Final Project Summary

Project title	Understanding resistance to decrease risk of severe phoma stem canker on oilseed rape		
Project number	RD-2009-3676	**Final Project Report**	PR606
Start date	1 October 2010	**End date**	1 October 2014
AHDB funding	£124,000 requested	**Total cost**	£1,127,000

What was the challenge/demand for the work?

Phoma stem canker (*Leptosphaeria maculans*) is the main disease on oilseed rape (*Brassica napus*) in the UK. It causes losses of more than £100M each year, despite use of fungicides. It is predicted that global warming will continue to increase the range and severity of phoma stem canker epidemics. Increased temperature, associated with climate change, is predicted to elevate losses in southern England by up to 50%, for susceptible cultivars.

There is evidence that temperature affects the operation of the two types of phoma resistance. Increased temperature renders ineffective some resistance (*R*) genes (e.g. *Rlm6*) that operate in leaves in autumn and decreases effectiveness of quantitative resistance (QR) that operates as the pathogen spreads down leaf stalk in autumn/winter and colonises stem tissues in spring and summer. Therefore, there is a need to understand effects of different factors (e.g. temperature and pathogen races) on effectiveness of resistance, especially resistance used in commercial cultivars in the UK.

The aim of this project was to decrease the risk of severe phoma stem canker on oilseed rape by improving understanding of host resistance against the causal pathogen *L. maculans*.

There were three tasks:

1. To identify *L. maculans* races in UK regions with different climates to optimise the deployment of oilseed rape resistance genes

2. To investigate phenotypes of *R* gene-mediated resistance and quantitative resistance (QR) against *L. maculans* in leaf and stem tissues in different environments

3. To develop an experimental system to investigate mechanisms of operation of *R* gene-mediated resistance and QR against *L. maculans*

How did the project address this?

The challenge of this project was addressed through the following three tasks.

1) *L. maculans* races in different regional environments were identified and used to guide deployment of different resistance genes

To detect differences in *L. maculans* races between regions (that differ in climate, cultivars and agricultural practices) and to optimise the deployment of different *R* genes, field experiments were conducted at sites. The experiments used the very susceptible winter oilseed rape cultivar Drakkar in three growing seasons (2010/11, 2011/12, 2012/13). Because Drakkar has no *R* gene, it acted as a trap crop for all *L. maculans* races in the local population. Phoma leaf spots on Drakkar, collected from different sites in autumn each season, were used to obtain *L. maculans* isolates. Isolate conidial suspensions were used to detect alleles of *Avr* genes using a set of cultivars/lines with known *R* genes. There were differences in race structure between different growing seasons and sites. There were differences between sites in frequencies of avirulent *AvrLm1, AvrLm4, AvrLm5* and *AvrLm6.* Populations of *L. maculans* were 100% virulent at *AvrLm2* and *AvrLm9* loci, suggesting that the corresponding resistance genes *Rlm2* and *Rlm9* are not effective against *L. maculans.* Frequency of *AvrLm7* was 100%, except in the 2011/2012 season when 3% of *L. maculans* isolates were virulent against *Rlm7*, indicating that the resistance gene *Rlm7* is still effective but there is a need to monitor the pathogen population, so that this *R* gene can be deployed effectively. In addition, Burkard seven-day spore samplers operated from September to February at four sites, over the three growing seasons, showed that timing and pattern of ascospore release differed between seasons and between sites. However, the date of maximum ascospore release differed by less than five days between sites.

2) Phenotypes of *R* gene-mediated resistance and quantitative resistance (QR) against *L. maculans* in leaf and stem tissues in different environments were investigated

To examine effects of environment (e.g. temperature) and background QR on effectiveness of *R* genes, six cultivars with different *R* genes (*Rlm1*, *Rlm4* or *Rlm7*) in backgrounds with/without QR, and two cultivars with QR but no known *R* genes, were used in field experiments at 11 sites. The two cultivars with *Rlm7* (Roxet and Excel) had less severe phoma stem canker than other cultivars. Cultivars DK Cabernet (*Rlm1* + QR) and Adriana (*Rlm4* + QR) had less severe phoma stem canker than cultivars Capitol (*Rlm1*) and Bilbao (*Rlm4*). Of the two cultivars with only QR, Es-Astrid had less severe stem canker than NK Grandia. This suggests that combining *R* genes with QR can provide effective control of phoma stem canker.

The effectiveness of different *R* genes (*Rlm1*, *Rlm4, Rlm6, Rlm7 and LepR3*) in different cultivars/lines was tested in controlled environment (CE) experiments at 20°C and 25°C. Cotyledons were inoculated with isolates with the matching *Avr* genes (i.e. the isolate producing a resistant phenotype on the cultivar with the corresponding *R* gene). To test the effectiveness of *Rlm1* and *Rlm4* in backgrounds with/without QR, different cultivars/lines with *Rlm1* and *Rlm4* were tested with different isolates at different temperatures.

There were differences between different *R* genes in sensitivity to temperature. There were also effects of QR on effectiveness of *R* genes at increased temperature. The resistance mediated by *Rlm4* in Jet Neuf and by *Rlm7* in Hearty was not sensitive to temperature; it was stable when temperature increased from 20 to 25°C. However, resistance mediated by *Rlm1* in Columbus and by *Rlm6* in DarmorMX was sensitive to temperature. Resistance mediated by *Rlm2* in Tapidor was stable when temperature increased from 20 to 25°C. By contrast, resistance mediated by *LepR3* in Surpass400 was not stable when temperature increased to 25°C. Adriana (*Rlm4* +QR) and Jet Neuf (*Rlm4*+QR) were moderately resistant while Bilbao (*Rlm4*) was susceptible at 25°C. DK Cabernet (*Rlm1* +QR) was moderately resistant, whereas Capitol (*Rlm1*) and Columbus (*Rlm1+Rlm3*) were susceptible at 25°C.

After leaf stalk inoculation, symptoms were observed near the inoculation sites at 10 days after inoculation (dpi) at 20°C and 25°C. By 18 dpi, stem canker symptoms were observed at the leaf scars on the stems of the susceptible control A30 at both 20°C and 25°C. By 49 dpi, severe phoma stem cankers developed on stems of susceptible A30, at both 20°C and 25°C. At 20°C, for cultivars with *Rlm1* (Capitol and DK Cabernet) or *Rlm4* (Bilbao and Adriana) in backgrounds with/without QR, no/limited stem canker symptoms were observed and the mean stem canker score was less than 1. However, at 25°C there were differences between these four cultivars in severity of stem canker. There were no/limited stem canker symptoms on stems of DK Cabernet and Adriana, whereas stem cankers developed on Bilbao and Capitol, though they were less severe than those on A30.

3) An experimental system to investigate mechanisms of operation of *R* gene-mediated resistance and quantitative resistance against *L. maculans* was developed

To investigate whether temperature has similar effects on quantitative resistance (operating in leaf stalk and stem tissues) to those on *R* gene-mediated resistance (operating in leaf tissues), CE experiments were done with sets of material with different *R* genes in the same background or the same *R* gene in different backgrounds. Cotyledon inoculation experiments with Tapidor (*Rlm2*), Bristol (*Rlm2*) or Surpass400 (*LepR3*), at 20°C and 25°C, showed that there were differences between these cultivars in their response to increased temperature. At 20°C, a resistant phenotype was observed on all these cultivars. However, when temperature was increased to 25°C, Surpass 400 showed a susceptible phenotype. The two cultivars with *Rlm2* (Tapidor and Bristol) showed different responses to temperature – Tapidor still showed a resistant phenotype, while Bristol showed a susceptible phenotype. This suggests that the host background resistance affects the effectiveness of *R* gene resistance at increased temperature.

To investigate host defence response and avoid host background effects, near-isogenic lines, carrying the resistance genes *Rlm4* or *LepR3* in the same Topas background, were tested at 20°C and 25°C. Results showed that there were differences between *Rlm4* and *LepR3* in their defence response. When Topas-*Rlm4* and Topas-*LepR3* were inoculated with isolates carrying the corresponding effector genes *AvrLm4* or *AvrLm1*, Topas-*Rlm4* produced a quicker, stronger

defence response than Topas-*LepR3,* at both 20°C and 25°C. At 25°C, the defence response of Topas-*Rlm4* was so strong that extensive cell death was observed at the inoculated site and a large lesion developed. When Topas-*Rlm4* and Topas-*LepR3* were inoculated with isolates without *AvrLm4* or *AvrLm1*, typical phoma leaf lesions were observed.

A new petiole method was developed to assess the effectiveness of *R* gene-mediated resistance in preventing development of cankers in stem tissues. The method was used to investigate whether the *R* gene-mediated resistance (operates at the leaf infection stage), also operates at the stem colonisation stage. It was also used to establish whether effects of temperature on its operation in leaf tissues are similar to those in stem tissues.

Leaf stalks of Tapidor (*Rlm2*) and Surpass400 (*LepR3*) were inoculated with *L. maculans* isolates, with the corresponding *Avr* genes, at 20°C and 25°C. There were no/limited stem canker symptoms observed on stems of Tapidor or Surpass400 at 20°C. However, at 25°C phoma stem cankers developed on stems of Surpass400 but there were no/limited stem canker symptoms on Tapidor (*Rlm2*). This suggests that *R* gene-mediated resistance also operates at the stem colonisation stage.

What outputs has the project delivered?

This project has identified methods to decrease the risk of severe phoma stem canker epidemics on UK winter oilseed rape crops, both in the short-term and the long-term. These methods have been communicated to the UK oilseed rape industry, including farmers, AHDB, crop advisors and breeders.

Who will benefit from this project and why?

The academic community, plant breeders, growers and the agricultural industry will benefit from this project. Six research papers related to this project have been published. The papers provided new information about current races of *L. maculans* in the UK and about effects of quantitative resistance on *R* gene-mediated against *L. maculans*.

This information will benefit the **academic community**, especially researchers working on resistance in brassicas. The results are also of generic relevant to scientists working on other pathosystems involving *R* gene resistance and quantitative resistance. The detection of *L. maculans* isolates virulent against the current effective *R* gene (i.e. *Rlm7*) in the UK, and the development of methods for assessing quantitative resistance in controlled environments, will be of benefit to **plant breeders**, because this information will help breeders to select pre-breeding material and guide their breeding strategies for development of suitable cultivars. The improved understanding of operation of host resistance and knowledge about regional *L. maculans* races for deployment of cultivars with effective resistance will benefit **growers**, by reducing yield losses caused by the disease and avoiding unnecessary fungicide use. Improved understanding of risks to current AHDB Recommended List cultivars from phoma stem canker (e.g. deployment of cultivars with effective resistant genes based on *L. maculans* races) will help to maintain yields so that oilseed rape can remain a profitable crop in the UK, which will benefit the **agriculture**

industry. In addition, the **public and the environment** will benefit from the outputs of this project, since reduced fungicide use, through improved guidance on deployment of host resistance, will not only reduce the greenhouse gas emission associated with disease control but also contribute to national food security.

If the challenge has not been specifically met, state why and how this could be overcome
Not applicable, as challenges have been met.

Lead partner	Professor Bruce David Ledger Fitt, University of Hertfordshire
Scientific partners	N/A
Industry partners	Co-operative Farms, DSV UK Ltd, Elsoms Seeds Ltd, Grainseed Ltd, Limagrain UK Ltd, LS Plant Breeding, Monsanto UK Limited, National Farmers Union, Pioneer-Hi-Bred Northern Europe GMBH, Saaten Union UK Ltd, Syngenta Seeds
Government sponsor	UK Biotechnology and Biological Sciences Research Council (BBSRC), Chadacre Agricultural Trust, Felix Thornley Cobbold Trust, Agriculture & Horticulture Development Board, Perry Foundation