This project “Understanding factors affecting durability of crop resistance genes” (Figure 1, acronym: “Durable resistance”, no: 302202) had three objectives: 1) To determine factors that influence durability of *R* genes in winter oilseed rape cropping, 2) to understand interactions between host and pathogen gene products in relation to durability/environmental stability of *R* genes, 3) to develop a scheme to describe the factors affecting the durability of *R* genes.

Figure 1: Project on “Understanding factors affecting durability of crop resistance genes (durable resistance)” at the University of Hertfordshire (tinyurl.com/pskgcnx).

Results

To address Objective 1, data sets on the breakdown of *R* gene (*Rlm6* and *LepR3*) resistance were used (Delourme et al., 2014; Van de Wouw et al., 2010). A weather-based model (Evans et al., 2008) was used to predict disease severity during trials in Northern France. Particular emphasis was on a sudden increase in disease severity irrespective of *Rlm6* and quantitative resistance during the 2010/2011 growing season. The existing weather-based model (Evans et al., 2008) could not explain this increase. Instead, changes in pathogen population structure may have caused this sudden increase in disease severity (Delourme et al., 2014). To better understand the disease epidemic in Australia, the distributions of virulent *avrLm1* and avirulent *AvrLm1* alleles were analysed before and after breakdown of *LepR3* resistance. Pathogen virulence significantly increased in frequency after this breakdown of disease resistance across Australia. Whereas a significant increase in frequency of the virulent *avrLm1* allele was observed after breakdown of *R* gene resistance in New South Wales and Victoria, this was not the case in South and Western Australia. Interestingly, the presence of the virulent allele was observed in all states before *LepR3* resistance broke down.

To better understand the interaction between pathogen effectors and *R* gene-encoded receptors (Objective 2), a docking model was generated using the known structure of the *Hyaloperonospora arabidopsidis* ATR1 effector (Chou et al., 2011) and the predicted model of the leucine-rich repeat (LRR) domain of the corresponding RPP1 receptor from its host *Arabidopsis thaliana*. The docking model suggests that effector binding involves two LRRs, but occurs slightly to the side of the LRR domain. This may not be unreasonable because the concave surface of the LRR domain may be bound to intramolecular domains (Takken and Goverse, 2012). This knowledge will be applied to the interaction between oilseed rape (*Brassica napus*) and *Leptosphaeria maculans* as soon as similarly detailed information about pathogen effector protein structures and corresponding *R* genes becomes available. To test the environmental stability of resistance (Objective 2), cultivars containing different *R* genes were exposed to *L. maculans* isolates at 20^{o}C and 25^{o}C. The *R* gene *Rlm4* was more sensitive to the higher temperature than *LepR3*. The *L. maculans* effector *AvrLm1* suppressed host defence responses, including *PR1* and *WRKY70* gene expression and hydrogen peroxide production, more effectively than the *AvrLm4* effector.

In support of Objective 3, effector-triggered defence against apoplastic fungal pathogens was defined to be dependent on recognition of extracellular effectors by corresponding receptor-like proteins (RLPs, Figure 2)(Stotz et al., 2014). Based on this new premise, the RLP complement of the *B. napus* genome was shown to contain more than 250 genes. This new information can be used to accelerate *R* gene cloning and learn about new approaches to increase the durability of resistance (Stotz et al., 2014).

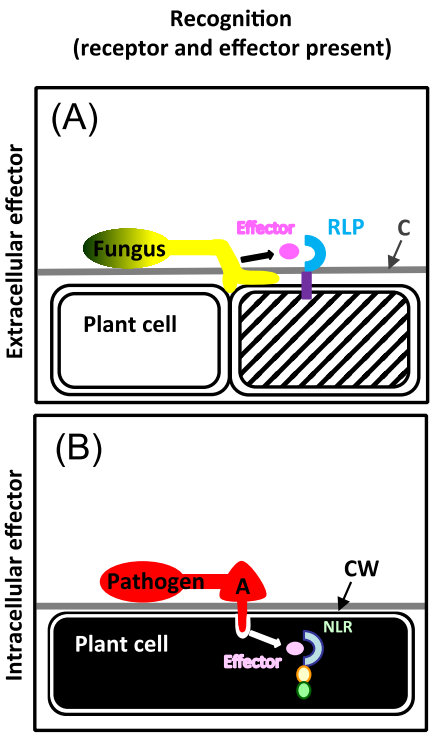

Figure 2: Defence and immune responses against apoplastic and haustoria-forming pathogens. (A) Apoplastic fungal pathogens secrete extracellular effectors that are recognised by receptor-like proteins (RLPs). Host cells do not undergo rapid death (cross hatched). The fungus stays alive (yellow to green). (B) Cell-penetrating pathogens filamentous pathogens target effectors to the cytoplasm where they are recognised by intracellular nucleotide-binding leucine-rich repeat receptors (NLRs). Hypersensitive cell death is indicated in black. The pathogen typically dies (red). Abbreviations: C, cuticle; CW, cell wall; A, appressorium.

Conclusions

New insights into *R* gene-mediated resistance have been obtained, especially when considering the dataset on breakdown of *LepR3* resistance (Van de Wouw et al., 2010); this has resulted in a better understanding of temporal and spatial spread of the Australian disease epidemic. The docking model of the predicted ATR1-RPP1 complex provides new information about the possible recognition of effectors by corresponding LRR-domain-containing receptors. This approach can help identify regions and amino acids that may be involved in recognition of pathogen effectors. The finding that RLPs play a critical role in recognition of extracellular effectors and their targets may cause a paradigm shift in thinking about resistance against extracellular pathogens, placing more emphasis on this class of membrane proteins (Stotz et al., 2014).

Impact

Findings have been disseminated in the form of publications (Fitt et al., 2013; Stotz et al., 2014), presentations at international meetings (ICPP2013 in Beijing, China, 11th EFPP Conference 2014 in Krakow, Poland, Agriculture and Climate Change Conference 2015, Amsterdam, Netherlands), press releases and websites (tinyurl.com/pskgcnx). A video has been produced for Trends in Plant Science to support the opinion article on effector-triggered defence (tinyurl.com/olbokek).

Target groups include plant breeders, distributors and growers. Work specifically with breeders will continue as a KTN BBSRC CASE PhD studentship on “Sustainable yield of oilseed rape through improved resistance against *Leptosphaeria maculans* (phoma stem canker)”. Phoma stem canker is likely influenced by climate change (Butterworth et al., 2010). *L. maculans* is a pathogen that threatens to invade China (Zhang et al., 2014). For the latter two reasons, new information about this pathogen should also be of interest to policy makers.

References

Butterworth, M.H., Semenov, M.A., Barnes, A., Moran, D., West, J.S., and Fitt, B.D. (2010). North-South divide: contrasting impacts of climate change on crop yields in Scotland and England. Journal of the Royal Society Interface *7*, 123-130.

Chou, S., Krasileva, K.V., Holton, J.M., Steinbrenner, A.D., Alber, T., and Staskawicz, B.J. (2011). Hyaloperonospora arabidopsidis ATR1 effector is a repeat protein with distributed recognition surfaces. Proc Natl Acad Sci U S A *108*, 13323-13328.

Delourme, R., Bousset, L., Ermel, E., Duffe, P., Besnard, A.L., Marquer, B., Fudal, I., Linglin, J., Chadoeuf, J., and Brun, H. (2014). Quantitative resistance affects the speed of frequency increase but not the diversity of the virulence alleles overcoming a major resistance gene to *Leptosphaeria maculans* in oilseed rape. Infection, Genetics and Evolution *27*, 490–499.

Evans, N., Baierl, A., Semenov, M.A., Gladders, P., and Fitt, B.D.L. (2008). Range and severity of a plant disease increased by global warming. Journal of the Royal Society Interface *5*, 525-531.

Fitt, B.D.L., Hughes, D., Stotz, H., McDonald, B.A., and Rouxel, T. (2013). Epidemiology and spread of *Leptosphaeria* species (phoma stem canker) on brassicas. Acta Phytopathologica Sinica *43*, 567.

Stotz, H.U., Mitrousia, G.K., de Wit, P.J., and Fitt, B.D. (2014). Effector-triggered defence against apoplastic fungal pathogens. Trends Plant Sci *19*, 491-500.

Takken, F.L., and Goverse, A. (2012). How to build a pathogen detector: structural basis of NB-LRR function. Current opinion in plant biology *15*, 375-384.

Van de Wouw, A.P., Cozijnsen, A.J., Hane, J.K., Brunner, P.C., McDonald, B.A., Oliver, R.P., and Howlett, B.J. (2010). Evolution of linked avirulence effectors in *Leptosphaeria maculans* is affected by genomic environment and exposure to resistance genes in host plants. PLoS Pathogens *6*, e1001180.

Zhang, X., White, R.P., Demir, E., Jedryczka, M., Lange, R.M., Islam, M., Li, Z.Q., Huang, Y.J., Hall, A.M., Zhou, G.*, et al.* (2014). *Leptosphaeria* spp., phoma stem canker and potential spread of *L. maculans* on oilseed rape crops in China. Plant Pathology *63*.