and wetness conditions (Huang et al., 2006a).

The field experiments done during the SECURE project (WP3) demonstrated the dynamic nature of the interaction between the phoma stem canker pathogen *L. maculans*, the *B. napus* oilseed rape host and the environment. The first two growing seasons (2002/03 and 2003/04) were characterised by a very dry autumn at some locations. Not only was the onset of the phoma leaf spot epidemic late in both seasons, but also poor emergence was a problem during the 2002/03 season, so that there was a late epidemic on small plants. In contrast to the first two seasons, the 2004/05 season was more typical, with moderate levels of phoma leaf spotting in the autumn and moderate to severe stem canker before harvest. The MX (*Rlm6*) resistance in field experiments was not rendered ineffective (Fig. 1) and the incidence and severity of phoma leaf spotting and stem canker were lower on plants with this resistance. From material tested, it appeared that leaf spots on MX lines were probably caused by the related pathogen *L. biglobosa*. Indeed, some of the planned experiments on pathogenicity in WP3.2 could not be done due to the scarcity of pseudothecia of *L. maculans* on the MX material. In addition, the race structure of 600+ isolates of *L. maculans* collected across Europe was characterised using a differential set of host lines (Stachowiak et al., 2006). This complimented data that had already been collected across France (Balesdent et al., 2006).

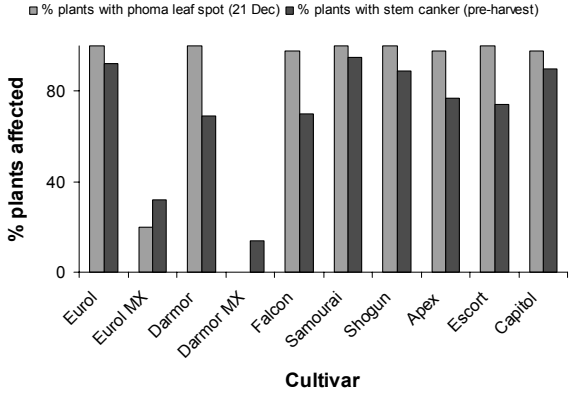

Figure 2. Incidence of phoma leaf spot in December and of stem canker in June, at Boxworth, UK during the 2004/05 winter oilseed rape growing season.

The results from work done to model durability of resistance (WP4) have provided some interesting conclusions. This work led to the development of a series of recommendations to policy makers and the breeding industry as to the best way to "manage" resistance genes to maintain durability of resistance (Pietravalle et al., 2006). Information was communicated to target user groups via an Internet site (www.secure.rothamsted.ac.uk), via the farming press and through the publication of peer-reviewed refereed papers (Gladders et al., 2006).

Discussion

SECURE has been a successful project with some important scientific results that have had a direct impact on many target groups including policymakers on a national and European scale. The field experiments highlighted the dynamic nature of the interaction between the phoma stem canker pathogen *L. maculans*, the oilseed rape host and the environment. Data gathered during the project were useful for validation of the phoma stem canker model developed early in the project. The cloning of *AvrLm1* (Gout et al., 2006) and *AvrLm6* (Fudal et al., 2006) was a major advance within the SECURE project as *L. maculans* is now one of the few fungal species for which two avirulence genes have been cloned. Subsequently, *AvrLm4* has also been cloned. In addition, data should become available from the *L. maculans* genome sequence project (http://www.genoscope.cns.fr/) during 2007/08, which should greatly help future understanding of the molecular genetics of *L. maculans*. This work, and the work done using transformed isolates of the pathogen to visualise the infection and colonisation process, have greatly improved our knowledge of the pathogen-host interaction and how the resistance mechanism operates at the physiological level.

The recommendations produced from the durability modelling guide future priorities and, for the first time, will allow quantification of changes in the pathogen population with respect to resistance gene release strategy (Pietravalle et al., 2006). Knowledge transfer to target audiences was a major priority during the project and it is interesting to note that aspects of the science done during the SECURE project have led to a change in policy in the UK, with the Department for Environment Food and Rural Affairs funding follow-on work involving the use of similar models. In addition, other aspects of SECURE work are being incorporated into the EU FP6 funded Network of Excellence ENDURE (European Network for the Durable Exploitation of crop protection strategies).

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References

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.

Delourme, R., Chevre, 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 *Leptosphaeria maculans* in oilseed rape (*Brassica napus*) European Journal of Plant Pathology, 114, 41-52.

Eckert, M., Maguire, K., Urban, M., Foster, S., Fitt, B., Lucas, J., 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.

Fudal, I., Ross, S., Gout, L., Blaise, F., Kuhn, M.L., Eckert, M.R., Cattolico, L., Bernard-Samain, S., Balesdent, M.H., Rouxel, T., 2006. Heterochromatin-like regions as ecological niches for avirulence genes in the *Leptosphaeria maculans* genome: map-based cloning of *AvrLm6*. Molecular Plant-Microbe Interactions, in press.

Gladders, P., Evans, N., Marcroft, S., 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) in oilseed rape cultivars. European Journal of Plant Pathology, 114, 117-126.

Gout, L., Fudal, I., Kuhn, M.-L., Blaise, F., Eckert, M., Cattolico, L., Balesdent, M.-H., Rouxel, T., 2006. Lost in the middle of nowhere: The *AvrLm1* avirulence gene of the Dothideomycete *Leptosphaeria maculans*. Molecular Microbiology, 60, 67-80.

Huang, Y.-J., Evans, N., Li, Z.-Q., Eckert, M., Chevre, A.-M., Renard, M., Fitt, B.D.L., 2006a. Temperature and leaf wetness duration affect phenotypic expression of *Rlm6*-mediated resistance to *Leptosphaeria maculans* in *Brassica napus*. New Phytologist, 170, 129-141.

Huang, Y.-J., Li, Z.-Q., Evans, N., Rouxel, T., Fitt, B.D.L., Balesdent, M.-H., 2006b. Fitness cost associated with loss of the *AvrLm4* avirulence function in *Leptosphaeria maculans* (phoma stem canker of oilseed rape). European Journal of Plant Pathology, 114, 77-89.

Kuhn, M.-L., Gout, L., Howlett, B.J., Melayah, D., Meyer, M., Balesdent, M.-H., Rouxel, T., 2006. Genetic linkage maps and genomic organization in *Leptosphaeria maculans*. Europpean Journal of Plant Pathology, 114: 17-31.

Pietravalle, S., Lemarié, S., van den Bosch, F., 2006. Durability of resistance and cost of virulence. European Journal of Plant Pathology, 114, 107-116.

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., Evans, N., 2006. Frequency of avirulence alleles in field populations of *Leptosphaeria maculans* in Europe. European Journal Plant Pathology, 114, 67-75.

van den Bosch, F., Gilligan, C.A., 2003. Measures of durability of resistance. Phytopathology, 93, 616-625.