Final Project Summary

Project title	Screening for costs of disease resistance caused by stomatal dysfunction		
Project number	RD-2009-3670	Final Project Report	PR557
Start date	October 2010	End date	June 2015
AHDB Cereals & Oilseeds funding	£112,000 (£97,000 cash & £15,000 in-kind)	Total cost	£889,546

What was the challenge/demand for the work?

Wheat varieties combining high yield and good resistance against septoria and rusts have proven elusive. There is significant evidence that some disease resistance genes impose a yield penalty. Hence, breeding for disease resistance can create 'yield drag' which slows the rate of yield improvement. Breeding for disease resistance is a high priority for plant breeders, and it would therefore be beneficial to select effective resistance genes during varietal development that do not exhibit a yield cost, to maximise yield potential.

The objective of this project was to identify resistance genes to septoria, yellow rust and brown rust which exhibit a yield penalty and to develop methods to minimise 'yield drag' associated with breeding for disease resistance. The specific focus of the project was to establish the role of stomatal dysfunction in causing deleterious yield effects associated with resistance genes and its use as an indicator of costs of resistance and potential germplasm screen to inform breeding programmes. The original project objectives are as follows:

1. Characterise key disease resistance genes for effects on stomatal function.
2. Relate stomatal dysfunction at a leaf level to impacts on radiation use efficiency at a canopy level and grain yield.
3. Test whether 'defeated' resistance genes carry a yield cost.
4. Test stomatal conductance as an indicator of yield potential in the UK.
5. Test improved porometry methods to increase screening throughput.

How did the project address this?

This project was a large collaborative project with significant contributions from nine different academic and industrial partners towards four project deliverables:

Deliverable 1. Quantification of yield penalties associated with widely used specific septoria tritici blotch, yellow rust and brown rust resistance genes.

Deliverable 2. Quantification of yield penalties associated with defeated resistance genes which are widely distributed in current UK wheat varieties, but no longer effective.

Deliverable 3. Identification and optimisation of methods to screen future resistance genes for yield penalties, either by direct measurement of stomatal dysfunction or by inference from their mode of operation.

Deliverable 4. Assessment of the scope to use fungicides to ameliorate costs of resistance, whilst deleterious genes are removed from UK breeding material.

During the course of the project it became evident that although stomatal dysfunction is likely to play a role in the deleterious effects of costs of resistance, it is likely to be one symptom of a wider metabolic disruption. Therefore, the project consortium broadened the remit of the project to investigate a range of physiological variables, in field and controlled environment conditions, which may act as indicators of yield costs, specifically: stomatal dysfunction, decreased radiation use efficiency (as quantified by the slope of the relationship of yield on healthy area duration (HAD) and canopy green area index integrated through the yield forming period; Parker *et al.,* 2004) and changes in plant metabolism associated with fitness costs i.e. measuring phytohormones, phenolics, and metabolites from the citric acid energy cycle. Field trials carried out during the project consisted of resistant and susceptible lines grown in high-risk septoria, yellow rust and brown rust sites. Resistant and susceptible plots were either fully treated with fungicide to control target and non-target disease to establish yield costs in the absence of disease challenge (uninoculated and fungicide treated) or untreated to establish yield costs in the presence of disease challenge (inoculated and no fungicide treatment). Yield, yield components (e.g grains/m^2, ears/m^2, grains/ear), canopy duration and radiation use efficiency were measured to determine fitness costs associated with selected resistance genes.

What outputs has the project delivered?

The key scientific messages are provided below for each of the four deliverables:

Quantification of yield penalties associated with resistance genes (Deliverable 1)

- Significant yield penalties (0.3 – 1.0t/ha) associated with septoria, yellow rust and brown rust resistance genes, were observed in spring and winter wheat during the course of this project. Such losses could have a serious impact on wheat productivity.
- Stacking multiple septoria resistance QTL does incur a significant cost of resistance, however, the yield costs does not appear to be greater than a line containing a single QTL.

- Not all rust resistance genes exhibit a yield cost in the absence of disease. It should be possible for breeders to increase yield, by prioritising the most effective disease resistance genes based on their productivity in both the presence and absence of pathogen challenge.

Quantification of yield penalties associated with 'defeated' resistance genes (Deliverable 2)

- *Lr37* exhibited significant yield costs when tested in three genetic backgrounds.
- Brown rust races dominant in the UK have changed in recent years and *Lr37* appears to be effective against current UK brown rust races and even with a modest epidemic the yield benefit can outweigh the cost.
- Wheat breeders should regularly review their varieties for deleterious yield losses and effectivity of resistance, to prioritise the most efficient resistance in breeding programmes.

Identification and optimisation of methods to screen future resistance for yield penalties, e.g. stomatal dysfunction, metabolic flux (Deliverable 3)

- Yield losses associated with septoria resistance QTL could be successfully quantified by measuring grain yield, grains/m^2, grains/ear, healthy area duration (HAD) and pre-anthesis radiation use efficiency (RUE). However, such methods are labour intensive and would not be suitable to screen for costs of resistance in early pre-breeding programmes.
- Significant stomatal dysfunction was associated with several *Lr* brown rust resistance genes in the presence and absence of challenge.
- Metabolomic analysis of a number of *Lr* resistance genes identified a significant accumulation of metabolites linked to plant pathogen defence. Such changes in metabolic flux may demonstrate a specific cost of resistance in the form of allocation costs, which may also impact stomatal opening and could explain yield losses associated with *Lr37.*
- Metabolomic analysis of key defence and energy metabolism compounds combined with stomatal conductance measurements, could be an effective technique for identifying costs of resistance in pre-breeding programmes. However, more research is required to establish whether clear effects can be reliably identified in pre-breeding genetic material.

Assessment of the scope to use fungicides to ameliorate costs of resistance (Deliverable 4)

- Fungicides can suppress yellow rust germination in susceptible lines to levels similar to resistant wheat lines and yield increases associated with fungicide treatment are predominantly associated with increased canopy duration.
- Certain fungicides were shown to significantly improve 'leaf greening' and photosynthetic rate of the crop canopy, but also an increase in auto fluorescence and localised cell death.

It appears that fungicide treatments can exert physiological effects on the host, many of which are considered to be beneficial to yield. However, more research is required to ascertain whether physiological responses can ameliorate the effects of costs of resistance.

Who will benefit from this project and why?

- Quantification of yield penalties associated with key wheat resistance genes (defeated and non-defeated) will help plant breeders improve varietal yield, allowing them to prioritise resistance genes/QTL with the highest yield in the presence and absence of disease challenge.
- Our consortium has made good progress on identifying screening methods to identify resistance genes which confer yield costs. In particular, metabolomics analysis of key defence and energy metabolism compounds, looks to be a promising technique for identifying material likely to exhibit yield costs in the field. If proven effective, this technique will allow plant breeders to screen their resistant varieties for fitness costs early in the pre-breeding process, allowing them to prioritise the highest yielding, resistant material.
- Farmers will benefit from the availability of commercial varieties with improved resistance, fitness and most importantly improved yield.

AHDBs return on investment:

Results from this project have shown that wheat resistance genes can confer yield costs of between 0.3t/ha – 1t/ha, but not all resistance genes are associated with a yield cost. It should therefore be possible for plant breeders to prioritise the R genes conferring the greatest fitness costs/yield in their breeding programmes, particularly if a costs of resistance screening method is made widely available. Selection of resistance genes with the greatest fitness is likely to provide large improvements in varietal yield. For illustration, if a single gene with a cost of 0.5 tonnes per hectare can be replaced/removed in varieties occupying half of the UK wheat area, then the economic benefit at the

farm gate would be approximately £50 million per annum (in round numbers: 0.5 tonnes per hectare * 1 million hectares * £100 per tonne) and would represent a significant return on the investment by AHDB during the course of this project.

If the challenge has not been specifically met, state why and how this could be overcome
All the project deliverables set out in the original proposal were met in full. However, during the course of the project it became evident that although stomatal dysfunction is likely to play a role in the deleterious effects of costs of resistance, it is just one of many factors involved in a complex story. Therefore, the project consortium broadened the remit of the project to investigate a range of physiological variables which may act as indicators of costs of resistance.

Lead partner	Lucy James and Neil Paveley, ADAS UK Ltd
Scientific partners	IBERS, Aberystwyth University, The John Innes Centre, The University of Nottingham
Industry partners	BASF plc, Delta-T Devices Ltd, RAGT Seeds Ltd, Limagrain UK Ltd, Syngenta Seeds Ltd,
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