Maximising disease escape, resistance and tolerance in wheat through genetic analysis and agronomy

by

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ABSTRACT

Three mechanisms can act in sequence to reduce yield loss caused by foliar diseases: escape inhibits spore transfer to the upper crop canopy, resistance reduces the capacity of spores which arrive on the upper leaves to infect and cause symptoms, and tolerance reduces the impact of symptoms on yield. This research tested the extent to which escape, resistance and tolerance might be improved in order to contribute to a reduction in disease and yield loss, and hence reduced dependence on fungicides.

**Disease escape:** Near-isogenic lines (NILs) of wheat were used to test the effect of major genes on escape traits against *Septoria tritici*. These lines differ in the presence or absence of single genes known to be of agronomic importance (dwarfing (Rht) genes and photoperiod insensitivity (Ppd) genes). So the effects of those single genes on candidate traits which might affect disease escape, could be measured in lines which share a common level of disease susceptibility. Field experiments demonstrated that the benefit from escape can be equivalent to a well timed full rate application of triazole fungicide. High levels of dwarfing from Rht3 and Rht12, cause increased spore transfer and lead to earlier epidemics. Rht2 reduced height to an agronomically acceptable extent, whilst maintaining reasonable escape. Rain shelter studies demonstrated that escape effects are robust, as they are expressed both in the presence and absence of rain-splash events which transfer spores. A cross was made between parents selected to contrast for traits thought to confer escape (Avalon and Cadenza), in order to create a mapping population, comprising large numbers of true-breeding (doubled-haploid) lines that vary for the candidate traits. Assessments demonstrated that improved escape could be achieved in taller plants, but, more importantly, some short lines demonstrated low disease.

**Disease resistance:** Most of the genetic resistance of wheat varieties against the important foliar diseases is partial and quantitative in effect. The field expression of variety resistance is subject to considerable environmental variation. This variation has implications for the assessment of resistance in breeding and variety evaluation, and for the exploitation of resistance by the industry. Pathogen growth is dependent on access to nutrients - principally nitrogen (N) and carbon (C) – via the tissues of the host crop. This project tested the extent to which variation in nutrient availability, due to differences in weather and agronomic inputs, might explain variation in the susceptibility of host tissues. Foliar pathogens with contrasting modes of nutrition - biotrophy and necrotrophy - were used for the studies. Variation in carbon (C) nutrition had only a small effect on epidemics. N, varied by crop nutrition, caused large and consistent effects on both *Phaeosphaeria (Septoria) nodorum* and *Puccinia striiformis*. Specific leaf N (g m-2) explained 61% of variation in the potential size of the epidemic. Clearly something other than availability of green leaf area was limiting disease, since low N leaves had significant green area remaining as disease progress slowed. This is hopeful in terms of constraining disease through crop N nutrition because 1.5 g m-2 specific leaf N, which substantially limited disease carrying capacities, is sufficient for efficient leaf photosynthesis in UK conditions. In laboratory experiments, *P. nodorum* levels increased with N supply, and ammonium nutrition
caused more disease than nitrate. Both effects were correlated with increases in leaf amino acid content. In vitro cultures indicated that growth and sporulation of *P. nodorum* is promoted by certain amino acids (particularly asparagine) and inhibited by others. Surprisingly, N effects on *P. nodorum* were consistent with effects on *P. striiformis*. Thus, similar effects on the important ‘hemi-biotroph’ *S. tritici* might be expected.

**Disease tolerance:** Evidence was found for significant variation in tolerance between elite wheat genotypes. There was an association between high attainable yield and greater loss per unit disease (intolerance), which may explain a proportion of the trend towards greater disease-induced losses – and hence greater fungicide requirement – in high yielding, modern wheats. Direct selection for tolerance in the large number of lines handled in breeding programmes is impractical. However, tolerant lines might ultimately be selected by phenotyping for tolerance traits (where these are visible and readily assessed) or by testing for genetic markers tightly linked to quantitative trait loci QTL. NILs were available from the JIC collection to test the effect on tolerance of the major genetic changes in wheat over recent decades. A doubled-haploid mapping population was derived from a cross between Cadenza x Lynx, known from previous work to contrast for traits thought to affect tolerance. The results suggest that most of the variation in tolerance relates to variation in resource capture by the leaf canopy. Other factors, such as stem carbohydrates and loss of green area per unit disease appear to be either neutral, deleterious or exhibit insufficient phenotypic variation to allow selection. The aim now is to identify determining mechanisms and traits of tolerance which are compatible with efficient resource capture, by quantifying the effects of the more influential traits.
SUMMARY

The Technical Details section (which consists of the peer reviewed papers listed at the end of the Summary) contains relevant references, which are excluded from the Summary for brevity.

DISEASE ESCAPE

Disease escape prevents or reduces contact between pathogen spores and the upper crop canopy that is critical for yield formation. Escape thus forms the first line of defence in winter wheat, and offers the opportunity to reduce dependence on fungicide treatment, particularly for splash-dispersed pathogens such as *Septoria tritici* and *Phaeosphaeria (Septoria) nodorum* (Lovell *et al.*, 2004).

Materials and methods

Two complementary approaches were used to identify and quantify the potential benefits of traits of wheat conferring disease escape:

**Near-isogenic lines (NILS)**

NILS are wheat plants which have been bred to differ for one particular gene, but are otherwise (as far as possible) genetically identical. Hence, they allow the effect of particular genes on traits to be measured. In the work reported here, NILS were used to quantify expression and value of disease escape, conferred by major genes of agronomic importance (*Rht* dwarfing and *Ppd* photoperiod). NILS of winter wheat cultivars Mercia, Maris Huntsman and Cappelle-Desprez, which varied for genes conferring different magnitudes of crop dwarfing or photoperiod response (which affects rate of crop growth and development), were grown in replicated field experiments at three sites (ADAS Rosemaund, Hereford; ADAS High Mowthorpe, Yorkshire; Long Ashton, Bristol). Measurements of crop growth and structure and proximity of infectious lesions to emerging culm leaves were recorded twice weekly from the start of stem extension. Foliar diseases were assessed weekly on 10 randomly selected main tillers per plot. Details can be found in Lovell *et al.* (2002).

**Doubled-haploid mapping population**

Two parental varieties (cv. Avalon and cv. Cadenza) were selected to provide the greatest contrast in canopy architecture traits thought to confer escape. These were crossed and 113 true breeding doubled-haploid lines were developed which segregated in all combinations for the traits of interest. The population was multiplied up from the few grains of seed produced for each line, firstly in the glass house and then in the field. Traits were measured in plots used to multiply seed at Long Ashton Research Station in 2002 and 2003. This dual approach (NILS and mapping population) was used because a single gene can be responsible for more than one trait, and several genes can control a single trait. At a practical level, the methods allow the value of putative beneficial traits to be quantified and provide a platform to link traits to genes and markers. The downside of the approach is that the time taken to develop NILS is such that the tests often have to be conducted in background varieties which are no longer of commercial importance.
Field experiments were located at sites providing temperature and rainfall contrasts, in order to test interactions between genotype and environment. The crucial interaction expected with rainfall was investigated further using unique mobile shelter field experiment facilities at ADAS Rosemaund, which allowed sheltering from, and supplementation of, ambient rainfall.

**Results and Discussion**

**Near-isogenic lines**

Experiments using NILS demonstrated unambiguously, for the first time, that disease escape is heritable. Rapid stem extension was associated with lower disease severity on the yield forming leaves. Presence of the *Rht2* dwarfing gene reduced crop height to an agronomically acceptable level, whilst maintaining most of the escape benefit of the taller *rht* line. Disease escape delayed epidemic progress by up to a full latent period (approximately 220 day degrees). This is equivalent to the effect observed from well-timed applications of triazole fungicide. Stronger dwarfing genes (e.g. *Rht3*) substantially increased disease. Observations in experiments using near isogenic lines support the hypothesis that disease escape delays epidemics whereas resistance affects rate.

The effect of dwarfing on the magnitude of epidemics (measured by area under the disease progress curve) on the final 3 leaf layers was found to be consistent across sites and seasons (Fig. 1). Epidemic development compared across rain-sheltered and ambient-rainfall treatments supported this observation. These results indicate that there is no interaction between the environment and disease escape conferred by crop height. Thus, whilst disease pressure (the amount of viable inoculum) differs substantially across sites and seasons, the benefits of escape are robust. Hence the economic optimum or appropriate dose of fungicide should be reduced by a predictable amount on cultivars with traits for good escape.

![Figure 1. Scatter plot showing area under the disease progress curve (AUDPC) on flag leaf ligule height for a range of isogenic lines of wheat varying for degrees of dwarfing, across a range of sites/seasons. The lines are regression slopes fitted using parallel curve analysis.](image-url)
Photoperiod (Ppd) genes had a large effect on the maturity of the crop and thus on the environment, and degree of infection, that leaves were exposed to during their emergence and expansion. Disease severity at a given calendar date, for a given leaf layer was consistently greater on the more mature lines due to the longer period of exposure. It is generally assumed that, when screening for resistance, this maturity effect can be adjusted for by regressing disease severity with date of heading. However, our observations suggest that this might only be appropriate if infection conditions are consistent over time, or vary progressively due to rainfall seasonality. In the UK, rainfall is sporadic. A more appropriate method of screening would therefore be to compare leaves that emerge concurrently and therefore share similar rainfall.

**Doubled-haploid mapping population**

Disease escape is dependent on increasing the distance between inoculum and the emerging yield forming leaves. However, this does not imply reliance upon tall crops to achieve the benefits from escape. Other canopy traits are known to increase the distance between diseased leaves and the final three leaf layers. The doubled-haploid mapping population (Avalon x Cadenza) developed by this project can be used to test the benefit from these traits. Preliminary measurements taken from unreplicated multiplication plots in 2003 indicate good variation across lines for the target traits. Crop height within this population correlates strongly with reduced *Septoria tritici* and glume blotch (caused by *P. nodorum*). Lines with flag leaf ligule heights above 90cm (in the absence of PGR) showed consistently very low disease severity, and ligule heights above 80cm substantially reduced disease (Fig. 2). However, such ligule heights result in unacceptably tall crop. All current HGCA Recommended List varieties are below 95cm total height and lodging risk and commercial acceptability make taller varieties undesirable. The mapping population contained a number of lines (in the bottom left of Fig. 2) which were short yet expressed low disease on the upper leaves. These lines are currently being tested in a Defra project, to distinguish between those which are resistant and those which are expressing escape due to other canopy characteristics than height.

![Figure 2](image-url)  
*Figure 2. Scatter plot showing the relationship between flag leaf ligule height and the severity of *Septoria tritici* on the flag leaf for 113 lines of a doubled-haploid mapping population derived from a cross between Avalon and Cadenza.*
The Avalon x Cadenza mapping population has been adopted by the UK Wheat Genetic Improvement Network as its reference population, as it has been found to segregate for a range of agronomically important traits (for example, resistance to Soil-Borne Wheat Mosaic Virus, grain quality and Hagberg falling number and nitrogen use efficiency) and is genetically diverse. As part of the Network, a high density of genetic markers will be identified. This will allow markers and QTL associated with traits of agronomic importance (including escape and resistance) to be located on the genome with precision, as an aid to marker assisted breeding.

**DISEASE RESISTANCE**

Resistance ratings attempt to quantify the genetic element of disease resistance, but they are subject to considerable variation due to environment. Thus for most diseases, for which resistance is only partial, growers cannot form a confident view of the degree to which their crops will resist disease, hence of the appropriate fungicide dose. Clarification and quantification of the environmental effect on resistance expression will increase the scope for resistance to be managed through crop husbandry, and improved through plant breeding.

Pathogens can be classed according to their mode of nutrition. Biotrophs, e.g. *Puccinia striiformis*, which grow on live host tissues, are particularly affected by the nitrogen (N) status of the host. With necrotrophs, which grow on host tissues that they have killed, mechanisms for environmental resistance are less well documented. Research on various crops shows that the sugar (carbon; C) status of the host may be more important than its N status. Increased sugar accumulation, has been noted to inhibit infection by various necrotrophs. Here we tested shading (such as by clouds) and crop nutrition, as the primary causes of variation in leaf C and N status respectively.

**Objectives and methods**

The aim was to test whether differences in resistance expression of wheat to a biotroph (*P. striiformis*) and a necrotroph (*Phaeosphaeria*, formerly *Septoria, nodorum*) can be explained by the nutrient status of the host. Experimentation was both in the field to ensure relevance to current cropping conditions, and under controlled conditions to allow more specific tests of proposed mechanisms for pathogen nutrition.

The two principal nutrients studied were carbon and nitrogen. Carbon status of the host was manipulated by shading; nitrogen status was manipulated with fertilisers. To seek comparability of disease across environments, assessments were made so that disease amounts in the field could be expressed in both proportional and absolute units. A data-review was conducted to derive a method of expression that would provide the best quantification of disease. Thus the sections of this report are arranged as follows:

1. Data-review: Absolute assessment of foliar disease and resistance
2. *P. striiformis*: shading, by field experiment
3. *P. striiformis*: N nutrition, by field experiment
4. *P. nodorum*: shading, by field experiment
5. *P. nodorum*: shading, in controlled environments
6. *P. nodorum*: N nutrition, in controlled environments

**Units for quantifying foliar disease and resistance**

Disease can be defined as ‘departure from the healthy state’, taking health as the state of an organism with no discernible pathogenic symptoms or other harmful disorders. Foliar disease is conventionally assessed in terms of ‘severity’ which expresses the area of a leaf exhibiting specific symptoms as a proportion or percentage of the total leaf area. Assumptions associated with the use of severity include:

- the areas of healthy leaves do not vary between genotypes, between environments, within a plant, or through time,
- total leaf areas are not affected by disease, for instance, due to leaf shrinkage, and
- the area attributed to a pathogen includes all of the tissue affected by that pathogen, and no tissue affected by another pathogen or by natural senescence.

It seems likely that these assumptions are not just occasionally, but generally, flawed. Thus, there is reason to suspect that there are fundamental problems in using severity to obtain consistent measures of partial resistance of crop varieties to disease, and to explain variation in crop losses due to disease. In specific relation to the work reported here, it was likely that some of the treatments used to alter the nutritional environment encountered by the pathogen would also affect the size of the host. Hence it was important that any effects on the size of host area could be accounted for when interpreting effects on progress of disease.

**Results & Discussion**

Extant data were taken from Defra project CE0515, in which replicated field plots of four wheat cultivars were grown at locations across England. Data were used from 24 experiments where the epidemic observed was predominantly of one species. Disease was expressed in four ways; as either

(i) the percentage of total leaf area exhibiting symptoms (severity),
(ii) the absolute area showing effects typical of the pathogen’s presence (symptoms),
(iii) the absolute change in area of green tissue from that at full leaf expansion (green leaf loss; this is the sum of natural senescence and pathogen-induced green area loss), or
(iv) the absolute difference in area of green tissue from that of the fungicide treated and healthy crop (green area difference).
Fig. 3  Example epidemic of *P. striiformis* on the flag leaf of winter wheat cv. Slejpner at ADAS Terrington in 1997, showing total (open symbols) and green (closed symbols) leaf areas for treated (circles) and untreated (triangles) treatments, and the difference in their green areas (dashed line). Untreated symptom area (dotted line) and the severity of *P. striiformis* (full line) are also shown (there was none on treated plots).

Overall, it was clear that quantification of foliar diseases in terms of their severities involved substantial inaccuracies, such that there seems little point in treating the data as more than semi-quantitative, especially if they are from different leaf layers, sites or seasons, assessors, or even different dates at the same site and in the same season. The progress curves for a single epidemic expressed as ‘severity’ and as ‘green area loss’ showed marked differences in the extent of disease, both in terms of the area affected and its duration (Fig 3). Severity appeared to represent the early stages of the epidemic with reasonable accuracy; i.e. the timing of its onset is similar. However, in this example, the absolute rate with which the pathogen affects the flag leaf’s green area would be 1.18 cm$^2$ leaf$^{-1}$ d$^{-1}$, more than twice the rate at which its symptoms increased i.e. 0.53 cm$^2$ leaf$^{-1}$ d$^{-1}$. When symptoms are expressed as ‘severity’, there are obvious difficulties towards the climax of an epidemic, as a pathogen’s symptoms become difficult to distinguish from natural senescence, and as they cause premature shrinkage of the total area of the affected leaf. In Fig 3 severity decreases whilst symptom area increases, and there then follows a two to three week period when it is not possible to quantify disease as severity, even though the healthy leaf has apparently retained its full function.

These conclusions challenge the basis on which foliar disease is currently assessed, however, it cannot be said that severity has been unsuccessful in supporting improvements in the control of foliar pathogens over that time. It is thus concluded that semi-quantitative assessment of foliar diseases suffices for most practical purposes. It is when the aim is to predict the extent or duration of foliar disease, or to predict yield loss from disease, that assessment as ‘severity’ becomes problematic. In the research reported here, where mechanisms of partial resistance are being explored, absolute assessments of the rate at which normal leaf tissues are affected are essential if disease is to be related to knowledge of pathogen metabolism.
Effects of shading on expression of resistance of wheat to *Puccinia striiformis* under field conditions

Extant data were used to test for effects of reduction in C supply on epidemic progress. Data were from Defra project CE0512 in which mobile crop shades were used to mimic the effect of cloudy conditions on crop growth. The experiments were originally designed to test whether differences in solar radiation receipts during different crop development phases might affect the disease-yield loss relationship, via differential effects on carbohydrate source and sink capacity. Experiments were performed on the susceptible cultivar Slejpnar, in 1994, 1995 and 1996 at ADAS Terrington, where natural epidemics of yellow rust are common and severe. Mobile, automatic, crop shading devices were applied when ambient radiation levels exceeded a threshold of 250 J s\(^{-1}\) m\(^{-2}\) for a period of one hour. This minimised disruption to the shaded crop microclimate in terms of air movement, rainfall, leaf surface wetness and relative humidity. Shading treatments were applied as main plot treatments during six consecutive intervals of crop development between GS 31 and GS 87, plus an unshaded control.

Results and Discussion

Disease progress was largely unaffected by shading. A small delay (approximately one week) was seen in the development of the epidemic on some of the upper leaves in some years, when compared against the epidemic in unshaded plots. However, these delays were similar to delays observed in emergence of those leaves. As leaf tissue cannot be infected until it is emerged, the delay in the epidemic was probably a direct result of the delay in emergence, rather than an effect of carbon nutrition. Given that the shading treatments imposed were equivalent to the maximum degree of shading which could occur naturally (due to prolonged cloudy weather) it is unlikely that variation in C supply is responsible for site and seasonal variation in expression of resistance to *P. striiformis*.

Effects of nitrogen nutrition on resistance of wheat to *Puccinia striiformis* under field conditions.

Many winter wheat cultivars express a degree of quantitative, race non-specific resistance against *P. striiformis*. Nitrogen (N) nutrition is thought to be an important environmental factor affecting quantitative resistance: high N is associated with increased severities of some foliar diseases such as cereal rusts and mildew. However, little information is available on the effects of N on specific components of epidemic development. Quantitative resistance is often referred to as ‘slow rusting’ and is generally considered to limit the rate of epidemic development. However, the observed reduction in disease severity in low N crops could equally occur by means of a reduction in carrying capacity (the upper asymptote of a sigmoidal disease progress curve) or by delaying epidemic onset. This work investigated effects of timing and amount of fertilizer N on yellow rust epidemics on winter wheat in two site-seasons (2000 at Swineshead, Bedfordshire and in 2001 at ADAS Terrington, Norfolk). N treatments varied canopy size and leaf N content independently of each other, as far as possible, and effects on epidemic onset, rate and carrying capacity were quantified. Details can be found in Neumann *et al.* (in press).
Results & Discussion

Severe epidemics of yellow rust and substantial effects of N treatment on disease severity were observed in both years. The upper three leaves in high N treatments showed severe necrosis after the end of June in both years and disease assessments stopped. Logistic functions (in relation to thermal time) explained a high proportion of the variation in symptom areas ($r^2 >64.5\%$). Allowing one parameter only to vary in turn demonstrated that the treatment effects were predominantly expressed through variation in carrying capacity. Leaf N status affected the capacity of wheat to sustain epidemics of yellow rust, rather than the epidemic rate. Epidemics were curtailed, even on a cultivar noted for poor quantitative resistance and despite an abundance of remaining green area and inoculum, by limiting the N supply to the host. Differences in quantitative resistance between cultivars are generally thought to be expressed through the apparent infection rate ($r$) of disease increase. If this is correct, then our findings suggest that the N environment affects a different component of the epidemic. Considering N status of the infected tissues, specific leaf N (SLN) generally increased significantly ($P=0.05$) in the higher N treatments, with greatest values of 2.8 g m$^{-2}$ N for leaf II. Linear regression of carrying capacity on SLN over both years, all assessed leaves and all nine N treatments, indicated a slope of 0.29 m$^2$ of symptom area per g leaf N with no significant intercept ($r^2=52\%$).

Given that total leaf N vastly exceeds fungal requirements for growth, the most likely inference is that the pathogen is dependent on a sub-component of total N (say a particular free amino acid), and that this becomes depleted as infection increases. The results support our previous work suggesting that the effect of N on disease is mediated via the N content of the host tissue, rather than through changes in canopy microclimate. Agronomically, it seems that dependence on fungicides could be reduced if a compromise can be found between managing N to promote canopy expansion and minimising development of biotroph epidemic.

Effects of shading on resistance of wheat to Phaeosphaeria (Septoria) nodorum under field conditions

For necrotrophic fungal pathogens, there is evidence that the sugar status of crops may be more important than N status in determining quantitative resistance. ‘Sink induced susceptibility’ has been reported, where the appearance of organs acting as plant sinks reduced the sugar status of other organs and increased their susceptibility to necrotrophs. It was presumed that the increased availability of sugars allowed for employment of resistance mechanisms through the provision of precursors for specific catabolic process, or through the provision of energy (via respiration). If this is the case, the large, natural variation in sunlight occurring in the UK could affect sugar content of the host tissues and hence affect quantitative resistance of crop species to necrotrophs. The experiment described here used shading on winter wheat cv. Cadenza at ADAS Terrington in 1998-9 to determine whether differences in host sugar status can affect the progress of symptoms for the necrotrophic pathogen of wheat, Phaeosphaeria nodorum (previously known as Stagonospora nodorum and Septoria nodorum). Epidemic development was encouraged by inoculation and the daily application of automatic mist irrigation. A natural epidemic of Septoria tritici also developed.
Results & Discussion
Shading of the field crop immediately prior to inoculation with *P. nodorum* resulted in somewhat lower *Septoria* severity in late June on all top leaves than with no shading, or shading after inoculation. There appears to have been a slower rate of *Septoria* development on the flag leaf, and possibly also the second leaf, and it seems likely that the infection process, or the first stages of fungal invasion of the leaf tissue was reduced in the previously shaded plots. This cannot relate to a difference in temperature, hence it is likely to relate to the different leaf condition. The carbohydrate status of the leaves was decreased by shading. At the time when the main effect of shading on *P. nodorum* originated (immediately after inoculation on 17 May) the area of the most affected leaf layer (the flag leaf) was increased by 6-7 cm² (more than 20%) whilst leaf biomass was decreased by about 20%, thus the specific weight (g m⁻² green leaf area) of all laminae was decreased by about 40%. Hence, no positive link between carbohydrate status and necrotrophic resistance was found; in fact there was some evidence for a negative relationship, but probably not of sufficient magnitude to be of practical importance.

Effects of shading on resistance of wheat to *Phaeosphaeria nodorum* in a controlled environment
The supply of C to a phytopathogen may come directly as products of photosynthesis, as translocated C within the leaf or plant, or as products of degradation by the fungus. The favoured form of C for most fungi is glucose, whilst carbohydrate in plants is transported mainly as sucrose. The main hypothesis tested here arose from the supposition that soluble sugars promote resistance to infection, but promote susceptibility to fungal growth. Hence, reduced carbohydrate status of shaded leaves will increase their susceptibility to *P. nodorum* if the shading is applied prior to inoculation, but will decrease susceptibility if the shading is applied after inoculation.

Materials and methods
Three experiments were conducted on wheat seedlings in controlled environments, and in each treatment half of the plants were inoculated and half were uninoculated with *P. nodorum*. Plants were shaded as follows:

*Shading pre-inoculation*  Plants were shaded to approximately 100 µmol m⁻² s⁻¹ for the 48 h immediately prior to inoculation.

*Shading post-inoculation*  Plants were shaded to approximately 100 µmol m⁻² s⁻¹ for 48h, commencing immediately after the 48 h period of high RH that followed inoculation.

*No shading*  Plants were not shaded.

Leaves were assessed for lesion numbers and symptom area. The number of days after inoculation (dai) on which the first lesions appeared was noted and lesion numbers on each infected leaf were counted at each
harvest. Symptoms were assessed visually as the percentage of leaf area covered by lesions, plus associated chlorosis or necrosis.

Results & Discussion

Overall, although results were variable between the three experiments, it was generally the case that shading pre-inoculation increased susceptibility of seedlings to \textit{P. nodorum}, and shading post-inoculation reduced susceptibility. Shading was brief, so effects on weight and carbon status were generally small, short-lived, and often non-significant; as expected, the trend was for carbon status to be reduced, but it generally recovered from shading quickly. Leaf N content showed effects apparently in consequence of those on C status. Presumably dry matter, and particularly soluble sugars, was lost from leaves as a result of shading, without equivalent effects on total leaf N. There was a relationship between lesion number and leaf N status which raises the possibility of an unexpected mechanism, and implies that its primary effect may have been on infection, rather than fungal growth. However, it was concluded (in agreement with the field data) that the direct effects of shading on disease are too inconsistent and small to be pursued.

\textbf{Effects of nitrogen amount and form on wheat, and on \textit{Phaeosphaeria nodorum in vivo and in vitro}.}

It is energetically more costly for fungi to take up nitrate than ammonium, or amino acids formed from ammonium. Hence, the form and concentration of N applied to plants can significantly affect disease progression; usually nitrate decreases and ammonium increases disease incidence caused by necrotrophs. The severity of rust diseases (biotrophs) is generally greater with nitrate supply rather than ammonium. Increasing leaf N status can increase lignin, decrease storage carbohydrates (fructans) and sucrose, and reduce phenolics. Concentrations of free amino acids and soluble proteins in leaves, increase with increasing N and with ammonium, rather than nitrate. Plants supplied with ammonium also generally contain more glutamine or asparagine.

Nitrate reductase is only synthesized by \textit{P. nodorum} if nitrate is the only available source of N. Nitrate is probably taken up via direct diffusion into hyphae down the gradient generated by intracellular activities of nitrate and nitrite reductases. Mutants of \textit{P. nodorum} and other fungi which cannot use nitrate are fully pathogenic, suggesting that amino acids and other reduced N sources are alternative substrates to nitrate during infection. Ammonium and glutamate are the preferred N sources for most fungi, and ammonium is a major regulator of N metabolism. Fungi take up amino acids by active transport systems (the number of which varies between species) which have differing affinities for different amino acids; certain amino acids may be taken up in preference to others. The work described here used wheat seedlings grown in controlled environments and \textit{P. nodorum} grown \textit{in vitro} to investigate effects of form and amount of N supply on resistance of wheat to \textit{P. nodorum}. Methods, results and discussion of all the laboratory work on environmental effect on resistance expression are fully described by Nason (2004 PhD thesis, University of Wales, Bangor).
Results & Discussion

Increasing N supply increased net photosynthesis, chlorophyll content, dry matter production and partitioning of dry matter to shoot, and decreased specific leaf area, transpiration rate and water use efficiency. Supplying NO₃ increased net photosynthesis, had some effect on dry matter production and decreased water use efficiency compared to supplying NH₄. There was no significant difference in efflux of ¹⁴C or shoot:root allometry, and differences in chlorophyll content varied with leaf age. The form of nitrogen supplied to plants affected leaf chemistry, more than the concentration of nitrogen supplied. It appears that the greater availability of soluble carbohydrates, proteins, amino acids and amides in leaves of plants supplied NH₄ rather than NO₃ provides a more favourable environment for P. nodorum, rendering these plants more susceptible to infection. Plants supplied with very low amounts of nitrogen (0.04 mmol) are poor hosts for the fungus, probably due to a combination of low nutrient availability and high levels of phenolic defence compounds. Plants supplied nitrogen at concentrations above 2 mmol appear to acquire ‘luxury’ amounts of N, and the increased N availability does not significantly affect susceptibility to P. nodorum.

Sources of N for in vitro tests were chosen to reflect potential N sources found in vivo. Varying the form of N supply produced colonies that were consistently and strikingly different in size, morphology and sporulation (Plate 1).

![Plate 1. P. nodorum colonies at 14 dai grown on solid medium containing NH₄, NO₃, glycine, glutamine or asparagine as the sole N source, or no N.](image)

Growth, measured as colony diameter, was favoured by ala, asn, glu and glu + asn. Media that contained aspartate produced the smallest colonies (P<0.01). Of the four treatments producing the smallest colonies colonies grown on asp alone were significantly smaller than those grown on asp + glu (P<0.01), and colonies grown on asp alone were significantly smaller than those grown on glu + asp + asn (P < 0.01). Plants grown
with moderate and high concentrations of N were more susceptible to *P. nodorum*, and those supplied with nitrate were less susceptible to *P. nodorum* than plants fed with ammonium. Plants supplied with ammonium had increased concentrations of soluble carbohydrates, total amino acids, amides and soluble proteins, but differences in soluble polyphenols were not clear. It therefore appears that it is the nitrogenous substrates that are primarily responsible for the differences in susceptibility to *P. nodorum*.

Sporulation was very sensitive to N source. Pycnidial production by colonies grown on asparagine alone was over six times greater than by colonies grown on aspartate, and 100% greater than by colonies grown on glutamate. Test with combinations of amino acids showed that aspartate and glutamate have an inhibitory effect on pycnidial production. It appears that the increased susceptibility of wheat plants supplied N as NH₄ to *P. nodorum* is due, at least partly, to the high levels of free amino acids and particularly the high concentrations of the amide asparagine within leaves.

**DISEASE TOLERANCE**

Reducing fungicide use by selecting for tolerance in breeding lines would provide a sustainable disease management solution, for two reasons. Firstly, tolerance does not result in selective pressure on pathogen populations, so efficacy will be maintained and plant genetic resources will not be eroded by pathogen adaptation. Secondly, certain tolerance mechanisms are likely to be effective against a range of pathogen species.

The project was planned in a series of overlapping phases:

1. Existing data were used to quantify tolerance in a range of modern wheat cultivars. Aim: to quantify the extent of variation in elite germplasm and to look for possible associations between tolerance and physiological traits or major genetic differences.
2. Formation of hypotheses (from the literature, mechanistic reasoning, mathematical modelling and 1. above) to explain variation in tolerance and identify candidate tolerance traits.
3. The creation of a doubled-haploid mapping population from a cross between parents known (from earlier phenotyping) to vary for candidate tolerance traits. This phase started concurrently with phase 4, to allow time for seed multiplication to provide sufficient seed for field experiments in phase 5. Aim: to create a mapping population segregating for traits of interest.
4. Field experiments using near-isogenic lines. Aim: to test for any effects of major genetic changes in wheat (which have occurred through breeding over recent decades) on tolerance.
5. Preliminary field experiments to phenotype the doubled-haploid mapping population. Aims: firstly, to discard candidate traits which do not affect tolerance and allow work to focus on those traits which are most likely to determine tolerance, and secondly, to find preliminary linkages between traits and quantitative trait loci (QTL).
The following sections summarise each of these phases.

**Tolerance variation in elite germplasm**

The aim was to quantify variation in disease tolerance within commercial varieties of winter wheat grown in the UK and to see if tolerance was associated with particular genotypic or phenotypic characteristics. Full details are given by Parker et al. (2004).

**Data and analytical methods**

Data were obtained from experiments (from an earlier Defra project CE0513) in which twenty five varieties of winter wheat, variously susceptible to *Septoria tritici* were grown in field experiments at two sites in England (ADAS Rosemaund, Hereford and ADAS Starcross, Exeter) in each of three seasons. The original aim of the experiment was to quantify the effect of variety resistance on epidemic progress. The experiment was a split plot design using three replicates, with main plots of a full fungicide programme to control all disease, or untreated.

Symptom severity and percentage green leaf area were assessed at weekly intervals on all leaf layers. At each assessment date, the absolute areas of the assessed leaves were measured. These measurements, combined with fertile shoot counts, allowed green area and symptom area to be expressed as dimensionless index values (the planar area of green or symptom area, expressed per unit of ground area that they occupy). Areas under both the symptom area index progress curve (AUSAI) and green area index curve (Healthy Area Duration HAD) were calculated. An extended regression analysis was used to detect differences between varieties in the slope of the fitted straight lines between yield (t ha\(^{-1}\)) and both AUSAI and HAD. Mixed linear models were fitted using a residual maximum likelihood (REML) method.

**Results**

Plots of yield against HAD showed an approximately linear relationship. Variation about the linear relationship differed between sites. A likelihood ratio test showed strong evidence (change in residual likelihood of 16.2 on 2df, \(p<0.001\)) of significant variation between variety slopes. The correlation coefficient of the fitted values with the data was \(r=0.872\). The estimated slope for each variety, the deviation away from the mean slope (the Best Linear Unbiased Predictor, BLUP, for the variety) and the prediction error variance for the BLUPS were calculated. Positive BLUP values indicated larger than average slope and imply intolerant varieties, because disease-induced loss of green leaf area has a large effect on yield. In contrast, negative values indicated lower than average slope and imply tolerant varieties. Comparison of the variety slope BLUPS with their prediction standard error suggests that varieties Brigadier, Haven and Rialto show evidence of intolerance, whilst varieties Avalon, Mercia, Flame and Hunter show evidence of tolerance. A plot of the estimated slope for each variety against attainable yield (expressed as the mean treated yield) indicated that tolerance was associated weakly with low attainable yield.


**Discussion**

Evidence of differences in tolerance, measured as response of yield to disease-induced changes in HAD, was found across a comparison of commercial varieties. These differences were large enough to be of agronomic importance. For example, in Mercia the sensitivity of yield to green area loss was approximately 30% lower than in Brigadier. On a limited subset of the data, there was no evidence that tolerance, quantified through measurement of HAD, differed within variety between experiments. This indicates that genotypic differences were responsible for the differences in tolerance. It is likely that some traits conferring tolerance have associated yield penalties. Equally, however, some may have positive or neutral effects on attainable yield. In this study there was evidence for tolerance being associated with lower yield potential. However, the most tolerant varieties were also older varieties. Given the large increase in yield achieved through breeding in the past two decades, it is possible that the correlation of tolerance with low yield is spurious. Instead, breeding efforts might have inadvertently selected out tolerance traits that are compatible with high yield.

**Potential tolerance mechanisms and candidate traits**

It is difficult to determine which of the agronomically important traits, introduced by plant breeding over the past two decades, have reduced tolerance. Nevertheless, the evidence presented here was used in conjunction with arguments from first principles and results from sensitivity analysis to set-up testable hypotheses for the causes of disease tolerance. Thus, candidate traits to impart disease tolerance were defined as: 1) Low loss of green lamina area per unit symptom area (to maintain green lamina area to intercept photosynthetically active radiation (PAR), 2) Large green canopy area (to maintain fractional interception of PAR despite disease-induced loss of green lamina area, 3) High extinction coefficient (k) for PAR, to maintain fractional interception of PAR despite disease-induced loss of green lamina area, 4) Presence of awns, to maintain fractional interception of PAR despite disease-induced loss of green lamina area, 5) Enhanced radiation use efficiency (RUE) in presence of disease, to maintain dry matter production despite disease-induced loss of PAR interception, and 6) High water soluble carbohydrate stem reserves, to compensate for reduced post-anthesis dry matter assimilation by translocation to grain of WSC accumulated pre-anthesis.

Corroboration of the association between traits and tolerance could be achieved by studying isogenic lines that vary only for the major genes believed to associate with the tolerance traits and doubled haploid mapping populations from crosses between parents with high and low trait expression.

**Major genetic changes in wheat with potential to affect tolerance**

There have been several major genetic changes in wheat grown in north-western Europe in recent decades; some of which reflect world-wide trends. Long awns have been selected against in the UK (through
qualitative variation in an allele on chromosome 5A) and became rare in commercially popular cultivars. The Rht2 semi-dwarfing allele has been introgressed into the majority of cultivars, and the 1BL.1RS wheat-rye translocation (in which an arm of chromosome 1B of wheat has been replaced by an arm of 1R of rye) has been present in most feed wheat cultivars since the late 1980s. Besides these major genetic changes, there were visible changes in canopy architecture brought about by phenotypic selection; for example, flag leaves became smaller and culm leaves became more erect.

Experiments with isogenic lines tested the hypothesis that these changes might be responsible for the observed decline in disease tolerance. There was sufficient circumstantial evidence to make this plausible. Rht2, 1BL.1RS and short awns are commonly combined in modern high yielding feed wheat cultivars, which tend to demonstrated poor tolerance. However, this does not necessarily indicate a causal relationship.

The experiments reported here used pairs of isogenic lines which contrasted for Rht2/rht, +/- awns or +/- 1BL.1RS. The background cultivars in which the isogenic lines were developed, differed for date of commercial release, canopy architecture and yield. Hence, if the greatest differences in tolerance were found between isogenic lines within common backgrounds, this would indicate that one or more of the major genetic changes were responsible. Whereas, if differences in tolerance between backgrounds exceeded those between the pairwise isogenic contrasts, this would suggest that other genetic changes - perhaps several, each of small effect - predominated. Full details can be found in Foulkes et al. (submitted).

Materials and methods
The work quantified effects of the Rht-D1b (formerly Rht2) allele by utilizing two pairs of NILS of winter wheat, one in Mercia and one in Maris Huntsman, developed at the John Innes Centre. Experiments quantified the effects of awns by utilizing two pairs of NILs contrasting for an allele on chromosome 5A controlling presence of awns in a Hobbit sib background. At Rosemaund, two winter type NILs were used (ex RA4 awnless and RA21 awned), and at Terrington two spring type NILs (ex RA15 awned and ex RA18 awned). The effect of the presence of the 1BL.1RS wheat-rye translocation was examined using Weston (+1Bl.1RS) and Chaucer (-1BL.1RS), two commercial cultivars derived from a single F6 plant.

One field experiment was conducted at each of two sites, ADAS Rosemaund, Herefordshire and ADAS Terrington, Lincolnshire in each of two years, 1998/9 and 1999/2000. The target disease was septoria leaf blotch at Rosemaund and yellow rust at Terrington. Each experiment used a randomised split-plot design with four replicates.

Results
Disease in these experiments was quantified as the difference in the areas under the GLAI curve in the post-anthesis period (i.e. the Healthy Area Duration; HAD) under full and non-target disease control. Disease
tolerance was estimated as the slope of the linear relationship between grain yield and HAD across treatments of contrasting disease pressure. Greater slopes relate to greater intolerance. Overall the presence of awns decreased tolerance (0.0358 cf. 0.0286 t ha\(^{-1}\) HAD\(^{-1}\)) in the Hobbit NILs (P< 0.005). Neither the \textit{Rht-D1b} allele (in Mercia or Maris Huntsman NILs) nor the 1BL.1RS contrast (Weston +1BL.1RS cf. Chaucer -1BL.1RS), however, had a significant effect on tolerance. Interestingly, there were differences between the genetic backgrounds (P< 0.05), with the modern cultivars Weston and Chaucer (average of 0.0337 t ha\(^{-1}\) HAD\(^{-1}\)) exhibiting decreased tolerance compared to the Maris Huntsman \textit{Rht} NILs (average of 0.0263 t ha\(^{-1}\) HAD\(^{-1}\)) and the Hobbit unawned NIL (0.0267 t ha\(^{-1}\) HAD\(^{-1}\)). The Mercia Rht NILs showed intermediate values.

Discussion
The analysis showed that differences in tolerance detected between backgrounds exceeded those between the pairwise NIL contrasts. This suggests that genetic changes other than those related to semi-dwarfing, awns or 1BL.1RS are responsible for the observed reduction of tolerance in high yielding, modern wheat cultivars.

The \textit{Rht-D1b} semi-dwarfing allele did not result in an increase of either water soluble carbohydrate stem reserves or tolerance. The increase in the percentage of the stem dry matter partitioned as WSC in the semi-dwarf NILs was counteracted by a corresponding reduction in total stem dry matter. Differences in the WSC accumulated between backgrounds did not appear to relate to tolerance; in fact the least tolerant cultivars (Weston and Chaucer) had the highest accumulation of WSC. A high proportion of the WSC reserves were utilized even when the post-anthesis duration of the canopy (and hence photosynthesis) was not constrained by disease. Hence, unlike in barley, accumulation and utilization of WSC (and hence the benefit to yield) appear similar in the presence or absence of disease. This agrees with the data of Parker et al. (2004) who found no relationship between WSC reserves and tolerance. Hence, the evidence available to date suggests that stem reserves can be discounted as being beneficial to tolerance. The negative association found with tolerance may be due to auto correlation, as increases in WSC through breeding may have occurred in parallel with other phenotypic changes which are deleterious to tolerance.

The awned NILs exhibited greater disease susceptibility, substantially increased attainable yield and decreased tolerance compared with the unawned lines. It is conceivable that these differences were due to the presence of awns. However, an increase in attainable yield with long awns would not be expected under UK conditions in the absence of abiotic stress. Hence, it is possible that the Hobbit lines were not completely isogenic and QTL deleterious to yield are tightly linked to the short awn allele on chromosome 5A. However, no such linkage has been reported previously.

There were trends for increased attainable yield and decreased tolerance with 1B.1R. Although these effects were not statistically significant or consistent, they agree with previous evidence. The finding that tolerance
associates with attainable yield also fits with previous work. The cultivar backgrounds in which the NILs were developed showed substantial phenotypic differences in radiation use efficiency, green canopy area, distribution of green laminae area by culm leaf layer (and hence distribution of PAR interception through the canopy) and extinction coefficient. Some of these traits appear to relate to differences in tolerance.

The cultivar backgrounds in which the NILs were developed showed substantial phenotypic differences. The more modern backgrounds had significantly higher WSC dry matter, radiation use efficiency and grains per m². No differences were found in GAI, but more modern cultivars had a smaller proportion of their total GAI in the flag leaves. These finding are in agreement with those of Shearman at Nottingham, who studied wheat cultivars released over a 30 year period. Of these phenotypic differences, soluble carbohydrate, and grains per m² were negatively correlated with tolerance, and flag leaf area was positively associated. Such correlations do not necessarily imply a causal link, but there are plausible mechanistic explanations for the effects observed.

Provided there is no counteracting change in grain size, the higher grains per m² in the modern backgrounds represent an increase in grain sink capacity for assimilate. All else being equal, a low sink capacity would be expected to associate with tolerance. Yield is determined by whichever is the lower of assimilate source and sink. For a given source capacity, a more sink-limited cultivar could lose more source to disease (through loss of green canopy and hence fractional PAR interception) before source became limiting to yield – leading to a shallower disease-yield loss relationship. In the material tested here, GAI, extinction coefficient and RUE (which collectively determine source capacity) differed little between backgrounds, whereas grains per m² differed substantially, suggesting a substantial change in source:sink balance, leading to intolerance.

Creating and phenotyping the Cadenza x Lynx mapping population

Materials and methods

A true breeding mapping population was created at the John Innes Centre from a cross between cultivars Cadenza and Lynx. The lines were multiplied over several generations, firstly in the glasshouse, then in the field. One field experiment was conducted at each of two sites, ADAS Mamhead, Devon, and ADAS Rosemaund, Herefordshire in each of two seasons, 2001/2 and 2002/3. Work in 2003 was funded by Defra project AR0511. The target disease was S. tritici. The experiments used a randomised split-plot design with three replicates.

Results and Discussion

Differences were detected between the Cadanza x Lynx lines in their tolerance to Septoria tritici. Taking mean data across the experiments in 2002 and 2003, intolerance varied from 0.13 to 0.06 t ha⁻¹ per unit of area under the green leaf area index progress curve. The results of previous studies (described above) suggest that tolerance is determined by a combination of a number of factors, each of which may explain
only a proportion of the tolerance variation. There was a significant regression in both years, with a negative slope, of intolerance on green leaf area index. Across the two years, variation in green area explained 22% of the intolerance variation. There was also a highly significant negative relationship between green canopy area and the extinction coefficient (kPAR), suggesting that those lines with less green area tended to have less erect leaves or higher chlorophyll content per unit leaf area (and hence lower light transmission). Green area was positively associated with treated yield in both years. These data provide the first indication that tolerance is not inextricably linked with low attainable yield. Hence traits might be identified – such as green leaf area index - which benefit tolerance and are compatible with, or beneficial to, productivity.

Mapping
The parents of the mapping population (Cadenza and Lynx) were screened at the John Innes Centre, using publicly available primer pairs to establish polymorphic microsatellite markers. Polymorphisms between the two parents proved less frequent than normal, so resources were focussed on obtaining an adequate number of polymorphic markers, rather than AFLPs. Eighty two markers were established, covering all 21 chromosomes, and all the lines were characterised for those markers. Preliminary QTL analysis revealed significant associations (P<0.05) between phenotypic variation in green leaf area index and markers on the long arm of chromosome 5D and 7BL, and between intolerance and 1BL, the short arm of 6A, 7BL and the short arm of 7D.

PROJECT CONCLUSIONS AND IMPLICATIONS
Disease escape
• Disease escape provides a valuable source of durable ‘field resistance’ against Septoria tritici.
• Similar benefits have been found for control of Septoria nodorum, which is a serious threat to yield and grain quality in the South West.
• The maximum expression of disease escape can control disease to a level equivalent to a label dose of broad-spectrum systemic fungicide.
• The effect of escape is consistent across environments. It is therefore predictable and should be exploited in crop management decisions.
• The separation distance between the highest sporulating lesion of Septoria and newly emerging upper leaves, provides a useful indicator of disease risk. Poor separation represents high risk.
• Resistance screening methods which use regressions of disease severity on heading-date, are unlikely to be reliable under UK conditions. More appropriate comparisons are possible by comparing disease on leaves that emerge concurrently.
• Epidemic development before GS31 is dependent on temperature. This phase of the epidemic, previously considered non-limiting to the summer epidemic, appears crucial in determining seasonal disease pressure at a given site, and hence the need for fungicide treatment.
Disease resistance

- Exploitation of the N effects on epidemic development found here will depend on their integration with other mechanisms of disease resistance.

- In relation to crop management, it will be necessary to reconcile the counter-acting effects of fertiliser N on green canopy expansion and on symptom development.

- In relation to plant breeding, the optimist’s approach will be to genetically constrain the N compounds which promote pathogen development, for example asparagine, without constraining N effects on canopy expansion and photosynthesis. However, preliminary work must test the N effect to confirm its general applicability, using sources of variation in leaf N status other than from fertiliser.

- N status may, for example, explain the observed increase in resistance to *P. striiformis* with leaf age. It may be possible to study mapping populations showing contrasts in specific leaf N, to test whether these are associated with QTL for disease resistances.

- Determination of an N-requirement of the disease (m² g⁻¹) should allow prediction of symptoms, and opens the way for optimisation of N nutrition, balancing the positive effect of N on canopy expansion with its negative effects on disease susceptibility.

Disease tolerance

- Tolerance has been shown to vary in elite UK wheat germplasm. There is evidence that tolerance is negatively associated with high attainable yield. Thus, modern high yielding cultivars are highly dependent on fungicides because even low or moderate levels of disease impact substantially on yield.

- Data from a range of elite germplasm showed that, when measured in absolute (rather than percentage) units, each unit of leaf area occupied by septoria leaf blotch symptoms caused between a two and four fold loss of green canopy area. There was no evidence that cultivars varied consistently for the amount of green area loss per unit symptom area. Hence, differences in tolerance related to differences in yield response to loss of green area.

- One hypothesis was that the decline in tolerance with increasing yield might be associated with the introduction of major alleles in wheat in recent decades; namely the introduction of the *Rht2* semi-dwarfing allele, the 1B.1R translocation, or selection against presence of an allele for long awns.

- The data from experiments with the NILs showed that the introduction of major alleles in wheat were not predominantly responsible for loss of tolerance.

- The NIL backgrounds varied significantly for tolerance, with higher yielding more modern backgrounds being more intolerant. There was some evidence that this was associated with variation in traits related to PAR interception and sink capacity.

- Tolerance was not associated with high stem storage of water soluble carbohydrate, pre-anthesis. In fact, high stem storage was associated with high attainable yield and low tolerance.
• A doubled-haploid mapping population was created from a cross between parents (Cadenza and Lynx) selected to vary, primarily, for candidate tolerance traits; canopy area and light extinction coefficient.

• Preliminary phenotyping suggests that the population varies substantially for the traits of interest.

• Increased green leaf area index associated with both improved tolerance and improved attainable yield. This is the first evidence that the linkage between high yield and poor tolerance can be broken.

• Improved tolerance would complement disease resistance and disease escape in reducing dependency on fungicide inputs.

• Preliminary phenotyping has identified QTL on the short arm of chromosome 7D consistently associated with tolerance in experiments in 2002 and 2003, which may represent a novel source of disease tolerance for breeders to exploit and for gene discovery.

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TECHNICAL DETAILS
Details of the research can be found in the following peer reviewed publications:


