Project Report No. 557

Screening for 'costs of disease resistance'

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The contributions of many people to the project are acknowledged, particularly the following: Simon Berry[6], Tim Boor[1], Ruth Bryant[4], Paul Fenwick[6], David Feuerhelm[5], Ed Flatman[6], John Foulkes[7], *Mike Grimmer[5], Peter Jack[4], Chris Nicholl[9], Adam Talbot[1], Steve Waterhouse[8].

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This is the final report of a 48 month project (RD-2009-3670) which started in October 2010. The work was funded by DEFRA and a contract for £97,000 from AHDB Cereals & Oilseeds through the Sustainable Arable LINK Programme.

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1. Abstract

Wheat varieties combining the highest yields 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 creates '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 aim of this project was to identify resistance genes or QTL which do, or do not, exhibit a yield penalty and develop methods to minimise 'yield drag' associated with breeding for disease resistance. Specifically four deliverables were addressed. (1) Quantify yield penalties associated with resistance genes/QTL effective against *Zymoseptoria tritici* (septoria tritici blotch), *Puccinia striiformis* (yellow rust) and *Puccinia triticina* (brown rust); (2) Quantify yield penalties associated with 'defeated' resistance genes/QTL; (3) Identify/optimise methods to screen future resistance genes/QTL for yield penalties; (4) Assess scope for using fungicides to ameliorate deleterious effects of host resistance responses. Significant yield costs associated with genetic resistance to septoria tritici blotch, yellow rust and brown rust were identified. Yield penalties were found to range between 0.3 – 1 t/ha depending on the resistance gene/QTL and genetic background of the variety. Significant yield costs could be quantified by the measurement of yield, healthy area duration (HAD) of the crop canopy and pre-anthesis radiation use efficiency (RUE). Lr37, a 'defeated' brown rust resistance gene, exhibited yield losses in the absence of disease in three genetic backgrounds. Lines containing three septoria resistance QTL did not exhibit significantly greater yield losses than lines containing a single QTL, suggesting that 'stacking' of septoria resistance QTL within a variety may not increase yield costs. Not all resistance genes or QTL tested exhibited deleterious yield effects. It should therefore be possible to prioritise resistance genes in breeding programmes by selecting high productivity in the presence and absence of disease. A significant decrease in stomatal conductance and yield was associated with several *Lr* brown rust resistance genes (inc. *Lr37*) tested in the Thatcher background, in the presence or absence of pathogen challenge. In addition, metabolic analysis identified a number of Lr genes, including Lr37, as being associated with significant metabolic changes even in the absence of pathogen challenge. Detection of changes in host metabolism (from which changes in stomatal conductance may result in some cases) could prove a useful pre-breeding technique to screen for resistance genes which are at risk of exhibiting deleterious yield effects. There was indirect evidence from field trials suggesting that certain fungicides may ameliorate physiological costs of resistance responses, where spore germination is reduced.

2. Introduction

Over the past 60 years there has been a significant increase in wheat productivity in Western Europe, due to improvements in both agronomy and cultivars via plant breeding (Silvey, 1986; Mackay *et al.,* 2011). UK plant breeders have focused their efforts on four main traits; yield, grain quality, agronomic traits (e.g. sowing and harvest dates, vernalisation requirement, plant height and straw length) and lastly, biotic resistance to key pests and diseases (Summers and Brown, 2013). A major contribution to the increased productivity is excellent control of disease due to effective use of fungicides and the use of cultivars carrying resistance to the major foliar diseases such as *Zymoseptoria tritici* (septoria tritici blotch), *Puccinia striiformis* (yellow rust), *Puccinia triticina* (brown rust) and *Blumeria graminis* f.sp. *tritici* (powdery mildew) (Summers and Brown, 2013; Mackay *et al.,* 2011). Host resistance to all diseases in wheat is generally viewed as ranking fourth in importance of breeding target traits, after yield, quality and agronomic traits. However, farmers are increasingly demanding stable resistance in order to reduce expenditure on fungicides and to reduce losses which occur when weather conditions are conducive to disease, but do not permit spray application. At present breeders introgress disease resistance genes into their germplasm in order to meet minimum disease resistance standards required for the UK Recommended List (RL). However, as EU regulations on pesticides and the Sustainable Use Directive (Directive 2009/128/EC) tighten from now until 2020, it is likely that farmers will demand higher and more durable resistance in wheat cultivars, whilst still maintaining productivity.

In their natural environment, plants are under continuous biotic stress caused by a variety of pests and pathogens. Plants defend themselves against pathogens via a two-step mechanism. The first step qualitatively blocks infection, and if this fails, the second step restricts pathogen growth and reproduction in a quantitative manner (Dodds and Rathjen 2010). These defences can be defined as constitutive or inducible. Constitutive defences include structural components and barriers to block infection (Veronese *et al*., 2003; Ferreira *et al.,* 2006) or accumulation of antimicrobial secondary metabolites which provide protection at the outer plant cell layers (Osbourn, 1999). Inducible defences occur after a pathogen attack and have become a major focus of scientific research, with some of the best characterised induced responses found to be triggered by the interaction between host resistance and pathogen-encoded avirulence (*AVR)* genes (Dangle & Jones, 2001; Nùrnberger *et al*., 2004; Ferreira *et al.,* 2006). This interaction often leads to activation of the hypersensitive response, including the elicitation of localised cell death, induction of defence genes and production of antimicrobial secondary metabolites such as phytoalexins (Mur *et al.,* 2008; Dickman & Fluhr, 2013).

Resistance genes can be categorised into two main types:

(i) Qualitative major R genes which are usually responsible for race-specific pathogen recognition and induction of defence mechanisms which provide complete control (Bergelson *et al.,* 2001)
(ii) Quantitative/partial/non-race specific resistance (usually identified as quantitative trait loci; QTL) which are considered to be under polygenic control (Poland *et al.,* 2009).

To achieve more durable disease resistance against the rusts, or to obtain sufficiently effective control where resistance is partial, two or more genes/QTL are often 'pyramided' in a variety for each disease. As result of pyramiding, and the range of pathogen species to be controlled, wheat varieties contain large numbers of resistance genes, some of which remain effective and others (usually major genes) which have been 'defeated' by pathogens evolving virulence (Boyd, 2005). The latter sometimes results in segments of alien chromosome remaining in breeding material, despite their original function having been lost. Resistance genes can be introduced into elite wheat varieties from related grass species (alien introgression) or from diverse hexaploid wheat germplasm. In both cases there is often linkage to undesirable alleles, which has to be minimised in subsequent breeding. In general, current literature suggests that breeding for resistance may have deleterious effects on yield (Bergelsen and Purrington, 1996; Purrington, 2000; Brown, 2002; Brown and Rant, 2013) caused by:

(i) selection for resistant phenotypes during early generations of breeding programmes reducing the number of high yielding lines from which to select in later generations
(ii) linkage between resistance genes and deleterious alleles,
(iii) deleterious effects from introgressing segments of alien chromosome,
(iv) pleiotopic increase in susceptibility to pathogens of a different trophic group (Kliebenstein & Rowe, 2008),
(v) yield costs associated with the energy-costly defences induced by pathogen activation of resistance genes or
(vi) by a physiological cost to the plant directly associated with a resistance gene, even in the absence of pathogen challenge.

The last is particularly difficult to measure and there is conflicting evidence in the literature. However, even if the yield cost of each individual resistance gene is small, the cumulative effect of pyramiding genes in varieties could be a significant constraint on current yield progression. This imposes a commercial limitation on breeding varieties with better disease resistance (Fenwick and Berry, 2008). It is therefore important that breeders strive to understand the complex interaction between disease resistance and productivity, in order to continue to improve yield and grain quality and feed.

Costs of disease resistance

Diseases reduce the fitness of host plants and therefore cause natural selection for disease resistance in host populations. However, not all plants exhibit host resistance to pathogens and there is much debate among evolutionary biologists regarding the existence of susceptible alleles in host populations. One theory is that resistance alleles exhibit fitness costs and the other is that costs of resistance are necessary to account for polymorphism at resistance loci. A cost of disease resistance can be defined as a negative association with another desirable trait, such as yield, quality or agronomic property of a variety in the absence of disease (Brown and Rant, 2013).

Research into fitness costs associated with breeding disease resistant crops has been of interest for many years and several examples of fitness costs associated with disease resistance introgressions in wheat and barley have been identified. *Ym4* in barley, which confers resistance to *barley mild mosaic virus* (BaMMV) and strain 1 of *barley yellow mosaic virus* (BaYMV-1), was found to exhibit a 2% yield penalty across eight trials (Le Gouis *et al.,* 1999). In wheat, *Wsm1,* which confers resistance to *wheat streak mosaic virus,* was identified to have a mean yield reduction of 21% (Sharp *et al.,* 2002) and *Sr26* conferring stem rust resistance was shown to exhibit a 9% yield penalty (Latter, *et al.,* 1998). Both Lr34 (combined with other Lr genes) and Lr9, which confer resistance to brown rust in wheat, exhibit a reduction in grain yield of up to 6% and 12%, respectively (Singh and Huerta-Espino, 1997; Ortelli *et al.,* 1996). In 1996, Bergelson and Purrington carried out a meta-analysis of 88 published studies to estimate the mean fitness of a plant simply containing a resistance gene. It was estimated that the mean fitness cost of a resistance gene is a 3.5% reduction in productivity when compared to susceptible plants. The authors acknowledged a number of limitations to the analysis, but they concluded that the presence of a resistance gene does generally incur a fitness cost to the plant.

Interestingly, not all introgressed segments have proven to incur a yield penalty in wheat. A *T.intermedium* translocation carrying resistance to *barley yellow dwarf virus*, conferred no significant reduction in grain yield, plant biomass or grain size (thousand grain weight, TGW) of uninfected plants (Ayala *et al.,* 2001). In addition, the 1RS chromosome arm from rye, which carries several disease resistance genes (some of them subsequently defeated), is associated with increased yield in the presence and absence of disease (Villareal *et al.,* 1998). A number of powdery mildew resistance genes in barley (Kjaer *et al.,* 1991) and rye (Welz *et al.,* 1995) have also been shown to have no significant deleterious effects on yield. In addition, it has been shown that priming of plant defences by perception of microbes or microbe-associated molecular pattern (MAMP) molecules produces a faster response to subsequent pathogen attack (Conrath, 2011), but has a significantly lower fitness cost than that associated with defences induced in response to chemical elicitors (van Hulten *et al.,* 2006).

Fitness costs associated with disease resistance are typically thought to occur due to the metabolic demands made by defence mechanisms (Walters & Boyle, 2005; Kliebenstein & Rowe, 2008; Herms & Mattson, 1992) and costs of resistance have been linked to altered photosynthesis, respiration or metabolite deficiency, as host resources are subsequently diverted to resistance responses (reviewed in Prats *et al.,* 2013). In addition, studies on the barley-powdery mildew, *Blumeria graminis* f.sp. *hordei* (*Bgh*) pathosystem have highlighted a link between stomata and resistance-derived costs. Work in the Defra Fellowship of Tim Carver (AR0712) at IBERS showed that inoculated barley cv. P01 (*Mla1*, HR-mediated resistance) plants were more susceptible to drought, suggesting a possible effect of disease resistance on the function of stomata. Leaf water conductance and measurement of stomatal apertures found that stomata close to hypersensitive response (HR) cells were unable to close fully (termed 'lock-open') in response to darkness or water stress – despite the stomatal guard cells remaining alive (Prats *et al*., 2006). The association between the resistance (R) gene (*Mla*) and the effect on stomatal conductance appeared to be causal, rather than due to linkage; the effect was confirmed in near-isogenic lines (NILs) in more than one background, and the affected stomata were close to HR cells and became impaired shortly after pathogen challenge. In addition, other researchers have recorded that infections by various pathogens (viruses, biotrophic and necrotrophic fungi and oomycetes) effected stomatal function in a range of crop species (reviewed in Grimmer *et al.,* 2012). Subsequent work by Prats *et al.,* (2007) tested the effect of major R genes in barley and wheat expressing resistance to brown rust (*Puccinia triticina* and *P. hordei*) involving mesophyll cell death and found – conversely to the mildew case – that stomata were unable to open fully in light (termed 'lock-shut'). Such closure is likely to affect both gas exchange and directly impact photosynthetic CO_2 fixation which would lead to conditions of excess excitation energy and increases in oxidative stress (Osmond et al., 1997; Mateo et al., 2004). Stomatal closure would also impede the transpiration stream, resulting in increased canopy temperature, contrasting with higher yielding cereals which are associated with cooler canopies (Fischer *et al.,* 1997). Thus stomatal locking is likely to cause a yield cost associated with resistance particularly in high-light or drought prone environments.

Resistance genes have a clear benefit, through improved disease control and reduced fungicide inputs, which usually outweighs any yield cost (and makes yield costs difficult to detect). However, scientific research has identified numerous resistance genes which are associated with host fitness costs. Such costs of resistance are widely considered to be the result of wider metabolic disruption due to the re-direction of host resources to host defence responses and are also linked to altered photosynthetic activity, respiration and stomatal dysfunction. Consequently, breeding for disease resistance can create 'yield drag' which slows the rate of yield improvement. Therefore, to inform resistance introgression and selection decisions in breeding programmes it would be beneficial to:

(i) Select effective resistance genes with the lowest yield cost,
(ii) Not carry 'defeated' genes which provide no benefit, if they are shown to carry a cost,

(iii) Confirm which deleterious trait is most indicative of costs of resistance in crops,
(iv) Develop a sensitive method to measure deleterious effects associated with particular genes/QTL.

Project Objectives

The objective of this project was to identify resistance genes to septoria and rusts 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 objectives were as follows:

(i) Characterise key disease resistance genes for effects on stomatal function.

(ii) Relate stomatal dysfunction at a leaf level to impacts on radiation use efficiency at a canopy level and grain yield.

(iii) Test whether 'defeated' resistance genes carry a yield cost.

(iv) Test stomatal conductance as an indicator of yield potential in the light-limited environment of the UK.

(v) Test improved porometry methods to increase screening throughput.

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 which may act as indicators of costs of resistance, specifically: stomatal dysfunction, decreased radiation use efficiency (as indicated by the slope of the relationship of yield on healthy area duration (HAD; 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. Therefore this report will look broadly at indicators for costs of resistance and is structured based on the four original deliverables of the project:

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

(ii) Quantification of yield penalties associated with defeated resistance genes which are widely distributed in current UK wheat varieties, but no longer effective (and could hence be removed)

(iii) 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.

(iv) Assessment of the scope to use fungicides to ameliorate costs of resistance (i.e. stomatal conductance and yield), whilst deleterious genes are removed from UK breeding material.

This project was a large collaborative project with significant contributions from nine different academic and industrial partners. Delivery of the project was carried out as follows:

- Yield trials required to quantify yield penalties associated with 'defeated' and effective brown rust, yellow rust and septoria resistance genes were delivered by industrial partners RAGT Seeds, Limagrain and Syngenta Seeds and ADAS (Deliverables 1 & 2).
- Physiological field and controlled environment trials to establish indicators of costs of resistance, were delivered by academic partners IBERs, Aberystwyth University, The John Innes Centre, The University of Nottingham and ADAS (Deliverable 3).
- Physiological field trial and controlled environment studies to determine the scope of using fungicides to ameliorate costs of resistance were delivered by academic partner IBERS, Aberystwyth University and industrial partner BASF and ADAS (Deliverable 4).

3. Materials and Methods

3.1. Plant material

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

Yield penalties were measured by comparison between wheat lines with and without the resistance gene or QTL of interest. Where near-isogenic lines contrasting for the relevant genes were available, these were used, even where the background cultivar was not bred for the UK environment. Development of NILs contrasting for the resistance QTL tested would have delayed the start of the work by some years, so mapping population lines were selected which contrasted for presence or absence of the resistance allele. Two approaches were used. Firstly, a small sub-set of lines were selected from each population for detailed phenotyping for yield penalty and physiological variables. Secondly, where possible, the findings from the sub-set of lines were corroborated by analysis of yield data from the whole population.

Option x Claire (OxC) yellow rust doubled-haploid (DH) mapping population (Winter wheat, 2D and 4D)

Lines carrying yellow rust resistance loci on 2D and 4D (donor = Claire) separately and in combination were provided by RAGT alongside the corresponding null line.

Avalon x Cadenza (AxC) DH mapping population (winter wheat, yellow rust 2B & 6B resistance QTL)

The AxC population of DH individuals were provided from Rothamsted Research (WGIN), which were derived from F1 progeny of a cross between cultivars Avalon and Cadenza (developed by Clare Ellerbrook, Liz Sayers and the late Tony Worland (John Innes Centre), as part of a Defra funded project led by ADAS). The parents were originally chosen (to contrast for canopy architecture traits) by Steve Parker (CSL), the late Tony Worland and Darren Lovell (Rothamsted Research).

Four pairs of AxC lines contrasting for two yellow rust resistance loci (identified during the project; see 4.1.1) which mapped to chromosome 2B and 6B (separately and in combination) were selected. All pairs were also matched based on similarity of phenotypic traits such as mean plant height, presence/absence of dwarfing alleles at the *Rht8* and *Rht2* loci mapping to chromosome 2D and 4D, respectively and early or late anthesis (see Table 1 for details).

YrQ Alcedo NILs (winter wheat, yellow rust 2D and 4B resistance QTL)

Near-isogenic lines (Brigadier background) carrying yellow rust resistance genes on chromosome 2D and 4B (from Alcedo) and the corresponding susceptible control, were provided by Limagrain for testing during this project.

Hereford x Player (HxP) brown rust resistant DH mapping population (winter wheat)

Fourteen lines matched into seven pairs contrasting for alleles of the brown rust resistance locus on 2A (Player as donor) were selected from the winter wheat HxP DH mapping population, provided by Syngenta UK.

Lr brown rust resistance genes (spring wheat Thatcher NILs)

Twenty-six near-isogenic lines (NILs) carrying different Lr resistance genes (and recurrent Thatcher parent (TcS)) in the Thatcher background (spring wheat) were provided by Jim Kolner (USDA). Development of the near-isogenic lines of the bread wheat cultivar Thatcher was carried out by Dyck and Samborski (1970), Samborski and Dyck (1976) and Dyck (1977). Dominant leaf rust resistance genes (Lr) were introduced by backcrosses into the recurrent parent Thatcher. See Table 2 for further details.

Table 1. Wheat lines from Avalon x Cadenza mapping population matched for contrasting 2B or 6B yellow rust resistance allele and matching phenotypic and genotypic traits

	Pair	AxC	YR resistance Ch 2B	YR resistance Ch 6B	Mean YR %	Spring/Winter	Rht8 wPt-9997 2D	Rht2 RhtMrkD 1 4D	Height wmc505b 3A	Anthesis wPt-6560 1Dt	Mean Height (cm; JIC data)
2B	**1**	31	Res.	Susc.	0.7	Winter	Res/Tall	Short	Tall	Late	87.83
		181	Susc.	Susc.	3.0	Winter	Res/Tall	Short	Short	Late	86.76
	2	52	Res.	Susc.	1.0	Spring	Res/Tall	Short	Tall	Late	78.36
		96	Susc.	Susc.	3.3	Spring	Susc/Short	Tall	Tall	Late	77.89
	3	167	Res.	Susc.	0.3	Spring	Susc/Short	Short	Short	Late	66.63
		193	Susc.	Susc.	1.7	Spring	Susc/Short	Short	Short	Early	64.25
	4	185	Res.	Susc.	0.3	Winter	Susc/Short	Short	Tall	Early	81.31
		3	Susc.	Susc.	5.3	Winter	Susc/Short	Short	Tall	Late	80.89
6B	1	30	Susc.	Res.	0.3	Winter	Susc/Short	Short	Tall	Late	71.74
		25	Susc.	Susc.	3.0	Winter	Susc/Short	Short	Tall	Late	71.55
	2	138	Susc.	Res.	1.0	Spring	Res/Tall	Short	Short	Late	74.75
		186	Susc.	Susc.	5.0	Spring	Res/Tall	Short	Short	Early	76.86
	3	184	Susc.	Res.	1.0	Winter	Susc/Short	Short	Short	Late	79.13
		3	Susc.	Susc.	5.3	Winter	Susc/Short	Short	Tall	Late	80.89
	4	192	Susc.	Res.	0.3	Spring	Susc/Short	Short	Short	Early	67.25
		196	Susc.	Susc.	8.7	Spring	Susc/Short	Short	Short	Early	68.18
2B & 6B	1	27	Res	Res	0.3	Spring	Res/Tall	Short	Short	Late	69.83
		130	Susc	Susc	3.0	Spring	Res/Tall	Short	Short	Late	65.68
	2	47	Res	Res	0.3	Winter	Res/Tall	Short	Short	Early	73.25
		175	Susc	Susc	3.7	Winter	Res/Tall	Short	Short	Early	73.68
	3	61	Res	Res	1.0	Winter	Susc/Short	Short	Tall	Late	77.25
		204	Susc	Susc	2.0	Winter	Susc/Short	Short	Tall	Late	79.44
	4	199	Res	Res	0.3	Spring	Susc/Short	Short	Short	Late	73.45
		176	Susc	Susc	3.7	Spring	Susc/Short	Short	Short	Late	70.31

Table 2. Thatcher NIL wheat varieties carrying brown rust resistance alleles ('Lr gene'). 'Expression' indicates whether the allele confers seedling resistance (SR) or adult plant resistance (APR) to brown rust.

Gene	Expression	Origin	Used in UK
TcS	Susceptible	Wheat	
LrB	SR	Wheat	
Lr1	SR	Wheat	Yes
Lr2a	SR	Wheat	
Lr2c	SR	Wheat	
Lr3	SR	Wheat	
Lr3bg	SR	Wheat	
Lr3ka	SR	Wheat	
Lr9	SR	Alien	
Lr10	SR	Wheat	Yes
Lr11	SR	Wheat	
Lr12	APR	Wheat	
Lr13	APR	Wheat	Yes
Lr14a	SR	Alien	Yes
Lr14b	SR	Wheat	
Lr16	SR	Wheat	
Lr17	SR	Wheat	Yes (*Lr17b*)
Lr18	SR	Alien	
Lr20	SR	Wheat	
Lr22a	APR	Alien	
Lr24	SR	Alien	
Lr26	SR	Alien	Yes
Lr28	SR	Alien	
Lr30	SR	Wheat	
Lr34	APR	Wheat	
Lr35	APR	Alien	
Lr37	APR	Alien	Yes

AxC DH mapping population (winter wheat, 3B, 5A and 5D septoria resistance QTL)

During a previous ADAS led project, QTL controlling septoria disease, canopy and harvest traits were identified using phenotypic data generated by ADAS and AxC marker data provided by the WGIN project (Grimmer *et al.,* unpublished data). Three QTL controlling septoria severity were selected for further analysis during this project. Four pairs of lines were selected which contrasted for each of the novel loci separately and in combination (ch 3B (early severity trait), 5A (late severity trait) and 5D (late severity trait). All pairs were matched where possible by phenotypic traits such as mean plant height and presence or absence of dwarfing alleles at the *Rht8* and *Rht2* loci mapping to chromosome 2D and 4D, respectively (see Table 3 for details).

Table 3. Wheat varieties from Avalon x Cadenza mapping population matched for contrasting 3B, 5A or 5D septoria resistance allele and matching phenotypic and genotypic traits

	Pair	AxC	STB early 3B	STB late 5A	STB late 5D	Rht8 wPt-9997 2D	Rht2 RhtMrkD 1 4D	Height wmc505b 3A	Mean Height (JIC)	Spring/Winter
3B	1	119	Res.	Susc.	Susc.	Susc./Short	Tall	Short	71.58	Spring
		159	Susc.	Susc,	Susc.	Susc./Shorty	Short	Tall	72.88	Spring
	2	186	Res.	Susc.	Susc.	Short	Short	Short	76.86	Spring
		202	Susc.	Susc.	Susc.	Tall	Tall	Short	78.56	Spring
	3	187	Res.	Susc.	Susc.	Tall	Tall	Tall	83.18	Winter
		3	Susc.	Susc.	Susc.	Short	Short	Tall	80.89	Winter
	4	138	Res.	Susc.	Susc.	Short	Short	Short	74.75	Spring
		70	Susc.	Susc.	Susc.	Short	Short	Short	73.56	Spring
5A	1	122	Susc.	Res.	Susc.	Susc./Short	Tall	Tall	78.89	Spring
		3	Susc.	Susc.	Susc.	Susc,/Short	Short	Tall	80.89	Winter
	2	63	Susc.	Res.	Susc.	Susc./Short	Tall	Short	69	Winter
		35	Susc.	Susc.	Susc.	Susc./Short	Short	Short	69	Winter
	3	12	Susc.	Res.	Susc.	Susc./Short	Tall	Tall	87.58	Winter
		8	Susc.	Susc.	Susc.	Res./Tall	Short	Tall	82.61	Spring
	4	57	Susc.	Res.	Susc.	Susc./Short	Tall	Tall	78.43	Winter
		202	Susc.	Susc.	Susc.	Res./Tall	Tall	Short	78.56	Spring
5D	1	152	Susc.	Susc.	Res.	Res./Tall	Short	Tall	81.24	Spring
		8	Susc.	Susc,	Susc,	Res/Tall	Short	Tall	82.61	Spring
	2	25	Susc.	Susc.	Res.	Susc./Short	Short	Tall	71.55	Winter
		159	Susc.	Susc.	Susc.	Susc./Short	Short	Tall	72.88	Spring
	3	96	Susc.	Susc.	Res.	Susc./Short	Tall	Tall	77.89	Spring
		202	Susc.	Susc.	Susc.	Res./Tall	Tall	Short	78.56	Spring
	4	89	Susc.	Susc.	Res.	Susc./Short	Tall	Tall	82.06	Winter
		3	Susc.	Susc.	Susc.	Susc./Short	Short	Tall	80.89	Winter
3B, 5A & 5D	1	60	Res.	Res.	Res.	Short	Tall	Short	77.4	Winter
		202	Susc.	Susc.	Susc	Tall	Tall	Short	78.6	Spring
	2	83	Res.	Res.	Res.	Tall	Tall	Short	88.7	Winter
		41	Susc.	Susc.	Susc.	Tall	Tall	Tall	91.1	Spring
	3	127	Res.	Res.	Res.	Tall	Short	Tall	81.9	Winter
		8	Susc.	Susc.	Susc.	Tall	Short	Tall	82.6	Spring
	4	204	Res.	Res.	Res.	Short	Short	Tall	79.4	Winter
		3	Susc.	Susc.	Susc.	Short	Short	Tall	80.9	Winter

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

Option x Potent (OxP) DH mapping population (winter wheat Lr37+/- matched pairs)

Eight lines were selected from the OxP DH mapping population and were matched into four pairs, contrasting for the Lr37 brown rust resistance locus. Individual pairs may vary for some background loci, so to limit the effect of such differences multiple pairs were assessed, with lines matched as

closely as possible based on their genotypic and phenotypic similarity. OxP pairs were matched based on similarity of height (cm), date of ear emergence (days from earliest line) and the absence of the high yielding 1RS loci (see Table 4 for further details).

Table 4. Wheat varieties from Option x Potent mapping population matched for contrasting Lr37 brown rust resistance allele and matching phenotypic and genotypic traits

Pair	OxP Code	Gene	1BS (=A), 1RS (=B),	Lr37 Res (Potent) or Sus (Option)	Height 2006 (cm)	Ear Emergence mean (2006 + 2007) (days from earliest line)
1	11	Lr37 +	A	R	80.0	4.75
	4	Lr37 -	A	S	80.5	4.0
2	57	Lr37 +	A	R	79.8	4.25
	75	Lr37 -	A	S	78.5	4
3	78	Lr37 +	A	R	77.8	4
	77	Lr37 -	A	S	78.5	4.5
4	22	Lr37 +	A	R	72.8	2.75
	54	Lr37 -	A	S	78.5	3.5

Lr37+/- Viscount (Winter wheat)

The cultivar Viscount +/- the brown rust Lr37 resistance gene was provided by Limagrain UK, for use during this project.

3.2. Sites and experimental design

3.2.1. Experimental design and treatments of ADAS phenotypic core field trials

Current evidence suggests that resistance genes/QTL differ in the circumstances in which a physiological effect is expressed, specifically:

(i) In the presence of challenge from avirulent strains of the pathogen, which elicit a resistance response.

(ii) In the absence of challenge from avirulent strains.

The latter may be due to a pleiotropic linkage cost to the plant from carrying the gene, or challenge by non-host air spora eliciting a costly resistance response

In order to distinguish between these possibilities all ADAS phenotypic core field trials were treated as follows:

- Contrasting inoculum density, to generate differing levels of resistance responses, was achieved by presence ('challenged' treatments) or absence ('unchallenged' treatments) of artificial inoculation, applied directly and/or via spreader plots. Target and non-target diseases were controlled as required for challenged and unchallenged treatments using appropriate fungicides, as stated in the methods sections below.

A split-plot design was used for all ADAS phenotypic core field trials. The main plot factor was '+/- disease challenge' and the sub-plot factor was 'line'. Each trial contained four replicates, with plot sizes of 12m x 2m. Wide guard areas of resistant crops were used to minimise inoculum cross-contamination between 'challenged' and 'unchallenged' main plots. Plots of wheat were drilled at a viable seed rate of 400 seeds m^{-2}. Fertilizer N was applied at rates recommended for high yielding crops based on previous cropping and/or soil analysis. Fertilizer P, K and S were applied according to soil mineral analysis and anticipated crop demand. Micronutrients, herbicides and insecticides were applied to all plots as per standard farm practice to avoid nutrient deficiency and providing robust weed and pest control. PGRs were applied as standard practice.

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

AxC DH mapping population (winter wheat, yellow rust 2B & 6B resistance QTL)

ADAS core field trials

A field experiment testing matched pairs contrasting for alleles of 2B and 6B QTL in combination were conducted in 2014 at ADAS, Terrington (Norfolk). The pathosystem used to test for yield costs associated with disease resistance was winter wheat – yellow rust (*Puccinia striiformis*). The experimental design was set out as described in 3.2.1. Spreader plots of susceptible cultivar were sown between each 'challenged' plot and around each block of 'challenged' plots. All lines were tested in the presence and absence of yellow rust challenge to generate differing levels of resistance responses. Contrasting treatments ('challenged vs unchallenged') were achieved via natural infection of yellow rust and appropriate fungicide programmes. High risk sites for natural epidemics of yellow rust were selected, with a one year break from cereal crops. Non-target diseases such as powdery mildew and septoria tritici blotch (*Zymoseptoria tritici)* were controlled in all of the experimental plots using cyflufenamid (Cyflamid @ 0.5 l ha^{-1}; GS 30/59) and chlorothalonil (Bravo @ 2.0 l ha^{-1}; GS30 and 1.0 l ha^{-1}; GS30, 32, 39 and 59), respectively. Brown rust and yellow rust was controlled within the 'unchallenged' plots (uninoculated/treated) using tebuconazole (Folicur @ 0.5 l ha^{-1}; GS30, 32, 39 and 59) and yellow rust 'challenged' plots were left untreated.

Industry partner trials

Yield trials consisting of Avalon x Cadenza wheat lines matched for presence or absence of yellow rust resistance alleles on chromosomes 2B and 6B were conducted in Cambridgeshire by Limagrain (2012) and RAGT (2012 & 2013). The experimental design was a randomised block using split plots, with disease/fungicide treatment as main plots and matched pairs and gene (R or S) as subplots. All lines were tested in the presence and absence of yellow rust challenge to generate differing levels of resistance responses. Contrasting treatments ('challenged vs unchallenged') were achieved via natural infection of yellow rust and application of appropriate fungicides for control of target and non-

target diseases.

Option x Claire double haploid mapping population (2D and 4D)

Industry partner trials

Yield trials consisting of Option x Claire lines differing in yellow rust resistance 2D and 4D QTLs were conducted in Cambridgeshire by Syngenta and RAGT (2014). All target and non-target diseases were controlled in all plots using an appropriate fungicide programme, to achieve optimal yields in all selected lines. The trial design was set out as a randomised block of six replicates.

YrQ Alcedo NILs (winter wheat, yellow rust 2D and 4B resistance QTL)

Industry partner trials

Yield trials consisting of YrQ Brigadier NILs differing in yellow rust resistance 2D and/or 4B QTL were conducted in Cambridgeshire by Limagrain (2014). All target and non-target diseases were controlled in all plots using an appropriate fungicide programme, to achieve optimal yields in all selected lines. The trial design was set out as a randomised block of six replicates.

Hereford x Player mapping population (winter wheat)

Industry partner trials

Yield trials consisting of Hereford x Player matched pairs, differing for brown rust resistance allele at 2A, were conducted in Cambridgeshire by Syngenta (2013 & 2014). All target and non-target diseases were controlled in all plots using an appropriate fungicide programme to achieve optimal yields in all selected lines. The trial design was set out as a randomised block of six replicates.

Lr brown rust resistance genes (spring wheat; Thatcher NILs)

ADAS core field trials

All NILs were multiplied as single plots between 2010 and 2013, with yield being measured in 2013. One replicated yield trial (24 m^2 plots in a randomised block design) was conducted in 2014 at ADAS Terrington (Norfolk) to identify yield costs associated with major Lr brown rust resistance genes. All lines were tested in the absence of brown rust challenge using appropriate fungicides to control target and non-target diseases. PGRs were applied as standard practice. Thatcher NILs included in the multiplication yield trial and replicated yield trial can be seen in Table 2.

AxC DH mapping population (winter wheat, 3B, 5A and 5D septoria resistance QTL)

ADAS core field trials

A field experiment testing matched pairs contrasting for alleles of 3B, 5A, 5D septoria resistance QTL in combination were conducted in 2011 at ADAS Rosemaund (Herefordshire). The pathosystem

used to test for yield costs associated with disease resistance was winter wheat – Septoria tritici blotch (*Zymoseptoria tritici*). The general experimental design was set out as described in 3.2.1. Spreader plots of susceptible cultivar were sown between each inoculated plot and around each block of inoculated plots. All lines were tested in the presence and absence of septoria challenge to generate differing levels of resistance responses. Contrasting treatments ('challenged vs unchallenged') were achieved via natural infection of septoria tritici blotch and application of epoxiconazole and metaconazole (Brutus @ 2.0 l/ha at GS31, GS32, GS33 and GS39) on 'unchallenged' plots. High risk sites for natural epidemics of septoria tritici blotch were selected, with a one year break from cereal crops. Non-target diseases such as powdery mildew were controlled in all of the experimental plots using cyflufenamid and proquinazid (Talius @ 0.25 l/ha at GS31; Cyflamid @ 0.5 l/ha at GS39). Yellow rust (*Puccinia striiformis*) and brown rust (*Puccinia triticina*) were controlled when necessary using tebuconazole (Folicur @ 0.25 l/ha).

Industry partner trials

Yield trials consisting of Avalon x Cadenza wheat lines matched for presence or absence of septoria resistance alleles on chromosomes 3B, 5A2 and 5D were conducted in the Cambridgeshire area by Limagrain in 2013. The experimental design was a randomised block using split plots, with disease/fungicide treatment as main plots and matched pairs and gene (R or S) as subplots. All lines were tested in the presence and absence of septoria challenge to generate differing levels of resistance responses. Contrasting treatments were achieved via natural infection of septoria tritici blotch ('challenged'/untreated plots) and application of appropriate fungicides for control of target and non-target diseases.

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

Option x Potent mapping population (winter wheat Lr37 +/- matched pairs)

ADAS core field trials

Two field experiments were conducted during 2011/2012 and 2013/2014. Both experiments were located at ADAS Boxworth and the pathosystem used to test for yield costs associated with Lr37 disease resistance was winter wheat – brown rust (*Puccinia triticina*). High risk sites for natural epidemics of brown rust were selected, with a one year break from cereal crops.

The design for both experiments was set out as described in 3.2.1. All lines were tested in the presence and absence of brown rust challenge to generate differing levels of resistance responses. Spreader plots of susceptible cultivar were sown between each inoculated plot and around each block of inoculated plots. Contrasting treatments were achieved via natural infection of brown rust or artificial field inoculation using mixed races (provided by NIAB). Brown rust and yellow rust was

controlled within the 'unchallenged'/treated plots using tebuconazole (Folicur @ 0.5 l ha^{-1}; GS30, 32, 39 and 59) and plots 'challenged' with brown rust were left untreated. Artificial inoculation was carried out due to a low brown rust epidemic at the trial locations in 2012 and 2014. Five pots containing actively sporulating seedlings (cv. Target) infected with brown rust (mixed races) provided by NIAB, were placed into each plot within the inoculated treatment. Non-target diseases such as powdery mildew and septoria tritici blotch (*Zymoseptoria tritici*) were controlled in all of the experimental plots using cyflufenamid (Cyflamid @ 0.5 l ha^{-1}; GS 30/59) and chlorothalonil (Bravo @ 2.0 l ha^{-1}; GS30 and 1.0 l ha^{-1}; GS30, 32, 39 and 59), respectively.

Industry partner trials

Yield trials consisting of four OxP matched pairs (Lr37+/-) drilled as 18 m^2 plots were conducted in the Cambridgeshire area by Syngenta (2013), Limagrain (2014) and RAGT (2014). All target and non-target diseases were controlled in all plots using an appropriate fungicide programme to achieve optimal yields in all selected lines (see Table 4 for details of matched pairs). The trial design was set out as a randomised block of six replicates.

Lr37 +/- Viscount (Winter wheat)

ADAS core field trials

Two field experiments were conducted during 2010/2011 and 2011/2012. Both experiments were located at ADAS Boxworth and the pathosystem used to test for yield costs associated with Lr37 disease resistance was winter wheat – brown rust (*Puccinia triticina*).

The design for both experiments was set out as described in 3.2.1. Plots (24 m^2) of winter wheat were drilled at a viable seed rate of 400 seeds m^{-2}. Spreader plots of susceptible cultivars were sown between each inoculated plot and around each block of inoculated plots. Lr37+/- lines were tested in the presence and absence of brown rust challenge to generate differing levels of resistance responses. Contrasting treatments were achieved via natural infection of brown rust or artificial inoculation using mixed races (provided by NIAB). Artificial inoculation was carried out (as described previously) during both experiments due to a low brown rust epidemic at the trial locations in 2011 and 2012. Brown rust and yellow rust was controlled within the 'unchallenged'/treated plots using pyraclostrobin (Comet 200 @ 0.625 l ha^{-1} GS31, 32, and 33) and tebuconazole (Folicur @ 0.5 l ha^{-1}; GS30, 32, 39 and 59) in 2012. 'Challenged' plots were left untreated. Non-target diseases such as powdery mildew and septoria tritici were controlled in all of the experimental plots using cyflufenamid (*2011:* Cyflamid @ 0.5 l ha^{-1} GS31, 0.35 l ha^{-1} GS32/33; *2012:* Cyflamid @ 0.5 l ha^{-1} GS30/59) and chlorothalonil (*2011:* Bravo @ 1.0 l ha^{-1} GS30, 31, 32, 33, 39; *2012*: Bravo @ 2.0 l ha^{-1};GS30 and 1.0 l ha^{-1};GS30, 32, 39 and 59), respectively.

Industry partner trials

Yield trials consisting of Lr37+/- Viscount lines were drilled as 18 m^2 plots were conducted in Cambridgeshire by Syngenta (2013), Limagrain (2014) and RAGT (2014). All target and non-target diseases were controlled in all plots using an appropriate fungicide programme, to achieve optimal yields in all selected lines (see 3.1.1 for details of matched pairs). The trial design was set out as a randomised block of six replicates.

3.2.4. Identification and optimisation of methods to screen future resistance genes for yield penalties (Deliverable 3)

Porometry of wheat infected with septoria tritici blotch (JIC, Norwich)

Porometry experiments were carried out on plants infected with *Zymoseptoria tritici*, the fungus which causes septoria tritici leaf blotch (STB) using the isolates IPO323 and IPO88004. The wheat cultivars used formed a balanced set of varieties resistant and susceptible to these isolates (Table 5).

Table 5. Wheat cultivars used in experiments on porometry of plants infected by Zymoseptoria tritici (septoria tritici blotch). [1] Arraiano and Brown (2006).

	Growth	Response to *Zymoseptoria tritici* Isolate[1]	
Baldus	Spring	Susceptible	Resistant
Flame	Winter	Resistant	Susceptible
Longbow	Winter	Susceptible	Resistant
Maris Dove	Spring	Resistant	Susceptible

Porometry experiments with barley mildew used the cultivar Pallas with the mildew-resistance gene *Mla8*, and lines P-01 (*Mla1*) and P-10 (*Mla12*; Kølster *et al.* 1986), inoculated with *Blumeria graminis* f.sp. *hordei* (*Bgh*) isolate CC66, which is virulent to *Mla8* but avirulent to *Mla1* and *Mla12*. Inoculated plants were compared with uninoculated control plants. Between 4 and 8 leaves were assessed per combination of plant line and treatment.

Z. tritici treatments and mock treatments were applied by spray inoculation (Arriano *et al.* 2001). The first set of experiments, conducted on STB alone, used a standard method of inoculating plants with *Z. tritici*. All four wheat varieties were used. The four treatments used were: no spray at all, mock-inoculation with a solution of 0.01% Tween 20 in water, inoculation with IPO323 at a density of 10^7 spores ml^{-1} suspended in water with 0.01% Tween 20, and a similar inoculation with IPO88004. Following inoculation, plants were covered with black polythene sheets for 48h to maintain humidity and promote fungal infection.

In the second set of experiments, in which both STB and barley mildew were studied, six treatments

were applied: water, IPO323 or IPO88004 with Tween 20, or the same three treatments without Tween 20. Only the varieties Flame and Longbow were used. Between 7 and 12 plants were used per combination of line and treatment in each experiment.

Plants were grown in a controlled environment (CE) chamber with 18°C day, 12° night and a 16/8 hour day/night cycle. They were inoculated with *Z. tritici* at 14 days old, covered with black plastic to maintain humidity, and kept in the dark for the following 48h to promote infection. In the first set of experiments, stomatal conductance (SC) was measured with a porometer (AP4 model, Delta-T Devices Ltd) at 1 day before inoculation (dbi) and 3, 7, 11 or 12 (depending on the experiment), and 16 days after inoculation (dai). Readings were taken mid-way through the light period and 1h after the start of the subsequent dark period. In the second set of experiments, experiments on STB of wheat and mildew of barley were done in the same controlled environment (CE) cabinet at the same time. Porometry readings were taken for all plants 1 dbi in the dark and light periods, then 1 hour before the end of the dark period at 16, 40, 64 and 84 hours after inoculation (hai) and in the middle of the light period at 24, 48 and 72 hai. Further readings on the plants in the STB tests taken 7, 9 and 11 dai, commensurate with the long latent period of *Z. tritici*.

3.2.5. Assessment of the scope to use fungicides to ameliorate yield costs associated with host resistance responses (Deliverable 4)

Plant material

For assessment of stomatal conductance under pathogen attack, three varieties of wheat were used. The Thatcher and Option x Potent (OxP) varieties carry genes for resistance to leaf rust and were therefore used to determine its effects on conductance; the Thatcher variety consists of Near Isogenic Lines and the OxP lines are breeder varieties. The Thatcher variety has a susceptible line (TcS) which was used as a control for comparison in assessment of this variety.

Lines were sown in 50 ml falcon tubes, with drainage holes drilled, in compost (Levington F2) at a rate of two seeds per tube. The tubes were placed in trays of compost to ensure they remained upright at a rate of 15 tubes per tray; these were cultivated in the greenhouse for 10 days, until approximately growth stage 12. The seedlings were then transferred to a growth chamber and separated into two groups; one that remained uninoculated and one that was inoculated with rust spores. The conditions of the growth chamber were set at 14 hours light/8 hours dark; a light period temperature of 21°C and a dark period temperature of 11°C; relative humidity of 50%; PAR of 300.

Inoculation

For inoculation of plant material, spores that had been stored at -80°C were mixed with laboratory grade talcum powder (Sigma) at a ratio of approximately 1:16 (spores:talc). The trays containing the plant material were placed in large clear plastic bags and sprayed with the pre-prepared inoculation material using a pipette tip and a squeezable rubber disperser. The containers were then

sealed in order to create a humid, controlled environment as favoured by rust spores (Hubbard, Amelia, NIAB, February 2014). Plants inoculated with leaf rust were immediately placed back in the same growth chamber as uninoculated plants and remained sealed until 72 hours after infection.

Microphenotyping wheat – fungal interactions using aniline blue staining and microscopy (IBERS, Aberystwyth)

Aniline blue epifluorescence stains the glucan component of fungal cell walls and callose (ß-1-3-glucans) in the plant. Leaves were cut into 1.5 cm lengths and autoclaved at 121°C in 5 ml of 1M KOH for 2 min. The samples were rinsed three times in sterile distilled water and then mounted on glass slides in several drops of stain solution. The stain solution (0.05 % water soluble analine blue dye, No. 12642 George T. Gurr Ltd. London, Leaves were viewed under a Nikon (Diaphot, Nikon, Tokyo, Japan) or Zeiss microscope (Axioplan, Welwyn Garden City, UK) with white light or UV illumination (excitation 355nm, emission 450nm). UV light was also filtered with a rhodamine filter (excitation 530nm, emission 615 nm).

Estimating cell death through electrolyte leakage (IBERS, Aberystwyth)

Estimation of cell-viability by electrolyte leakage was determined by measuring the conductivity of 500 uL10 mM phosphate pH 7 buffer in 24 multi-well dishes (Nunc) containing a leaf explant (1 cm diameter core) using a Horiba conductivity meter (B-173) over a 24 h period.

Assessment of stomatal conductance (IBERS, Aberystwyth)

The rate of plant stomatal conductance was measured using an AP-4 porometer (Delta-T, Cambridge, UK) set to measure conductance in mmol m^{-2} s^{-1}. Readings were taken twice daily, once in the light period and once in the dark period; a period of two hours was left between the end of the previous photoperiod and the taking of readings to ensure stomata were given time react to light alterations. Readings commenced 72 hours after inoculation (hai) and continued to 144 hai; there were seven sample points in total. Eight readings were taken per genotype at each sample point. Readings were taken from the first leaf where possible in order to improve consistency.

Metabolite extraction (IBERS, Aberystwyth)

Leaf samples (40 mg ± 1 mg) were excised from plants and placed into 2 ml microcentrifuge tubes each containing a single acetone washed stainless steel ball. Samples were immediately flash-frozen in liquid nitrogen, homogenised using a ball mill and put on ice. Then 1 ml of chloroform: methanol: dH_2O (1: 2.5: 1) solution was added to each sample. Samples were vortexed in a cold room at 4 °C for 15 min and returned to ice before being centrifuged at 4°C at 5000 x *g* for 3 minutes. Aliquots of the supernatant were then decanted into clean microcentrifuge tubes and dried using rotary evaporator. Once dried, 1 ml of 75% aqueous methanol was added to the samples and they were prepared for flow injection electrospray mass spectrometry (FIE-MS) analysis. FIE-MS analysis was carried out according to the method described by Favé *et al.* 2011.

ADAS/BASF/IBERS controlled environment trials

Two experiments were carried out in 2012, under controlled environment (CE) conditions, using winter wheat cultivar Brigadier and the NIL, YrQ. Seedlings were grown in a nutrient-balanced, peat-based compost (Levington M2) and one seed per pot (5 cm diameter) was sown. No additional inputs were applied. Plants were watered freely throughout the experiment and maintained at 20ºC with ambient relative humidity and 12h photoperiods. Seedlings were grown and maintained in a clean environment in order to test for direct effects of fungicides in the absence of pathogen challenge. The seedlings remained free from disease symptoms for the duration of the experiment. Eight replicates were used and fungicides were applied at the equivalent of 200 litres water ha^{-1}, when the second emerged leaf was fully expanded (Tables 6 and 7). It should be noted that Filan is NOT approved for use as a solo product in wheat and was selected for experimental purposes only, in order to compare two SDHIs directly. The experiment was carried out twice.

A LI-6400XT portable photosynthesis system (Li-Cor) was used to measure stomatal conductance, transpiration rate (mmol m^{-2} s^{-1}) and net photosynthetic rate (µmol m^{-2} s^{-1}) on emerged leaf 2, at five days post fungicide application. One measurement per plant was made during the light period.

Table 6. Treatment list for the controlled environment winter wheat experiments, 2012.

Treatment	Inoculation	Genotype	Fungicide	Rate applied
1	Uninoculated	Brigadier	Untreated	–
2	Uninoculated	Brigadier	Filan	0.35 kg ha-1
3	Uninoculated	Brigadier	Ignite	0.75 l ha-1
4	Uninoculated	Brigadier	Ceriax	1.50 l ha-1
5	Uninoculated	YrQ	Untreated	–
6	Uninoculated	YrQ	Filan	0.35 kg ha-1
7	Uninoculated	YrQ	Ignite	0.75 l ha-1
8	Uninoculated	YrQ	Ceriax	1.50 l ha-1

Table 7. Active ingredients for fungicide products.

Fungicide	Active ingredient	w/w and g/l	Fungicide group
Untreated	–	–	–
Ceriax	Epoxiconazole Pyraclostrobin Fluxapyroxad	4%, 41.6 g/l 6.4%, 66.6 g/l 4.0%, 41.6 g/l	SDHI
Comet	Pyraclostrobin	19.2%, 200.0 g/l	Strobilurin
Filan	Boscalid	50%, 500 g/kg	SDHI
Imtrex	Fluxapyroxad	6%, 62.5 g/l	SDHI
Ignite	Epoxiconazole	8%, 83.0 g/l	Azole

ADAS/BASF core field trials

Field experiments were carried out in 2009-10 at ADAS Boxworth, Cambridgeshire and in 2010-11 at ADAS Rosemaund, Herefordshire, using the winter wheat–yellow (stripe) rust (*Puccinia striiformis* f. sp. *tritici*) pathosystem. Two resistant near-isogenic lines (NILs) YrQ1 and YrQ2, both carrying the same resistance gene and backcrossed into the winter wheat cultivar Brigadier, were challenged by an epidemic of yellow rust, Robigus race. Susceptible cultivar Brigadier was included as a control. Three fungicide treatments, Filan (boscalid, SDHI), Comet (pyraclostrobin, strobilurin) and Imtrex (fluxapyroxad, SDHI) were compared with an untreated control for their effects on stomatal conductance. It should be noted that Filan is NOT approved for use as a solo product in wheat and was selected for experimental purposes only. Fungicide applications in the field experiments were timed to coincide with the emergence of each of the top four culm leaves.

The design for both field ex periments was a randomised block using split plots, with inoculation as main plots and a combination of genotype*fungicide as sub-plots (Table 8). There were four replicates and sub-plot size was approximately 24 m^2. Each group of main plots was separated by a 10 m discard area of resistant cultivar to minimise the pathogen spread between plots of different disease levels. Spreader plots of a susceptible cultivar were sown around each block of inoculated plots.

Resistant YrQ lines 1 and 2 were sown as separate plots in 2010. Evidence gained during 2010 and discussions with wheat breeders suggested there was no reason for the different NILs carrying a resistance allele to behave differently. Thus, for the 2011 experiment seed from NILs YrQ1 and YrQ2 were mixed together prior to drilling to create batches of resistant material, referred to as 'YrQ'. Data values for 2010 are means across both NILs.

Table 8. Treatment list for winter wheat – yellow rust field experiments, 2010 & 2011.

Treatment	Inoculation	Genotype	Fungicide	Rate at each application
1	Inoculated	Brigadier	Untreated	–
2	Inoculated	Brigadier	Comet	0.63 l ha^{-1}
3	Inoculated	Brigadier	Filan	0.35 kg ha^{-1}
4	Inoculated	Brigadier	Imtrex	1.00 l ha^{-1}
5	Inoculated	YrQ	Untreated	–
6	Inoculated	YrQ	Comet	0.63 l ha^{-1}
7	Inoculated	YrQ	Filan	0.35 kg ha^{-1}
8	Inoculated	YrQ	Imtrex	1.00 l ha^{-1}
9	Uninoculated	Brigadier	Untreated	–
10	Uninoculated	Brigadier	Comet	0.63 l ha^{-1}
11	Uninoculated	Brigadier	Filan	0.35 kg ha^{-1}
12	Uninoculated	Brigadier	Imtrex	1.00 l ha^{-1}
13	Uninoculated	YrQ	Untreated	–
14	Uninoculated	YrQ	Comet	0.63 l ha^{-1}
15	Uninoculated	YrQ	Filan	0.35 kg ha^{-1}
16	Uninoculated	YrQ	Imtrex	1.00 l ha^{-1}

In 2011, a supporting field experiment was carried out using spring wheat lines from the Avalon x Cadenza (AxC) doubled-haploid mapping population. Eight resistant and eight susceptible AxC lines were selected using a combination of phenotypic and genotypic data and the lines within each category were matched, as far as possible, for phenotypic factors such as height and flowering date (as outlined in Table 9). Seed from the eight lines was mixed together prior to drilling to create batches of resistant or susceptible material. Pathogen challenge was provided by naturally occurring *Zymoseptoria tritici* causal agent of septoria tritici blotch.

Table 9. Treatment list for spring wheat – septoria field experiment, 2011.

Treatment	Inoculation	Genotype	Fungicide	Rate at each application
1	Inoculated	Susceptible	Untreated	–
2	Inoculated	Susceptible	Comet	0.63 l ha^{-1}
3	Inoculated	Susceptible	Filan	0.35 kg ha^{-1}
4	Inoculated	Susceptible	Imtrex	1.00 l ha^{-1}
5	Inoculated	Resistant	Untreated	–
6	Inoculated	Resistant	Comet	0.63 l ha^{-1}
7	Inoculated	Resistant	Filan	0.35 kg ha^{-1}
8	Inoculated	Resistant	Imtrex	1.00 l ha^{-1}
9	Uninoculated	Susceptible	Untreated	–
10	Uninoculated	Susceptible	Comet	0.63 l ha^{-1}
11	Uninoculated	Susceptible	Filan	0.35 kg ha^{-1}
12	Uninoculated	Susceptible	Imtrex	1.00 l ha^{-1}
13	Uninoculated	Resistant	Untreated	–
14	Uninoculated	Resistant	Comet	0.63 l ha^{-1}
15	Uninoculated	Resistant	Filan	0.35 kg ha^{-1}
16	Uninoculated	Resistant	Imtrex	1.00 l ha^{-1}

3.2.6. Measurements

ADAS core field trials

Background disease

At the time of each fungicide application foliar diseases were assessed on 25 tillers from across all the plots for each genotype. The percentage leaf area affected by each disease was recorded along with the percentage green leaf area, on all leaf layers with green tissue.

Main disease assessment, visual green leaf area and leaf emergence

Disease incidence and severity were assessed on all green leaves on ten randomly selected plants from each plot. Foliar disease severity was assessed as percentage 'pathogen' area (the area containing viable sporulating structures), by individual leaf layer, according to ADAS standard identification keys. Green leaf area was assessed as a percentage of leaf area alongside disease scores. Disease severity and % green leaf area were assessed between GS32 and GS75, see results section for details. At approximately GS75, the stem bases were assessed for penetrating eyespot and the ears assessed for disease which may adversely affect yield. A random sample of 50 shoots

were assessed from across the experimental plots.

Crop measurements

Healthy area duration

The relationship between percent disease severity and yield loss is subject to considerable variation across sites and seasons. Waggoner & Berger (1987) demonstrated for a range of foliar pathogens that effects of disease on yield were consistent if accounted for via the effects on crop canopy green area index, accumulated light interception and the resulting dry matter accumulation and partitioning.

At each main disease assessment in the field experiments the absolute areas of the assessed leaves were measured on a sub-set of four shoots per plot, by the method of Bryson *et al*. (1997). Disease severity data were used to adjust for the total green leaf area and a count of fertile shoots in the form of ears m^{-2} was carried out at GS85, for each plot. This enabled disease to be quantified as disease induced loss of green canopy area and converted to green lamina area indices (planar area of green lamina per unit of ground area). The date of canopy senescence was defined as the date when percentage green leaf area was less than 15% (equating to GAI <1) and was recorded for each plot.

The green leaf area index progress curve was plotted for each plot from GS59 to canopy senescence and the area under the curve was calculated across the top four leaves using the trapezoidal method Parker *et al.,* 2004. This integral was termed healthy area duration (HAD).

Crop growth stage

The growth stage of the crop and any variation within the plots was recorded at each visit using the decimal code (Tottman *et al.,* 1987)

Crop measurements

At harvest maturity all plots were harvested by a plot combine harvester and a sample of grains taken for determination of mean grain weight and moisture content.

Toxicity

Effects attributable to phytotoxicity were assessed within two weeks of treatment application. Effects could potentially include spotting, chlorosis, scorch of foliage or plant growth effects. Assessments were made using a 1–9 scale where 1 was considered a very slight effect and 9 a severe effect.

Meteorological and edaphic records

On the date of fungicide application, precipitation (type and amount in mm) and temperature (max, min and average °C) were recorded at each the site. Any significant change in weather was noted, particularly in relation to time of application and any extreme weather conditions were recorded throughout the trial.

Statistical analyses

Unless otherwise stated, grain yields are expressed on the basis of 85% dry matter. Statistical analysis was by ANOVA for split-plot designs using Genstat 14th edition (VSN International Ltd., Hemel Hempstead, UK). Residuals were checked for homogeneity of variance and normality of distribution and transformed where necessary.

Yellow rust resistance QTL mapping analysis of Avalon x Cadenza DH mapping population

Genetic data for 202 lines of the Avalon x Cadenza DH population was obtained from the Wheat Genetic Improvement (WGIN) website (wgin.org.uk). Segregation data for known genes (e.g. *Glu-D1*) and SSR/DArT molecular markers was obtained, along with their map distances along each linkage group. DArT markers had the prefix wPt. The linkage map was drawn using MapChart software (Voorrips 2001). Linkage group 4BD was known to contain a dwarfing gene and linkage group 5A2 was known to contain a gene determining vernalisation requirement. Linkage groups were tested for the presence of segregating QTL using the interval mapping (Lander and Botstein, 1989; van Ooijen, 1992) function of MapQTL software 4.0 (van Ooijen *et al*., 2002). Default parameters were used except the mapping step size was set to 1.0 cM. The significance threshold for QTL detection on each linkage group was determined by performing 1000 permutations of the marker-trait data ($\alpha = 0.05$) (Churchill and Doerge, 1994; Doerge and Churchill, 1996). Use of a linkage group-wide threshold, as opposed to a higher genome-wide threshold, provides an output of 'suggestive QTL' (van Ooijen, 1999). This is an inclusive method which facilitates the detection of patterns in multiple sets of trait data.

ADAS/BASF field trials

Disease assessment, green leaf area and leaf emergence

Disease incidence and severity was assessed on all green leaves on ten randomly selected plants from each plot, for all foliar diseases present in the field experiments. Disease was assessed as the percentage leaf area affected, according to ADAS standard identification keys. Assessments of remaining green leaf area, as the percentage of leaf area, were carried out alongside disease scores.

In the 2010 winter wheat-yellow rust experiment, full assessments were carried out at GS32 (12 May), GS59 (15 June) and GS71 (28 June). In 2011, assessments for the winter wheat-yellow rust experiment were carried out at GS32 (27 April) and further disease assessments were carried out at GS59 (03 June) and GS71 (22 June). An additional assessment of percentage green leaf area was carried out on 05 July. For the 2011 spring wheat–septoria experiment, assessments were carried out at GS32 (30 May), GS59 (29 June) and GS71 (18 July) with an additional assessment of percentage green leaf area on 01 August.

At GS31 the youngest fully expanded leaf was tagged on 10 plants per genetic line, from across the range of replicates and treatments. For subsequent assessments up to GS39 the leaf number of each tagged leaf was recorded, thus ensuring that each disease assessment could be related to the

position that the leaves would eventually occupy on the mature culm, reported here by eventual leaf (EL) number, where EL1 is the flag leaf, EL2 the next leaf down the stem, etc.

Crop measurements

Healthy area duration

Healthy area duration (HAD) measurements were carried out as outlined in ADAS core field trials.

Canopy 'greenness' measurements

Leaf 'greenness' was measured using a SPAD 502Plus Chlorophyll Meter (Spectrum Technologies Inc.) in both of the winter wheat-yellow rust field experiments described above. Two sets of measurements were made on the flag leaf at timings of 7–10 days post-fungicide application and 21–28 days post application. Care was taken to place the SPAD meter clip in the approximate centre of the leaf but avoiding diseased tissue. Three readings per plot were taken from all plots.

Photosynthetic rate measurements

A LI-6400XT portable photosynthesis system (Li-Cor) was used to measure photosynthetic rate of the flag leaves, on one occasion in June, in 2011. Measurements were taken before visible disease symptoms or natural senescence were present. Three readings per plot were taken.

Stomatal conductance readings

Stomatal aperture, as leaf conductance to water vapour (mmol m^{-2} s^{-1}), was measured using an AP4 cycling porometer (Delta-t Devices, Cambridge) in the three experiments described above. Measurements were made on eventual culm leaf 3 (EL3), EL2 and EL1 (flag leaf).

Readings were made on a leaf layer within two weeks of emergence, before disease symptoms were expressed. All readings were taken from the upper surface and simultaneous light and leaf temperature readings were also taken by the porometer. Higher conductance values equate to open stomata.

In the 2010 winter wheat-yellow rust experiment, five sets of readings were taken from the flag leaf and six sets from EL3. In the 2011 winter wheat–yellow rust experiment, five sets of readings were taken from each of EL1, EL2 and EL3. In the 2011 spring wheat–septoria experiment, eight sets of readings were taken from EL1 and EL2 and three sets were collected from EL3. Mean values for stomatal conductance were calculated from across all of the leaf layers for each experiment and subject to analysis of variance (ANOVA). Each individual set of readings was also analysed using ANOVA.

Yield measurements

Prior to harvest the percentage plot area with lodged or leaning plants was assessed in all plots, in all field experiments. Plots were harvested using a Sampo plot combine harvester and grain yields were measured on approximately 20 m^2 area of each plot. A 1.0 kg sub-sample of grain from each

plot was retained for the determination of moisture content and yields were adjusted to 85% dry matter. Thousand grain weight and specific weight were recorded for each plot.

4. Results and Discussion

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

4.1.1. Yield costs associated with genetic resistance to yellow rust *(Puccinia striiformis)*

Identification of AxC yellow rust resistance QTL

There were 202 DH lines from the Avalon x Cadenza mapping population assessed for yellow rust severity during trials at Limagrain 2010, Syngenta 2010 and RAGT 2012. Crop canopies were assessed for yellow rust severity using a 0–10 score and data transformed (log10) when a normal distribution was not observed. Assessments were taken during the month of June, to achieve an early, mid and late assessment in the Limagrain and RAGT trial. Only one assessment was taken during the Syngenta trial in 2010. Genetic data for 202 lines of the Avalon x Cadenza DH population was obtained from the Wheat Genetic Improvement (WGIN) website (wgin.org.uk) and available markers (317 markers covering a total of 1735.4cM with an average distance between markers of ~5.5 cM) analysed for association with resistance to YR using composite interval mapping.

Additive effects and t-test P values for markers underlying yellow rust resistance QTL LOD peaks (using a threshold of LOD score 2.5) as determined by composite interval mapping and interval mapping analysis across the 3 trials (Limagrain 2010, Syngenta 2010 and RAGT 2012), can be seen in Table 10. A number of suggestive QTL on chromosomes 2A, 2B, 3B, 4B1, 5A1, 5A3, 5D, 6A and 6B were found, several of which were corroborated through growth stages and/or across sites. QTL mapped to chromosomes 2B and 6B were considered particularly consistent across sites, seasons and growth stages and exhibited the highest level of significance across the assessments. AxC lines carrying the resistance allele of both QTL separately and in combination, showed significantly lower yellow rust severity (<0.001) in 2010 industrial trials (Figure 1). Yellow rust resistance QTL identified on chromosomes 2B and 6B were therefore selected for further analysis during the project, to corroborate the yellow rust resistance observed and to establish whether yield costs are associated with the resistance QTL.

Table 10. Additive effect (and t-test P value) for markers underlying yellow rust resistance QTL LOD peaks as determined by composite interval mapping and interval mapping analysis across 3 trials (Limagrain 2010, Syngenta 2010 and RAGT 2012). For the Limagrain and RAGT trials, assessments were made at an early and late point in the infection. Yellow signifies P <0.05, orange signifies P <0.001 based on t-test of presence or absence of resistance allele.

	Chromosome arm								
	2A	2B	3B	4B1	5A1	5A3	5D	6A	6B
Lim 2010 early	0.25 (0.007)	0.60 (<.001)	-0.10 (0.2)	0.30 (0.003)	0.30 (0.001)	-0.15 (0.2)	-0.15 (0.1)	0.25 (0.02)	0.30 (0.009)
Lim 2010 late	0.35 (0.01)	0.40 (<.001)	-0.20 (0.2)	0.40 (0.004)	0.30 (0.04)	-0.30 (0.08)	-0.25 (0.07)	0.45 (0.005)	0.40 (0.004)
Syn 2010	0.35 (0.01)	0.95 (<.001)	-0.05 (0.6)	0.40 (0.002)	0.30 (0.02)	-0.30 (0.03)	-0.25 (0.07)	0.25 (0.08)	0.45 (<.001)
RAGT 2012 early	0.10 (0.1)	0.15 (0.02)	-0.25 (<.001)	0.10 (0.1)	0.10 (0.1)	-0.25 (<.001)	-0.20 (<.001)	0.30 (<.001)	0.00 (0.9)
RAGT 2012 late	0.20 (0.1)	0.30 (0.004)	-0.25 0.01	0.10 (0.4)	0.30 (0.009)	-0.40 (<.001	-0.30 (0.007)	0.50 (<.001)	0.20 (0.03)

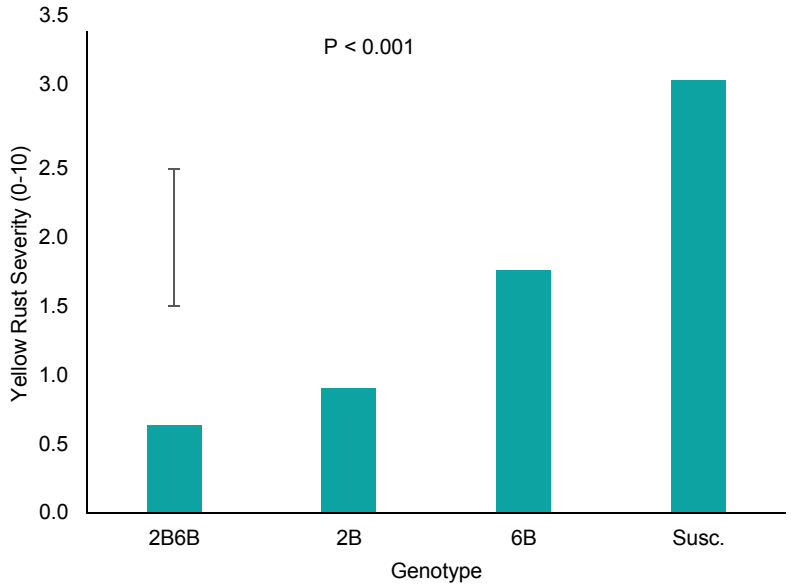


Figure 1. Severity of yellow rust (0–10 scale) of AxC wheat varieties carrying resistance alleles on chromosome 2B, 6B or both in trials at Limagrain (2010) and Syngenta (2010) (P = <0.001). Vertical bar represents least significant difference at 5% level from an unbalanced ANOVA.

Yield costs associated with Avalon x Cadenza 2B and 6B yellow rust resistance QTL

Sixteen lines were selected from the AxC DH mapping population and were matched into eight pairs, four pairs contrasting for each of the two alleles, as outlined in Table 1. A replicated yield trial was conducted by Limagrain (2012) and RAGT (2012 and 2013) to test the 2B and 6B AxC matched pairs for yield costs. The trial consisted of 'challenged' (inoculated and fungicide untreated) and 'unchallenged' (uninoculated and fungicide treated) plots of each matched pair (resistant and susceptible lines). The lines 2B and 6B +/- were tested in the presence and absence of yellow rust challenge to generate differing levels of resistance responses.

A significant difference in yield (mean values of fungicide treated and inoculated plots) was identified between plots contrasting for 2B and 6B yellow rust resistance, with plots carrying 2B and 6B resistance alleles exhibiting a significant yield benefit of 0.428 t/ha and 0.804 t/ha, respectively, when compared to those carrying the susceptible alleles (Table 11). Data was analysed by cross-site ANOVA. The yield benefit associated with 2B resistance was observed in two out of the four matched pairs, with pair 1 and 4 showing increased average yield (t/ha) in the resistant lines in the presence or absence of disease. In addition, the yield benefit associated with 6B resistance was evident across all four matched pairs with resistant treated and untreated plots exhibiting higher yield than the corresponding susceptible lines (Figure 2). Interestingly, this suggests that the lines carrying the 2B and 6B resistance alleles are inherently higher yielding than those carrying the susceptible alleles irrespective of yellow rust challenge. 'Unchallenged' (uninoculated/treated) plots exhibited significantly higher yield than 'challenged' (inoculated/untreated) plots (P = 0.004), suggesting a good level of disease challenge in inoculated/untreated plots throughout the trial. Significant differences in yield between matched pairs were also identified (P = <0.001) for both the 2B and 6B alleles, suggesting that the matched pairs did vary in their yield performance. No Treatment x Pair x Gene interactions were observed for 2B or 6B matched pairs (Figure 2). In summary, trials carried out by Limagrain and RAGT showed a significant yield benefit associated with 2B and 6B resistance alleles, suggesting that no cost of resistance is associated with the yellow rust resistance QTL.

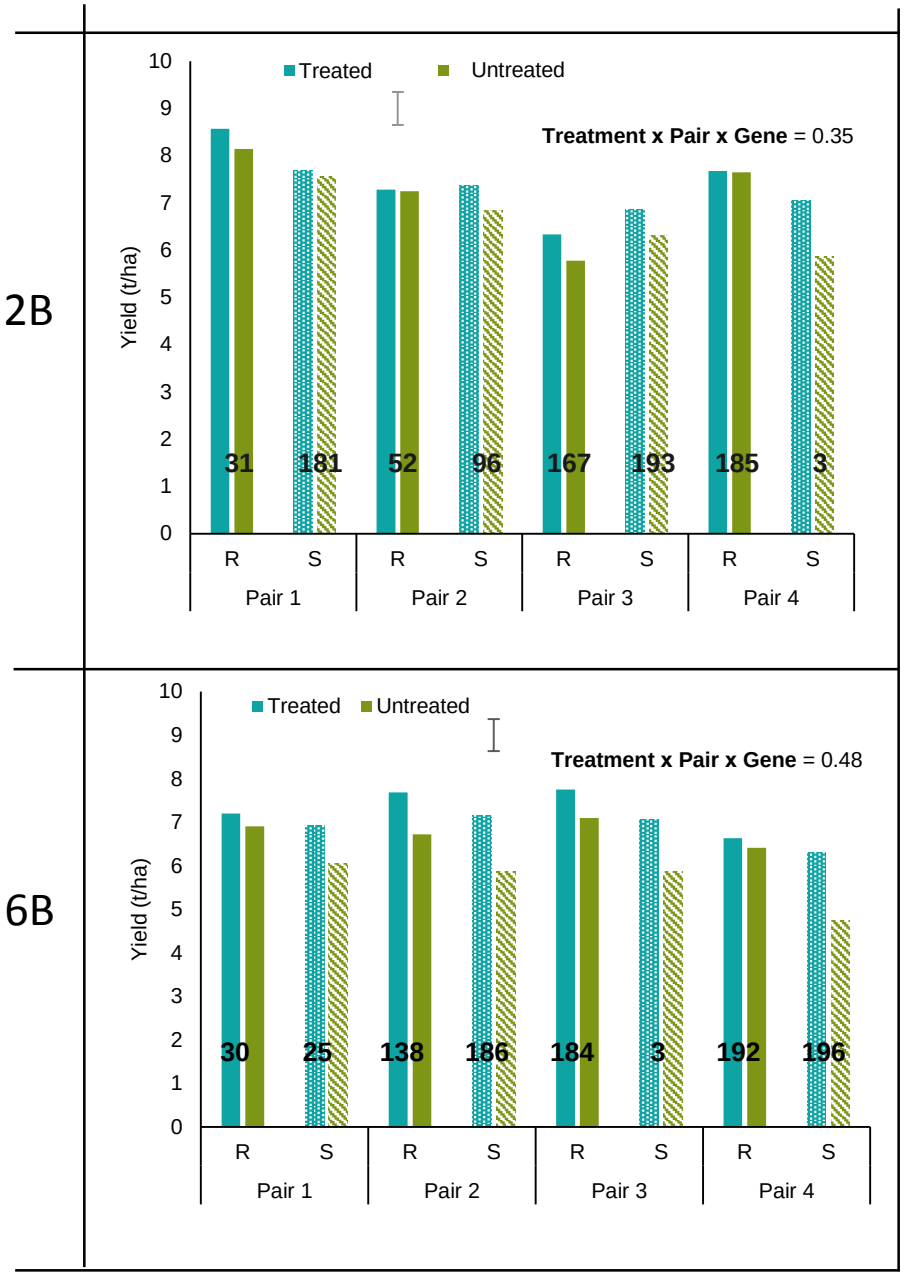


Figure 2. Yields (t/ha 85% DM) of Avalon x Cadenza wheat lines (line numbers superimposed on chart) matched for 2B or 6B yellow rust resistance allele across Limagrain (2012) and RAGT (2012 & 2013) trials. Vertical bar represents least significant difference based on a cross-site ANOVA (n=3).

Table 11. Yields (t/ha 85% DM) of Avalon x Cadenza matched wheat varieties differing for presence or absence of yellow rust resistance alleles on chromosome 2B and 6B across Limagrain (2012) and RAGT (2012 & 2013) trials (mean values of fungicide treated and inoculated plots).. Including least significant difference, degrees of freedom and P-value from a cross-site ANOVA.

		2B	6B
Yield (t/ha)	Resistant	7.372	7.05
	Susceptible	6.944	6.246
	L.S.D.	0.2455	0.2368
	Degrees of Freedom	112	112
	P-value	<0.001	<0.001

In 2014 a replicated yield trial was conducted by ADAS at a site at Terrington (Norfolk) to test for yield costs associated with 2B, 6B alleles when stacked within AxC lines. The trial consisted of eight additional lines which were selected and matched into four pairs contrasting for both of the resistance alleles (resistant lines contained resistance alleles from each of the QTL), as described and outlined in Table 1. The trial was a randomised block design using split plots, with 'challenged' (inoculated and fungicide untreated) and 'unchallenged' (uninoculated and fungicide treated) as main plots and matched pairs and gene (R or S) as subplots. AxC 2B and 6B +/- lines were tested in the presence and absence of yellow rust to generate differing levels of resistance responses.

At GS61+10 days 'challenged' plots had significantly lower % green leaf area (GLA%) than 'unchallenged' plots (*P* = <0.001), suggesting that a good level of yellow rust infection had established in the inoculated plots. Lines carrying both resistance QTL showed a significantly higher average GLA% (*P* = 0.006) than those carrying the susceptible alleles, suggesting that 2B/6B combined resistance alleles were providing good resistance to yellow rust. When paired lines were examined under a three-way ANOVA no significant Treatment x Pair x Gene interaction was observed. All treated resistant and susceptible plots within the four pairs exhibited >40% GLA% suggesting good fungicide control of yellow rust in the treated plots (Figure 3).

Significant yield differences were identified between treatments (*P* = <0.001), with 'unchallenged' plots exhibiting higher yield than 'challenged' suggesting that a good level of yellow rust had established within the trial. Significant differences in average yield (mean values of fungicide treated and inoculated plots) were observed between lines which were carrying the combined 2B/6B alleles compared to its corresponding susceptible lines, with lines carrying both resistance alleles showing a 0.42 t/ha yield benefit (Table 12). The yield benefit associated with 2B/6B combined resistance was evident across all four matched pairs with resistant treated and untreated plots exhibiting higher yield than the corresponding susceptible lines (Figure 3). Interestingly, this suggests that the lines carrying both the 2B and 6B resistance alleles are inherently higher yielding than those carrying the susceptible alleles irrespective of yellow rust challenge and supports data suggesting that both 2B and 6B as single QTL exhibit a yield benefit resulting from trials conducted by Limagrain (2012) and

RAGT (2012 and 2013).

Table 12. Yields (t/ha 85% DM) of Avalon x Cadenza matched wheat lines differing for presence or absence of yellow rust resistance alleles on chromosome 2B/6B of ADAS 2014 Terrington trial (mean values of fungicide treated and inoculated plots). Including least significant difference, degrees of freedom and P-value from an ANOVA.

		2B/6B
Yield (t/ha)	Resistant	6.11
	Susceptible	5.69
	L.S.D	0.2835
	Degrees of Freedom	31
	P-value	0.005

An additional replicated yield trial was conducted in 2014 by ADAS at a site at Boxworth (Cambridgeshire) to test for yield costs associated with 2B, 6B alleles in the absence of disease when stacked within AxC lines. Similar to the 2014 Terrington trial, the trial consisted of the same eight lines selected and matched into four pairs contrasting for both of the 2B/6B resistance alleles. All plots were fungicide treated in order to assess yield costs in the absence of yellow rust challenge.

No significant differences in average yield (t/ha) were observed when an ANOVA test was conducted (P = 0.587) between plots carrying the 2B/6B resistance alleles when compared to those carrying the resistance allele (data not shown). In summary, no evidence of a yield cost associated with 2B/6B resistance alleles in combination were identified in the absence of yellow rust challenge. The findings of this trial supports those of both the industrial replicated yield trial and the ADAS split-plot yield trial. It is therefore highly unlikely that the 2B or 6B yellow rust resistance alleles cause any form of yield loss in lines where the alleles were inherited as a single QTL or in combination and they confer a significant yield benefit in the presence of disease.

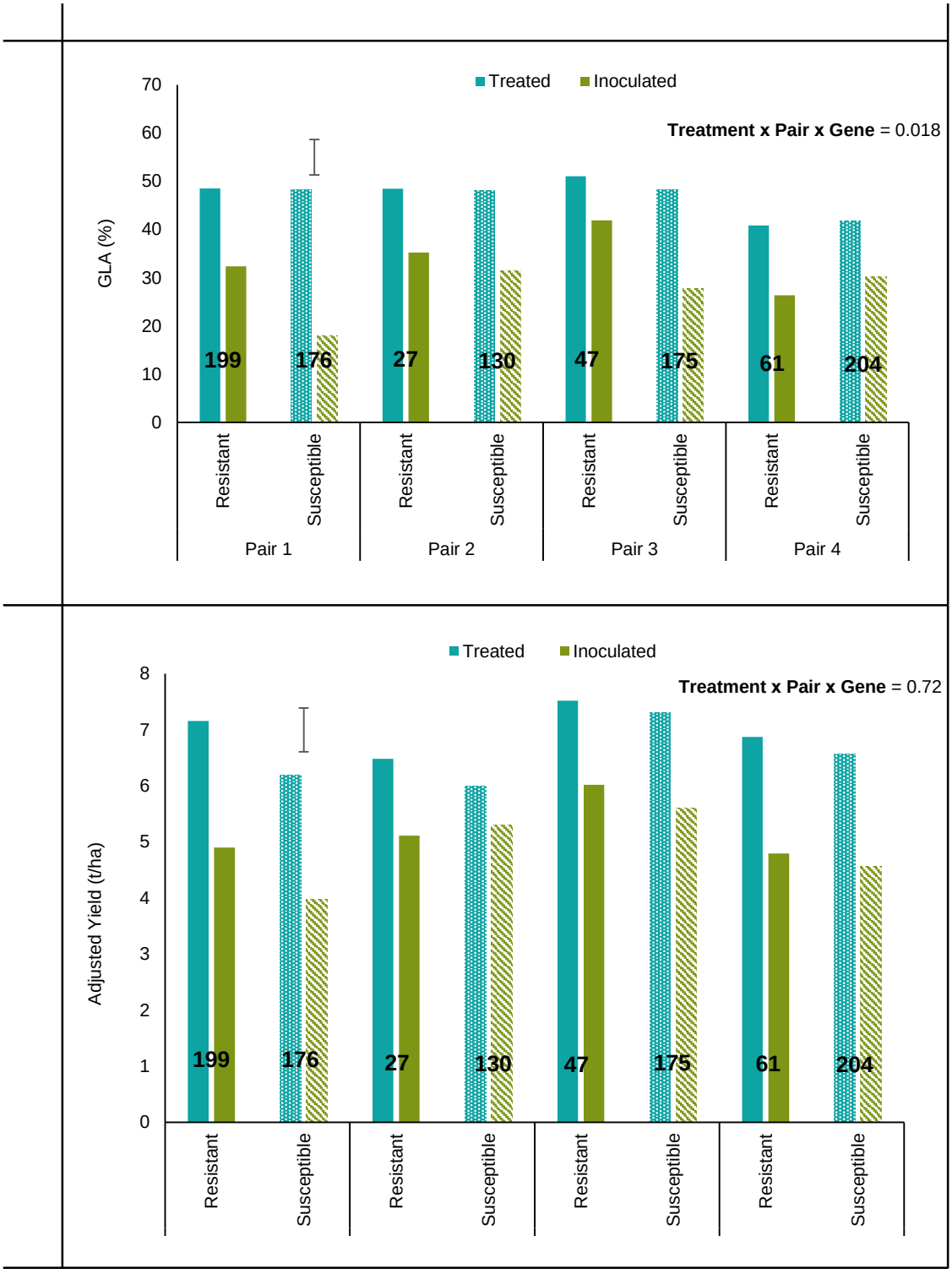


Figure 3. Green Leaf Area (%) and yields (t/ha) of Avalon x Cadenza matched pairs selected for presence or absence of yellow rust resistance alleles on chromosome 2B and 6B at Terrington yield trial 2014. Vertical bar represents least significant difference at 5% level based on a split-plot ANOVA (n=4).

Corroboration of yield costs/benefits with yellow rust resistance QTL on chromosome 2B/6B from WGIN data

Yield data collected from the AxC DH population (202 individuals) during the WGIN project (2005–2008) was available for download from the WGIN website during the course of the project. AxC DH lines which had inherited the 2B and 6B resistance QTL as single and double QTL combinations were compared against lines which had not inherited the QTL and the corresponding yield data was statistically analysed by ANOVA. In addition, grain yield (t/ha) measurements of the Avalon x Cadenza mapping population collected by the WGIN programme between 2005–2008, was subdivided into groups of lines which had inherited varying numbers of yellow rust resistance alleles previously identified within the project (e.g. 0–3, 4, 5, 6–8 yellow rust resistance alleles). Statistical differences in yield associated with number of yellow rust QTL present was calculated via ANOVA.

In 2007 significant differences in yield were observed between the lines carrying the 2B and 6B alleles and the susceptible lines (Table 13), with lines carrying the 2B and 6B alleles as a single QTL exhibiting higher yield than the susceptible lines. Lines carrying the two resistance alleles in combination showed the greatest yield benefit. No significant differences were identified in years 2005, 2006 and 2008, however in general all lines carrying the resistance alleles in single or double combinations produced higher yield than the susceptible lines. This trend is also reflected in the mean yield values across the four trial years, however the differences in yield were not statistically significant. In summary, no evidence was found to suggest that the 2B or 6B resistance alleles identified within this project from the AxC DH population are associated with a yield cost when inherited as single or double QTL combinations. Therefore the yield data collected during the WGIN project between 2005 and 2008, also supports the findings of all the field trials conducted within this project.

*Table 13. Average yield (t/ha 85% DM) of Avalon x Cadenza DH population (Church farm, JIC, Norwich 2005 – 2008 *all data downloaded from WGIN website). Standard error of difference and P-value from an ANOVA using presence or absence of 2B and 6B resistance alleles as a factor are shown. Colour indicates yield per year with red indicating a low yield and green indicating a high yield.*

		2005	2006	2007	2008	Mean
Yield (t/ha)	Susc.	8.14	7.2	8.5	9.23	8.28
	2B	8.21	7.3	8.7	9.41	8.43
	6B	8.01	7.4	8.7	9.21	8.36
	2B6B	8.35	7.4	9.3	9.33	8.61
	P-value	0.516	0.8	0.0	0.82	0.30
	SED	0.22	0.2	0.2	0.24	0.18

In 2005 significant differences in yield were observed between the lines carrying varying numbers of yellow rust resistance alleles (P = 0.029) (Table 14). Lines carrying 6–8 yellow rust resistance alleles exhibited the highest yields, with a yield benefit of 0.48 t/ha when compared to lines which had

inherited 0–3 resistance alleles. This trend was also present in yield data collected in 2006, 2007 and 2008, however the results were not statistically significant. However, when the mean yield (t/ha) of all four years was examined a significant difference in yield was observed (*P* = 0.02), with lines carrying 6–8 alleles showing significantly higher yield than those carrying just 0–3 alleles. This data suggests that the yellow rust resistance QTL identified during this project do not appear to confer a yield cost and 'stacking' multiple resistance alleles results in a yield benefit rather than a yield deficit.

*Table 14. Average yield (t/ha 85% DM) of Avalon x Cadenza DH lines with varying numbers of yellow rust resistance alleles in WGIN yield trials 2005–2008. (Church farm, JIC, Norwich 2005 *all data downloaded from WGIN website). Standard error of difference and P-value from an ANOVA are shown. Colour indicates yield per year with red indicating a low yield and green indicating a high yield.*

	No. QTLs	2005	2006	2007	2008	Mean
Yield t/ha	0–3	8.05	7.05	8.75	9.16	8.25
	4	8.25	7.37	8.68	9.21	8.38
	5	7.89	7.40	8.69	9.14	8.28
	6 to 8	8.53	7.67	9.19	9.71	8.77
	P-value	0.029	0.087	0.122	0.065	0.02
	SED	0.22	0.24	0.24	0.24	0.18

Yield costs associated with Option x Claire 2D and 4D yellow rust resistance QTL

Three lines carrying the yellow rust resistant loci on 2D and 4D (donor = Claire) separately and in combination were provided by RAGT alongside the corresponding null line. Replicated yield trials were conducted in the Cambridgeshire area by RAGT and Syngenta (2014) and all plots were fungicide treated in order to assess yield costs in the absence of yellow rust challenge. A cross-site analysis of data from 2014 sites (Table 15) revealed a significant difference between plots carrying the 2D, 4D and 2D/4D yellow rust resistance alleles and those carrying the susceptible allele (*P* = <0.001). Single site analysis via ANOVA, showed that a significant yield cost was evident at the Syngenta site (*P* = 0.001), with the resistant lines exhibiting a yield deficit of 0.42–0.54 t/ha. No significant yield cost was identified within the RAGT trial, however all three resistant lines produced lower yield than the corresponding null line. No evidence was found to suggest that combining 2D/4D QTL within a line significantly increases the yield deficit when compared to lines carrying a single QTL. In summary, evidence was found to suggest that 2D and 4D resistance QTL in the Option x Claire background are associated with significant yield costs, but the occurrence of yield costs can be variable between sites and seasons.

Table 15. Yields (t/ha 85% DM) of Option x Claire wheat varieties differing for presence or absence of yellow rust resistance alleles on chromosome 2D and/or 4D (all plots fungicide treated). Least significant difference, degrees of freedom and p-values from unbalanced ANOVA shown.

		Syngenta	RAGT	Cross-Site
Yield (t/ha)	Susc.	0.34	10.99	10.99
	2D	9.86	10.77	10.77
	4D	9.80	10.88	10.88
	2D4D	9.92	10.72	10.72
	L.S.D.	0.28	NS	0.095
	D.O.F.	85	87	48
	P-value	0.001	0.164	< 0.001

Yield costs associated with YrQ 2D and 4B yellow rust resistance QTL

Near-isogenic lines (Brigadier susceptible background) carrying yellow rust resistance genes on chromosome 2D and 4B (donor = Alcedo) and the corresponding susceptible control, were provided by Limagrain for testing during this project. Replicated yield trials were conducted in the Cambridgeshire area by Limgrain (2014) and all plots were fungicide treated in order to assess yield costs in the absence of yellow rust challenge. No significant differences in yield were identified between lines carrying the 2D, 4B and 2D/4B yellow rust resistance alleles and those carrying the susceptible allele (P = 0.587) when an ANOVA was performed, however all three resistant lines produced lower yield than the corresponding null line (Table 16). In 2009, a field experiment was conducted to assess the YrQ NILs (DEFRA project IF0129) for costs of resistance. Yield was found to be ~0.8t/ha lower in the resistant NILs in both challenged and unchallenged treatments, when compared with unchallenged Brigadier. Indicating that there was a significant cost to having the resistance QTL even in the absence of yellow rust challenge. No additional evidence was found during the course of this project to suggest that 2D and 4B resistance QTL (YrQ NILs) are associated with significant yield costs, suggesting environmental variation between sites and seasons can have a significant impact on the yield performance of the resistant lines.

Table 16. Yields (t/ha 85% DM) of YrQ Brigadier NILs differing for presence or absence of yellow rust resistance alleles on chromosome 2D and/or 4B (all plots fungicide treated). Least significant difference, degrees of freedom and p-values from unbalanced ANOVA shown.

		Limagrain 2014
Yield (t/ha)	Susc.	13.165
	2D	12.387
	4B	12.598
	2D4B	12.564
	L.S.D.	NS
	Degrees of Freedom	49
	P-value	0.587

4.1.2. Yield costs associated with genetic resistance to brown rust (*Puccinia triticina)*

Yield costs of Thatcher NILs containing brown rust resistance genes

In 2013, a single replicate of sixteen Thatcher NILs carrying *Lr* brown resistance genes were drilled by ADAS for seed multiplication, alongside the recurrent Thatcher parent (TcS). Statistical analysis of the yield data (t/ha) could not be conducted as the multiplication trial was not replicated. However, all sixteen NILs showed lower grain yield than the Thatcher parent with each resistant line exhibiting yield losses of >1 t/ha when compared to the TcS control (Figure 4). All plots were treated with fungicide and therefore the yield losses were not associated with disease challenge. This data, suggests that all the NILs exhibit a yield cost which associated with the *Lr* resistance genes.

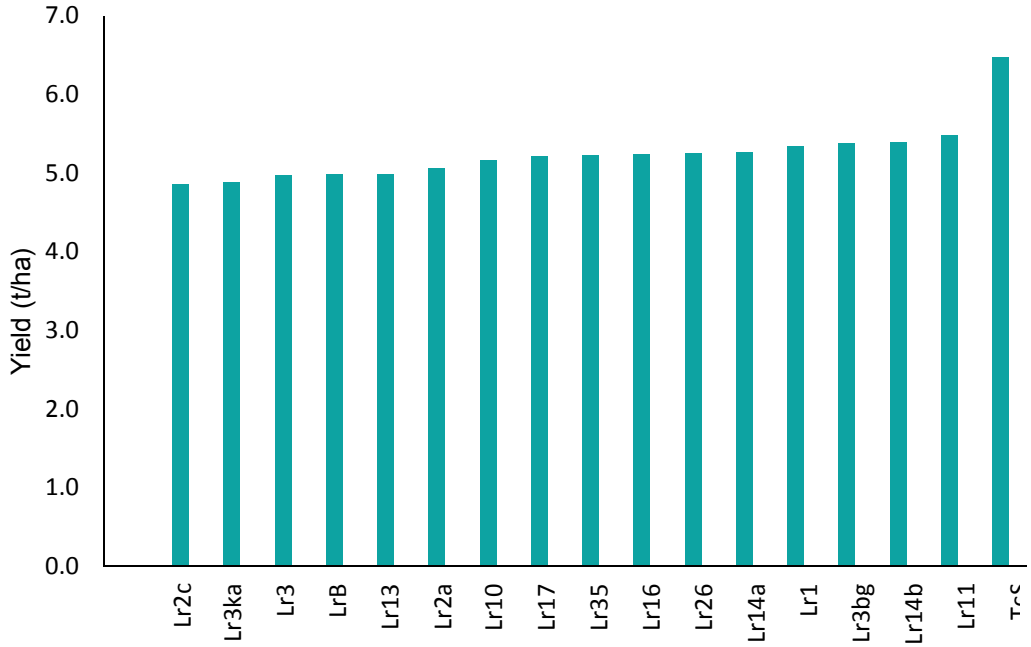


Figure 4. Yields (t/ha 85% DM) of Thatcher NILs in 2013 ADAS multiplication plots (n=1).

One replicated yield trial (24 m^2 plots in a randomised block design) was conducted in 2014 by ADAS, to identify yield costs associated with major *Lr* brown rust resistance genes. All twenty-six NILs and the Thatcher control (TcS) were tested in the absence of brown rust challenge using appropriate fungicides to control target and non-target diseases. All NILs were found to exhibit significantly lower thousand grain weight (TGW) than the Thatcher line (P = <0.001), with the exception of *Lr2c* (Figure 5a and Table 17). In addition, as observed in the non-replicated multiplication trial, all twenty-six NILs exhibited significantly lower yield (t/ha) than the Thatcher control (P = 0.017) (Figure 5b). *Lr2c* was found to be the lowest yielding NIL in both the multiplication trial and the replicated yield trial and *Lr11* the highest yielding NIL. Data from both the multiplication trial and the yield trial suggest that the Thatcher NILs exhibit a significant yield cost associated with the major *Lr* brown rust resistance genes, in the absence of challenge, when compared to the TcS control.

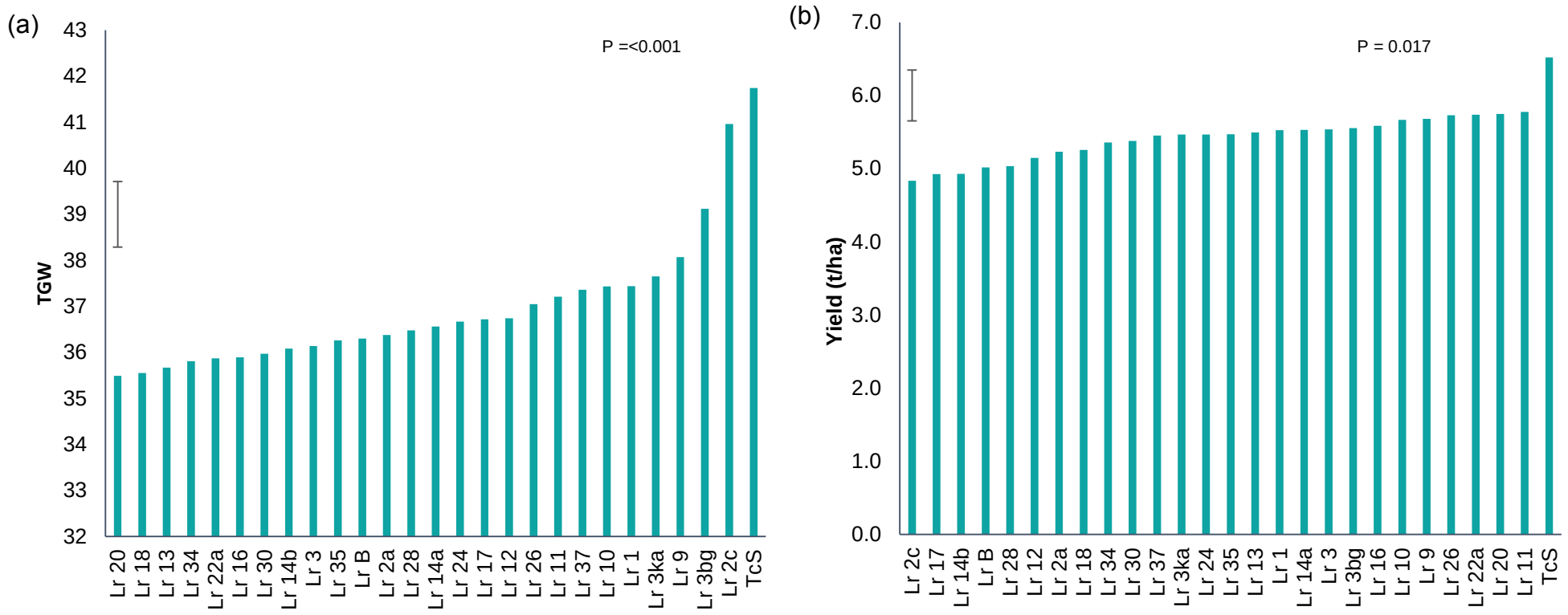


Figure 5. (a) Thousand grain weight (grams) and (b) Yields (t/ha 85% DM) of Thatcher NILs in ADAS yield trial 2014 (n=4). Vertical bar represents least significant difference at 5% level based on ANOVA using genotype as factor.

Table 17. Least significant difference, degrees of freedom and P-value from an ANOVA of Thatcher NIL thousand grain weight (TGW) and yield (t/ha 85% DM) in 2014 ADAS yield trial.

	TGW	Yield
L.S.D.	1.4277	0.6984
Degrees of Freedom	52	52
P-value	< 0.001	0.017

Yield costs associated with 2A brown rust resistance QTL

Fourteen lines matched into seven pairs contrasting for alleles of the brown rust resistance locus on 2A (Player as donor) were selected from the Hereford x Player DH mapping population, for yield testing. Replicated yield trials were conducted in Cambridgeshire by Syngenta (2013 and 2014) and all plots were fungicide treated in order to assess yield costs in the absence of pathogen challenge. A cross-site analysis of both 2013 and 2014 data sets (Table 18) revealed that there was no significant difference between plots carrying the 2A brown rust resistance allele and those carrying the susceptible allele (P = 0.96). Single site analysis via ANOVA, showed that a significant yield cost was evident in 2013 (P = 0.009), but the effect was slight with an average yield decrease of just 0.11 t/ha. In 2014, no significant yield costs were identified (P = 0.431). Overall, it appears that if a yield cost is associated with the 2A brown rust resistance QTL, it is slight and is not observed consistently between sites and seasons.

Table 18. Yields (t/ha) 85% DM) of Hereford x Player wheat in 2013 & 2014 Syngenta trial with least significant difference, degrees of freedom and P-value from an ANOVA using BR2A as factor (i.e. no line effects) (all plots fungicide treated).

		2013	2014	Cross-site
Yield (t/ha)	Resistant	8.618	10.34	9.479
	Susceptible	8.508	10.442	9.475
	L.S.D.	0.0682	NS	NS
	Degrees of Freedom	5	5	11
	P-value	0.009	0.431	0.96

4.1.3. Yield costs associated with resistance to septoria tritici blotch (*Zymoseptoria tritici*)

Yield costs associated with septoria resistance 3B, 5A and 5D QTL

Between 2007 and 2010, an independent project was funded by DEFRA (IF0129) where novel QTL segregating in the Avalon x Cadenza (AxC) DH mapping population (202 lines) were identified, which control canopy, harvest and septoria severity traits. Avalon x Cadenza lines were grown at three trial sites (High Mowthorpe, Long Ashton and Rosemaund) and assessed for canopy, harvest and septoria severity traits. A number of suggestive QTL were identified, several of which were corroborated through growth stages and/or across sites. Three significant QTL (>2.5 LOD)

associated with septoria resistance were selected for further analysis during the current project; a QTL showing early expression on 3B and two QTL on 5A and 5D exhibiting late expression.

Twenty-four lines were selected from the AxC DH mapping population and were matched into twelve pairs, four pairs contrasting for each of the three alleles, as outlined in Table 3. A replicated yield trial was conducted by Limagrain in 2013 to test the 3B, 5A and 5D AxC matched pairs for yield costs. The replicated trial consisted of 'challenged' (inoculated and fungicide untreated) and 'unchallenged' (uninoculated and fungicide treated) plots of each matched pair (resistant and susceptible lines). The 3B, 5A and 5D +/- lines were tested in the presence and absence of septoria tritici blotch challenge to generate differing levels of resistance responses.

A significant difference in yield was identified between plots contrasting for 3B, 5A and 5D resistance and plots which were treated or untreated with fungicide, with 'unchallenged' (treated) plots exhibiting higher yield than those which were untreated suggesting a good level of disease challenge in 'challenged' (inoculated/untreated) plots throughout the trial. In addition, significant differences in yield between matched pairs was identified for the 3B, 5A and 5D alleles, suggesting that matched pairs did vary in their yield performance.

No significant differences in yield were observed between lines which were carrying the 3B resistance allele when compared to the susceptible lines. However, the average yield was lower in the resistant lines than the susceptible (Table 19), indicating that a slight cost of resistance maybe evident. No significant Treatment x Pair x QTL interaction was observed (P = 0.605). Matched pairs 2 and 3 showed lower yield in treated and untreated plots containing the resistance allele 3B, suggesting a yield cost associated with 3B in these matched pairs. However, pairs 1 and 4 did not behave in a similar manner with the resistant lines in the treated plots exhibiting higher yield and thus a yield benefit (Figure 6).

Significant differences in average yield were observed between lines which were carrying the 5A compared to its corresponding susceptible lines, with lines carrying the 5A allele showing a 0.553 t/ha yield deficit (Table 19) and a significant Treatment x Pair x QTL was identified (P = 0.018). All matched pairs showed lower yield in both treated and untreated plots containing the resistance allele 5A, suggesting a yield costs associated with 5A in all four matched pairs (Figure 6).

Significant differences in average yield were not observed between lines which were carrying the 5D compared to lines carrying the susceptible allele, however lines carrying the 5D allele did show a 0.41 t/ha yield deficit (Table 19) and a significant Treatment x Pair x QTL interaction was identified (P = <0.001). Matched pairs 3 and 4 showed lower yield in treated and untreated plots containing the resistance allele 5D, suggesting a yield cost associated with 5D in these matched pairs. However, in matched pairs 1 and 2 yield costs were not evident (Figure 6).

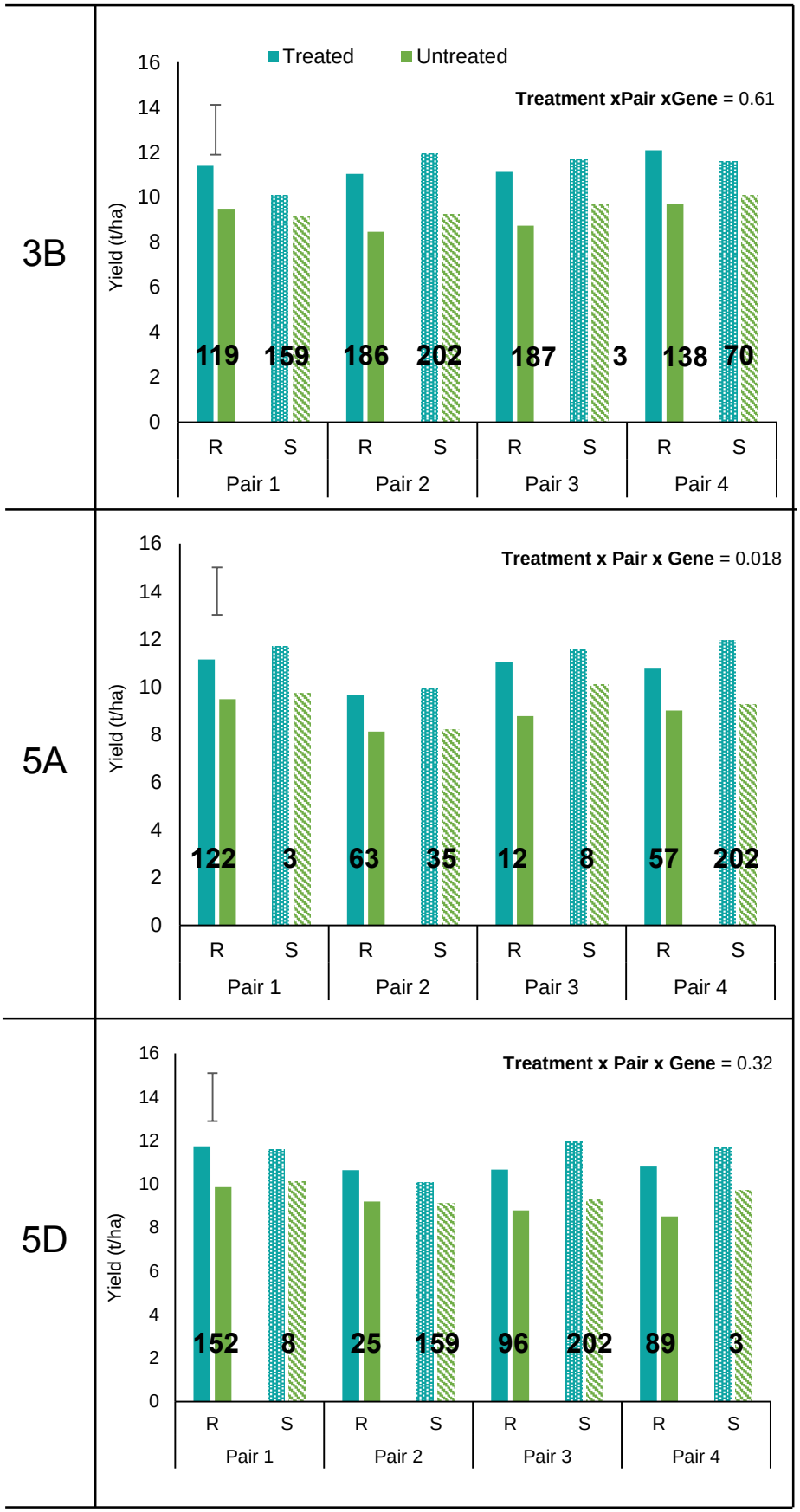


Figure 6. Yields (t/ha 85% DM) of Avalon x Cadenza wheat varieties matched for presence or absence of septoria resistance allele on chromosome 3B, 5A2 and 5D industrial yields trials at Limagrain (2013). Vertical bar represents least significant difference at 5% level based on split-plot ANOVA (n=3).

Table 19. Yields (t/ha 85% DM) of Avalon x Cadenza wheat varieties matched for presence or absence of septoria resistance allele on chromosome 3B, 5A and 5D in yield trials at Limagrain (2013) (mean values of fungicide treated and inoculated plots) . Least significant difference, degrees of freedom and P-value from split-plot ANOVA (n=3).

		QTL for Septoria Resistance		
		3B	5A	5D
Yield (t/ha)	Resistant	10.255	9.748	10.025
	Susceptible	10.435	10.301	10.435
	L.S.D.	NS	0.337	NS
	Degrees of	2	2	2
	P-value	0.344	0.024	0.058

In 2011 a replicated yield trial was conducted by ADAS, to test for yield costs associated with 3B, 5A and 5D alleles when stacked within AxC lines. The trial consisted of eight additional lines which were selected and matched into four pairs contrasting for all three of the resistance alleles (resistant lines contained resistance alleles from each of the three QTL), as outlined in Table 3. The trial was a randomised block design using split plots, with challenged (inoculated and fungicide untreated) and unchallenged (uninoculated and fungicide treated) as main plots and matched pairs and gene (R or S) as subplots. AxC 3B, 5A and 5D +/- lines were tested in the presence and absence of septoria to generate differing levels of resistance responses.

At GS72 'challenged' (inoculated/untreated) plots had significantly more septoria (average % coverage) than 'unchallenged' (uninoculated/treated) plots (P = <0.001), suggesting that the infection of the 'challenged' (inoculated) plots was successful. Lines carrying all three resistance QTL showed lower average septoria levels than those carrying the susceptible alleles, however the effect was not statistically significant (P = 0.083). In addition, differences in septoria severity in untreated susceptible plots was higher in three out of the four matched pairs, suggesting that the resistance QTL when stacked were providing some resistance to the disease. When paired lines were examined under a three-way ANOVA no significant Treatment x Pair x Gene interaction was observed. All treated resistant and susceptible plots within the four pairs exhibited low average levels of septoria (<2% disease severity) suggesting good fungicide control of septoria in the treated plots (Figure 7).

Significant differences in average yield were observed between lines which were carrying the 3B/ 5A/5D alleles compared to its corresponding susceptible lines, with lines carrying all three alleles showing a 0.33 t/ha yield deficit (Table 20). Significant yield differences were identified between treatments (P = <0.001), with 'unchallenged' (uninoculated/treated) plots exhibiting higher yield than 'challenged' (inoculated/untreated) suggesting that the inoculation with septoria was successful. A significant Treatment x Pair x Gene interaction (P = 0.03) was observed. All matched pairs showed lower yield in treated plots containing the resistance 3B/5A/5D alleles with exception of Pair 4, suggesting a yield cost is associated with 3B/5A/5D alleles (Figure 7). Several additional yield components supported this finding. Lines carrying 3B/5A/5D resistance alleles were found to have

significantly fewer grains/m^2 and significantly fewer grains per ear (Table 20), suggesting that the 3B/5A/5D resistance alleles, when combined, have a significant effect on the number of grains it can produce per ear within the crop canopy. Interestingly, there was no significant difference in ears/m^2 between lines carrying the three resistance alleles and their corresponding susceptible lines, suggesting that the resistance alleles do not affect the number of ears produced within the canopy. In addition, thousand grain weight (TGW) is also not affected (Table 20). The yield deficit associated with the resistance alleles when stacked within a variety does not appear to be greater than the yield decrease observed in lines carrying a single resistance QTL.

In order to measure differences in canopy size and duration between lines carrying the resistance alleles and those carrying the susceptible alleles, the green lamina area index (GLAI) progress curve was plotted for each plot from GS61 until canopy senescence. The area under the curve was then calculated using the trapezoidal method, across the four top leaves, and expressed as the healthy area duration (HAD) of the canopy. The effect of foliar disease was quantified as the difference in HAD between the healthy and diseased treatments and the effect of fungicide was quantified as the difference in HAD between the untreated and treated treatments. HAD was calculated for each plot and subject to ANOVA. A significant difference in HAD value was identified between lines carrying the resistance alleles with those carrying the susceptible alleles (Table 20), with the resistant lines showing significantly higher HAD than lines carrying the susceptible alleles. Thus, suggesting the canopy of plots carrying the resistance alleles 3B/5A/5D had a longer duration and larger size than those carrying the susceptible alleles. This effect is likely to be largely due to the beneficial effect of the resistance QTL in the presence of septoria challenge in the inoculated/untreated plots. Lines carrying the resistance alleles exhibited significantly higher HAD/grain than the susceptible lines. Grain number is determined primarily pre-anthesis, whereas HAD differences occur primarily post-anthesis, hence differences in HAD/grain are likely to occur when HAD varies by treatment (Table 20). When the relationship between yield and HAD (GS61–senescence) was calculated it was discovered that for every unit of HAD the resistant lines produced significantly lower yield than the susceptible lines, suggesting that the canopy of the resistant lines was not as efficient at producing yield as the susceptible lines, despite the canopy of the resistant lines having a larger size and longer duration. In addition, when pre-anthesis radiation use efficiency (RUE) was calculated, the resistant lines carrying 3B/5A/5D had significantly lower RUE than those carrying the susceptible alleles, suggesting the inefficiency of the canopy maybe due to loss of radiation use efficiency.

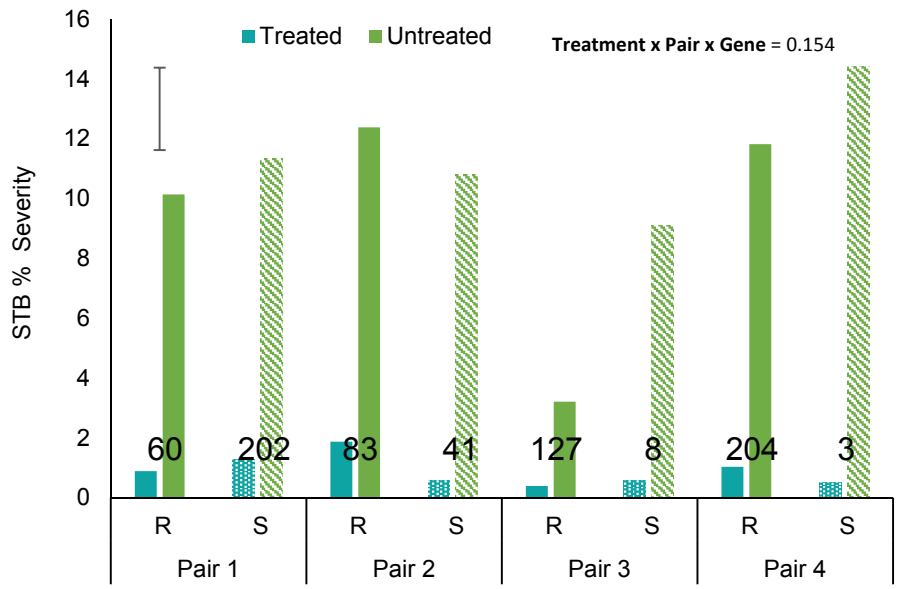
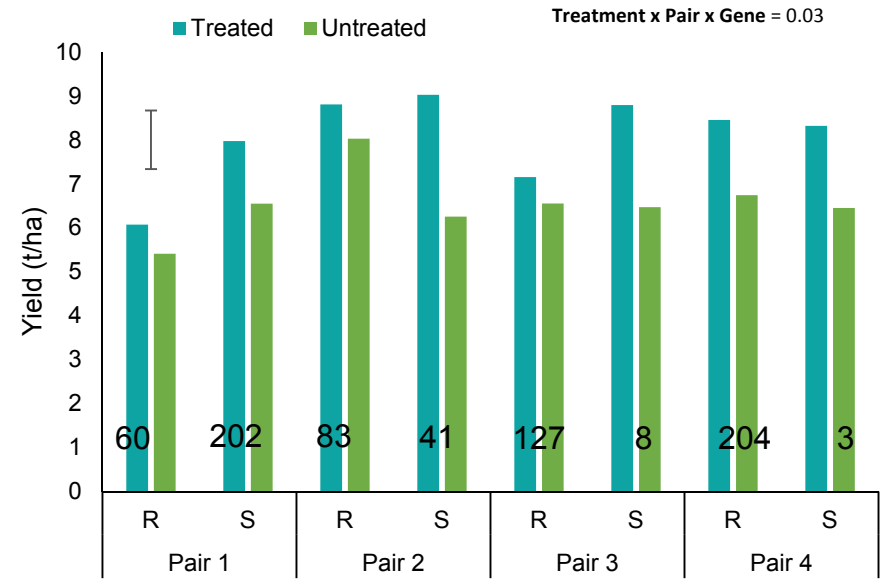
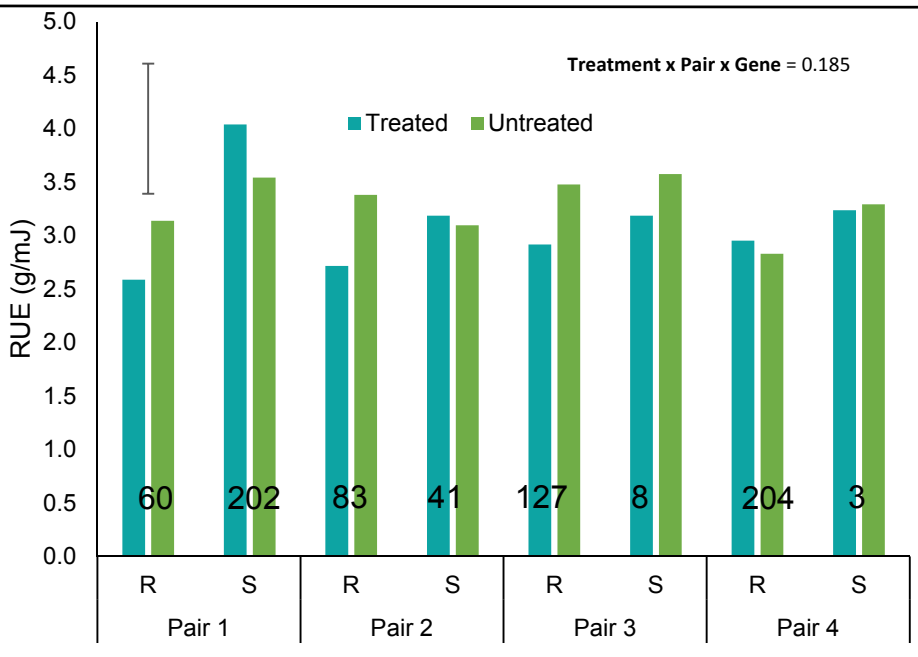
Disease	
Yield	
Radiation Use Efficiency	

Figure 7. Average septoria severity (% leaf coverage), radiation use efficiency (g/mJ) and yield (t/ha 85% DM) of Avalon x Cadenza wheat varieties matched for presence or absence of three septoria resistance QTLs on chromosome 3B, 5A2 and 5D in 2011 ADAS phenotypic trial. Vertical bar represents least significant difference at 5% level from an ANOVA.

Table 20. Quantification of yield costs associated with Avalon X Cadenza 3B/5A2/5D1 QTL via quantitative physiological traits of Avalon x Cadenza matched pairs (mean values of fungicide treated and inoculated plots).

Trait	Resistant	Susceptible	df	LSD 5%	P-value
Yield (t/ha)	7.15	7.48	6	0.306	0.04
Grains/m^2	14953	16098	6	1053.7	0.038
Ears/m^2	371.5	352	6	NS	0.086
Grains/ear	40.9	46.3	6	3.72	0.11
TGW	48.01	46.37	6	NS	0.055
HAD GS61 -Sen	73.2	64.7	6	6.31	0.017
HAD/grain	0.0049	0.004	6	0.00052	0.004
AGDM GS61 (g/m^2)	867	977	6	76.2	0.012
Pre-anthesis RUE	3.013	3.42	6	0.2473	0.002

Corroboration of yield costs/benefits with septoria resistance QTL on chromosome 3B/5A/5D and WGIN data

Yield data collected from the AxC DH population (202 individuals) by the WGIN consortium (2005–2008) was available for download on the WGIN website during this project. AxC DH lines which had inherited the 3B, 5A and 5D resistance QTL as single, double and triple QTL combinations were compared against lines which had not inherited the QTL and the corresponding yield data was statistically analysed by ANOVA. In 2005 and 2006 significant differences in yield were observed between the lines carrying the 3B, 5A and 5D alleles and the susceptible lines (Table 21). Lines carrying the 3B, 5A and 5D alleles as a single QTL exhibited lower yield than the susceptible lines in 2005 and 2006. Resistance allele 5A had the greatest yield decrease, followed by 5D and then 3B, as was seen in the Limagrain yield trials in 2013. WGIN data from 2007 and 2008 showed a similar trend, with 5A exhibiting the largest yield decrease. However, the differences in yield observed within these trials were not statistically significant (Table 21). In summary, lines carrying the 3B, 5A and 5D resistance alleles have lower average yield when compared to susceptible lines, with 5A exhibiting the greatest yield cost associated with a resistance QTL. This trend is also reflected in the mean yield values across the four trial years, where differences in yield between the lines were found to be significant. When QTL were stacked within varieties in combinations of two and three alleles, decreases in yield were also evident when compared to lines carrying susceptible alleles. Interestingly, lines carrying stacked QTL do not generally exhibit higher yield deficits than lines carrying single QTL, suggesting that yield costs do not increase as QTL are accumulated within a line. This also supports findings observed iEn the 2011 ADAS trial where lines carrying 3B/5A2/5D1 alleles were compared to corresponding susceptible lines.

Table 21. Average yield (t/ha 85% DM) of Avalon x Cadenza DH population (Church farm, JIC, Norwich 2005 – 2008). Least significant difference, degrees of freedom and P-value from an ANOVA using presence or absence of resistance alleles as a factor are shown. Colour indicates yield per year with red indicating a low yield and green indicating a high yield. Annual average STB % leaf area on leaf 2 from the national WW survey (www.cropmonitor.co.uk).

		2005	2006	2007	2008	Mean
Yield (t/ha)	**Susc**	8.47	7.76	9.35	9.76	8.83
	3B	8.22	7.38	8.81	9.18	8.4
	5A	7.36	6.68	8.09	8.6	7.68
	5D	7.89	7.09	8.87	9.2	8.26
	3B5A	8.41	7.39	8.8	9.56	8.54
	3B5D	8.17	7.92	8.88	9.51	8.62
	5A5D	8.11	7.36	9.01	9.09	8.39
	3B5A5D	8.55	7.23	8.6	9.26	8.41
	~STB%	4.5	3.75	5.1	3	2.9
	P-value	0.009	0.026	0.088	0.092	0.01
	S.E.D	0.31	0.35	0.35	0.35	0.26

Discussion

Cost of resistance associated with yellow rust resistance genes

Between 2010 and 2012 industrial partners carried out three phenotypic trials to identify novel yellow rust resistance QTL in the Avalon x Cadenza (AxC) DH mapping population (202 lines). A number of suggestive QTL were found, several of which were corroborated through growth stages and/or across sites. Two significant QTL (>2.5 LOD) thought to control yellow rust resistance were selected for further analysis during the project; a QTL showing early and late expression on 2B and a QTL exhibiting early expression on 6B.

No costs of resistance were associated with the novel Avalon x Cadenza 2B and 6B yellow rust resistance QTL, when inherited as single QTL within a line or when stacked within a line during field trials conducted between 2012 and 2013. Avalon x Cadenza yield data available from the WGIN project (2005–2008) was used to assess the yield impacts on lines which have inherited the 2B and 6B resistance QTL as single and double QTL combinations. No evidence of a yield cost was found to be associated with either the 2B or 6B QTL and lines where both QTL were stacked were found to exhibit a yield benefit. This data therefore corroborated the findings of the 2011, 2012 and 2014 trials carried out by Limagrain, RAGT and ADAS. It was also discovered that the yellow rust resistance QTL identified during this project do not appear to confer a yield cost and 'stacking' multiple resistance alleles results in a yield benefit rather than a yield deficit.

No evidence was found during the course of this project to suggest that 2D and 4B resistance QTL (YrQ NILs) are associated with significant yield costs. However, a small yield cost of 0.22 t/ha and 1.11 t/ha was associated with the 2D and 4B resistance genes, respectively, in the Option x Claire

background in the absence of disease.

Yield penalties associated with brown rust *Lr* resistance genes

In 2013 and 2014 yield trials were conducted by Syngenta to establish whether yield costs are associated with the 2A resistance QTL in the Hereford x Player material. In conclusion, yield costs associated with the brown rust 2A resistance QTL were slight and inconsistent between sites and seasons, suggesting that any costs of resistance associated with the QTL are likely to be minor and not of practical importance.

Twenty-six near-isogenic lines containing *Lr* major resistance genes in the Thatcher background were tested in the 2014 ADAS yield trial. All 26 genes were associated with a significant decrease in grain yield (t/ha) and grain size (TGW) in the absence of brown rust challenge when compared to the susceptible Thatcher control. Among the *Lr* resistance genes exhibiting yield costs were *Lr9* and *Lr34*; both resistance genes showed a significant yield cost in the Thatcher background supporting previous publications reporting deleterious yield costs associated with both major resistance genes (Singh and Huerta-Espino, 1997; Ortelli *et al.,* 1996).

In summary, it appears that *Lr* resistance genes are associated with deleterious yield effects in the absence of disease challenge when compared to the Thatcher cultivar, suggesting that the major resistance genes do exhibit fitness costs. However, caution should be taken when comparing NIL lines with the recurrent parent. In some instances NILs have exhibited smaller isogenicity to the recurrent parent than predicted. This can be due to several factors:

1. 'Linkage drag', whereby the donor genes as well as the donor target gene are introgressed into the recurrent parent (Brinkman and Frey, 1977).
2. The presence of random donor genes, not linked with the target gene, which have still not been removed.
3. The unconscious selection of the breeder during the backcrossing process which may bias the outcome.
4. The availability of more than one genotype for the recurrent parent, thus the selection of the correct parent for comparison is critical.

However, in 1986, Zeven and Waninge conducted a study to establish the phenotypic resemblance of the Thatcher NILs and the wheat Thatcher cultivar. During this study it was concluded that the NILs tested had good phenotypic resemblance to Thatcher when measuring coleoptile colour, grain colour after phenol treatment, dead flag leaf point, yield (1982 and 1983) and disease symptoms.

However, it should be noted that several NILs exhibited significantly different yield (kg/ha) when compared to Thatcher, with six NILs showing significantly higher yield and five NILs exhibiting significantly lower yield to Thatcher during trials conducted in 1981. It is possible that the variation in yield was due to varying levels of disease challenge throughout the trial and the effectiveness of the resistance gene. In summary, it appears that all the major brown rust *Lr* genes confer a significant yield cost when compared to the recurrent parent with yield losses exceeding 1 t/ha in some cases, however, it may be necessary to confirm that yield differences between the NILs and the Thatcher line are due to costs of resistance rather than lack of isogenicity.

Yield penalties associated with septoria resistance genes

Yield trials conducted by the industrial partners, showed that the line containing individual resistance allele 5A achieved significantly lower yield (t/ha) in the absence of disease than lines carrying the susceptible alleles, (0.55 t/ha yield deficit). Lines carrying resistance alleles 5D and 3A also showed lower yield (0.41 t/ha and 0.18 t/ha yield loss, respectively) in the absence of disease than lines carrying the susceptible alleles, however the effect was not statistically significant. A core phenotypic trial conducted by ADAS in 2011 showed that lines carrying the three septoria QTL in combination exhibit good resistance to septoria. Lines carrying all three QTL did not exhibit higher yield losses than lines carrying single QTL. Deleterious yield costs associated with the 3B, 5A and 5D QTL, could be quantified by yield components such as grain yield (t/ha), grains/m^2 and grains/ear. However, no significant differences in thousand grain weight (TGW) and ears/m^2 were identified between lines carrying the three resistant alleles, compared to the corresponding susceptible alleles. In addition, costs of resistance could be quantified by healthy area duration (HAD) and radiation use efficiency measurements.

Yield data collected from the AxC DH population (202 individuals) during the WGIN project (2005 – 2008) was available for download from the WGIN website during this project. AxC DH lines which had inherited the 3B, 5A and 5D resistance QTL as single, double and triple QTL combinations were compared against susceptible lines and the corresponding yield data statistically analysed. In conclusion significant yield costs associated with 3B, 5A and 5D resistance QTL, were identified across the trials, with the 3B early expressed QTL showing the lowest mean yield decrease (0.43 t/ha) and the 5A late expressed QTL exhibiting the highest yield loss (1.15 t/ha). No evidence was found to suggest that accumulating septoria resistance alleles in AxC DH lines increases yield in a cumulative manner.

In summary, novel QTL on 3B, 5A and 5D confer significant resistance to septoria in the presence of disease, when stacked within matched pairs. Trials conducted during the project concluded that the resistance QTL are also associated with a significant decrease in yield when expressed as single QTL or when stacked, in the absence of septoria challenge. These findings were corroborated when yield data generated by the WGIN project of the AxC DH mapping population were analysed, based on inheritance of the 3B, 5A and 5D resistance alleles. In addition, data from both SCORE and

WGIN projects suggests that the deleterious yield costs associated with this QTL are not cumulative when alleles are stacked and can be quantified by measuring grain yield and yield components such as grains/m^2 and grains/ear. Yield costs associated with stacked 3B/5A/5D resistance alleles were found to be linked to significantly decreased yield per unit of HAD and decreased radiation efficiency, despite the canopy exhibiting significantly increased HAD. Therefore, yield costs are likely to be due to loss of RUE in lines carrying the resistance QTL which is expressed as lower grain per ear.

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

Yield costs of Viscount NILs carrying Lr37

A cross-site analysis of replicated yield trials carried out by Syngenta (2013), Limagrain (2014) and RAGT (2014), was conducted to establish whether deleterious yield costs are associated with *Lr37* resistance in the absence of disease using appropriate fungicides to control target and non-target disease. A cross-site analysis of 2013 and 2014 trials was conducted and no significant differences in yield (t/ha) (*P* = 0.824) were detected between plots carrying the *Lr37* brown rust resistance gene compared to those which were carrying the susceptible allele. In addition, no significant differences in yield (t/ha) were identified when data from individual trials was statistically analysed via ANOVA (Table 22).

Table 22. Average yield (t/ha 85% DM) of Viscount wheat varieties carrying resistant or susceptible Lr37 allele in 2013 and 2014 Industrial trials. Cross site analysis of Syngenta 2013 yield data and Limagrain and RAGT 2014 yield data (all plots treated with fungicide). Least significant difference, degrees of freedom and P-value at 5% level based on an ANOVA using Lr37 as a factor are shown.

		Limagrain 2014	RAGT 2014	Syngenta 2013	Cross-site
Yield (t/ha)	Resistant (Lr37+)	12.87	11.24	8.46	10.86
	Susceptible (Lr37-)	13.16	11.16	8.34	10.89
	L.S.D.	NS	NS	NS	NS
	Degrees of Freedom	5	5	5	24.5
	P-value	0.495	0.409	0.69	0.824

Two field experiments were conducted during 2010/2011 and 2011/2012 by ADAS, to test for yield costs associated with *Lr37* disease resistance in the Viscount background. *Lr37*+/- lines were tested in the presence and absence of brown rust challenge to generate differing levels of resistance responses. In 2011, disease levels were particularly low, therefore all plots were considered free from pathogen challenge. In the absence of disease, lines carrying the resistance gene *Lr37* were found to exhibit significantly lower yield (t/ha) when compared to the susceptible lines (*P* = 0.002), with resistant lines exhibiting an average yield loss of 0.74 t/ha when compared to susceptible plots

(Figure 8, Table 23). When the relationship between yield and HAD (GS61–senescence) was calculated it was discovered that for every unit of HAD the resistant lines carrying *Lr37* resistance produced significantly lower yield than the susceptible lines (Figure 9), suggesting that the canopy of the *Lr37* resistant lines was not as efficient at producing yield as the susceptible lines, as was observed during the AxC septoria trial in 2011. However, no significant difference in yield (t/ha) was evident in the 2012 trial, when comparing the *Lr37* resistant and susceptible lines (P = 0.445), in treated or untreated plots (Figure 8, Table 23).

In summary, evidence to suggest that costs of resistance are associated with *Lr37* resistance in the Viscount background is limited. In 2011, a clear yield deficit was observed in the *Lr37* resistant lines when compared to those carrying the susceptible allele in the absence of disease and was also reflected in the relationship between HAD and yield. However, these results were not repeated during industrial yield trials carried out in 2013 and 2014 (in the absence of disease) or during the split-plot brown rust inoculated yield trial carried out in 2012. In conclusion, it appears that yield losses can be linked to the *Lr37* resistant gene in the Viscount background, however such effects have proven difficult to repeat in further trials.

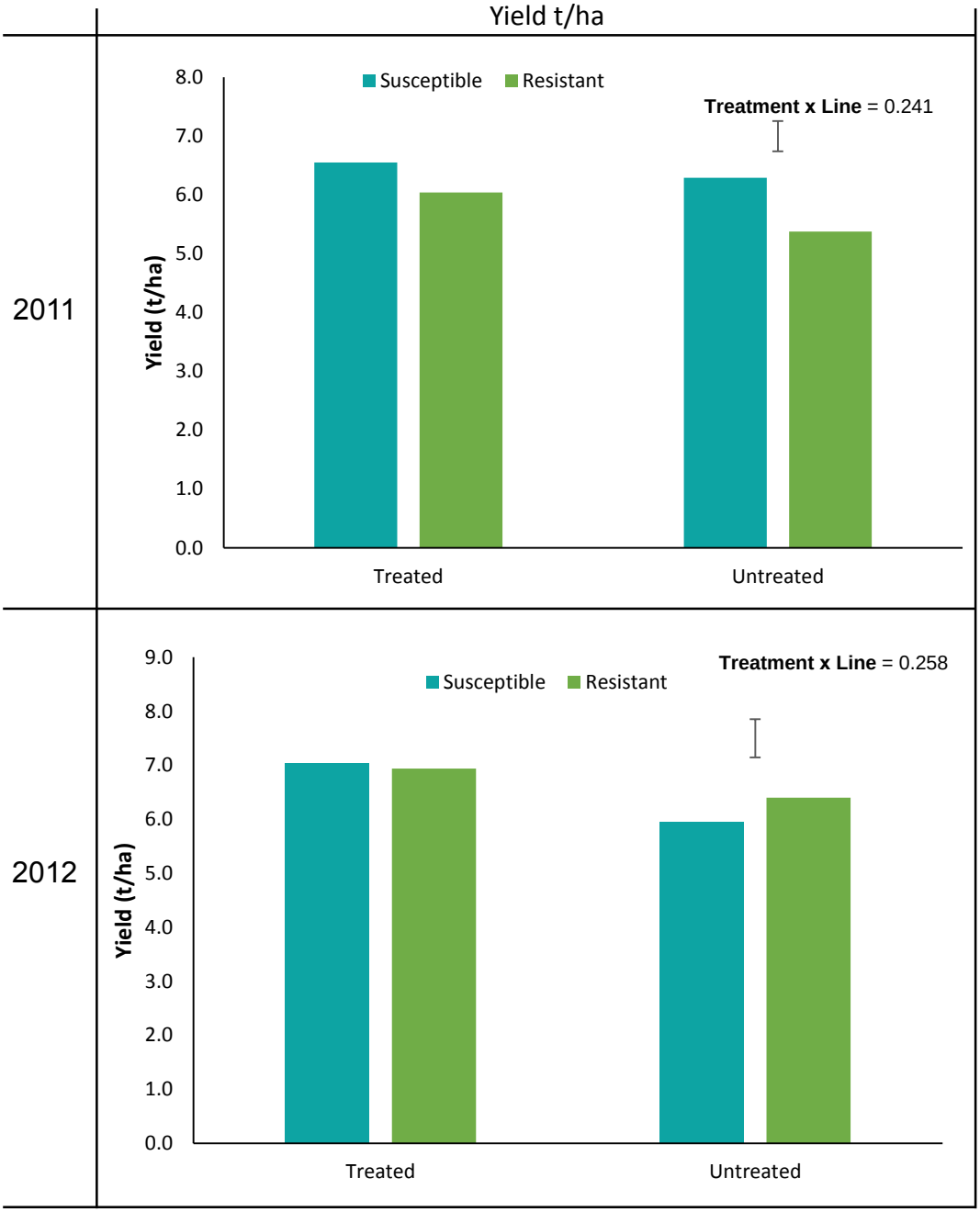


Figure 8. Average yields (t/ha 85% DM) of Viscount wheat varieties carrying resistant or susceptible Lr37 allele in 2011 and 2012 ADAS phenotypic trial. Least significant difference at 5% level from an ANOVA as shown as vertical bar.

Table 23. Average yields of Viscount wheat varieties containing resistant or susceptible alleles of Lr37 resistance QTL (mean values of fungicide treated and inoculated plots).

		2011	2012
Yield (t/ha)	Resistant (+Lr37)	5.70	6.67
	Susceptible (-Lr37)	6.44	6.49
	L.S.D.	0.391	NS
	D.O.F.	9	9
	P-value	0.002	0.445

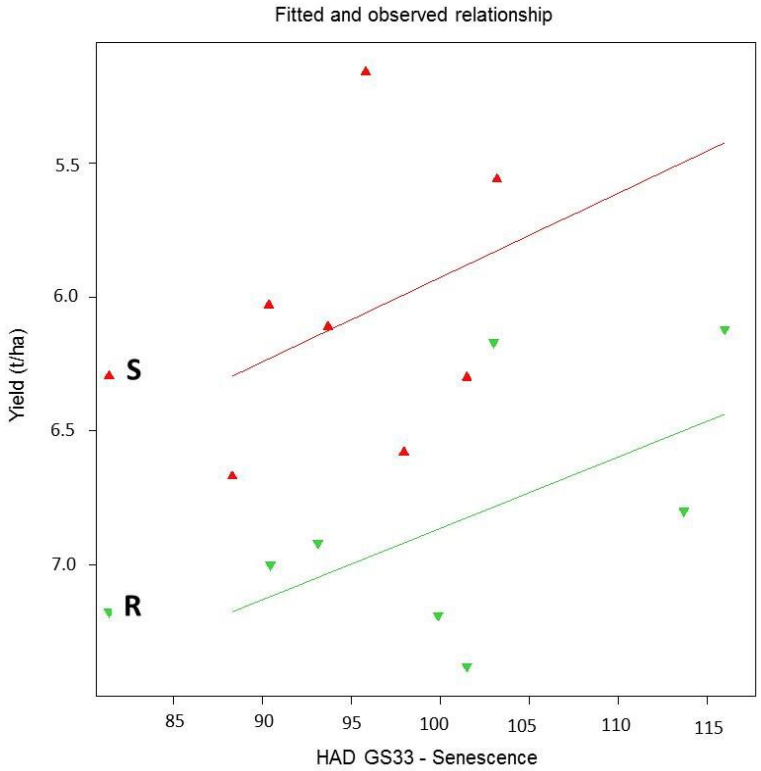


Figure 9. Relationship between yield (t/ha) and Healthy area duration (HAD) between GS33 and senescence (P = 0.025; 40% variance accounted) of Viscount lines +/- Lr37 in 2011 ADAS phenotypic trial.

Yield costs in Option x Potent wheat carrying Lr37

Eight lines were selected from the Option x Potent DH mapping population and were matched into four pairs contrasting for the *Lr37* brown rust resistance alleles. Replicated yield trials were conducted by Syngenta (2013), Limagrain (2014) and RAGT (2014) and all plots were treated with fungicide in order to assess yield costs associated with the *Lr37* gene in the absence of disease. A cross-site analysis via ANOVA, showed that there was a significant yield cost associated with lines which carried *Lr37* when compared to lines which carried the susceptible allele (*P* = <0.001), with resistant lines exhibiting an average yield deficit of 0.32 t/ha when compared to susceptible lines. Trials conducted by Syngenta in 2013 and Limagrain 2014 showed significantly lower yield in the resistant lines when compared to susceptible lines when single-site analyses were carried out (Table 24). However, there was no significant difference in average yield between resistant and susceptible lines in the RAGT trial, although the resistant lines did show lower yield when compared to the susceptible lines. In summary, there does appear to be a significant yield cost associated with the *Lr37* gene in the Option x Potent background in the absence of disease, with two out of three trials showing a yield deficit in the lines carrying *Lr37* resistance.

Table 24. Average yield (t/ha 85% DM) of Option x Potent wheat lines carrying resistant or susceptible Lr37 allele in 2013 and 2014 industry partner trials (all plots treated with fungicide). Cross site analysis of Syngenta 2013 yield data and Limagrain and RAGT 2014 yield data. Least significant difference, degrees of freedom and P-value at 5% level based on an ANOVA using Lr37 as a factor are shown.

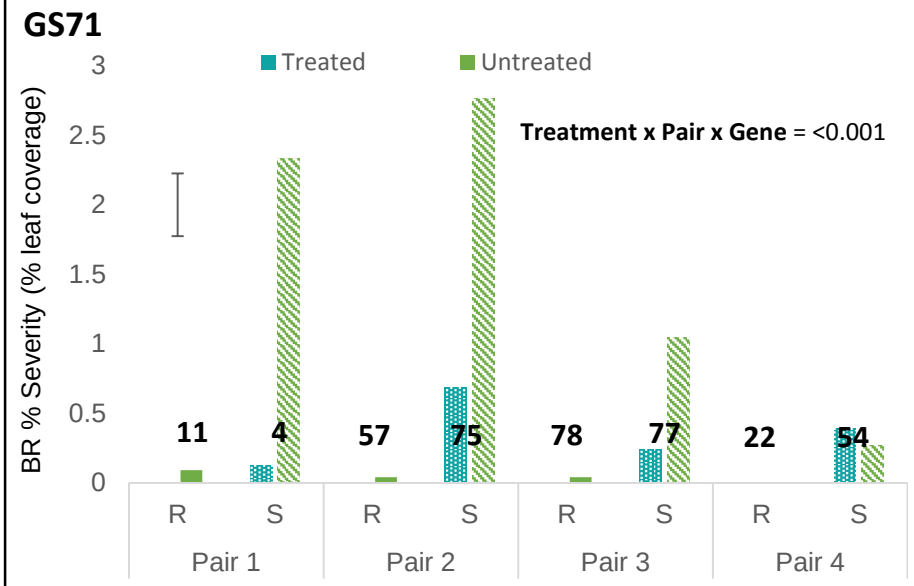
		Syngenta	Limagrain	RAGT	Cross-site
Yield (t/ha)	Resistant (+Lr37)	7.798	10.074	11.66	9.844
	Susceptible (-Lr37)	8.027	10.617	11.829	10.159
	L.S.D.	0.189	0.1197	NS	0.0883
	P-value	0.019	< 0.001	0.106	<0.001

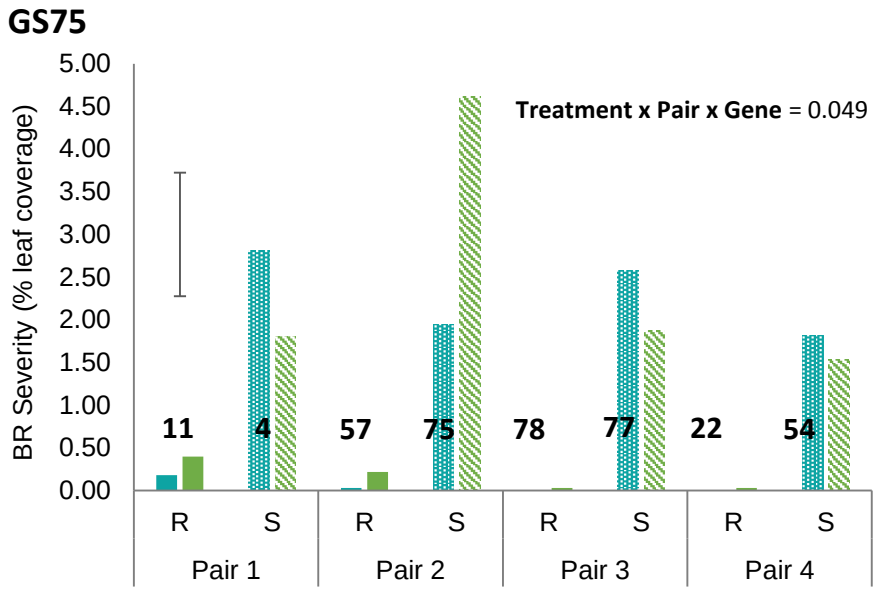
Two field experiments were conducted during 2011/2012 and 2013/2014 by ADAS, to test for yield costs associated with *Lr37* disease resistance in the Option x Potent background. Both trials tested eight lines which were selected from the OxP DH mapping population and matched into four pairs, contrasting for the *Lr37* brown rust resistance locus. Both experiments were a randomised block design using split plots, with 'challenged' (inoculated and fungicide untreated)/'unchallenged' (uninoculated and fungicide treated) as main plots and matched pairs and gene (R or S) as subplots. *Lr37*+/- lines were tested in the presence and absence of brown rust challenge to generate differing levels of resistance responses, as outlined in 3.2.2.

In 2012, brown rust was present at all disease assessments (GS32, GS71 and GS75) in 'challenged' (untreated) plots, suggesting that the artificial inoculation was successful, however levels were low with % leaf coverage recorded at <5%. Yellow rust and septoria tritici blotch were also present at low levels, at GS32, at <0.25% average leaf coverage. Yellow rust levels remained low throughout the duration of the trial, however septoria levels did peak at GS71, with ~9% leaf coverage on leaf 3. At GS71 brown rust severity in 'challenged' (untreated) susceptible plots was significantly higher than resistant plots (P = <0.001), suggesting that the resistant gene was activated within the trial. When paired lines were examined under a three-way ANOVA, a significant Treatment x Pair x QTL interaction was evident (P= <0.001). All 'unchallenged' (treated) resistant and susceptible plots within the four pairs exhibited low average levels of brown rust (<1% disease severity) suggesting good fungicide control of brown rust in treated plots. At GS75 no significant difference was seen between 'challenged' plots and 'unchallenged' plots. In 'challenged' plots the resistant lines showed considerably lower brown rust than the susceptible lines across all the paired lines, suggesting *Lr37* remained active during the trial. When paired lines were examined under a three-way ANOVA, a significant Treatment x Pair x Gene interaction was evident (P = 0.049). All 'unchallenged' resistant plots within the four pairs exhibited low average levels of brown rust (<1% disease severity). However, 'unchallenged' (treated) resistant plots exhibited higher levels of brown rust symptoms than the treated resistant plots, suggesting that full disease control had been lost between GS71 and GS75 which could potentially have an impact on yield (See Figure 10 for disease severity data). In 2014, brown rust was present at all disease assessments (GS32, GS61, GS71) in 'challenged' (untreated) plots, suggesting that the artificial inoculation was successful, however levels were low with % leaf coverage recorded at <5%. Yellow rust and septoria tritici blotch were also present at low levels, at

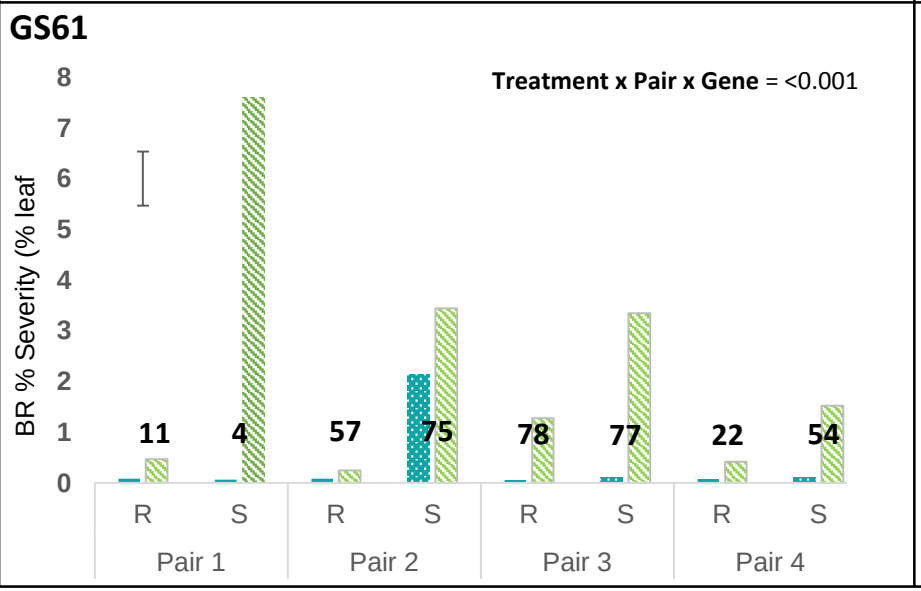
GS32, at <0.25% average leaf coverage. At GS61 'challenged' (inoculated/untreated) plots had significantly more brown rust (average % coverage) than 'unchallenged' (uninoculated/treated) plots (P = <0.001). In addition, differences in brown rust severity in untreated susceptible plots was significantly higher than resistant plots (P = <0.001), suggesting that the resistance gene was activated within the trial. When paired lines were examined under a three-way ANOVA a significant Treatment x Pair x Gene interaction was evident (P = <0.001). All treated resistant and susceptible plots within the four pairs exhibited low average levels of brown rust (<1% disease severity) suggesting good fungicide control of brown rust in treated plots. At GS71 no significant difference was seen between 'challenged' (inoculated/untreated) plots and 'unchallenged' (uninoculated/treated) plots or between plots carrying the resistant and susceptible alleles, indicating that there was a low level of disease across all the trial plots at GS71 (Figure 10).

2012


GS71
Treated
Untreated
Treatment x Pair x Gene = <0.001
BR % Severity (% leaf coverage)
3
2.5
2
1.5
1
0.5
0
11
4
57
75
78
77
22
54
R
S
Pair 1
Pair 2
Pair 3
Pair 4


GS75
Treatment x Pair x Gene = 0.049
BR Severity (% leaf coverage)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
11
4
57
75
78
77
22
54
R
S
Pair 1
Pair 2
Pair 3
Pair 4

2014


GS61
Treatment x Pair x Gene = <0.001
BR % Severity (% leaf
8
7
6
5
4
3
2
1
0
11
4
57
75
78
77
22
54
R
S
Pair 1
Pair 2
Pair 3
Pair 4

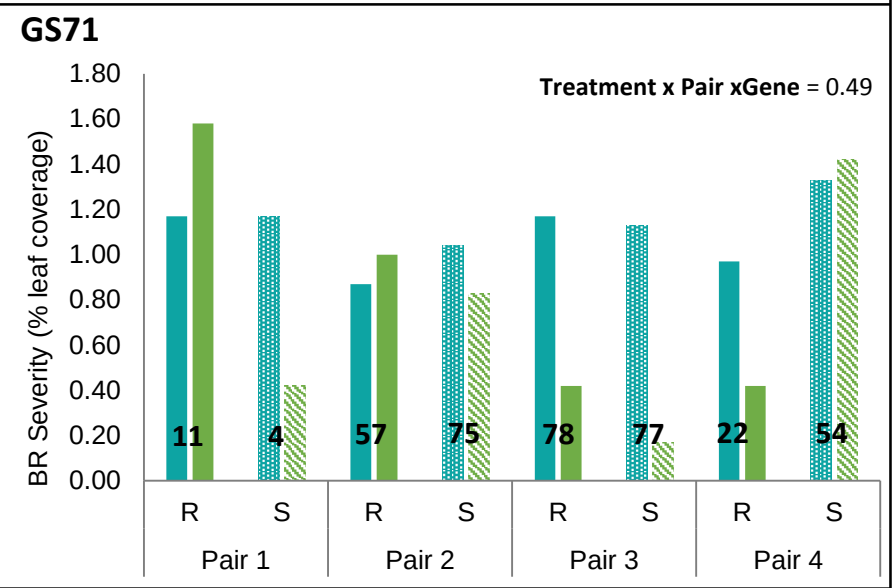

GS71
Treatment x Pair xGene = 0.49
BR Severity (% leaf coverage)
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
11
4
57
75
78
77
22
54
R
S
Pair 1
Pair 2
Pair 3
Pair 4

Figure 10. Average disease severity (% leaf coverage at GS71 and GS75 (2012) and GS61 and GS71 (2014)) and yields (t/ha 85% DM) of Option x Potent Wheat lines carrying resistant or susceptible Lr37 allele in 2012 and 2014 ADAS phenotypic trial (n = 4). Vertical bar represents least significant difference at 5% level from an ANOVA...

In 2013 and 2014 three yield trials were conducted by Syngenta, Limagrain and RAGT, where resistant lines carrying *Lr37* in the OxP background exhibited a significant yield deficit when compared to the susceptible lines. However, in trials conducted by ADAS in 2012 and 2014 the opposite trend was observed, with the resistant lines carrying the *Lr37* gene exhibiting a significant yield benefit (*P* = <0.001) when compared to lines carrying the susceptible allele in both trials (Table 25).

In 2012, resistant lines of matched pairs 2 and 4 exhibited more yield than susceptible lines in 'unchallenged' (treated) and 'challenged' (untreated) plots, suggesting that *Lr37* had been activated in untreated plots and was responsible for a yield benefit and that either there is no yield cost associated with *Lr37* in 'unchallenged' (treated) plots or that late infection of treated susceptible plots at GS75 has significantly lowered yield in these plots. Resistant lines of pair 1 exhibited more yield than susceptible in 'challenged' plots, suggesting that the *Lr37* gene had been activated. However, resistant lines in pair 1 had the same average yield as susceptible lines when treated with fungicide, suggesting that either there is no yield cost associated with *Lr37* or that late infection of treated susceptible at GS75 has significantly lowered yield in these plots. Resistant lines in pair 3 exhibited lower average yield than susceptible lines in 'unchallenged' (treated) plots, suggesting that a yield cost is associated with *Lr37* in pair 3. Both the resistant and susceptible lines showed the same levels of yield in the 'challenged' (untreated) plots, suggesting that activation of *Lr37* had minimal yield effect in pair 3 (Figure 11).

In 2014, resistant lines of pair 1, 2 and 4 showed more average yield than susceptible lines under 'challenged' (untreated) conditions, suggesting that *Lr37* had been activated in untreated plots. In addition, resistant lines of pair 1, 2 and 4 showed more average yield than susceptible lines under treated conditions, suggesting that either there is no yield cost associated with *Lr37* in treated plots or that late infection of treated susceptible plots at GS71 has significantly lowered yield in these plots. In 2014, pair 3 performed similarly to the 2012 trial, suggesting that a yield cost is associated with *Lr37* in pair 3 (Figure 11).

In summary, data from 2012 and 2014 trials suggest that the *Lr37* gene in the OxP background is effective against current brown rust races, as a yield benefit was observed in the resistant untreated and treated plots in the presence of just a small amount of disease. Previously, Lr37 had been considered defeated, suggesting that a significant change in brown rust races has occurred within the natural population over recent years. Loss of brown rust control in 'unchallenged' (treated) susceptible plots may have confounded the data by lowering the yield of 'unchallenged' susceptible plots and subsequently masking any yield costs associated with *Lr37*. These trials show the importance of controlling disease throughout the duration of the trial, as late expression of the disease can induce *Lr37* expression in the 'unchallenged' susceptible plots and the subsequent

yield benefit can then mask any costs of resistance associated with the resistance gene.

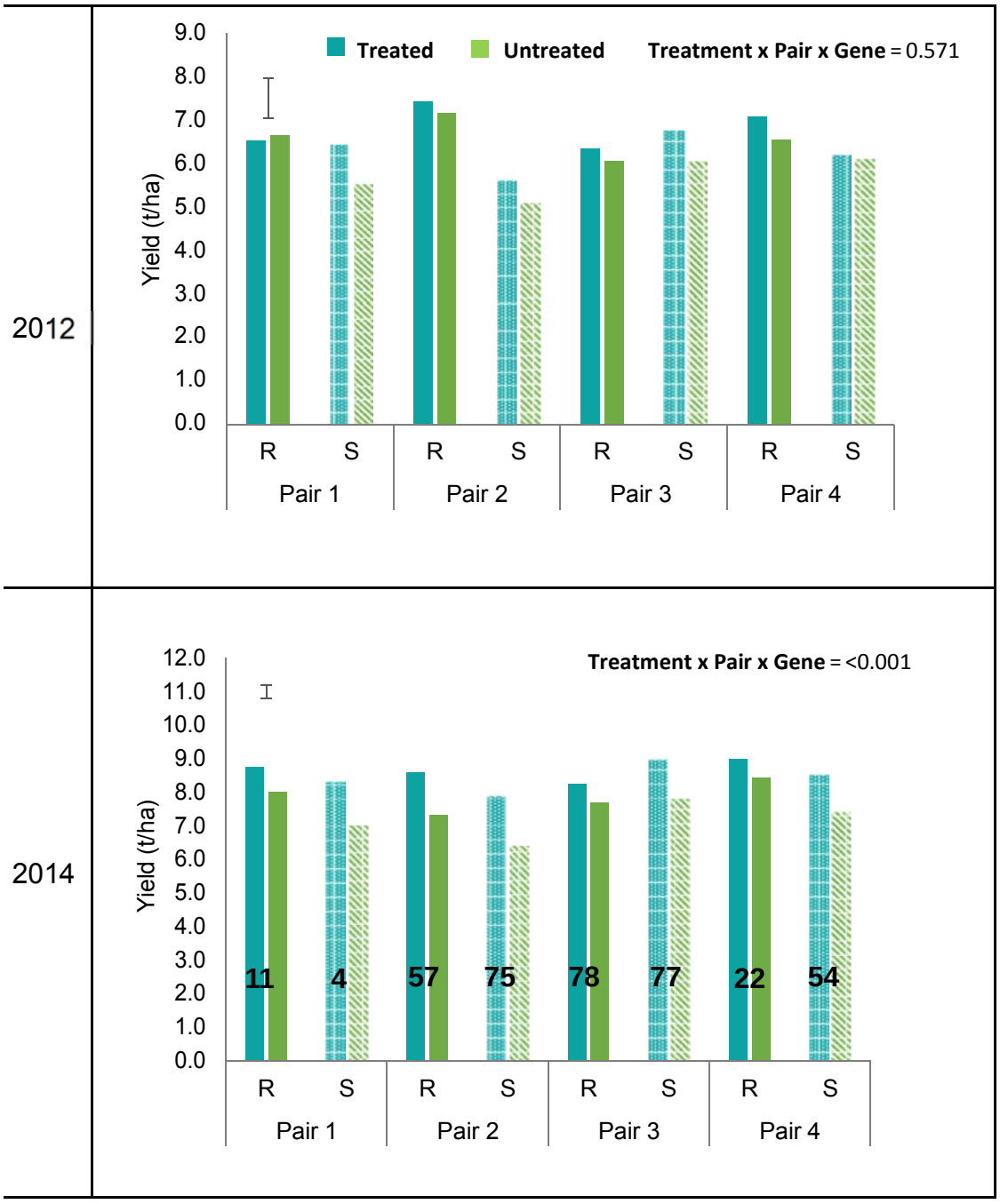


Figure 11. Average yields (t/ha 85% DM) of Option x Potent wheat lines carrying resistant or susceptible Lr37 allele in 2012 and 2014 ADAS phenotypic trial (n = 4). Vertical bar represents least significant difference at 5% level from an ANOVA.

Table 25. Yields (t/ha 85% DM) of Option x Potent wheat lines containing resistant or susceptible allele of Lr37 brown rust resistance QTL (mean values of fungicide treated and inoculated plots). Least significant difference, degrees of freedom and P-value based on ANOVA is shown.

		2012	2014
Yield (t/ha)	Resistant (+Lr37)	6.713	8.254
	Susceptible (-Lr37)	6.024	7.842
	L.S.D.	0.3123	0.1386
	Degrees of Freedom	42	42
	P-value	< 0.001	< 0.001

Discussion

Quantification of yield penalties associated with defeated resistance genes

In summary, trials conducted between 2011 and 2014 testing the 'defeated' *Lr37* brown rust resistance gene for deleterious yield effects have provided evidence to suggest that *Lr37* is associated with yield costs in the absence of disease. Evidence of yield costs associated with *Lr37* have been identified in three genetic backgrounds, Viscount, OxP and Thatcher. Yield costs of between 0.32 t/ha and 0.74 t/ha in the OxP and Viscount backgrounds have been observed, however, such effects have not proven repeatable across sites and season, due to variation in abiotic and biotic stress.

At the start of this project *Lr37* was considered 'defeated' and thus ineffective against current UK brown rust races. However, trials conducted in 2012 and 2014 by ADAS where *Lr37* was tested in the Option x Potent background suggests that the brown rust resistance gene is effective against current UK races, with resistant lines producing an average yield benefit of between 0.4 t/ha and 1.69 t/ha when compared to susceptible lines. The perceived yield benefit associated with lines carrying the *Lr37* resistance gene may be due to the unexpected loss of yield in susceptible treated plots caused by a late brown rust infection that fungicide treatments failed to completely control. It appears that even a modest infection in the field can produce a significant yield benefit.

In conclusion, *Lr37* does appear to be associated with deleterious yield costs in the absence of disease. Although it's thought to be 'defeated' *Lr37* does appear effective, even in modest epidemics. Therefore, it may not be advantageous for breeders to select against this gene within their breeding programmes, as it appears to provide good protection against yield losses during small brown rust epidemics. However, if in the future *Lr37* is found to be ineffective, varietal yield maybe improved by removing this resistance gene from future breeding lines.

4.3. Identification and optimisation of methods to screen future resistance genes for yield penalties (Deliverable 3)

Development of methods to screen for costs of resistance associated with brown rust resistance genes in wheat (IBERS Aberystwyth University).

The cost of a plant deploying its defences against invading pathogens has been linked to altered photosynthesis, respiration or metabolite deficiency as resources are diverted towards defence. IBERS has led research showing that defence responses have also been shown to alter stomatal function. During *R*-gene elicited resistance responses of barley and oat to powdery mildew, stomata lock in an open configuration; displaying little or no closure in response to diurnal rhythms or abscisic acid. This IBERS-led work package investigated the following:

1. If stomatal perturbation is a feature of all R gene-mediated defence responses against brown rust and whether measuring stomatal dysfunction could be used as a screening method for costs of resistance in wheat germplasm.
2. To assess the metabolomic impact of R-genes and investigate changes in metabolic flux of resistant wheat lines as a possible indicator of costs of resistance.

Screening for stomatal perturbation R gene-mediated defence responses against brown rust

Thatcher (Tc) near-isogenic lines with differing leaf rust (*Lr*), R genes were infected with field-isolated brown rust and the impact on stomatal conductance was assessed using a Delta-T porometer (Figure 12).

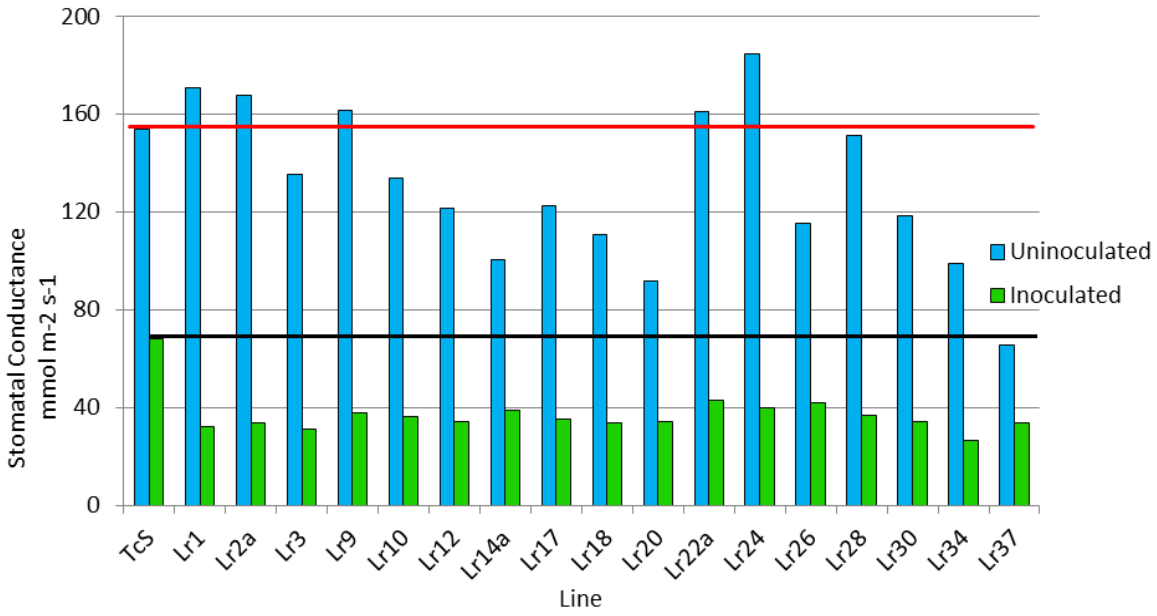


Figure 12. Average light period stomatal conductance of uninoculated and inoculated Thatcher near isogenic lines (see text for detail).

Average stomatal conductance in the uninoculated group during the light period varied with different genotypes. The conductance level of the uninoculated susceptible line (TcS) is highlighted by the red line; and a majority (71%) of *Lr*-encoding lines exhibited a lower average stomatal conductance level. Conductance level of inoculated TcS is indicated by the black line and it can be seen that all *Lr* lines show a lower stomatal conductance following challenge with brown rust which was suggestive of a reduced stomatal opening.

Statistical analysis of the data demonstrated a significant difference ($P<0.001$) between treatment groups. Post-hoc tests of uninoculated plants showed that three *Lr* lines were significantly lower than TcS, i.e. *Lr20* ($P = 0.019$), *Lr34* ($P = 0.049$), and *Lr37* ($P = 0.002$). In the inoculated datasets, post-hoc tests showed differences between TcS and all *Lr* lines ($P<0.001$).

Follow-up analyses focused on examining the stomatal responses of paired lines of Option x Potent (O x P) following inoculation with brown rust and in uninoculated controls.

Analysis of O x P matched pairs demonstrated variability in stomatal conductance level in un-inoculated lines. In the three pairs, lines with resistance gene *Lr37* (+37, Figure 13) showed a significantly ($P = 0.01$) higher rate of stomatal conductance than that of the corresponding line without *Lr37* (-37). However, in pair four, the +37 line showed a lower level of conductance compared to the -37 line. Considering the effects of inoculation, all lines demonstrated reduced stomatal conductance, irrespective of possession of the *Lr37* gene.

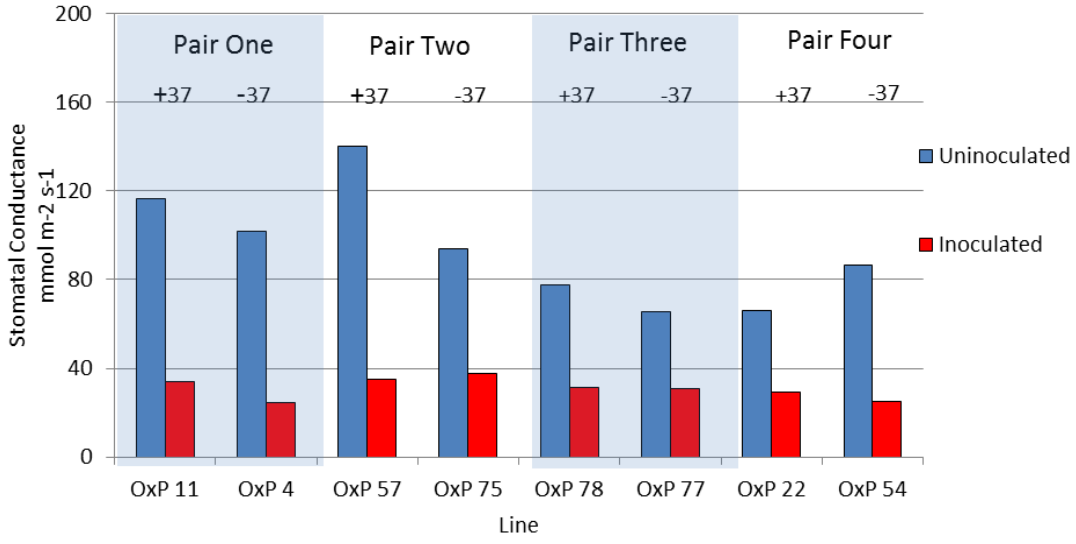


Figure 13. Average light period stomatal conductance of Option x Potent matched pairs +/- Lr37 resistance gene.

To assess the metabolomic impact of R-genes and investigate changes in metabolic flux of resistant wheat lines as a possible indicator of costs of resistance.

Classically, *R* genes are thought to be triggered through their interaction with pathogen-triggered avriulence gene products. However, data such as provided in Figure 12 suggested that R genes or linked loci are influencing stomatal opening in the absence of pathogen challenge. The regulation of stomatal opening is influenced by both carbon and nitrogen fluxes which would be reflected as changes in primary and secondary metabolism. Therefore, we tested the hypothesis that "background" R genes effects were influencing metabolism in such a way as to influence stomatal function.

Two week old seedlings of six genotypes selected from the previous stomatal conductance study were sampled and polar and non-polar extractions were profiled by mass spectroscopy. The derived profiles were analysed by flow-infusion electrospray-mass spectroscopy (FIE-MS). The resulting profiles were analysed using multivariate statistics (Figure 14). These suggested three clusters of genotypes displaying broadly similar metabolomic patterns; one group comprising *Lr10*/*Lr20* another *Lr9*, *Lr24* and *Lr37* and a final group comprising a single genotype *Lr34.*

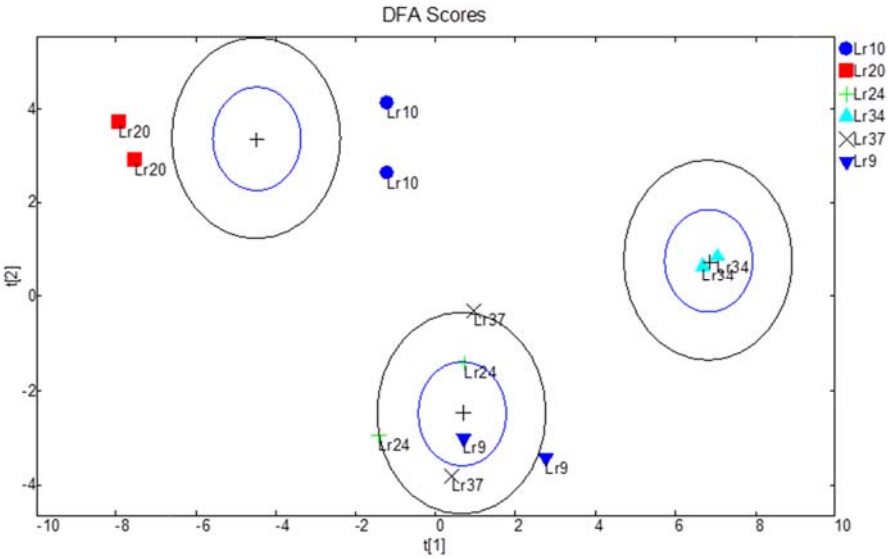


Figure 14. Discriminant function analysis of selected uninoculated Lr lines. Circles represent 90% and 95% confidence intervals.

Interrogation of the major sources of variation between the clusters particularly targeted differences in the accumulation of metabolites linked to the tricarboxylic acid (TCA) cycle and core phenylpropanoid pathway; a source of defence- related phenolic compounds. To display these changes heat-maps of the log transformed levels of metabolites linked to TCA (Figure 15A) or core phenylpropanoid pathway (Figure 15B) were derived. In both heat-maps, lines *Lr34* and *Lr37* can be seen to generally be showing an increase in metabolites associated with both TCA and phenylpropanoids compared to other lines. *Lr10* shows some increases in levels of phenolic

compounds and in TCA associated metabolites, however there is also evidence of lower levels in some compounds. The general trend for other lines is a relative reduction in the levels of these metabolites.

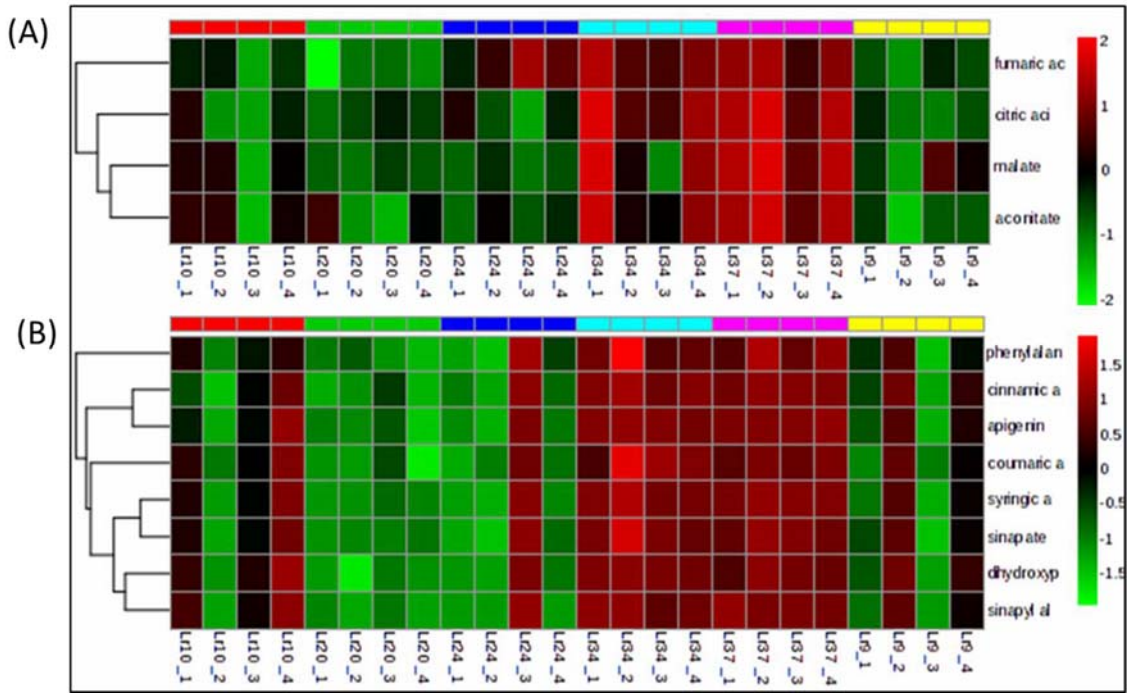


Figure 15. Heat-maps of metabolite increases/decreases in selected uninoculated Thatcher lines. A) Heat map of log transformed levels of metabolites linked to tricarboxylic acid (TCA) cycle. B) Heat map of log transformed levels of metabolites linked to the core phenylpropanoid pathway.

Porometry of wheat infected with septoria tritici blotch (JIC, Norwich)

In the first of two experiments to test the effect of septoria tritici blotch on stomatal conductance (SC), the inoculation process greatly increased SC in both the dark and the light. In the light, mean scores varied between experiments, cultivars, treatments and time after inoculation but no single cultivar, treatment or time had consistently the highest or lowest SC. In the dark, these effects of most experimental factors were similarly variable, although the four varieties varied with some degree of consistency. Flame had the lowest SC in the dark (Baldus 41, Flame 23, Longbow 51, Maris Dove 36 mol m^{-2} s^{-1}; standard error of $\log_{10}$ SC: 0.013 mol m^{-2} s^{-1}). The principal conclusion of these experiments is that the process of inoculating plants with *Z. tritici* has profound effects on the physiology of wheat, which overrides relatively small and somewhat variable effects relating to inoculation of the plant or the type of interaction with the host, compatible or incompatible.

In a second set of experiments, barley plants inoculated with *Blumeria graminis f. sp. hordei* (*Bgh)* (or mock-inoculated) were grown at the same time in the same CE chamber as wheat plants, to check that the environmental conditions used produced results comparable to published data on barley mildew. Data relating to barley mildew were consistent with previous experiments (Prats *et al.,* 2006, 2010), with reduced SC ('lock-shut') in light periods at 24, 48 and 72 hai in infected plants,

whether the *Bgh* isolate was virulent or avirulent. There was increased SC ('lock-open') in the dark periods at 40, 64 and 84 hai in incompatible interactions between barley and the avirulent *Bgh* isolate but not in compatible interactions (Figure 16).

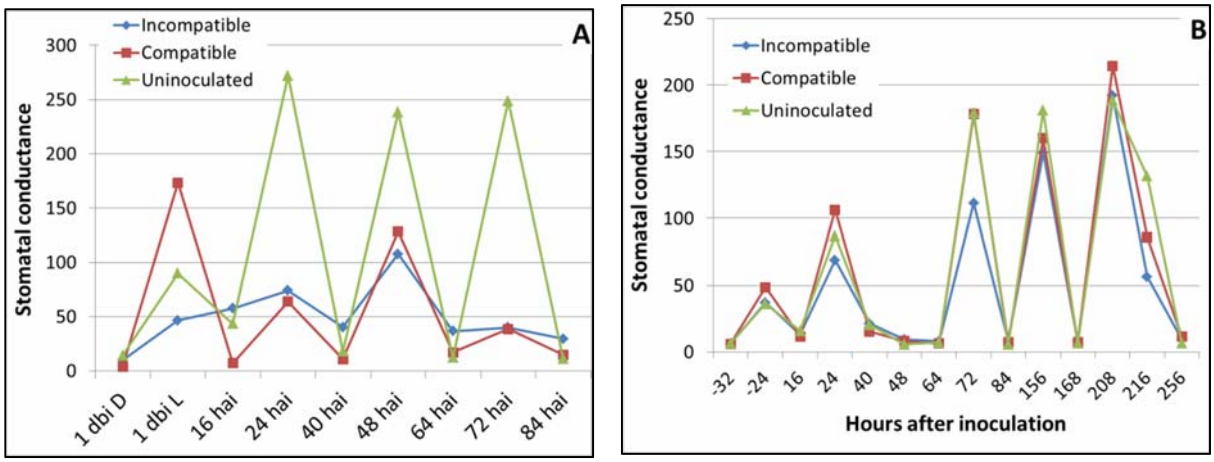


Figure 16. Stomatal conductance (mol m^{-2} s^{-1}) of cereal varieties avirulent or virulent pathogens, resulting in incompatible or compatible interactions, or mock-inoculated. (A) Barley powdery mildew. (B) Septoria tritici blotch of wheat.

Tween 20 is a detergent used in many plant pathology experiments to aid suspension of spores for use as inoculum and to allow an aqueous inoculum mix to spread over the waxy surface of leaves. The design of the *Z. tritici* experiment allowed the effect of Tween on SC to be assessed. It had a large and highly significant effect on causing 'lock-open' of stomata in the dark, with means higher than all other factors of 27.6 and 9.4 mol m^{-2} s^{-1} with and without Tween 20, respectively. Mean SC in the light was 100 mol m^{-2} s^{-1} with or without Tween. All effects of factors other than the use of Tween 20, the contrast between day and night, and the interaction between Tween and time of day, were comparatively minor. Therefore, further analysis was done of treatments in which Tween 20 was not used.

As in the first set of experiments, the inoculation process increased SC in the first day after inoculation, regardless of whether the isolate was avirulent or virulent, or the plants were mock-inoculated. While SC showed a diurnal cycle, as expected, there was no evidence for stomata being either locked open at night or locked shut in the daytime. As in the first set of experiments, Flame, the more resistant variety, had a lower SC than Longbow, although this was more evident in the light than the dark (dark: Flame 9.1, Longbow 9.6 mol m^{-2} s^{-1}; light: Flame 83, Longbow 122 mol m^{-2} s^{-1}; standard error of $\log_{10}$ SC: dark 0.023, light 0.025 mol m^{-2} s^{-1}; *P*-value for difference between varieties: dark 0.4, light 0.003).

Discussion

Screening for stomatal perturbation *R* gene-mediated defence responses against brown rust

Controlled environment studies concluded that brown rust challenge can induce stomatal dysfunction in wheat. In addition, the majority (71%) of Thatcher NILs with *Lr* resistance genes demonstrated lower stomatal conductance than the susceptible variety in the absence of disease, with three *Lr* lines (Lr20, Lr34, Lr37) exhibiting significantly lower stomatal conductance than the susceptible control. In the inoculated datasets, post-hoc tests showed significant differences between TcS and all *Lr* lines. It can be hypothesised that the perturbed stomatal regulation seen with or without brown rust infection could lead to a reduction in overall plant fitness and therefore impact on yield. This notion is supported by ADAS field trial data which showed that corresponding Thatcher NILs returned lower yields than the TcS susceptible variety. However, 29% of uninoculated Lr lines did show an increased rate of stomatal conductance in comparison with the susceptible line, yet they also returned a lower yield in the field trials. This variability would suggest that reduced stomatal conductance is one of many factors contributing to yield losses in uninoculated lines. Therefore, it would not be possible to use stomatal conductance alone, as a predictor of yield costs associated with genetic resistance.

To assess the metabolomic impact of R-genes and investigate changes in metabolic flux of resistant wheat lines as a possible indicator of costs of resistance

Metabolomic analysis of *Lr10*, *Lr34* and *Lr37* identified a significant accumulation of metabolites linked to the tricarboxylic acid (TCA) cycle and core phenylpropanoid pathway; a source of defence related phenolic compounds. The findings from metabolomic analysis would suggest that lines *Lr10*, *Lr34,* and *Lr37* may demonstrate a specific cost of resistance in the form of allocation costs. The production of constitutive defence-related phenolic compounds (Figure 15) is energy demanding and could be linked to the increase in metabolites associated with the TCA cycle. The latter in particular would result in altered malate content and bioenergetic metabolism; particularly, the utilisation of hexose sugars (data not shown). Such biochemical changes would affect stomatal opening as well as contributing to the energetic requirements of a defence response. Thus, stomatal locking would be seen as a wider impact of defence deployment that are conferred by certain *R* genes even under uninfected conditions. It would appear from studies carried out during this project that *Lr37* is likely to confer a constitutive cost associated with metabolic flux and stomatal dysfunction in the absence of disease. These results support the data from field trials conducted during the trial, which suggests that *Lr37* confers a yield cost in the absence of disease in three different genetic backgrounds.

Results from this project suggest that metabolomic analysis of key defence and energy metabolism compounds, could prove to be an effective technique for identifying costs of resistance in pre-breeding programmes.

Porometry of wheat infected with septoria tritici blotch (JIC, Norwich)

The results from both experiments suggest that the procedure for inoculation, particularly the use of Tween 20 as a detergent, causes substantial effects on the physiology of wheat plants which perturb stomatal conductance (SC). A recommendation for future research on the effect of *Z. tritici* on SC is that inoculation should be done in as natural a way as possible. Even in the absence of Tween 20, however, there was no evidence that inoculation with *Z. tritici*, whether avirulent or virulent, had a substantial, repeatable effect on SC. This was the case even in environmental conditions which reproduced the expected pattern of variation in SC in barley attacked by powdery mildew. It is therefore unlikely that septoria tritici blotch has a substantial effect on SC. It also appears unlikely that any yield penalty of resistance to STB is likely to be the result of inappropriate regulation of SC.

Among the cultivars, Flame had the lowest SC in both sets of experiments on *Z. tritici*, particularly in the dark in the first series and in the light in the second series. It may be notable that of the cultivars used in these experiments, Flame is the most resistant to STB in the field (Arraiano and Brown, 2006; Arraiano *et al.*, 2009) but a rigorous conclusion about the relationship of SC to resistance cannot be drawn from this small set of cultivars.

4.4. Assessment of the scope to use fungicides to ameliorate yield costs associated with host resistance responses (Deliverable 4)

Assessment of the impact of fungicide-treatments on wheat (IBERS, Aberystwyth University)

An initial study examined the effectiveness of three fungicide treatments on yellow rust infections of two near-isogenic wheat genotypes; Brigadier and *YrQ*. Examining the results from wheat plants that had not been treated with fungicide indicated that the *YrQ R* gene was effective in suppressing the germination of rust uredospores (Figure 17 A). Each fungal treatment was also able to suppress rust, uredospore germination to levels equivalent to those seen on the *YrQ* lines (Figure 17 B, C and D). In any fungicide treatment there were always ~ 5% of uredospores which still germinated but did not lead to a successful infection.

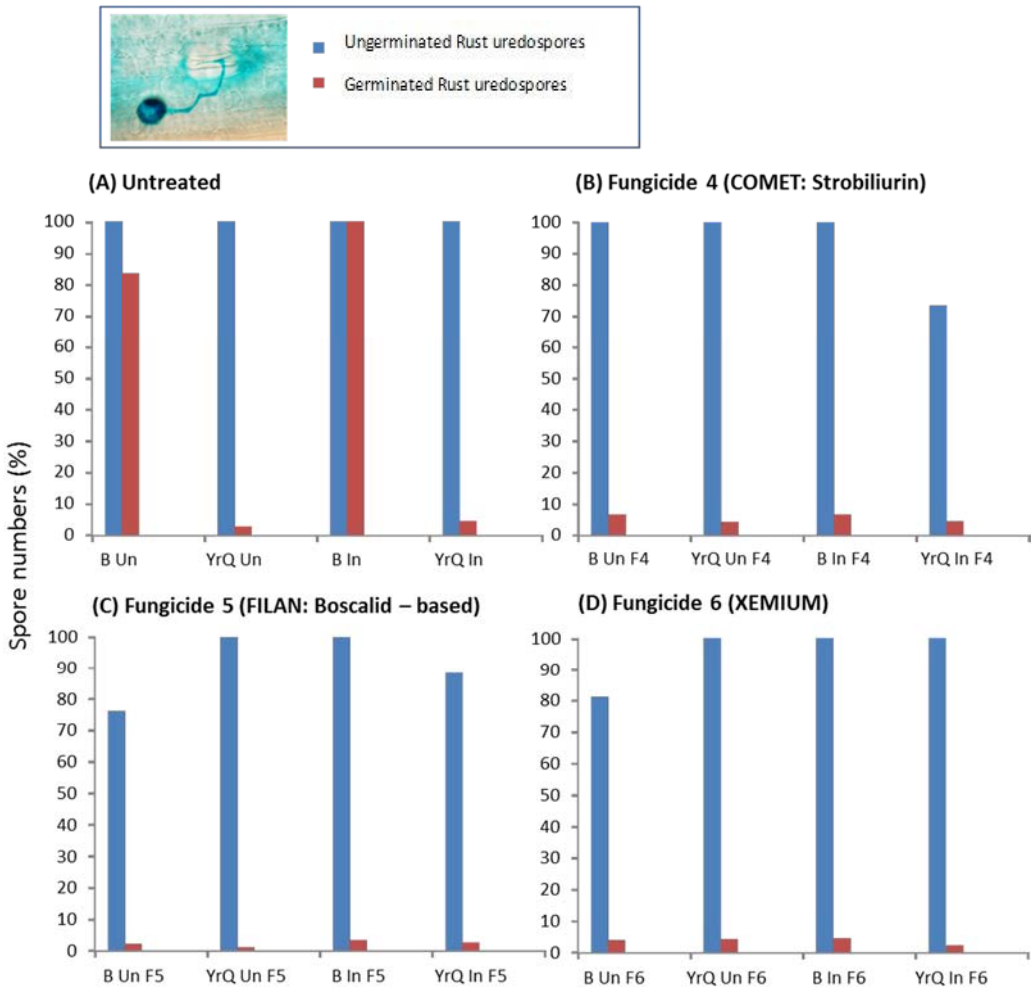


Figure 17. Proportion of germinated and ungerminated P. striiformis *uredospores Brigadier (B) and YrQ in uninfected (Un) or infected (In) field plots under different fungicide regimes.*

To begin to determine any impact of fungicide on plant physiology, field samples (n = 50 each genotype) from uninfected plants of cv. Brigadier and YrQ were assessed for the deposition of phenolics by aniline blue staining coupled with UV-microscopy (Figure 18 A). The resulting patterns of auto fluorescence were classified into difference scoring categories from 1 to 3 inclusive (Figure 18 A). Plotting the average scores for each genotype following treatment with each fungicide suggested that there was increased auto fluorescence; particularly with Filan and Xemium compared to untreated controls (Figure 18 B). Following transformation to statistical normality, both genotype and fungicide were shown to significantly (P<0.001) affect the degree of auto fluorescence in field grown wheat.

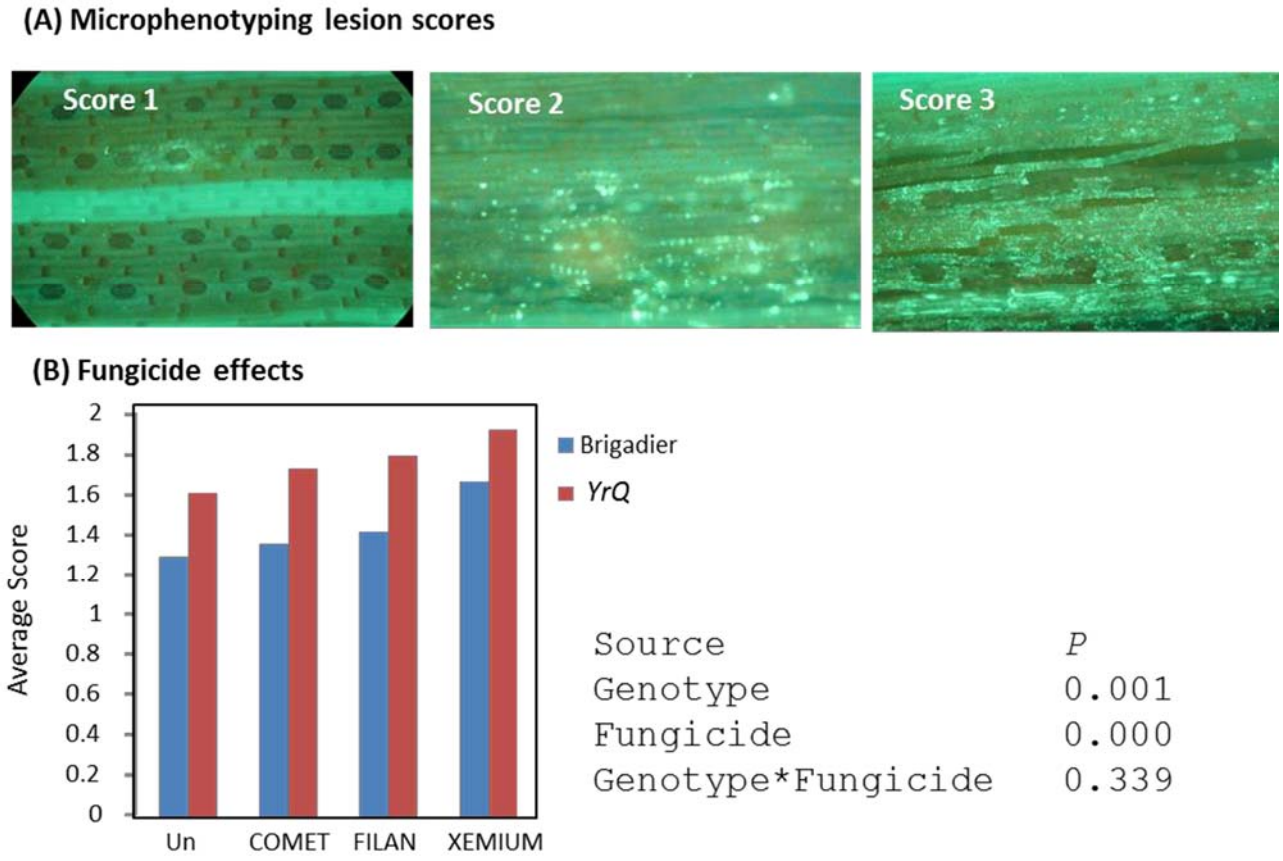


Figure 18. Assessing auto fluorescence in field grown wheat under different fungicide regimes.

Such auto fluorescence are typical of the after-effects of challenge with pathogens which we have linked to stomatal dysfunction. To assess if this was also the case following treatment with fungicides, 2 week old Brigadier seedlings (Figure 19 A) were treated with field-equivalent concentrations of fungicide and after 16 h stomatal conductivity was assessed using a Delta-T porometer (Figure 19 B) sampling over 4 days in both light and dark periods. The seedlings were grown under a 12 h light period. Treatment with each fungicide resulted in significantly (P <0.001) reduced stomatal opening in the light periods compared to controls. Interestingly, the extent of reduced stomatal opening was negatively correlated with the average auto fluorescence scores (see Figure 19C). Xemium, for example, has the highest auto fluorescence scores but the lowest stomatal opening in the light.

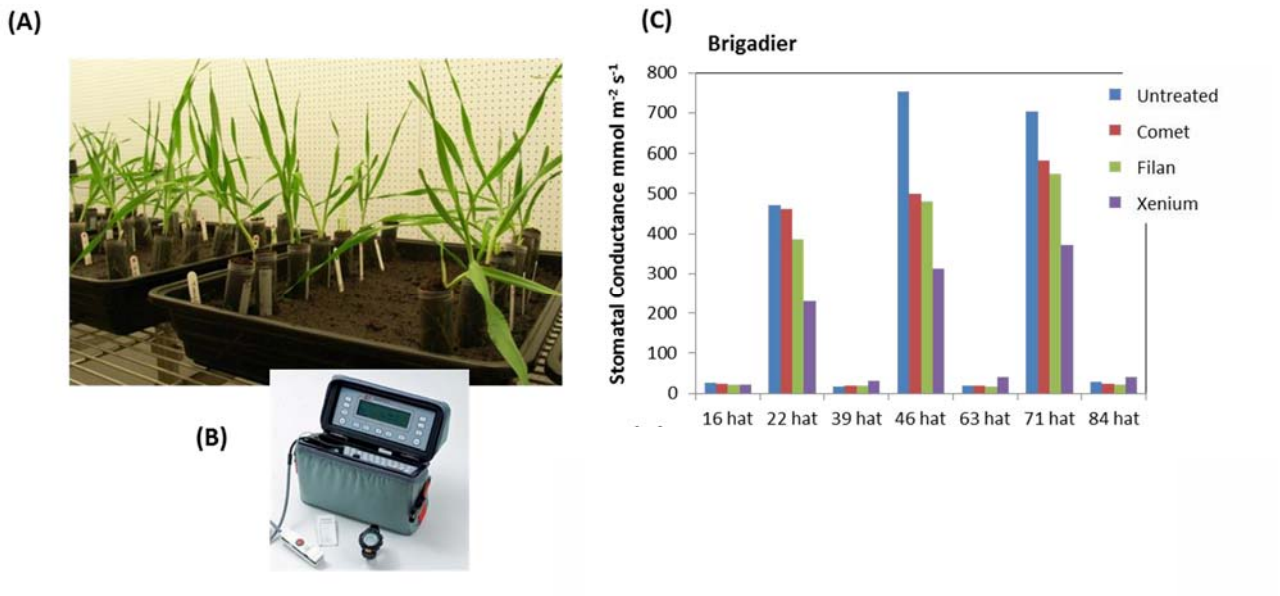


Figure 19. The effects of fungicide treatment on stomatal performance in Brigadier seedlings grown under controlled environmental conditions.

Two experiments were carried out in 2012, under controlled environment (CE) conditions, using winter wheat cultivar Brigadier and the NIL, YrQ. Seedlings were grown and maintained in a clean environment in order to test for direct effects of fungicides in the absence of pathogen challenge. The seedlings remained free from disease symptoms for the duration of the experiment. A LI-6400XT portable photosynthesis system (Li-Cor) was used to measure stomatal conductance, transpiration rate (mmol m^{-2} s^{-1}) and net photosynthetic rate (µmol m^{-2} s^{-1}) on emerged leaf 2, at five days post fungicide application.

Significant differences in stomatal conductance were not found between the susceptible (Brigadier) and resistant (YrQ) genotypes but significant (*P* = <0.001) differences were found between fungicides on both occasions (Figure 20). On each occasion, all fungicide products significantly decreased conductance when compared with the untreated control (Experiment 1: untreated= 144.0, Filan= 110.8, Ignite= 105.0, Ceriax= 59.2, sed= 6.03; Experiment 2: untreated= 150.2, Filan= 98.7, Ignite= 107.6, Ceriax= 76.1, sed= 8.45) and conductance in the Ceriax treatment was significantly lower than for the other fungicide products.

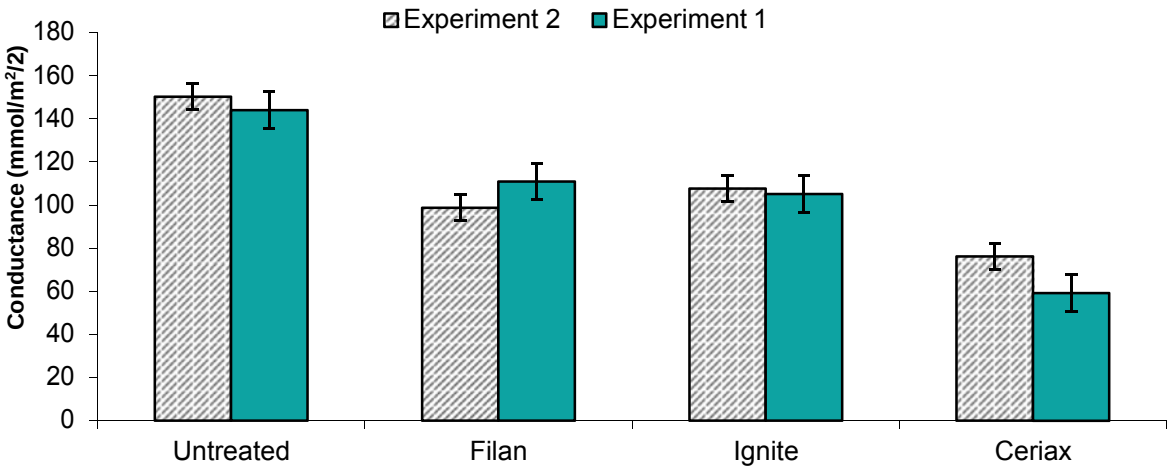


Figure 20. Stomatal conductance measured on emerged leaf 2 of winter wheat seedlings under controlled environment conditions. The solid bars represent data from experiment 1 and the hatched bars represent data from experiment 2. Data are mean values from across both genotypes.

Relatively little information exists about scaling up the effects of leaf stomatal conductance to canopy conductance. However, methods of scaling up are offered by Bailey and Davies, 1981, Whitehead *et al*., 1981 and Sinclair *et al*., 1976, and there is evidence to suggest that effects on conductance at the leaf level could follow through and be detected at the canopy level.

Although auto fluorescence has been linked to increased cell death, we sought to quantify this by measurement of electrolyte leakage (as indicative of membrane distribution linked to cell death) from explants of fungicide treated seedlings. This was measured through changes in the conductivity of the solution bathing explants which were sampled at 16 h following treatment with the each fungicide (Figure 21). In each case, there were significant (P <0.001) increases in electrolyte leakage following treatment with each fungicide.

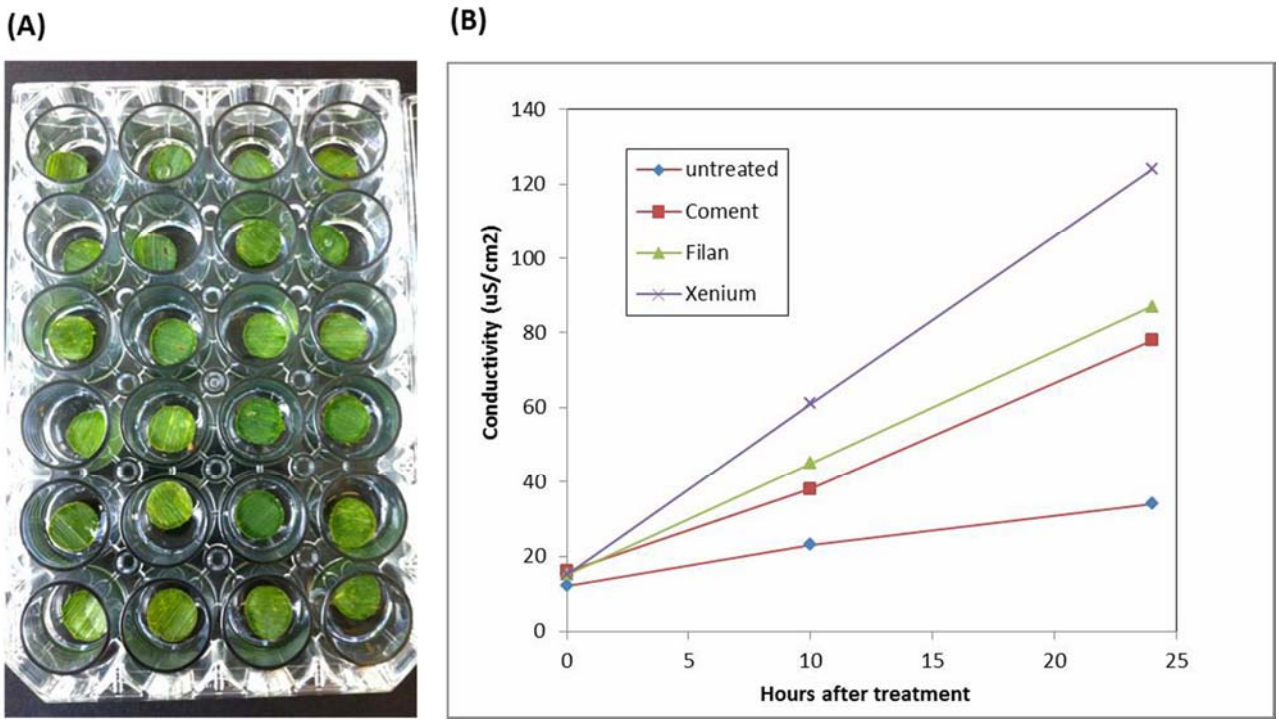


Figure 21. Electrolyte leakage from explants from seedlings of wheat cv Brigadier treated with fungicide.

ADAS/BASF 2010/2011 field trials

Winter wheat – yellow rust experiment 2010.

The crop was sown on 25 September 2009. By 06 May (GS31) a natural yellow rust epidemic was established in the field and disease foci were seen in the plots on eventual leaf 4 (EL4) (counting down the shoot, with the flag leaf being eventual leaf 1), EL5 and EL6. Disease progressed through the canopy and by late June the severity had increased to approximately 20% on the flag leaf (Figure 22).

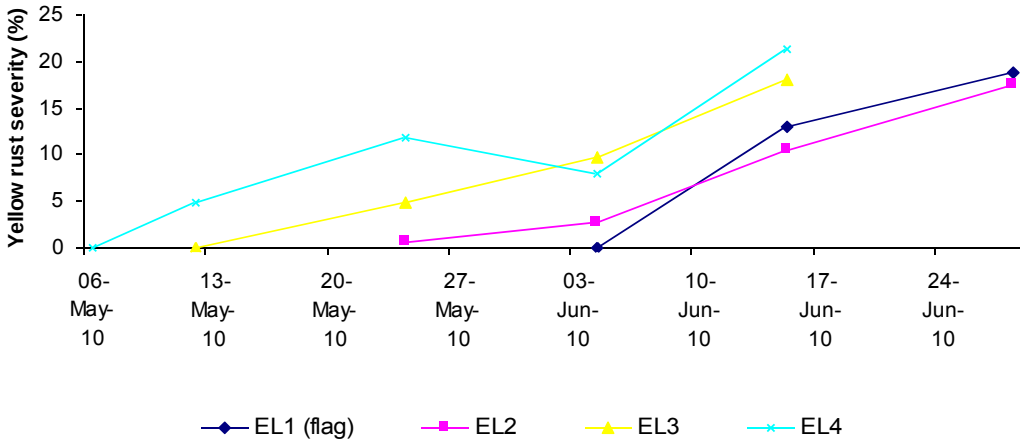


Figure 22. Yellow rust severity in inoculated, untreated Brigadier, 2010, ADAS Boxworth.

There was spread of yellow rust between inoculated and uninoculated treatments (there was no significant difference between these treatments) and disease symptoms were seen throughout the experiment. However, by GS59 (15 June), significant differences (*P* = <0.001) in yellow rust severity were seen between genotypes and between fungicide treatments (Table 26).

Table 26. Yellow rust severity on 15 June 2010 (GS59), ADAS Boxworth.

Factor	YR EL1 (%)	YR EL2 (%)	YR EL3 (%)
Brigadier	9.03	7.31	12.62
YrQ1	0.22	0.00	0.03
YrQ2	0.12	0.11	0.00
P value	*<0.001*	*<0.001*	*<0.001*
SED (66df)	*1.002*	*0.830*	*1.342*
Untreated	5.56	4.40	7.38
Comet	1.12	0.21	0.00
Filan	5.72	4.88	9.42
Imtrex	0.08	0.42	0.08
P value	*<0.001*	*<0.001*	*<0.001*
SED (66 df)	*1.157*	*0.959*	*1.549*

Winter wheat – yellow rust experiment 2011.

The crop was sown on 18 October 2010 and disease was first seen in the experiment on 27 April 2011 (GS31–32). Yellow rust severity was lower than in 2010 and reached approximately 7% on EL4, in late June (Figure 23).

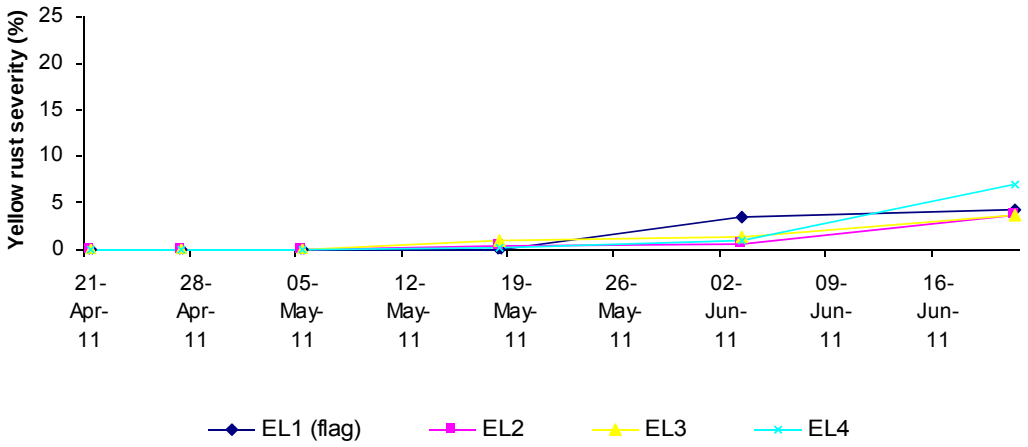


Figure 23. Yellow rust severity in inoculated untreated Brigadier, 2011.

On 03 June (GS59) inoculated treatments had more disease than uninoculated treatments and differences were significant for EL1 (*P* = 0.004). The susceptible genotype Brigadier had significantly more (*P* = <0.001) yellow rust than the resistant YrQ line. When looking across fungicide treatments, disease levels were similar for the untreated and the Filan treatments, and were significantly lower (*P* = <0.001) in the Comet and Imtrex treatments (Table 27).

Table 27. Yellow rust levels on 03 June 2011 (GS59), ADAS Rosemaund.

Factor	YR EL1 (%)	YR EL2 (%)
Inoculated	1.18	0.18
Uninoculated	0.54	0.09
P value	*0.004*	*NS (0.056)*
SED (45df)	*0.213*	*0.047*
Brigadier	1.54	0.26
YrQ	0.18	0.01
P value	*<0.001*	*<0.001*
SED (45df)	*0.213*	*0.047*
Untreated	1.30	0.18
Comet	0.26	0.00
Filan	1.81	0.34
Imtrex	**0.08**	**0.01**
P value	*<0.001*	*<0.001*
SED (45df)	*0.301*	*0.066*

Spring wheat – septoria experiment 2011.

The crop was sown on 21 March 2011. The predominant disease throughout the experiment was septoria leaf blotch. Septoria was seen in the plots at GS32 (31 May) on EL4 and EL5 but disease severity was very low <1%. Disease increased slowly but by GS71 (18 July) disease severity had only increased to approximately 2%. Significant differences were found between inoculation treatments, fungicides and genotypes at GS71 but because disease levels were very low these differences are of little practical interest and data are therefore not shown.

Canopy size and duration

In 2010 the date of canopy senescence was extended by two days by YrQ when compared with Brigadier, and by Comet and Imtrex when compared to the untreated control (data not shown). HAD was influenced strongly by disease severity and the benefit of the resistant genotype was demonstrated within the untreated contrasts whereby values for HAD in YrQ were approximately twice as large as for susceptible Brigadier (Table 28). Applications of Comet and Imtrex significantly increased HAD in Brigadier (sed = 2.243) but did not significantly improve HAD in YrQ treatments.

The pattern of effects was similar in 2011. YrQ delayed canopy senescence by two days when compared with the Brigadier plots and Imtrex delayed senescence by one week when compared to untreated plots across both genotypes (data not shown). HAD was significantly increased (*P* = <0.001) by the YrQ treatments when compared with the susceptible Brigadier plots. A significant genotype*fungicide interaction was also seen and HAD was increased following application of Imtrex and Comet to susceptible Brigadier plots (Brigadier untreated = 78.8, Brigadier Imtrex = 110.6, Brigadier Comet = 104.1; sed = 6.44). Disease severity was low in the spring wheat–septoria experiment but differences in HAD were significant between inoculation treatments (*P* = 0.009) and between genotypes (*P* = <0.001) with the resistant line showing an increased HAD when compared

to the susceptible treatment (resistant = 137.8, susceptible = 114.8). Differences in HAD between fungicide treatments were not significant but the trend was similar to the yellow rust experiments with Imtrex and Comet increasing HAD when compared with the untreated control (Table 28).

Table 28. Healthy area duration for field experiments 2010 and 2011 (GS59 – Senescence).

	Healthy area duration (HAD)		
Factor	**Winter wheat Yellow rust 2010**	**Winter wheat Yellow rust 2011**	**Spring wheat Septoria 2011**
Inoculated	47.8	98.9	121.7
Uninoculated	47.4	104.7	130.9
P value	*NS (0.692)*	*NS (0.077)*	*0.009*
SED	*1.12*	*3.22*	*3.37*
*Susceptible	40.1	93.1	114.8
*Resistant	55.1	110.5	137.8
P value	*<0.001*	*<0.001*	*<0.001*
SED	*1.12*	*3.22*	*3.37*
Untreated	39.6	93.1	119.6
Comet	54.0	100.9	128.9
Filan	40.7	101.0	126.8
Imtrex	56.2	112.3	129.9
P value	*<0.001*	*0.002*	*NS*
SED	*1.19*	*4.55*	*4.76*
**Susceptible*			
Untreated	23.8	78.8	109.1
Comet	53.7	104.1	116.4
Filan	25.7	79.1	110.8
Imtrex	57.4	110.6	122.9
**Resistant*			
Untreated	55.3	107.3	130.1
Comet	54.4	97.7	141.5
Filan	55.6	123.0	142.2
Imtrex	54.9	114.0	136.9
*Genotype*fung P value*	*<0.001*	*<0.001*	*NS*
SED	*2.24*	*6.44*	*6.73*

**Susceptible refers to Brigadier (winter wheat –yellow rust 2010 & 2011) and mixed batches of AxC lines labelled as 'susceptible' (spring wheat – septoria 2011).*

**Resistant refers to YrQ (winter wheat –yellow rust 2010 & 2011) and mixed batches of AxC lines labelled as 'resistant' (spring wheat – septoria 2011).*

Yield

In each of the field experiments the resistant genotype yielded higher than the susceptible genotype and this was significant (*P* = <0.001) for both of the winter wheat–yellow rust experiments (Table 29). Significant yield differences were found between fungicide treatments in all of the field experiments. In the 2010 winter wheat–yellow rust experiment significant differences (*P* = <0.001) were found between fungicides and Imtrex and Comet products gave higher yields than the untreated or Filan treated plots (sed = 0.092). A significant genotype*fungicide interaction (*P* = <0.001) showed that yield in the susceptible (Brigadier) plots was increased by Imtrex and Comet to a level that was comparable to the untreated YrQ plots (Brigadier untreated = 7.01, Brigadier strobilurin = 9.60,

Brigadier candidate = 9.53, YrQ untreated = 9.74, sed = 0.130). Within the YrQ genotype, all fungicide treatments had increased yield when compared to the untreated control but the only significant improvement in yield was from Comet (YrQ untreated = 9.74, YrQ Comet = 10.06, sed = 0.130).

The pattern of effects was similar in the 2011 winter wheat–yellow rust experiment, except that in this year all of the fungicide products significantly (*P* = <0.001) increased yield when compared to the untreated control (sed= 0.312). A significant genotype*fungicide interaction (*P* = <0.001) showed that yield in the susceptible (Brigadier) plots was increased by Imtrex and Comet products to a level that was comparable to the untreated YrQ plots (Brigadier untreated = 8.84, Brigadier Comet = 11.24, Brigadier Imtrex = 10.78, YrQ untreated = 10.58, sed = 0.441).

No significant differences were found between the susceptible and resistant genotypes in the spring wheat–septoria experiment although yield was slightly higher for the resistant type (resistant= 5.82 t/ha, susceptible= 5.59 t/ha). Despite the very low disease levels in this experiment, all fungicide treatments significantly (*P* = 0.018) improved yield by >0.5 t/ha when compared with the untreated plots (untreated = 5.31, Comet = 5.79, Filan = 5.93, Imtrex = 5.79, sed = 0.201). Yields in the susceptible genotype were increased by Comet, Filan and Imtrex treatments to levels which were comparable to the resistant genotypes. Small improvements in yield were seen following application of all fungicides within the resistant genotype but these improvements were not significant (sed = 0.284).

Table 29. Yield expressed at 85% dry matter, 2010 and 2011.

Factor	Yield @ 85% dry matter (t ha^{-1})		
	Winter wheat Yellow rust 2010	**Winter wheat Yellow rust 2011**	**Spring wheat Septoria 2011**
Inoculated	9.07	10.14	5.67
Uninoculated	9.10	10.95	5.75
P value	*NS (0.874)*	*<0.001*	*NS*
SED	*0.156*	*0.221*	*0.142*
* Susceptible	8.29	10.03	5.59
* Resistant	9.88	11.06	5.82
P value	*<0.001*	*<0.001*	*NS*
SED	*0.065*	*0.221*	*0.142*
Untreated	8.37	9.71	5.31
Comet	9.83	10.87	5.79
Filan	8.44	10.54	5.93
Imtrex	9.69	11.05	5.79
P value	*<0.001*	*<0.001*	*0.018*
SED	*0.092*	*0.312*	*0.201*
**Susceptible*			
Untreated	7.01	8.84	5.01
Comet	9.60	11.24	5.79
Filan	7.01	9.24	5.96
Imtrex	9.53	10.78	5.93
**Resistant*			
Untreated	9.74	10.58	5.61
Comet	10.06	10.49	5.79
Filan	9.87	11.84	5.90
Imtrex	9.86	11.31	5.99
*Genotype*fung P value*	*<0.001*	*<0.001*	*NS*
SED	*0.130*	*0.441*	*0.284*

SPAD readings

SPAD readings were significantly different (*P* = <0.001) between lines on both assessment dates in 2010, with the lowest SPAD values being present within the Brigadier plots (Table 30). Differences between fungicide treatments were also significant (*P* = <0.001) for both dates and the same pattern of effects was seen; untreated had the lowest SPAD values and Comet and Imtrex were similar and produced the highest values. This effect may have been a result of effective disease control in the Brigadier plots, because regression analysis showed that percentage green leaf area was related to SPAD values (16 June R^2 = 0.41, 25 June R^2 = 0.73). However, the leaf clip of the SPAD meter had specifically been placed on parts of the leaf that were green and not showing disease symptoms which suggests that other factors could also be affecting chlorophyll content in apparently healthy leaf tissue.

In 2011, at 21–28 days post fungicide application YrQ treatments had significantly higher SPAD values than Brigadier plots (*P* = 0.005, YrQ = 47.55, Brigadier = 46.17, sed = 0.477). There was also a significant (*P* = 0.022) genotype*fungicide interaction whereby canopy greenness was

improved in the untreated plots by the presence of YrQ. Trends in the data were in agreement with findings from 2010.

Table 30. SPAD readings for the winter wheat–yellow rust experiments in 2010 and 2011.

	Winter wheat–yellow rust, 2010		Winter wheat–yellow rust, 2011	
	7–10 days post fungicide	**21–28 days post fungicide**	**7–10 days post fungicide**	**21–28 days post fungicide**
Brigadier	42.18	41.33	47.25	46.17
YrQ	50.47	50.22	46.9	47.55
P value	*<0.001*	*<0.001*	*NS (0.523)*	*0.005*
SED	*0.96*	*0.919*	*0.546*	*0.477*
Untreated	43.4	44.42	46.96	46.29
Comet	51.04	48.83	47.35	46.40
Filan	45.34	45.19	46.86	47.29
Imtrex	51.03	50.58	47.14	47.46
P value	*<0.001*	*<0.001*	*NS (0.924)*	*NS (0.203)*
SED	*1.108*	*1.061*	*0.772*	*0.674*
Brigadier				
Untreated	30.84	33.12	47.09	45.00
Comet	50.63	48.09	47.27	46.99
Filan	35.51	34.55	46.73	45.98
Imtrex	51.74	49.58	47.92	46.71
YrQ				
Untreated	49.68	50.08	46.82	47.58
Comet	51.24	49.20	47.44	45.81
Filan	50.27	50.52	46.99	48.60
Imtrex	50.68	51.08	46.35	48.21
*P Line*Fung*	*<0.001*	*<0.001*	*NS (0.620)*	*0.022*
SED	*1.92*	*1.838*	*1.092*	*0.953*

Photosynthetic rate

Differences in photosynthetic rate between fungicides were not significant (P = 0.071) (Figure 24) although Imtrex and Comet treatments increased photosynthetic rate when compared with untreated and Filan plots. No other treatments effects were found in the data.

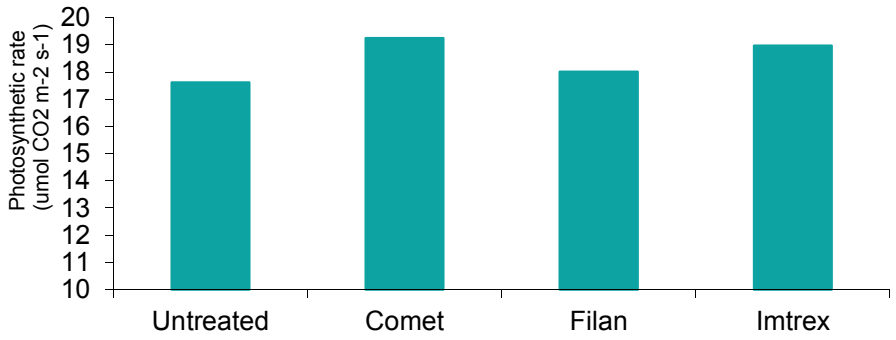


Figure 24. Photosynthetic rate of flag leaves in mid-June, 2011. Data are mean values across resistant and susceptible genotypes.

Stomatal conductance

In the 2010 yellow rust experiment, significant differences were not found between fungicide treatments (P = 0.346) for the mean data, but conductance was lower following application of Imtrex (Figure 25). This was a consistent trend and was also seen within the data for individual sets of readings across different leaf layers (data not shown). The same pattern of effects was seen across the top three leaf layers during the 2011 experiment (Figure 25). Significant (P = 0.016) differences in conductance between fungicide treatments were seen (untreated 345.1, Comet 332.6, Filan 335.6, Imtrex 326.3, sed = 5.96). This pattern of effects was highly consistent and was observed for individual sets of readings across different leaf layers (data not shown).

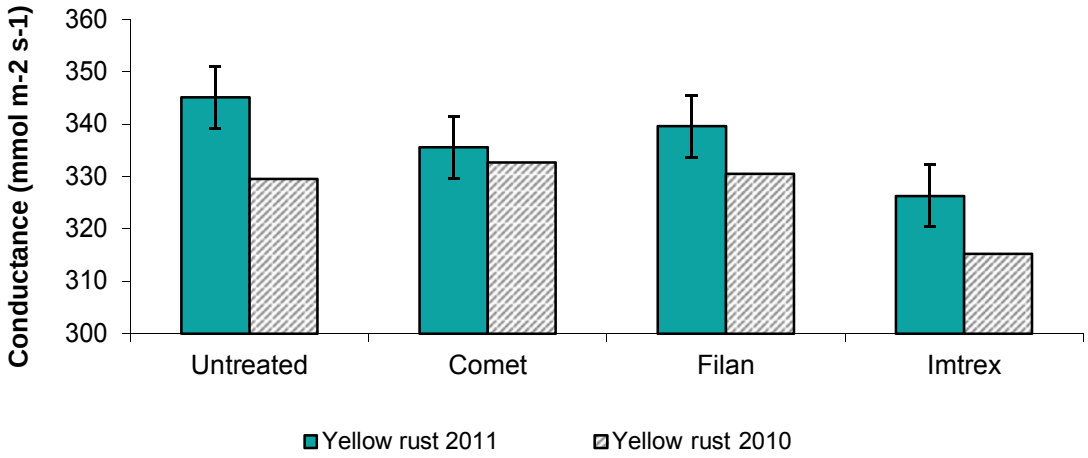


Figure 25. Stomatal conductance in winter wheat-yellow rust experiments 2010 & 2011. Data are mean values across resistant and susceptible genotypes, with SED bars.

In the spring wheat–septoria experiment differences between fungicide treatments were not significant for the mean data but, similar to the yellow rust experiments, there was a broadly comparable rank order of fungicide products with Imtrex usually giving the lowest conductance values (Figure 26).

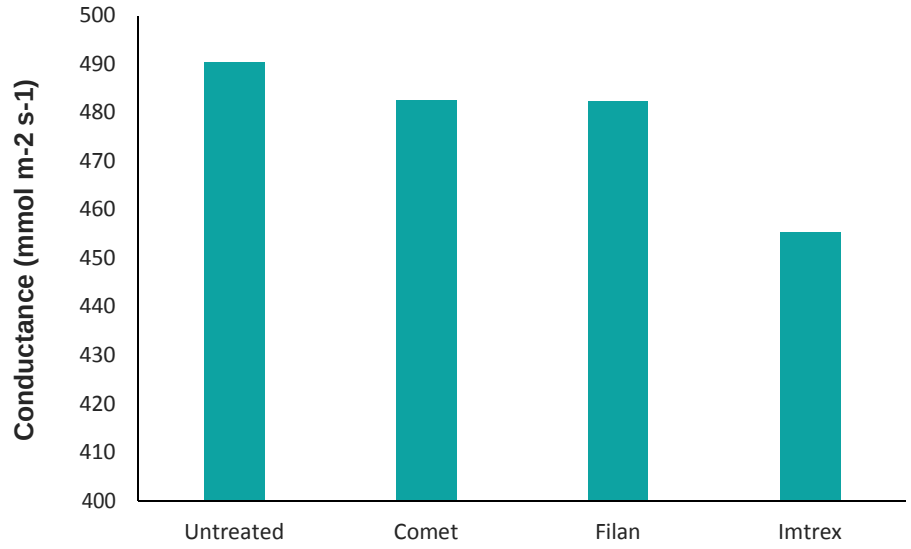


Figure 26. Stomatal conductance in spring wheat–septoria experiment 2011. Data are means of assessments across the top three culm leaves.

Discussion

Assessment of the impact of fungicide treatments (IBERS, Aberystwyth University)

A number of laboratory assessments were carried out by IBERS to assess the impact of fungicide treatments on wheat grown in the field and in controlled environment conditions. All fungicides tested were shown to suppress yellow rust uredospore germination in the susceptible cv. Brigadier to a similar level as the resistant untreated inoculated YrQ line, suggesting that fungicide treatments have a similar impact on disease as an activated R-gene with regards to spore germination. In addition, it was observed that both Filan and Xemium significantly increase auto fluorescence, and electrolyte leakage studies suggested that the microphenotype associated with fungicide use is likely to be indicative of localised cell death. All fungicides tested throughout the project were found to significantly decrease stomatal conductance. In summary, it would appear that treatment with a range of fungicides will increase auto fluorescence and (presumably) localised cell death which could contribute to the elicitation of plant defence mechanisms.

ADAS/BASF Field trials

Field trials conducted in 2010 and 2011 investigated the impact of yellow rust and septoria tritici blotch infection on the canopies of resistant and susceptible varieties, measuring canopy duration (HAD), canopy 'greenness', photosynthetic rate, stomatal conductance and subsequent yield. Resistant lines infected with yellow rust and septoria tritici blotch had significantly higher yield and HAD than susceptible lines. In addition, applications of Comet and Imtrex significantly increased HAD in susceptible lines infected with yellow rust in the 2010 trial, but did not significantly improve HAD in resistant lines. Differences in HAD between fungicide treatments were not significant in the 2011 septoria tritici blotch experiment, but the trend was similar to the yellow rust experiments with Imtrex and Comet increasing HAD when compared with the untreated control. Comet and Imtrex

were also found to significantly increase photosynthetic rate and 'leaf greening' of the crop canopy when measured by a LiCOR and SPAD meter, respectively. In controlled environment conditions all fungicide products were found to significantly decrease stomatal conductance when measured by a porometer.

In each of the field experiments the resistant genotype yielded higher than the susceptible genotype and this was significant for both of the winter wheat–yellow rust experiments. Significant yield differences were found between fungicide treatments in all of the field experiments. In both yellow rust trials, Imtrex and Comet products significantly increased yield in the susceptible lines to a level that was comparable to the untreated resistant lines. Despite the very low disease levels in the septoria tritici blotch experiment, all fungicide treatments significantly improved yield by >0.5 t/ha when compared with the untreated plots. Yields in the susceptible genotype were increased by all fungicide treatments to levels which were comparable to the resistant genotypes. Control of yellow rust by Imtrex and Comet was also shown to deliver a significant improvement in yield.

To conclude, it appears that fungicide treatments can exert direct physiological effects on the host, many of which are considered to be beneficial to yield. However, it was unclear whether these physiological responses can directly ameliorate the effects of costs of resistance in the field. Whilst different classes of modern fungicides are broadly similar in their efficacy in controlling disease symptoms, their effects on disease challenge may differ. For example, the strobilurins and SDHIs prevent spore germination, germ tube elongation and penetration and thus would be expected to reduce triggering of the hyposensitive cell death resistance response. Conversely, the triazole fungicides are active against mycelial growth within the host tissue and may therefore allow the host resistance response to be triggered and any resulting penalty to be expressed. The resulting effect might be that neither of the treated crops display disease symptoms but a yield penalty may still be incurred by the latter azole treated crop. The experimental design in 2010 and 2011 did not allow a proper test of the hypothesis because it lacked an azole comparison. This was rectified in 2012 when Ignite (epoxiconazole) was included in the treatment list. However, poor summer weather conditions during 2012 meant that reliable data could not be obtained from the field. Therefore, the hypothesis that certain fungicides can directly ameliorate the deleterious effects of resistance responses could not be tested. This remains a pertinent question.

5. Key Messages

5.1. Quantification of yield penalties associated with septoria, yellow rust and brown rust resistance genes (Deliverable 1)

- Significant yield penalties associated with septoria, yellow rust and brown rust resistance genes (including genes recently identified and those which are already widely used within UK breeding programmes) were observed during the course of this project.

- Costs of resistance associated with the disease resistance genes tested in wheat caused yield losses of between 0.3–1.0 t/ha. Such losses could have a serious impact on wheat productivity in the absence of pathogen challenge, particularly if this scale of yield impact accumulates for genes targeted against each of a number of key diseases.
- Stacking multiple septoria resistance QTL did not incur a yield cost that was significantly greater than a variety containing a single QTL. This finding needs to be corroborated with other populations and QTL, before being considered as generally true.
- Not all rust resistance genes exhibit a yield cost in the absence of disease. It should, therefore, be feasible for breeders to increase variety yield, by prioritising the most effective disease resistance genes based on their productivity in both the presence and absence of pathogen challenge.
- A table summarising the costs of resistance identified during the project can be seen below:

Table 31. Summary of material tested during the project and yield penalties associated with resistance genes/QTL

Disease	Gene/QTL	Cost	Yield Penalty	Corroboration
Zymoseptoria tritici (septoria tritici)	Avalon x Cadenza 3B (early expression)	Y	0.28 – 0.43 t/ha	2 sites/seasons (4 matched pairs) *nb. industrial field trial did not exhibit a yield penalty*, WGIN yield data (2005–2008)
	Avalon x Cadenza 5A (late expression)	Y	0.55 – 1.15 t/ha	2 sites/seasons (4 matched pairs), *nb. industrial field trial did not exhibit a yield penalty*, WGIN yield data (2005–2008)
	Avalon x Cadenza 5D (late expression)	Y	0.41 – 0.57 t/ha	2 sites/seasons (4 matched pairs), WGIN yield data (2005–2008)
Puccinia triticina (Brown rust)	Hereford x Player QTL (2A)	N	N/A	2 seasons (7 matched pairs)
	Lr37	Y	0.32 – 0.71 t/ha	3 genetic backgrounds
	Major Lr genes	Y	>1 t/ha	2 sites/seasons
Puccinia striiformis (Yellow rust)	Option x Claire (2D & 4D)	Y	0.35 t/ha	1 of 2 sites showing significant yield cost
	Avalon x Cadenza 2B	N	N/A	2 sites, one season
	Avalon x Cadenza 6B	N	N/A	2 sites, one season
	YrQ (Brigadier NILs) 2D & 4D	N	N/A	5 seasons

5.2. Quantification of yield penalties associated with 'defeated' resistance genes widely distributed in current UK wheat varieties (Deliverable 2)

- Prior to the work the brown rust resistance gene *Lr37* was considered 'defeated' by virulent strains in the UK.
- *Lr37* exhibited significant yield costs in the absence of disease, 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.
- For continued yield improvement in the UK, wheat breeders should identify key resistance genes/QTL which do, or do not, exhibit yield costs in the absence of challenge, and review deleterious yield losses against effectiveness of resistance regularly, in order to prioritise the most efficient resistance in future breeding programmes.

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

- No evidence was found to suggest that septoria tritici blotch induces stomatal dysfunction in wheat in controlled environment conditions or that stomatal dysfunction plays a role in yield losses associated with septoria resistance genes in field conditions.
- 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 costly and would therefore not be suitable to identify costs of resistance in early pre-breeding programmes. An alternative, less labour intensive approach, could be to use high-throughput spectral reflectance (NDVI) as an indicator of GAI, in order to calculate HAD.
- Controlled environment studies concluded that brown rust challenge can induce stomatal dysfunction in wheat.
- Significant stomatal dysfunction was identified in all of the Thatcher NILs carrying *Lr* brown rust resistance genes in the presence of challenge, when compared to the recurrent parent.
- In addition, three NILs (*Lr20, Lr34* and *Lr37*) exhibited significantly lower stomatal conductance than Thatcher in the absence of disease, suggesting that these *Lr* genes are constitutively expressed.

- Metabolomic analysis of *Lr10, Lr34* and *Lr37* NILs identified a significant accumulation of metabolites linked to the tricarboxylic acid (TCA) cycle and core phenylpropanoid pathway; a source of defence related phenolic compounds. 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 help explain yield losses associated with *Lr37* in field trials.
- Metabolomic analysis of key defence and energy metabolism compounds combined with stomatal conductance measurements, could potentially be an effective technique for identifying costs of resistance in pre-breeding programmes.

5.4. Assessment of the scope to use fungicides to ameliorate deleterious effects of host resistance responses (Deliverable 4)

- Fungicides can suppress yellow rust germination and infection in susceptible lines to levels similar to resistant wheat lines.
- Yield increases associated with fungicide treatment are predominantly associated with increased healthy area duration of the crop canopy.
- Certain fungicides can significantly improve 'leaf greening' and photosynthetic rate of the crop canopy.
- However, certain fungicides can increase auto fluorescence and localised cell death, associated with elicitation of plant defence mechanisms.
- Certain fungicides can significantly and consistently lower stomatal conductance.
- To conclude, it appears that fungicide treatments can exert direct physiological effects on the host, many of which are considered to be beneficial to yield. However, it remains unclear whether these physiological responses can directly ameliorate the effects of costs of resistance.

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7. Acknowledgements

The following organisations and companies participated in the SCORE LINK project:

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ADAS UK Ltd
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Delta-T
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SCORE was supported by funding from the AHDB Cereals & oilseeds, BBSRC and DEFRA

The authors thank their many colleagues for their work delivering a challenging programme of experiments.