

Section 1: PROJECT IDENTIFICATION		NOT CONFIDENTIAL
Information to be provided for project identification		
Title of the project: StEm Canker of oilseed rape: molecular tools and mathematical modelling to deploy dUurable Resistance		
Acronym of the project: SECURE		
Type of contract: Shared cost		Total project cost (in euro) €3200117
Contract number QLK5-CT-2002-01813	Duration (in months) 48 Months + 4 month extension	EU contribution (in euro) €1705677
Commencement date 1 September 2002		End date 31 December 2006
<u>PROJECT COORDINATOR</u>		
Name: Neal Evans	Title: Dr	Address: Rothamsted Research, Harpenden, Herts., AL5 2JQ, UK
Telephone: +44 (0)1582 763133	Telefax: +44 (0)1582 760981	E-mail address: neal.evans@bbsrc.ac.uk
Key words (5 maximum - Please include specific keywords that best describe the project.). Durable resistance, Stem canker, <i>Leptosphaeria maculans</i> , Oilseed rape, Modelling		
World wide web address (the project's www address) http://www.secure.rothamsted.ac.uk/		
List of participants Provide all partners' details including their legal status in the contract i.e.,contractor, assistant contractor (to which contractor?). P1 RRES (Contractor and Coordinator): Dr Neal Evans, Rothamsted Research, Harpenden, Herts., AL5 2JQ, UK. neal.evans@bbsrc.ac.uk , Tel: +44 (0)1582 763133, Fax: +44 (0)1582 760981 WWW: www.rothamsted.ac.uk P2 INRA PMDV (Contractor): Dr Thierry Rouxel, Institut National de la Recherche Agronomique, Unité PMDV, Route de St Cyr, Versailles 78026, France. rouxel@versailles.inra.fr , Tel: +33 (0)1 30 83 32 29, Fax: +33 (0)1 30 83 31 95, WWW: www-pmdv.versailles.inra.fr/projets/lepto/lepto.htm P3 UMR BiO3P (Contractor): Dr Hortense Brun, Domaine de la Motte BP 35327, Le Rheu Cedex 35653, France. hortense.brun@rennes.inra.fr , Tel: +33 (223) 485 185, Fax: +33 (223) 485 180, WWW: www.rennes.inra.fr/bio3p/ P4 ADAS (Contractor): Dr Peter Gladders, ADAS Boxworth, Battlegate Road, Boxworth, Cambs. CB3 8NN, UK. peter.gladders@adas.co.uk , Tel: +44 (0)1954 268230, Fax: +44 (0)1954 267659, WWW: www.adas.co.uk P5 CETIOM (Contractor): Dr Xavier Pinochet, CETIOM, Centre de Grignon, B.P. no. 4, Thiverval-Grignon 78850, France. pinochet@cetiom.fr , Tel: +33 (0)1 30 79 95 00 Fax: +33 (0) 1 30 79 95 90, WWW: www.cetiom.fr P 6 IGR (Contractor): Dr Małgorzata Jędryczka, Instytut Genetyki Roslin - PAN, Strzeszynska 34, Poznan, 60-479, Poland. mjed@igr.poznan.pl , Tel: +48 (61) 65 50 248, Fax: +48 (61) 65 50 301, WWW: www.igr.poznan.pl P7 SWAB (Contractor): Dr Ingrid Happstadius, Svalöf Weibull AB, Svalöv, SE-268 81, Sweden. ingrid.happstadius@swseed.com , Tel: +46 (0)418 66 73 66, Fax: +46 (0)418 66 73 49, WWW: www.swseed.com UMR 118 INRA/ENSAR APBV (Assistant Contractor [to UMR BiO3P]): Domaine de la Motte BP 35327, Le Rheu Cedex 35653, France. Dr. Michel Renard (renard@rennes.inra.fr) Instytut Uprawy (Assistant Contractor [to IGR-PAN]): Nawozenia i Gleboznawstwa, Czartoryskich 8, 24-100 Pulawy, Poland. Dr Anna Podlesna (ap@iung.pulawy.pl) Dept. of Phytopathology, August Cieszkowski Agricultural University (Assistant Contractor [to IGR-PAN]), Dabrowskiego 159, 60-594 Poznan, Poland. Dr Zbigniew Karolewski (karolew@au.poznan.pl)		

Objectives:

Objective 1: To model the life cycle of *Leptosphaeria maculans*, the causal agent of stem canker of oilseed rape.

Objective 2: To investigate the effects of pathogen variation at Avr (avirulence) loci on durability of resistance.

Objective 3: To investigate the effects of genotype/environment interactions on durability of resistance.

Objective 4: To develop a strategy for sustainable deployment of durable resistance to stem canker for oilseed rape.

Project Summary:

Epidemics of stem canker (*Leptosphaeria maculans*), the most serious disease of oilseed rape (*Brassica napus*) in Europe, can cause substantial yield loss (>40%). New sources of oilseed rape cultivar resistance usually break down in a few seasons. Control of the disease relies on fungicides in many countries. Environmental and economic considerations, in line with EU policy, preclude routine fungicide treatment against the disease. To optimise use of novel cultivar resistance so that it does not break down quickly and minimise use of fungicides for controlling the disease, the main aim of SECURE was to model factors affecting durability of resistance and produce strategies for sustainable management of durable resistance to stem canker.

Durability of cultivar resistance is affected by the ability of the pathogen to shift from avirulence to virulence and by the effect of the resulting loss in fitness on the ability of the pathogen to complete its life cycle. The epidemiology of the *L. maculans*/*B. napus* host-pathosystem is influenced by both plant genetic background and the environment. A major aim of the project was to investigate the effect of these factors on durability of resistance. To do this, SECURE utilised a unique set of research tools. Firstly, a set of four oilseed rape cultivars. This consisted of two cultivars with different *B. napus* genetic backgrounds, Darmor (with quantitative background resistance to *L. maculans*) and Eurol (without background resistance). The second two were the novel cultivars DarmorMX and EurolMX that had the same genetic background/are near isogenic to Darmor and Eurol except they contained the major (qualitative) resistance gene *Rlm6* (originating from *B. juncea*). The significance of this material was that *Rlm6* had never been commercially released. Secondly, a series of *L. maculans* isolates were produced that were genetically the same except for alleles at one specific target effector locus (*AvrLm1*, *AvrLm4* or *AvrLm6*) that corresponded with the *Rlm1*, *Rlm4* and *Rlm6* resistance genes in the host plant material. These materials were used in a series of field, glasshouse, controlled environment and laboratory studies to measure aspects of the host-pathogen interaction.

In addition to the field and laboratory work, mathematical modelling work was done. During the first three years of SECURE, a life-cycle model was developed that was designed to describe the progress of phoma stem canker epidemics, as they complete different stages from ascospore (infective particle) production, leaf infection, phoma leaf spot development, systemic growth of the pathogen along the leaf petiole to the stem and stem canker development. Data were assimilated from datasets that pre-existed the project and model testing and validation was done using datasets collected during the course of the project across the main oilseed rape growing region of Europe (UK, France, Germany, Sweden and Poland). Model fit to data was generally very good, with 50% of models fitted to datasets having an $R^2 < 80\%$. From a total of 14 data sets the model fitted well to 9 data sets. A description of the model development and results from this work were published in a paper in the Proceedings of the 12th International Rapeseed Congress held in Wuhan, China (Evans et al., 2007).

Molecular work investigated various aspects of the genetic mechanisms involved in the host-pathogen interaction and the possible consequences of these changes on durability of resistance genes. During SECURE, two effector genes, *AvrLm6* (corresponding to *Rlm6* the main resistance gene used in SECURE), and *AvrLm1*, located in dissimilar genome environments, were analysed and cloned. Both genes corresponded to small secreted proteins that fully restored the differential avirulent phenotypic response on a set of plant genotypes with the *Rlm1*, or *Rlm6* genes, respectively. This and subsequent work since SECURE ensured that SECURE was extremely successful, since *L. maculans* is one of the few fungal species for which four effector genes have been cloned. Sequence data on

AvrLm1, *AvrLm6* and BAC clone sequences in the *AvrLm1-AvrLm6* region were uploaded to international databases and two papers were published (Fudal et al., 2007; Gout et al., 2006).

The successful cloning of *AvrLm1* and *AvrLm6* during SECURE allowed us to study in detail the molecular events responsible for the gain of virulence (loss of AVR function) in natural populations. The work revealed that two main molecular events occurred during the loss of the avirulent allele in the French *L. maculans* populations. Evolution to virulence at the *AvrLm1* locus occurred mainly through large-scale deletion of the surrounding genomic region (Gout et al., 2007). However, other events involved in the loss of *AvrLm6* function were identified and these included deletion and RIP (repeat induced point) mutation events. A paper submitted to a peer-reviewed journal is under review.

Work to measure the biological consequences of loss of avirulence in terms of fitness of the invading fungus involved a number of field, laboratory and controlled environment experiments. These fitness studies investigated how pathogen isolates that had lost *AvrLm1* or *AvrLm4* functions could compete against those that had not. The studies indicated that there was a fitness cost associated with evolution from avirulence to virulence at both the *AvrLm1* and *AvrLm4* loci and that this affected the ability of the fungus to produce leaf symptoms, grow systemically along the petiole to reach the stem base and to colonise the basal plant tissues. This suggests that when there is no selection (i.e. when crop cultivars are grown that do not contain the corresponding resistance gene), the frequency of avirulent alleles will increase in the population. Since the effect was stronger for *AvrLm4*, we suggested that this process would probably occur more rapidly for the *AvrLm4* isolates in comparison to those avirulent at the *AvrLm1* locus. These results highlighted two major factors affecting durability of resistance. The larger fitness cost at the *AvrLm1* locus than at the *AvrLm4* locus suggested that the corresponding resistance gene *Rlm4* may be more durable than *Rlm1*. It also suggested that the *Rlm1* and *Rlm4* resistance genes could be re-cycled in future new crop cultivars when the corresponding avirulent isolates again become predominant in local *L. maculans* populations. Therefore, the differences in fitness cost between different AVR loci can be used to predict the durability of different resistance genes. Work on *AvrLm4* was published (Huang et al., 2006). A publication on the *AvrLm1* work has been submitted to a peer-reviewed journal and is currently under review.

A European-wide survey of *L. maculans* was completed during the first and second year and all 603 isolates collected were tested for avirulence gene combinations. Isolates were collected from the spring rape cultivar Drakkar (no known genes for resistance against *L. maculans*). Collections were made at six experimental sites in the UK (two sites; Hertfordshire and Cambridgeshire), Germany (Saxony), Sweden (Svalöv) and Poland (two sites; Wielkopolska and Lublin). The results indicated that the *L. maculans* population was much less diverse than had been anticipated, since approximately 90% of all isolates belonged to one of two races (combinations of avirulence alleles) Av5-6-7 (77% of isolates) or Av6-7 (12%). In total, eight races were identified, with four races at frequencies less than 1%. Results of this work were published on the SECURE website (www.secure.rothamsted.ac.uk) and in a peer-reviewed scientific paper (Stachowiak et al., 2006).

Other field experiments at the SECURE field sites investigated the response of a set of oilseed rape cultivars, including Eurol, EurolMX, Darmor and Darmor MX. These were assessed under field conditions and although the MX-containing lines were significantly less susceptible to canker, damaging cankers were still observed. However, when tested in culture and by PCR assay, it was found that infections on MX lines were caused by the related species *L. biglobosa*. The experiments were very useful in highlighting the need to consider breeding for resistance against both *Leptosphaeria* species (Gladders et al., 2006).

Recurrent selection experiments were done in the field in France (Rennes) and in Poland (Wielkopolska and Lublin). Eight oilseed rape lines, including Eurol, EurolMX, Darmor and Darmor MX were grown in isolated blocks (at least 1 km between fields). Inoculum from the four experimental lines was retained from one season for use in the next and used to inoculate the specific blocks to increase selection for that particular genotypic line. These were assessed for leaf and stem canker lesions during each season. Even though the MX resistance gave better canker control, disease did occur (but was significantly less severe in comparison with cultivars without the MX resistance). Again these experiments highlighted the importance of *L. biglobosa* in the canker disease complex.

Sustaining durability of resistance to *L. maculans* requires a strategy to extend the life of available resistance genes and to efficiently deploy novel resistance genes. The second piece of mathematical modelling work was the development of a model that was used to evaluate three measures of durability. This was used to compare different breeding strategies (e.g. benefits of sequential release of resistance genes in different cultivars versus pyramided deployment). The model indicated that the cost of virulence to the pathogen greatly influenced which deployment strategy gave the greatest additional yield. If cost of virulence was low, sequential deployment was best. However, if cost of virulence was high, pyramiding resistance genes gave a better benefit. The model also indicated that length of deployment time of the resistant crop does not influence which strategy gives the greatest additional yield. Models developed during SECURE are currently being exploited and developed further through two spin-off projects. Therefore, the innovative and technically challenging models developed during SECURE will be published in the near future after further refinement.

In conclusion, breeders assume that pyramiding resistance genes will improve durability of resistance since they believe that the “additive effect” of a complex of resistance genes requires the pathogen to accumulate a corresponding complex of virulence factors (the pathogen would need to lose avirulence function for each respective resistance gene). However, results for SECURE show that in most cases, single gene resistance is sufficient for the life of a commercially viable cultivar. Pyramiding can give a lower return/shorter disease resistance durability so the recommendation is that pyramiding might not always be the most efficient use of a resistance gene. Results from the project have been widely disseminated at major international and national meetings, through radio and TV interviews and on the SECURE website. Fourteen peer-reviewed scientific papers have been produced from the project to date, with more to come. These are listed on the project website. Much of the work done during SECURE was published in a special issue of the European Journal of Plant Pathology (Volume 114, issue 1), which was also published as a book (Fitt et al., 2006).

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