Cereal Pathosystems

Edited by Graham Jellis and Clive Edwards

Papers from BSPP Presidential Meeting,
Queen Mary, University of London
16-17 December 2008
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Preface

The rise in food prices over the past year or two has made developed countries appreciate that its food supplies cannot be taken for granted and that food security is a real issue - something the rest of the world has been all too aware of for decades. Today, global average wheat yields are about 2.5t/ha but an estimated 4.0t/ha will be needed to sustain the world in 2020 (Sears, 2006). At the Oxford Farming Conference in January 2008 we were reminded a number of times that by the middle of the 21st Century the world will need to produce twice as much food as now, at a time when climate change is likely to considerably reduce the production potential of some regions.

With an average yield of over 8t/ha, the UK is in the top league of wheat producers, something our industry can be proud of. We are also major users of fungicides and other pesticides and we depend on these to maintain yield. At present the European Commission and Parliament are negotiating the revision of EC directive 91/414/EEC which specifies the approval process for pesticides. ADAS have carried out an evaluation of the impact of this legislation, as it appeared in May 2008, on UK agriculture for the European Crop Protection Association. They concluded that loss in wheat production due to the withdrawal of many fungicides, including triazoles, could be between 20-32%, requiring between an extra 513-928 thousand hectares of land to produce the same crop (Clarke, 2008). Even if the final legislation does not lead to such a major withdrawal, this study demonstrates how reliant we are on relatively few groups of fungicides and the serious impact that fungal diseases have on crop productivity in the UK. We therefore urgently need alternative means of controlling diseases.

The papers published here by an international group of cereal pathologists, breeders and biotechnologists were among those presented at the 2008 Presidential Meeting of the British Society for Plant Pathology. They examine pathosystems relating to two major small-grain cereals, wheat and barley, and
their important fungal and viral pathogens. Progress in understanding the
development of pathogen virulence to resistant cultivars and of fungicide
resistance, strategies for breeding for durable resistance and for tolerance, and
disease management systems using reduced fungicide inputs are described.
Much of this work uses the molecular tools that are now an every-day part of
many plant pathologists’ research programmes. One exciting development is the
use of PCR amplification of host and pathogen DNA to examine the history of
pathosystems and to learn lessons from the past. I hope you find the collection of
papers as interesting as I have.

I am grateful to Gerry Saddler, Kim Hammond-Kosack and Neil Paveley for their
help in planning the conference and to all those who contributed to the
publication, including my co-editor Clive Edwards and the production team
comprising of Michele Charlton, Steven Burgner and Ray Ellis.

Graham Jellis

President, BSPP 2008

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Durable resistance to rust diseases of wheat: from theory to breeding application

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The rust diseases of wheat pose a recurring threat to sustainable wheat production and thus food security worldwide. The most promising long-term control strategy is to breed and deploy cultivars carrying durable adult plant resistance based on minor, slow-rusting genes with additive effects. Combining four to five slow-rusting resistance genes result in a high level of resistance, comparable to immunity. Traditional genetic and molecular mapping studies have demonstrated high genetic diversity for such minor genes in wheat germplasm and often resistance to brown and yellow rusts is under pleiotropic genetic control. Cloning of gene Lr34 has shown that this pleiotropic slow-rusting resistance gene is different from race-specific resistance genes. Although, marker-assisted breeding remains impractical in the absence of tightly linked markers for a majority of slow-rusting resistance genes, over thirty years of effort in selecting for such resistance in the field at CIMMYT has resulted in the development of high-yielding wheat germplasm with high levels of resistance to brown and yellow rusts. Adequate levels of adult plant resistance to the Ug99 race of black rust is present in about 5% of CIMMYT improved spring wheat and high emphasis is being given to breeding high-yielding wheat germplasm with a high level of resistance to all three rusts.

Introduction

Wheat is the primary source of calories for millions of people worldwide and is grown on about 225 million hectares, producing over 600 million tonnes annually. It can be grown from the equator to latitudes of 60°N and is found worldwide at altitudes ranging from sea level to more than 3,000 m. The three rust diseases (black (or stem), brown (or leaf) and yellow (or stripe)), caused by the fungi
*Puccinia graminis* f. sp. *tritici*, *P. triticina* and *P. striiformis* f. sp. *tritici*) continue to cause losses, often major, in various parts of the world.

Rust fungi are obligate parasites and must survive on living plants. Survival during the off-season occurs on either self-sown (or volunteer) wheat plants or other grass species that can also be infected. High-input, irrigated agriculture and the existence of cool highlands in different parts of the world promote the carryover of inoculum between seasons. Moreover, conditions favorable to disease development (i.e. temperature and high humidity leading to dew formation) occur in most years throughout the growing season in a majority of the area. Races capable of overcoming different resistance genes, or their combinations, are known for all three rust fungi and numerous races are now known to occur worldwide. New races may arise through sexual recombination (not known for *P. striiformis*), mutation, and somatic hybridization followed by selection if a new race has a selective advantage. Being an airborne pathogen, new races may also be introduced into a new area through migration. Therefore, if a new race arises in an area, given time it could spread throughout the epidemiologic region or beyond.

The phenomenon of the erosion of race-specific resistance genes, or their combinations, led scientists to look for alternative approaches to resistance management. Van der Plank (1963) was the first epidemiologist to clearly define the theoretical basis of the concepts of resistance. In the late 1960s and 1970s, there was a revival of the concept of general (race-nonspecific) resistance and its application in crop improvement (Caldwell, 1968). This approach was widely used for breeding brown-rust resistance by Caldwell (1968), black-rust resistance by N.E. Borlaug, and yellow-rust resistance by Johnson (1988). The wide application of such a concept in breeding for brown-rust resistance, commonly known as slow rusting, has dominated in CIMMYT’s bread wheat improvement programme for almost 30 years. The genetic basis of durable resistance to rust diseases is understood better today, and this knowledge is being applied in breeding.
Development and deployment of such resistance should provide long-term genetic control of rust diseases.

**Lr34, Lr46 and other minor genes for durable resistance to brown rust**

The Mexican-Rockefeller Program first used the Brazilian variety ‘Frontana’ in the 1950s in its breeding programme. This variety is known to carry durable resistance. Semi-dwarf derivatives of ‘Frontana’, such as ‘Penjamo 62’, ‘Torim 73’, and ‘Kalyan/Bluebird’ show slow-rusting characteristics. Genetic analysis of Frontana and several CIMMYT wheats possessing excellent slow-rusting resistance to brown rust worldwide has indicated that such adult plant resistance is based on the additive interaction of *Lr34* and two or three additional slow-rusting genes (Singh & Rajaram, 1992). Brown rust severity observed in Mexico on most slow-rusting varieties could be related to the number of minor genes they carry (Figure 1). Varieties with *Lr34* and two or four additional genes, referred to as ‘near immune’ (Singh et al., 2000) show a stable response in all environments tested so far, with final brown rust ratings lower than 10%. The presence of *Lr34* can be indicated by leaf tip necrosis in adult plants, which is closely linked with it. Gene *Lr34*, located in chromosome arm 7DS, was cloned recently.
Figure 1. Graphical representation of the additive effects from estimated number of minor genes in retarding rust progress in the field.

Slow-rusting resistance to brown rust is common in wheat germplasm and at least 10-12 slow-rusting genes are involved in the adult plant resistance of CIMMYT spring wheats. Lines where *Lr34* is absent but still possess a high level of slow-rusting resistance are also identified indicating that durable resistance is feasible even in the absence of *Lr34*. The second slow-rusting resistance gene *Lr46* was identified in wheat cultivar ‘Pavon 76’ and located on chromosome arm 1BL (William et al., 2003) and attempts are underway to clone it. A third undesignated slow-rusting resistance gene, located on chromosome arm 7BL in CIMMYT wheat ‘Parula’, is under fine mapping after developing a single-gene based mapping population.

**Yr18, Yr29 and other minor genes for durable resistance to yellow rust**

The moderate level of durable adult-plant resistance to yellow rust of the CIMMYT-derived U.S. wheat cultivar ‘Anza’ and winter wheats, such as ‘Bezostaja’, is controlled in part by the *Yr18* gene (Singh, 1992; McIntosh, 1992). This gene is pleiotropic to *Lr34* gene. The level of resistance it confers by itself is usually not adequate. However, combinations of *Yr18* and 3-4 additional slow-rusting genes result in adequate resistance levels in most environments (Singh and Rajaram, 1994). Genes *Lr34* and *Yr18* occur frequently in germplasm
developed at CIMMYT and in various countries. Recently identified slow-rusting gene \textit{Yr29} is pleiotropic to slow brown rusting gene \textit{Lr46} (William et al., 2003).

Low disease severity to yellow rust is usually associated with at least some reduction in infection type because the yellow rust fungus grows systemically in leaf tissues. This phenomenon results in chlorotic or necrotic stripes and therefore creates difficulties in distinguishing slow-rusting resistance from race-specific resistance. Durability and acceptance of adult plant resistance can be expected if the cultivar’s low disease severity is due to the additive interaction of several (4 to 5) partially effective genes.

**New threat and challenge posed by the Ug99 race of the black rust pathogen**

Black rust is historically known to cause severe losses to wheat production. However, it has been controlled effectively through the use of genetic resistance in cultivars associated with the green revolution during the 1960s and 1970s. Over 80% of the spring wheat area in developing countries is currently sown to cultivars either derived directly from CIMMYT germplasm or from CIMMYT germplasm used as parents. For more than 30 years, a major proportion of the CIMMYT wheat germplasm and germplasm developed by other breeding programmes have remained resistant to black rust. Resistance gene \textit{Sr31}, located on rye translocation 1B.1R contributed to the high level of resistance in several wheat cultivars developed worldwide in recent years.

Detection in 1998 of race Ug99 of the black rust pathogen in Uganda with broad virulence, including the virulence for \textit{Sr31}, and its migration to Kenya, Ethiopia, Yemen, Sudan and Iran has been recognised as a highly significant event and led to the launch of ‘Global Rust Initiative’ during 2005, now known as ‘Borlaug Global Rust Initiative’. Several major cultivars currently grown in North Africa, the Middle East and Asia are moderately or highly susceptible to this race. Predominant wind patterns or human errors are likely to lead to further migration
in the region and beyond. Identification of two new variants of Ug99 in Kenya, with virulence for resistance genes $Sr_{24}$ and $Sr_{36}$, show that Ug99 is not only migrating but also evolving. The major challenge to wheat breeding is identifying or developing and releasing adapted resistant cultivars that will have durable resistance.

Although some race-specific resistance genes, mostly of alien origin, *viz.*, $Sr_{22}, 25, 26, 27, 29, 32, 33, 35, 39, 40, 44, R$ (1A.1R translocation) and $Tmp$, can provide effective control; not all can be used in developing cultivars as some of these alien translocations are associated with negative effects on grain yield or quality. Shortening of these alien segments could help their utilization. The best strategy therefore is to reconstitute the durable adult-plant resistance that once protected the ‘green revolution’ wheat varieties and subsequent varieties.

**$Sr_{2}$ and other minor genes for durable resistance to black rust**

The durable black rust resistance of some older US, Australian and CIMMYT spring wheats is believed to be due to the deployment of $Sr_{2}$ in conjunction with other unknown minor, additive genes. McFadden transferred Gene $Sr_{2}$ to hexaploid wheat in the 1920s from the tetraploid emmer wheat cultivar ‘Yaroslav’. The slow-rusting gene $Sr_{2}$ confers by itself only moderate levels of resistance, insufficient to reduce losses to non-significant levels under high rust pressure. Its presence can be detected through its complete linkage with the pseudo-black chaff phenotype. The adult-plant resistance of some old, tall Kenyan varieties and CIMMYT-derived semidwarf variety ‘Pavon 76’ and breeding lines ‘Kingbird’, ‘Kiritati’, etc. was thought to be based on $Sr_{2}$ and other unknown minor genes (Singh *et al.*, 2008). Testing of breeding materials in the field in Kenya under high black rust pressure and in the greenhouse with Ug99 has identified various sources of adult plant resistance that were distributed worldwide through ‘Stem Rust Resistance Screening Nurseries’ (Table 1).
Table 1. Stem rust resistance of entries included in 1st, 2nd and 3rd SRRSN (Stem Rust Resistance Screening Nursery).

<table>
<thead>
<tr>
<th>Resistance Category</th>
<th>1stSRRSN</th>
<th>2ndSRRSN</th>
<th>3rdSRRSN</th>
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<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td><strong>Adult plant</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R (5-10% severity)</td>
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<td>4</td>
<td>0</td>
</tr>
<tr>
<td>R-MR (15-20% severity)</td>
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<td>18</td>
<td>26</td>
</tr>
<tr>
<td>MR (30% severity)</td>
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<td>6</td>
<td>22</td>
</tr>
<tr>
<td>MR-MS (40% severity)</td>
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<td>2</td>
<td>15</td>
</tr>
<tr>
<td>MS (50-60% severity)</td>
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<td>0</td>
<td>17</td>
</tr>
<tr>
<td>S (70-100% severity)</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td><strong>Race-specific</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sr24</td>
<td>39</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>Sr25</td>
<td>17</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Sr36 (+Sr24)</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sr1A.1R (+Sr24)</td>
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<td>2</td>
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<tr>
<td>SrTmp</td>
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</tr>
<tr>
<td>SrSynt</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>SrSha7</td>
<td>9</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>SrND643</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>SrUnknown</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

**Total** 103  128  104

a Adult plant resistance lines include some that are susceptible in seedling greenhouse tests but have the highest rating (R) during multiple years/seasons of testing (2005-2007) when the susceptible entries had 100% stem rust severity, based on the modified Cobb Scale.
Breeding for durable resistance to rust diseases

Breeding for durable resistance based on minor additive genes has been challenging and often slow, for several reasons: 1) an insufficient number of minor genes may be present in a single source genotype, 2) the source genotype may be poorly adapted, 3) there may be confounding effects from the segregation of both major and minor genes in the population, 4) rust pressure in the field may be variable or inadequate and the right races may not be present, 5) crossing and selection schemes and population sizes are more suitable for selecting major genes, 6) reliable molecular markers for several minor genes are unavailable, and 7) the cost associated with identifying and utilising multiple markers is high. One suggested approach is to use recurrent selection schemes to accumulate several minor genes in a single genetic background. Such selection schemes have often been more of scientific interest than actually being applied in breeding. Selection for resistance alone will not generate important popular cultivars, unless it is simultaneously combined with other traits, such as high yield and quality. However, such germplasm carrying combinations of minor genes should be very useful in transferring these genes to modern cultivars.

A successful example of breeding for resistance based on minor genes is the resistance to brown and yellow rusts in wheat, which took about 30 years of continuous effort at CIMMYT. In the early 1970s, S. Rajaram, influenced by the concept of slow-rusting resistance in wheat proposed by R. Caldwell and partial resistance to late blight of potato by J. Niederhauser, made a strategic decision: to initiate selection for slow-rusting resistance to brown rust in CIMMYT spring wheat germplasm. In the early phase of breeding he maintained plants and lines in segregating populations that would show 20-30% rust severity with a compatible infection type. This strategy led to the release of several successful wheat varieties, such as ‘Pavon 76’ and ‘Nacozari 76’, in Mexico and other countries. These slow-rusting lines were used extensively in the crossing programme and resulted in the wide distribution of minor genes within CIMMYT spring wheat germplasm.
The genetic basis of such resistance started to become clear in the early 1990s. High-yielding lines that combine four or five additive, minor genes for both brown and yellow rusts and show near-immune levels of resistance were developed in the 1990s (Singh et al., 2000). Three or four lines carrying different minor genes were crossed (3-way and 4-way crosses), and plants in large segregating populations were selected under artificially created rust epidemics. Races of pathogens that have virulence for race-specific resistance genes present in the parents were used to create the epidemics. The resulting highly resistant lines are now being used in a planned manner to transfer these minor resistance genes to adapted varieties and other important breeding materials. Based on genetic information on the number of additive, minor genes that must be transferred to achieve the desired level of resistance, the crossing and selection scheme described below was developed and applied. This strategy has allowed simultaneous transfer, not only of resistance genes but also other minor genes with small effects that increase the yield potential or improve the grain quality of adapted materials.

To transfer minor gene-based resistance into a susceptible adapted cultivar or any selected genotype, we use a ‘single backcross-selected bulk’ scheme, where the cultivar/genotype is crossed with a group of about 6-8 resistance donors; 20 spikes of the F1 plants from each cross are then backcrossed to obtain 400-500 BC1 seeds. Selection is practiced from the BC1 generation onwards for resistance and other agronomic features under high rust pressure. Because additive genes are partially dominant, BC1 plants carrying most of the genes show intermediate resistance and can be selected visually. About 1600 plants per cross are space-grown in the F2, whereas population sizes of about 800 plants are maintained in the F3-F5 populations. Plants with desirable agronomic features and low to moderate terminal disease severity in early generations (BC1, F2 and F3), and plants with low terminal severity in later generations (F4 and F5) are retained. We use a selected-bulk scheme where one spike from each selected plant is harvested as bulk until the F4 generation, and plants are harvested individually in
the F₅. Bulking of selected plants poses no restriction on the number of plants that can be selected in each generation, as harvesting and threshing are quick and inexpensive, and the next generation is derived from a sample of the bulked seed. Because high resistance levels require the presence of 4 to 5 additive genes, the level of homozygosity from the F₄ generation onwards is usually sufficient to identify plants that combine adequate resistance with good agronomic features. Moreover, selecting plants with low terminal disease severity under high disease pressure means that more additive genes may be present in those plants. Selection for seed characteristics is carried out on seeds obtained from individually harvested F₅ plants. Small plots of the F₆ lines are then evaluated for agronomic features, homozygosity of resistance etc, before conducting yield trials. It should be noted that having minor gene-based resistance in several backgrounds eases future selection for these resistance genes through intercrossing of these adapted parents.

The shuttle breeding between two field sites Ciudad Obregon in the northwestern Mexico and Toluca in the highlands near Mexico City, initiated by N.E. Borlaug in the 1940s favours the simultaneous selection of adult plant resistance to brown rust and yellow rust endemic at these sites, respectively. However, to incorporate adult plant resistance to the Ug99 race of the black rust pathogen, we have implemented a “Cd. Obreop-Toluca-Njoro-Cd. Obreop” shuttle breeding scheme where segregating materials in the ‘F₃ and F₄’ or ‘F₄ and F₅’ generations are selected at Njoro Research Station in Kenya under high pressure of Ug99. Advanced breeding lines from the first cycle of selection will be tested for yield performance in Mexico and resistance to black rust in Kenya during the current cropping season 2008-2009. Selection for adult plant resistance is at present only feasible under field conditions due to the lack of molecular markers linked to resistance genes.
References


The population and evolutionary biology of fungal necrotrophs

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Analysis of pathogen population genetics can provide novel insights into pathogen biology and disease epidemiology and can help us to understand the processes driving pathogen evolution. Besides providing fundamental knowledge about pathogen evolution, research oriented towards understanding the evolutionary biology of plant pathogens can help us to explain the emergence and spread of virulence against resistant cultivars and the emergence and spread of fungicide resistance. Here I will focus on these two problems using examples from three cereal plant pathosystems that have been studied for over 20 years in my laboratory, *Mycosphaerella graminicola* and *Phaeosphaeria nodorum* on wheat and *Rhynchosporium secalis* on barley.

**Introduction**

Why should we invest research effort into understanding the evolutionary biology of plant pathogens? Because pathogens evolve! Plant pathologists have witnessed this evolution many times during the 100 years since Biffen’s discovery of major resistance genes (Biffen, 1905). Careful studies of pathogen population genetics can provide important insights into pathogen biology and disease epidemiology and can help us to understand the processes driving pathogen evolution. Through understanding these processes, we may be able to design disease management strategies that are more likely to be “durable” in agro-ecosystems (McDonald & Linde, 2002; Stukenbrock & McDonald, 2008).

Understanding pathogen evolution becomes especially important as we seek to capitalise on investments made in crop biotechnology and prepare to release transgenic crops that carry genetically engineered forms of disease resistance. It would be naïve to assume that genetically engineered resistance will be more durable than "natural" resistance that has emerged through millions of years of
co-evolution with plant pathogens. Just as resistance against BT toxins in transgenic plants has emerged in some insects (eg Janmaat & Myers, 2003), and resistance against glyphosate herbicides has emerged in many weeds (Cerdeira & Duke, 2006), an inappropriate deployment of genes encoding pathogen resistance will be likely to drive the emergence of pathogen populations that can overcome the engineered resistance.

Besides providing fundamental knowledge about pathogen evolution, research oriented toward the population genetics and evolutionary biology of plant pathogens can help us to understand two particularly relevant problems in agro-ecosystems, namely the emergence and spread of virulence against resistant cultivars and the emergence and spread of fungicide resistance. Here I will focus on these two problems using examples from three plant pathosystems that have been studied for over 20 years in my laboratory, *Mycosphaerella graminicola* and *Phaeosphaeria nodorum* on wheat and *Rhynchosporium secalis* on barley.

**The evolution of fungicide resistance in *Mycosphaerella graminicola***

The first example will consider the evolution of fungicide resistance in European populations of the septoria tritici leaf blotch pathogen *M. graminicola*. Our most recent findings indicate that *M. graminicola* emerged about 10,500 years ago from an ancestral pathogen population during the domestication of wheat in the Fertile Crescent (Stukenbrock *et al.*, 2007). The ancestral pathogen population still exists in the Fertile Crescent and has a wide host range. Through genomic analyses of strains sampled from the “wild” ancestral population, we may be able to identify genes involved in host specialisation and sympatric speciation (E.H. Stukenbrock, personal communication). Contemporary populations of the “domesticated” wheat pathogen exhibit a highly recombining population structure with little clonality, indicating that sexual reproduction makes a significant contribution to their population genetics and to epidemic development and spread (Linde *et al.*, 2002). Because gene flow on a regional basis is very high (Zhan *et al.*, 2003), largely due to the wind dispersal of ascospores, Europe is
effectively a single population that appears to be at drift/migration equilibrium (Zhan and McDonald, 2004). Under these conditions, we predict that mutations encoding fungicide resistance (or virulence against major resistance genes) will be recombined into many different genetic backgrounds and spread rapidly throughout Europe in places where fungicides (or major R-genes) are used regularly.

We now have strong evidence supporting this prediction for two classes of fungicides. For the strobilurin class, we found that resistance encoded by the G143A point mutation in the mitochondrial cytb gene emerged independently at least 4 times in western Europe by 2003 (Torriani et al., in press). These mutations in different mtDNA lineages then spread eastward across Europe. Because the G143A mutation appears to carry no fitness cost, strobilurin fungicides are likely to have reduced efficacy, or at least a shorter useful life expectancy, even in areas where strobilurins have not been applied. For the DMI class of fungicides, we found evidence that there has been significant evolution of the Cyp51 gene that encodes the protein targeted by many DMI fungicides (Brunner et al., 2008). In European populations, the wild-type Cyp51 gene no longer exists while non-European populations carry the wild-type allele at very high frequencies. Many point mutations have been characterised in the Cyp51 gene and some of these mutations have been correlated with resistance to DMIs (eg Leroux et al., 2007). But by analysing the entire DNA sequence of a large portion of the Cyp51 gene in a large number of isolates, we were able to show how combinations of point mutations have emerged within particular Cyp51 haplotypes, probably contributing to the emergence of high levels of DMI resistance over time (Brunner et al., 2008). In this case, it appears that there has been a succession of selective sweeps, due to applications of different DMI fungicides, that removed the wild type allele and replaced it with a mosaic of Cyp51 variants carrying differing degrees of resistance to different DMIs. At least one of the successful variants appears to have arisen very recently as a result of a rare intragenic recombination event that brought together different point
mutations from different parts of the *Cyp51* gene. We proposed that these recombinants (now found in 16% of contemporary European isolates) are responsible for much of the emergence of highly resistant *M. graminicola* populations in western Europe (Brunner *et al*., 2008). We also proposed that these different *Cyp51* alleles are responsible for the majority of the quantitative variation in DMI sensitivity observed in *M. graminicola* across western Europe as well as the gradual increase in resistance to DMI fungicides over the last 30 years.

**The evolution of virulence in *Rhynchosporium secalis***

The second example will consider the evolution of host specialisation and virulence in the barley scald pathogen *Rhynchosporium secalis*. We showed recently that *R. secalis* is a complex of closely-related but host-specialised species (Zaffarano *et al*., 2008), with different pathogen species infecting barley, rye, and *Agropyron repens*. We also presented evidence that *Rhynchosporium* did not emerge through co-evolution with barley in the Fertile Crescent, but instead emerged in northern Europe about 1000 years after barley was introduced into Scandinavia, ie about 3500 years ago (Zaffarano *et al*., 2008). We interpreted these findings to mean that barley scald emerged via a “host jump” from a host species in northern Europe that has not yet been identified (Stukenbrock & McDonald, 2008; Zaffarano *et al*., 2008). All *Rhynchosporium* populations exhibit the “signature of sex”, including high genotypic diversity and low clonality, a recombining population structure, and with mating type alleles at equal frequencies (Linde *et al*., 2003; Zaffarano *et al*., 2006), but the teleomorph has not yet been identified. If the teleomorph exists, then we predict that it will produce airborne ascospores from small apothecia, as found in its closest known relative *Oculimacula (Tapesia) yallundae* (Goodwin, 2002). Epidemiological studies have not up until now considered the possibility of an airborne source of inoculum, but we believe that this could in part explain why regional populations exhibit a population structure consistent with a significant amount of gene flow (Zaffarano *et al*., 2006).
*Rhynchosporium* has a long history of evolving resistance to fungicides and virulence to major R-genes. The product of the *R. secalis* avirulence gene *Nip1* interacts with the product of the barley *Rrs1* resistance gene (R-gene) in a gene-for-gene interaction that determines compatibility (Rohe *et al.*, 1995). *Nip1* is also a toxin that acts as a pathogenicity factor. We showed that barley-infecting populations of *R. secalis* can become virulent on *Rrs1* barley either through point mutations in *Nip1* or by deleting *Nip1* entirely, with the mechanism of deletion seemingly favoured over point mutations (Schürch *et al.*, 2004). In some barley fields, the *Nip1* deletion was fixed, suggesting that the *Rrs1* gene had been widely used in the corresponding region, while in other fields most strains had wild-type *Nip1* sequences, suggesting that *Rrs1* had not been used extensively in that region. Point mutations conferring virulence occurred at least three times in *Nip1*, with independent mutation events in California, Europe, and the Middle East. Among the strains containing *Nip1*, an excess of non-synonymous mutations were found, consistent with the hypothesis of positive diversifying selection occurring at the *Nip1* locus (Schürch *et al.*, 2004). This finding suggests a co-evolutionary process between *Nip1* and *Rrs1* whereby *Nip1* accumulated mutations to avoid recognition by *Rrs1* while maintaining its toxin function. But the finding that *Nip1* deletions are very common indicates that the *Nip1* toxin activity is not needed for pathogenicity and there appears to be little fitness cost associated with losing this toxin. I speculate that major *R. secalis* R-genes identified in barley and its wild ancestor *Hordeum spontaneum* are less likely to be “durable” when introduced into agro-ecosystems because these R-genes have not been tested and selected through a long-lived co-evolutionary process with the pathogen. If this is true, then we should search for “durable” R-genes in the original co-evolved host of *Rhynchosporium*, which may still exist in northern Europe.
The evolution of host specific toxins in *Phaeosphaeria nodorum*

The third example considers the evolution of host-specific toxins in the wheat leaf and glume blotch pathogen *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum*). *P. nodorum* populations have the same genetic structure as *M. graminicola* and *R. secalis*, with highly diverse populations characterised by regular cycles of recombination and low levels of clonality (Stukenbrock *et al.*, 2006). A recent field experiment showed that sexual ascospores originating from within the field make a significant contribution to epidemic development during the growing season (Sommerhalder *et al.*, manuscript in review). Airborne ascospores are hypothesised to be the mechanism for the high gene flow observed regionally. The European population appears to be a single population at drift/migration equilibrium (Stukenbrock *et al.*, 2006). The centre of origin is not yet known for *P. nodorum*, but early indications from work in progress are consistent with emergence during domestication of wheat in the Fertile Crescent, i.e. an evolutionary history similar to *M. graminicola*.

The *ToxA* gene that was first characterised in the tan spot pathogen *Pyrenophora tritici-repentis* (Ciuffetti *et al.*, 1997) was recently found in the *P. nodorum* genome. *ToxA* is a host-specific toxin that interacts in a gene-for-gene manner with the *Tsn1* locus in wheat, with the dominant *Tsn1* allele conditioning sensitivity to *ToxA* and the recessive *tsn1* allele giving resistance to the toxin. Based on a population genetic analysis that showed high diversity at the *ToxA* locus in *P. nodorum* but little diversity in *P. tritici-repentis*, Friesen *et al.* (2006) proposed that the *ToxA* gene was shared as a result of a recent horizontal gene transfer from *P. nodorum* into *P. tritici-repentis*. We analysed the population genetics of *ToxA* in *P. nodorum* populations from around the world and found a pattern surprisingly similar to *Nip1*. The majority of *P. nodorum* strains carried a deletion at the *ToxA* locus, with some field populations carrying *ToxA* at a high frequency and other field populations carrying *ToxA* at a very low frequency (Stukenbrock and McDonald, 2007). Our interpretation was that the *ToxA* frequency corresponds with the frequency of the corresponding *Tsn1* allele in the
region where the *P. nodorum* populations were sampled, with a high frequency of ToxA expected in regions where most of the wheat carries *Tsn1* and a low frequency of ToxA expected in regions where most of the wheat carries *tsn1*. This hypothesis was supported by the analysis of Oliver *et al.* (2008) who showed that almost all Australian wheat cultivars grown over the last 100 years carried the *Tsn1* allele. A detailed analysis of diversity in the *ToxA* gene provided evidence for positive diversifying selection, as was found in *Nip1* (Stukenbrock & McDonald, 2007). The surprising similarity found between the effector proteins ToxA and Nip1 suggests that they have been subjected to the same type of selection in the agro-ecosystem. We hypothesised that *Tsn1* would be a major R-gene similar in function to the *Rrs1* gene in barley (Stukenbrock & McDonald, submitted). We also hypothesised that avirulence proteins and host-specific toxins may bind with the same R-gene proteins in plants, as proposed by Wolpert *et al.* (2002).

Wheat pathologists across Europe noticed that the incidence of *P. nodorum* has decreased while the incidence of *M. graminicola* has increased over the past 20 years. It is reasonable to postulate that these pathogens compete with each other in the agro-ecosystem because they occupy the same niche and have nearly identical life histories. Examination of the relative abundance of pathogen DNA found in wheat straw collected during the Broadbalk experiment showed that the cycling between these pathogens in the UK goes back over 150 years, with a correlation found between the observed cycle and SO₂ concentrations in the air (Bearchell *et al.*, 2005). I hypothesise that another possible cause for the long-term cycling between these pathogens is changes in the frequency of *Tsn1* in European wheat over this time period. Under this hypothesis, I predict that *P. nodorum* will increase in importance, relative to *M. graminicola*, during periods when more European wheat cultivars contain *Tsn1* and *P. nodorum* will decrease in importance when more cultivars carry *tsn1*. This hypothesis can be tested by determining which *Tsn1* alleles were deployed in European wheat over the last 100 years. It is possible that significant resistance to both *P. nodorum* and
P. tritici-repentis can be gained by screening current European wheat cultivars to remove the Tsn1 allele from European wheat breeding programmes.

**Cultivar mixtures slow pathogen evolution**

For all three pathogens, we have conducted replicated field experiments designed to detect differences in fitness among pathogen strains competing on different hosts grown in pure stand and in mixture (Abang et al., 2006; Sommerhalder et al., in review; Zhan et al., 2002). One of the most important shared outcomes from these experiments was the finding that cultivar mixtures significantly slowed pathogen evolution in each plant pathosystem. I consider this a significant finding that merits further research because it suggests that increased host diversity in the agro-ecosystem may offer our best solution for achieving durable disease control for rapidly evolving fungal pathogens. The increase in diversity does not need to diminish agronomic uniformity of the crop. Creation through genetic engineering of multilines carrying different major R-genes in the same genetic background could present an evolutionary dilemma for the pathogen population. Similarly, alternations or mixtures of fungicides are likely to have longer effective life-spans in agro-ecosystems when deployed properly.

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Controlling cereal disease with reduced agrochemical inputs  
- a challenge for both growers and advisers

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Danish farmers have a long tradition of minimising and optimising fungicide inputs in cereals based on several IPM (integrated pest management) principles. Despite this, problems with dissemination still exist. Complex knowledge in crop protection is often generated by scientists and compiled in Decision Support Systems (DSS) such as Crop Protection Online (CPO). However, the information often does not reach the farmer as it is presented in a way that does not correspond with the farmers’ way of thinking or the way things are done in practice. Based on a sociological investigation into the farmers’ way of making decisions in the area of crop protection, several constraints for broader use of thresholds and management systems were identified. The requirements for detailed assessments in the field as demanded by DSS are not compatible with the farmers’ current management practices. It was also recognised that there is a lack of economic incentives to change from today’s standard treatments to threshold-based systems. Farmers and advisers are generally not prepared to risk unintended disruption of their management system, which potentially could lead to economic losses due to weeds, diseases and pests.

Introduction

Legislation and reduction schemes have been imposed on the use of pesticides in Denmark for the past 20 years (Jørgensen & Kudsk, 2006). Following the
development of national action plans, it has become important to encourage low pesticide input strategies. Similar proposals to eliminate unnecessary use are also stated in the EU’s Thematic Strategy on the Sustainable Use of Pesticides (Anonymous, 2006), which includes recommendations to generate national action plans for pesticides. The strategy also intensifies the focus on reducing risks associated with pesticides, including a demand for management of plant protection according to the rules of Integrated Pest Management (IPM). This means turning away from a routine approach to a flexible handling of pesticides that is adapted to specific requirements.

The average yield in Danish wheat production is around 7.5 tonnes/ha and responses to disease control are today approximately 10% with a major variation (5-25%) caused by year, disease pressure and the susceptibility of cultivars. The comparable figures in spring barley are approximately 5 tonnes/ha and an average yearly fungicide response of 9%, varying between 2 and 14%.

**Tools supporting a low use of fungicides**

Danish agriculture is characterised by a relatively low use of chemical inputs while maintaining high crop yield. Danish farmers and advisers have access to several tools to assist them in selecting disease management options in the field.

1. Cultivar data describing the susceptibility to diseases and potential need for fungicides are given in a database (http://www.sortinfo.dk). Growing cultivars with good disease resistance is the most important means of reducing disease-induced yield losses in an integrated control strategy. For each disease in wheat, all cultivars are divided into four groups according to their resistance level. This grouping influences the control threshold and the recommended dose.

2. The Danish Monitoring Network is an important tool when recommendations have to be made and adjusted during the season. Advisers report typically once a week on the disease incidence in local fields for both susceptible and
more resistant cultivars. These data are summarised on a map of Denmark indicating the locations that currently need treatment. The data are available on the internet as well as in the farming press.

3. Thresholds for disease control as given by the Decision Support System (DDS) Crop Protection Online (CPO) (Hagelskjær et al., 2003). CPO requires input of current disease incidence at field level in order to provide an evaluation of the need for pesticide treatment. Furthermore, it ranks potential solutions according to either costs or Treatment Frequency Index (TFI). The potential of CPO for reducing fungicide use has to a large extent already been realised and the number of actual users is relatively low. However, information given in newsletters to farmers is often based on output from the system.

4. Strong systems for conducting field trials exist in Denmark. The objective of many of the trials is to test new active ingredients as well as plant protection strategies in farmers’ fields. Results from pesticide efficacy experiments are published on the internet 1-2 weeks after harvest and as a full publication 3 months after harvest. Furthermore, results are freely available to advisers and farmers and all results are published. Historical trials data form the basis for a detailed assessment of the strengths and weaknesses of different crop protection strategies including the possibilities of using reduced doses and optimising margin over pesticide cost. Focus has been on net yields rather than gross yields.

5. Fast dissemination of research results to farmers via advisers. Denmark has a comprehensive and independent agricultural advisory service (DAAS) targeting approximately 85% of Danish farmers and focusing on optimisation of economic output at farm level. A tradition of strong collaboration between agricultural scientists and the advisory service enables swift communication of recent research results to end-users and communication of research needs from end-users to scientists.
Variation in fungicide input across Europe

As part of the ENDURE project information on current fungicide use in winter wheat in several countries has been collected. The current use of fungicides in the different countries varies significantly measured in terms of fungicide input (TFI) and money spent on disease control (Table 1). Major differences in disease pressure exist in Europe and also the potential yield responses from chemical control measures vary to a great extent. The sources of information on fungicide use in specific crops are limited in some countries, particularly Hungary, Italy and Poland.

Table 1. Quantity and cost of fungicides used in Europe 2003-2007.

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of treatments</th>
<th>Total fungicide input (TFI)</th>
<th>% area treated</th>
<th>Money spent on fungicides €</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>2.7</td>
<td>1.7-2.4</td>
<td>&gt;95</td>
<td>66-80</td>
</tr>
<tr>
<td>France</td>
<td>2.1</td>
<td>1.3-2.0</td>
<td>&gt;95</td>
<td>69 (40-88)</td>
</tr>
<tr>
<td>Germany</td>
<td>2.7</td>
<td>1.3-1.5</td>
<td>&gt;95</td>
<td>80-100</td>
</tr>
<tr>
<td>Denmark</td>
<td>2.1</td>
<td>0.6-0.8</td>
<td>&gt;95</td>
<td>33-47</td>
</tr>
<tr>
<td>Netherlands</td>
<td>2</td>
<td>1.6</td>
<td>&gt;95</td>
<td>80-100</td>
</tr>
<tr>
<td>Hungary</td>
<td>0.7 (0-2)</td>
<td>0.5-0.7</td>
<td>60</td>
<td>20-30</td>
</tr>
<tr>
<td>Italy</td>
<td>0.25 (0-2)</td>
<td>0-1.5</td>
<td>15</td>
<td>0-60</td>
</tr>
<tr>
<td>Poland</td>
<td>0.75 (0-2)</td>
<td>0.7</td>
<td>60</td>
<td>20-55</td>
</tr>
</tbody>
</table>

Farmers’ attitudes to pesticide use

In 1999 and 2003 the economic optimum for use of pesticides was calculated in Denmark to be approximately 1.7 TFI. Since a politically fixed target of TFI=2 was reached in 2002 it has, however, proved more difficult to keep pesticide use as low as 2 and the farmers’ enthusiasm for keeping down inputs has reduced. As
an average of the last 3 years (2005-2007) TFI has increased to 2.33. The consequence of this increase in TFI is still unclear but the action plan is currently under evaluation. A new calculation of the economic pesticide optimum has shown a shift from 1.7 to 2.3 due to increasing problems with diseases, pests and weeds partly driven by intensified production of winter cereals and a milder climate (Ørum et al., 2008). Fungicide use in cereals has, however, been quite stable since 2000, based on data from pesticide statistics.

The reduction in pesticide use in Denmark has mainly relied on voluntary actions by farmers with support and encouragement from the national advisory services and results obtained from research. Farmers’ attitudes to adopting new methods or their actions in relation to crop protection issues have therefore become of major importance in order to reach the target of the pesticide action plans. At the start of Action Plan II, farmers were quite supportive of reaching the overall target of TFI=2. Farmer field groups, use of reduced doses, thresholds and intensive use of advisory support on crop protection, were some of the elements supporting the farmers’ crop protection strategies.

**Figure 1.** Development of TFI between 1990 and 2007 in winter cereals (mainly wheat) and spring cereals (mainly spring barley).
**Knowns and unknowns**

Despite the relatively low use of pesticides in Denmark, in certain situations there is still a tendency for farmers to overestimate the risk of weeds, pests and diseases and therefore to use a dose that is slightly higher than the optimum or to apply an extra spray. Farmers are generally not prepared to risk unintended disruption of their management system, which potentially could lead to economic losses due to weeds, diseases and pests.

In fairness to the farmer, part of the overestimated use is due to uncertain prediction tools. Although thresholds exist for most pests and diseases, the actual loss and optimal dose cannot be calculated at the time of application as this is dependent on the weather following the time of spraying. One example is in control of Septoria leaf blotch (*Septoria tritici*). The general recommendations rely, therefore, to a great extent on the average outcome of historical field trials. On the other hand, historical field trials in wheat have shown that application of early treatments before GS 32 only accounts for approximately 15% of the total yield increase from disease control in wheat. This is most likely too low a response to be profitable and should remind the farmers not to overestimate the need for early treatments.

The optimal input of fungicides in winter wheat has for several years been approximately 0.75 TFI. This is fully in line with actual use based on statistics and also the level which CPO is likely to recommend based on thresholds.

**Disease management and static yields**

There is no indication that less effective fungicides, too small doses of fungicides or increasing disease pressure have played a major role in the static wheat yields, which have been seen in recent years. More effective fungicides have between the early 1980s and today resulted in a potential increase in yield response of approximately 11-12 dt/ha (Figure 2). Net yield rather than gross yield has come into focus since the end of the 1980s and has led to widespread
use of reduced fungicide dosage. Calculations have shown that the gross yield may be reduced by 4-5 dt/ha due to the focus on economic optimums rather than gross yield. This means that the potential increase in yield during the last 25 years is approximately 6-8 dt/ha Application of reduced doses is, however, still seen as being of economic benefit to the farmers. Modern cultivars, introduced in the last 10 years, are more resistant than older ones. Septoria tritici arrived as a new serious disease at the beginning of the 1980s and has since been the major target for fungicide treatments. This disease is, however, generally found to be controlled satisfactorily at farm level and is not believed to be contributing to the stagnating yields.

**Figure 2.** Yield differences following fungicide treatment from 1998 to 2007, mainly based on trials with two-spray programmes using 1/3 rate of fungicides. The trials are from DJF in areas of relatively high disease pressure.

Columns 1-4: Originate from 8 trials in 2001-2002. Column 5 (*): Yield benefit from Opera, after strobilurin resistance (R) was widespread, was still 1.5 dt/ha compared to Opus team/Opus. Column 6 (**): The change from Opus/Opus team to Bell/Proline is based on registration trials with Bell compared to Opus team giving a benefit of 1.7 dt/ha using data from 2 x ½ rate trials.

The static yields are more likely to be caused by restrictions in nitrogen input, larger areas with second year wheat and wheat also being introduced on less strong soils.
Influence of grain price and fungicide resistance

Increases in grain price as seen in 2007 (10 € to 20 € per dt) have a major impact on the optimum input. For fungicides in wheat, the optimum input for control of Septoria has been found to increase by 50% from 0.5 to 0.75 in susceptible cultivars, see Figure 3 (Jørgensen et al., 2007). A major problem for farmers is that they have to decide on the actual input before the grain price is known. The latest drop in grain price again fully illustrates this dilemma.

Another element which influences the optimal input of fungicides is a gradual shift in sensitivity to fungicides. Since the introduction of epoxiconazole the efficacy from quarter rate has dropped compared to when the product was initially introduced. A limited range of fungicides is available compared to several of our neighbouring countries. The limited numbers of actives available does not make it possible to apply a specific anti-resistance strategy. At present we rely entirely on triazoles or triazoles+boscalid for control of septoria leaf blotch.

Figure 3. Calculated net yield gain in winter wheat in susceptible varieties for selected strategies using two prices for cereals; 10 or 20 € per dt. Based on data from the Sjælland region 1999-2003.

The legends are ranked according to the most beneficial solutions. Treatments A-E are defined as A: GS 25-31, B: GS 32-36, C: GS 37-50, D: GS 51-64 and E: GS 65-70.
Sociological behaviour behind decision-making

In order to try understanding the way farmers make decisions in the area of crop protection a project was initiated in 2005-06 including both a survey and focus group interviews. The method used to identify characteristic types was previously described by Weber (1998) and the study has been described in more detail by Langvad & Noe (2006) and Jørgensen et al., (2008). The project identified three types of farmers a) system-oriented farmers, b) experience-based farmers and c) advisory-oriented farmers (see Table 2).

The following hurdles were identified and are believed to be of major importance for a wider use of DSS like CPO.

1) DSS does not appeal at present to the farmer’s ways of making decisions on crop protection in general.
2) The requirement for carrying out detailed assessments at field level was seen as a major hurdle and often not found to be realistic in practice.
3) Many farmers have problems identifying specific diseases (and weeds) in the field.
4) Farm sizes are getting larger, leaving less time for decision-making in the individual field.
5) Lack of economic incentives to change from today’s standard treatments.

In future developments of DSS, it was recommended that farmers’ decision-making practices and the reasoning behind them is taken into account. Farmers have different ways of making decisions and it is therefore important that this is taken into account. Future development needs to take place in close dialogue and collaboration with user groups.
Table 2. Grouping of farmers based on decision-making strategies on crop protection issues.

<table>
<thead>
<tr>
<th>System-oriented farmers</th>
<th>Experience-based farmers</th>
<th>Advisory-based farmers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large farms.</td>
<td>Medium size farms.</td>
<td>No specific farm size.</td>
</tr>
<tr>
<td>Systematic approach to planning.</td>
<td>Very aware of problems in specific fields.</td>
<td>Often main income from animal production or other special production</td>
</tr>
<tr>
<td>Specialised arable farmers.</td>
<td>Have no specific spray plan prepared before season.</td>
<td>Less specific interest in arable farming.</td>
</tr>
<tr>
<td>Prepare a spraying plan in winter, which mostly will be followed during the season.</td>
<td>Have a “Learning by doing” approach.</td>
<td>Rely on recommendations from advisers.</td>
</tr>
<tr>
<td>Relatively high pesticide input.</td>
<td>Normal to slightly high pesticide input.</td>
<td>Relatively low pesticide input.</td>
</tr>
</tbody>
</table>

**Economic value rationale:**
- System-oriented farmers: Future multiplication of weeds and pests is avoided and safety margins are kept high.
- Experience-based farmers: The challenge of punctuality is the centre of experience-based weed and pest control.
- Advisory-based farmers: Crop protection is adjusted to more valuable tasks on the farm.
References


Crop production in NW Europe is highly dependent on fungicide treatment. The degree of fungicide dependence can be quantified, as it relates to the economic optimum total fungicide dose, which varies with disease severity and yield loss per unit of severity. The former can be reduced by disease resistance and the latter by improving tolerance. Resistance and tolerance can both be selected for during breeding and have complementary effects on fungicide dependence. Tolerance – the focus of this paper – has not been actively selected for in cereal breeding in NW Europe. Selection for yield over recent decades has been associated with a reduction in tolerance. Tolerance traits need to be identified which break this association. Candidate traits determining tolerance will differ between pathosystems, depending on the extent to which diseases constrain yield through crop dry matter ‘source’ or ‘sink’ components. Recent work on winter wheat has shown that: (i) there is tolerance variation in UK elite cultivars, (ii) transgressive segregation for tolerance can occur, and (iii) genotypes with high healthy canopy area duration per grain, in the absence of disease (i.e. a high source:sink balance) are better able to tolerate foliar disease.

**A brief history of fungicide dependence**

The introduction of new, highly-effective protectant and systemic fungicides during the 1960s and ‘70s changed the market for new wheat varieties. Disease susceptibility was no longer a major impediment to commercial success. This
allowed breeders to focus their finite resources primarily on quality and yield, in order to compete for selection to the UK Recommended List, where high fungicide-treated yield was the predominant driver.

Plant breeders did not neglect disease resistance during the 70s, 80s and 90s. Breeding lines continued to be screened in ‘disease nurseries’. But strong selection for resistance against all the major pathogens substantially constrains the size of the breeding population from which to select for desirable combinations of other, higher priority, traits. Physiological costs associated with certain sources of disease resistance (Brown, 2002) may also have limited progress. Breeders focussed predominantly on qualitative resistance under simple genetic control, because of the difficulties of tracking quantitative resistance in breeding lines. The ability of pathogen populations to overcome such resistance was best illustrated by the rapid breakdown of a succession of gene combinations in wheat against yellow rust (\textit{Puccinia striiformis}) (Boyd, 2005). However, the availability of effective fungicides meant that resistance weaknesses against specific diseases were not a great concern for growers. Disease resistance ranked no higher than fourth in growers criteria for choosing wheat varieties, after yield, grain quality and standing power.

By the end of the 1990s, the changes described above resulted in 98% of wheat crops being treated with fungicide, with approximately 35% of crops being sprayed twice and 45% being sprayed three or more times (Hardwick \textit{et al.}, 1998). The industry had become highly dependent on fungicides.

\textbf{Consequences of fungicide dependence}

Under the high selection pressure for fungicide insensitivity placed on pathogen populations by the repeated sprays required on susceptible varieties, a succession of fungicide modes of action lost efficacy against major pathogens. For the first time in thirty years there was real concern amongst cereal producers about maintaining control if disease pressure was high. There were no new curative modes of action with activity against Septoria leaf blotch
(Mycosphaerella graminicola), so the industry returned to the use of the protectant, chlorothalonil (first introduced in 1964), in mixtures with high doses of triazoles, to maintain control. Reducing disease risk by growing varieties with better resistance against *M. graminicola* became a higher priority and plant breeders realised that it would be more difficult to sell very susceptible varieties in future. There is now a consensus within the arable industry that a better balance is required between disease management through genetic improvement and fungicide use.

**Quantifying dependence**

Crop management decisions are driven by the need to minimise the unit cost of grain production in a competitive world commodities market. The effect of cereal foliar pathogens on unit cost is predominantly through yield, rather than marketability. Plotting yield on fungicide dose applied gives a dose-response curve which approximates to an exponential, with diminishing increments of yield response to each additional increment of dose. Input costs rise linearly with dose, so an optimum dose will be reached somewhere on the ‘shoulder’ of the response curve, where unit cost is minimised. A high optimum dose represents high fungicide dependence – multiple sprays and/or high doses per application are required to maximise economic efficiency. Conversely, a low optimum dose represents low dependence.

Producers or society could choose or regulate to restrict fungicide inputs to below the economic optimum. This would, however, have deleterious economic and greenhouse gas consequences (Berry *et al.*, 2008).

The optimum dose depends on the curvature of the response curve (a function of the distribution of ED50 values in the pathogen population) and the size of the preventable yield loss (the difference between the untreated yield and the asymptote of the yield response curve). Curvature is a characteristic of each pathogen/active substance combination and is governed, at introduction, by the skill of agrochemical developers and, subsequently, by the capacity of pathogen
populations to evolve towards insensitivity. As long as fungicide is applied at the best time (so that the vast majority of yield loss could, in theory, be prevented by a sufficiently high dose) preventable yield loss is the product of:

- disease severity (in the absence of treatment), and
- the effect of a unit of severity on yield.

These are therefore the targets to reduce dependence.

**Reducing disease severity**

Disease severity is determined by the balance between ‘disease pressure’ and ‘host resistance’. Others in this publication will summarise progress and prospects for disease resistance, so it will not be considered further here except to make two observations.

Firstly, the task is huge. The ecological niche occupied by the foliar pathogens of small grain cereals is vast and resource rich – approximately $2 \times 10^{11}$ m$^2$ planar area of leaf tissue (in the UK alone) rich in nitrogen and carbon in readily available forms. Reducing the occupation of such a niche is likely to remain a treadmill, as a result of evolution within pathogen species and competition between species. The greater the reduction in severity required, the greater the selective pressure exerted. If preventable yield loss were to be reduced solely through severity, then a large reduction would be required, because the relationship between preventable yield loss and optimum dose is not linear. Halving average severity from current levels would have only a modest effect on fungicide dependence, but progressively larger benefits would accrue with reductions below that level – provided these could be sustained.

Secondly, the more durable forms of resistance tend to be partial in effect. Hence, there will be some remaining disease for the plant to tolerate. For these two reasons, reducing the effect of a unit of severity on yield – i.e. improving tolerance – complements improvements in resistance. This will be the focus of the remainder of this paper.
Reducing yield loss per unit severity

Yields achieved by cultivated crops generally fall considerably short of their potential (Sylvester-Bradley & Wiseman, 2005). For small grain cereals, debate continues about whether yields are constrained predominantly by ‘source’ (as the capacity to assimilate dry matter through photosynthesis) or ‘sink’ (the capacity to store dry matter in grain). The inter-play between source and sink formation confounds many attempts to quantify which is smaller. Worldwide, the general view tends towards sink limitation (Fischer, 2007; Miralles & Slafer, 2007), with some exceptions (e.g. Sinclair & Jamieson et al., 2006). Under the light-limited conditions of the UK, where nutrient and water supplies are generally adequate for tillering and spikelet formation, the evidence suggests that, if diseases are well controlled, source and sink are in close balance in wheat (Beed et al., 2007; Shearman et al., 2005). In contrast, barley tends towards sink limitation (Bingham et al., 2007a&b), largely because barley ears contain only one fertile floret per spikelet. The contrast between the two crops increases when the effects of foliar disease on source and sink components are considered.

In wheat, foliar disease seldom has much effect on sink components (fertile shoots per m² or grains per ear), but substantially reduces post-anthesis source by reducing green canopy duration. As a result, diseased wheat crops tend towards source limitation. ‘Source-driven’ models, based on measurements of the effects of disease on source capacity during the yield forming period (Waggoner & Berger, 1987), tend to relate consistently to yield (e.g. Bryson et al., 1997).

In barley, in contrast, foliar disease is associated with significant reductions in sink components, particularly affecting the number of fertile shoots per m². It is uncertain whether the mechanism of the beneficial effect of fungicide treatment on sink capacity is through control of visible or asymptomatic disease, or through direct growth regulatory effects on the plant. Source-driven models tend to have poor predictive value, whereas sink-driven models relate more consistently to yield (Young et al., unpublished).
These differences between crop species in factors limiting yield in the healthy state and how diseases affect yield have important consequences for disease management. For example, adult plant resistance is likely to be of less value in barley, where fertile shoot numbers - and hence yield - are determined early in development, than in wheat, where control of disease post-anthesis is crucial to maintain source. The differences also mean that different traits are likely to be associated with disease tolerance in the two crop species. Candidate tolerance traits for barley are described in a recent review (Bingham et al., 2008), so we summarise here progress with determining tolerance traits for wheat.

**Traits for disease tolerance in wheat**

In Parker et al. (2004) we defined units for tolerance, expressed as yield loss per unit of disease-induced green canopy area loss. Green area was measured as an index of planar canopy area per unit ground area and integrated through time to quantify healthy area duration (HAD), as defined by Waggoner & Berger (1987). These tolerance units reduce the extent of extraneous variation, compared to previous methods relating yield to disease severity or area under the disease progress curve (AUDPC). Evidence was presented for significant tolerance variation between elite UK wheat cultivars. Tolerance has not been actively selected for in plant breeding programmes. In fact, an association between high attainable yield and greater loss per unit disease (intolerance) was found.

The units of tolerance combine yield and canopy measurements across a range of epidemic levels. Replication within experiments and across sites/seasons is required to discriminate between tolerant and intolerant genotypes. Hence, direct selection in the large number of lines handled in breeding programmes is impractical. Selecting for high untreated yield would tend to select for some combination of resistance and tolerance. Tolerant lines might ultimately be selected by phenotyping for tolerance traits (where these are visible and readily assessed) or by markers tightly linked to QTL.
The original hypothesis under test was that (provided sufficient variation could be found) tolerance would be conferred by the following candidate traits (identified using a model developed from the analysis reported in Paveley et al., 2001):

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 the presence of disease (to maintain dry matter production despite disease-induced loss of PAR interception).

6. High water soluble carbohydrate (WSC) stem reserves* (to compensate for reduced post-anthesis dry matter assimilation by translocation to grain of WSC accumulated pre-anthesis).

Further analysis of the data set used by Parker et al. (2004) showed little variation between cultivars in candidate trait 1. More variation may exist in wider material. Foulkes et al. (2006) measured tolerance against Septoria leaf blotch (*Mycosphaerella graminicola*) and yellow rust (*Puccinia striiformis*) across a range of near isogenic lines in contrasting backgrounds, from the John Innes Centre (JIC) collection. The work showed that candidate traits 4 (the presence of awns) and 6 (high WSC reserves) were neutral or negative in their association with tolerance. High WSC appears to be equally beneficial to yield in diseased and

* Low molecular weight fructans produced pre-anthesis, stored in the stem and then translocated to grain post-anthesis.
healthy crops, so does not reduce the rate of yield loss per unit of disease-induced green area loss or, consequently, the optimum fungicide dose.

No evidence of up-regulation of RUE in the presence of disease (candidate trait 5) has been found to date in European germplasm or in the Israeli material reported by Zuckerman et al. (1997) to exhibit this trait, when grown under UK conditions. In the absence of up-regulation, high intrinsic RUE tends to associate with poor tolerance, because each MJ of PAR interception lost to disease has a greater impact on dry matter accumulation. However, large flag leaf area, which intercepts more light before it reaches the more heavily diseased lower leaves, was associated with tolerance.

Relating these results to work investigating the mechanisms by which yield has increased in recent decades (Shearman et al., 2005) suggests that the negative effect of breeding on tolerance may be due to changes in the source and sink type traits of modern and old cultivars. Whereas green canopy area (affecting assimilate source) has changed little in cultivars released over a 30 year period, ear fertility of individual shoots (affecting sink capacity of grains) increased. These changes in sink size have been tracked by those in source size through improvement in RUE. Hence, modern cultivars are vulnerable to loss of green area to disease, as loss of source is reflected directly through to yield, whereas older sink limited cultivars could tolerate some loss of source before yield was compromised.

To test this, a doubled-haploid mapping population was derived (at the JIC) from a cross of cv. Cadenza X cv. Lynx, segregating for green canopy area, extinction coefficient, stem reserves and grains per ear, and hence source:sink balance (Foulkes et al., 1998). The population was used as a source of phenotypic variation for trait analysis within a restricted genotypic background, in order to test candidate traits. The parents of the population were screened at the JIC, using publicly available primer pairs, to establish polymorphic microsatellite markers and the lines were characterised for these markers, to allow key traits to
be associated with QTL in future. Seed of forty of the lines, plus parents, was available in sufficient quantity for preliminary, small scale, field phenotyping in harvest years 2002 and 2003. Small sub-populations of the most tolerant and intolerant lines were selected and phenotyped in detail in each of harvest years 2005 and 2006 at a site at ADAS Rosemaund, near Hereford. Non-QoI fungicide treatment was used to vary disease severity, and hence HAD loss, in randomised and replicated field plots. Disease progress, green leaf area index, and crop dry matter accumulation and partitioning were quantified by successive assessments and growth analysis at key developmental stages.

The predominant pathogen in all experiments was *Mycosphaerella graminicola*. The lines differed significantly for tolerance, despite there being no significant difference between the parents, suggesting transgressive segregation. The tolerant and intolerant groups selected from the preliminary 2002/2003 phenotyping were confirmed, with few lines deviating from their previous behaviour, indicating a reasonable degree of heritability. There was no relationship between tolerance and resistance (as measured through loss of HAD), hence the two appear to be independent. There was no significant association between tolerance and total above-ground biomass accumulation, but the trend was weakly positive, which counteracted the significant negative association between tolerance and harvest index. Hence, the relationship of healthy yield and tolerance was not significant - suggesting that tolerance and high yield are not inherently incompatible. Both green lamina area (measured at flowering) per grain and HAD per grain (measures of source:sink balance) were positively associated with tolerance and explained 37% and 40% of the variation in tolerance, respectively.
Conclusions

Disease resistance and tolerance could both be selected in plant breeding programmes and have complementary effects on fungicide dependence. The latter has not been actively selected in cereal breeding in NW Europe, where selection for yield in later generations is predominantly under fungicide treated conditions. Candidate traits for tolerance differ between pathosystems and environments, depending on the extent to which diseases constrain yield through source or sink. Experimental evidence from work on winter wheat under the light-limited conditions of the UK suggests that:

- Quantifying tolerance through the slope of the relationship of yield on HAD, rather than on AUDPC, removes much of the extraneous variation caused by the effects of site and season.
- Selection for high total dry matter productivity, harvest index and yield over recent decades has been associated with a reduction in tolerance. Tolerance traits need to be identified which break these associations.
- There is tolerance variation in UK elite cultivars.
- Transgressive segregation for tolerance has been demonstrated.
- Water soluble stem carbohydrates are similarly beneficial to yield in diseased and healthy crops, so do not affect the optimum dose/fungicide dependence.
- Tolerance is associated with high HAD per grain, i.e. a high source:sink balance.
- Selection for high yield without fungicide treatment in plant breeding programmes would select for a combination of resistance, tolerance and yield.

In further work we aim to quantify the physiological costs and benefits of changing source:sink balance by each of the components of HAD per grain. For example:
• HAD might be increased by canopy size, delayed and rapid senescence and/or later completion of senescence.

• The dry matter cost of allocating more assimilate to leaf tissue is relatively small, but may impact on ear fertility unless biomass overall is increased at flowering or canopy size is increased through a reduction in specific leaf dry matter (DM per unit area).

• The epidemiological consequences of prolonging the life of leaf tissue (i.e. more pathogen generations) may be managed through rate-limiting resistance, but there is limited scope to delay the end of senescence, as it may delay harvest.

• Increasing HAD by the above routes may require increased N uptake efficiency, unless it can be achieved by a reduction in specific leaf N (N per unit leaf area). Increases in specific leaf N should be avoided, due to deleterious effects on the severity of diseases caused by biotrophs (Neumann et al., 2004).

• High N uptake tends to increase the number of fertile shoots. High shoot number appears to be neutral or weakly beneficial to tolerance, whereas high grain numbers per ear appear deleterious.

Acknowledgements

Funding from Defra, the Sustainable Arable LINK programme and the HGCA is gratefully acknowledged. Thanks are due to John Snape, Tony Worland (deceased) and Clare Ellerbrook and colleagues at JIC for developing and genotyping the Cadenza x Lynx mapping population, Ian Bingham of SAC for physiological analysis of barley, and to colleagues in ADAS and SAC for conducting field experiments.
References


Molecular breeding for resistance to soil-borne viruses in cereals

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In Europe, soil-borne viruses, i.e. *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV) both belonging to the genus *Bymovirus* and *Soil-borne cereal mosaic virus* (SBCMV) which is a member of the *Furoviruses*, cause severe yield losses in barley and wheat, respectively. Due to transmission by the plasmodiophorid *Polymyxa graminis*, cultural practices as well as the application of chemicals as control measures are not effective. Therefore, the only way to prevent high yield losses is breeding and the growing of resistant cultivars. While at least eight loci concerning resistance to BaMMV/BaYMV have been identified or isolated (*rym4/rym5*), respectively, in barley, only two loci conferring resistance to SBCMV on chromosome 5DL and on chromosome 2BS of *Triticum aestivum* have been mapped up to now. In addition, a QTL has been identified on chromosome 2BS of *T. durum*. In this paper the state of marker development for respective resistance genes and their application in molecular breeding schemes, e.g. marker assisted backcrossing or pyramiding, is briefly reviewed.

**Barley yellow mosaic virus complex**

In Europe, barley yellow mosaic virus disease, which was first detected in Germany in 1978 (Huth & Lesemann, 1978) and later on in the UK (Hill & Evans, 1980), France (Lapierre, 1980) and many other European countries, is caused by two different strains of *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV, Huth & Adams, 1990; Hariri *et al*., 2003; Kanyuka *et al*., 2004a; Habekuß *et al*., 2008a). Out of these, BaYMV-2 is able to overcome the resistance gene *rym4* and BaMMV-SIL and BaMMV-Teik are able to infect cultivars carrying *rym5*. Due to a constant spread of the area infected and yield
losses up to 50% (Ordon et al., 2004) barley yellow mosaic disease is today recognised as one of the most important diseases of winter barley in Europe. Based on 2007 data the potential economic losses caused by BaMMV/BaYMV in Germany can be calculated as follows: The acreage of winter barley in Germany in 2007 was 1,424,100ha and the average yield was 5.81t resulting in a production of 827,4021t of barley. At that time the price for 1t of barley was about 180€ resulting in an economic value of 1,489,323,780€. According to Huth (1988) 50% of the barley growing area in Germany has to be considered as potentially infected with BaMMV/BaYMV, i.e. 712,050ha. Taking into account only a moderate yield loss of 25% this corresponds to a loss of 1,034,252t equivalent to 186,165,360€.

Resistant cultivars were identified within the set of released cultivars in Germany soon after the first discovery of this disease. By genetic analysis it turned out that the resistance of these cultivars is due to a single recessive gene, which was called \textit{rym4} and assigned to chromosome 3L of barley by trisomic and telo-trisomic analysis (Kaiser & Friedt, 1989, 1992). This gene was probably introduced by chance into German barley breeding via the Dalmatian landrace ‘Ragusa’ which has been used to improve resistance against powdery mildew since the 1930’s. However, at that time (1980’s) resistant cultivars were in general considerably lower yielding than susceptible ones (Table 1). Today barley breeding has achieved a combination of resistance to BaMMV/BaYMV and high yields as most of the released cultivars are resistant and out yield susceptible cultivars. Besides this, in 2005 5 cultivars already carried the resistance gene \textit{rym5} which in addition to \textit{rym4} also confers resistance to BaYMV-2.

Due to this narrow base of resistance, intensive screening programmes were conducted which revealed genotypic differences in the reaction to the different members of the barley yellow mosaic virus complex (Ordon et al., 1993). Some cultivars derived from this germplasm were also resistant to the newly detected strains BaMMV-Sil and BaMMV-Teik, i.e. to all strains of BaMMV and BaYMV known so far in Europe (Habekuß et al., 2008a, 2008b).
Table 1. Improvement in yield of BaMMV/BaYMV resistant and susceptible cultivars in Germany from 1986-2005 (Anonymous, 1986, 1995, 2005).

<table>
<thead>
<tr>
<th>Year</th>
<th>No. cultivars</th>
<th>Yield</th>
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<tr>
<td></td>
<td>Resistant</td>
<td>Susceptible</td>
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<tr>
<td>1986</td>
<td>6</td>
<td>37</td>
</tr>
<tr>
<td>1995</td>
<td>24</td>
<td>41</td>
</tr>
<tr>
<td>2005</td>
<td>52</td>
<td>23</td>
</tr>
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Genetic analyses of the resistance of this germplasm resulted in the identification of different recessive resistance genes within the primary gene pool of barley (Götz & Friedt, 1993; Ordon & Friedt, 1993) and using molecular techniques these genes were mapped on chromosomes 1H, 3H, 4H, 5H, and 6H and easy-to-handle PCR based markers were developed (for overview cf. Ordon et al., 2005; Friedt & Ordon, 2007, Figure 1). In addition to this, dominant resistance genes derived from Hordeum bulbosum were mapped on chromosome 2H and 6H (Ruge et al., 2003, 2006, Figure 1).

Figure 1. Localisation of resistance genes against BaMMV/BaYMV
Closely linked markers represent an efficient tool for barley breeding, since they facilitate the selection of resistant plants without phenotypic analysis which concerning BaMYV/BaYMV-2 to a large extent relies on the climatic conditions during winter and spring time. In practice, the availability of appropriate molecular markers allows doubled haploid populations (DHs) to be screened already in vitro and only those plantlets that carry the resistance encoding allele need to be transferred to the greenhouse.

Moreover, backcrossing procedures required to incorporate these resistance genes derived from low yielding exotic germplasm (Ordon & Friedt, 1994) into adapted high yielding cultivars can be considerably shortened by using molecular markers (Ordon et al., 2003). This holds especially true if genes only effective against BaYMV and BaYMV-2 have to be incorporated, because in contrast to BaMMV and BaMMV-Teik, no efficient screening for these viruses can be conducted at the single plant level by mechanical inoculation in the greenhouse (Ordon et al., 2006).

Besides this, these markers facilitate efficient pyramiding of resistance genes (Werner et al., 2005, 2006). Pyramiding may become of special importance in the future as many of the known recessive resistance genes are not effective against all strains of the barley yellow mosaic virus complex (Kanyuka et al., 2004a; Habekuß et al., 2008a, 2008b), e.g. rym4 is not effective against BaYMV-2, rym5 not against BaMMV-SIL/BaMMV-Teik, rym9 not against BaYMV/BaYMV-2. This approach will lead to an extended usability of these resistance genes in barley breeding, e.g. the combination of rym5, which at present is the sole basis of resistance to BaMMV, BaYMV and BaYMV-2 in European cultivars, with rym9 being effective against BaMMV, BaMMV-SIL and BaMMV-Teik (Kanyuka et al., 2004a; Habekuß et al., 2008) should result in resistance against all yellow mosaic inducing viruses known in Europe. Figure 2 shows a time efficient procedure to combine three recessive resistance genes with only one DH-step. In contrast to a procedure including two DH-line production steps, co-dominant
markers are needed for this procedure. For details see Werner et al. (2005, 2006).

**Figure 2.** Schematic diagram of a pyramiding strategy including one haploid step, for resistance genes *rym4*, *rym9* and *rym11*. Ellipse = resistance encoding allele, rectangle = susceptibility encoding allele (Werner *et al.*, 2005).

However, markers are based in general on polymorphisms around the locus of interest so recombination may lead to false selections. Based on a high resolution map comprising about 7000 meioses (Pellio *et al.*, 2005) and BAC sequence analysis of about 440kb (Wicker *et al.*, 2005), the *Rym4/Rym5* locus located on
chromosome 3H was isolated and comprised the translation initiation factor 4e (Hv-eIF4E, Stein et al., 2005). Specific SNPs in the nucleotide sequence of Hv-eIF4E confer resistance to the different strains of BaMMV and BaYMV (Stein et al., 2005, Kanyuka et al., 2005). Based on these SNPs efficient markers facilitating an allele-specific selection were developed, and analysis of a broadly-based barley germplasm collection resulted in the detection of different alleles of Hv-eIF4E which are being analysed for their specific reaction to the different members of the barley yellow mosaic virus complex (Stracke et al., 2007, Azhaguvel pers. comm.). In addition to this, new alleles of Hv-eIF4E, which up to now have not been detected by screening the natural variation present in gene banks, have been identified by a TILLING approach (Gottwald pers. comm.).

Other plant translation genes, besides Hv-eIF4E, are involved in potyvirus resistance (Robaglia & Caranta 2006); these are valuable candidate genes for different loci encoding resistance to the barley yellow mosaic virus complex. Mapping of these candidate genes is in progress but up to now no candidate gene has been mapped in the vicinity of a BaMMV/BaYMV resistance locus, e.g. nCBP has been mapped on chromosome 5HL where no BaMMV/BaYMV resistance gene is located (cf. Figure 1, Perovic, per. comm.).

**Soil-borne cereal mosaic virus**

*Soil-borne cereal mosaic virus* (SBCMV) is becoming increasingly important in many European wheat growing areas (Koenig & Huth, 2000; Yang et al., 2001, Clover et al., 2001; Budge et al., 2008). Resistance derived from the cultivar ’Cadenza’ (Sbm1), which is inherited in a monogenic manner and acting as a translocation resistance from the roots to the leaves (Kanyuka et al., 2004b), has recently been located on chromosome 5DL (Bass et al., 2006). Although not related by pedigree to ’Cadenza’, genetic analysis has shown that the resistance of cultivars ’Tremie’ and ’Claire’ also has a monogenic mode of inheritance (Perovic et al., 2005), and by using SSR- and bulked segregant analysis, resistance of these cultivars to SBCMV was also located on chromosome 5DL.
(Perovic et al., submitted). During this project an additional SSR marker \((Xgwm469-5D)\) co-segregating with this gene in the ‘Tremie’ and ‘Cadenza’ population and located 1cM distally in the ‘Claire’ population was developed and has proved to be of high diagnostic value. In a set of 99 wheat cultivars analysed, all susceptible cultivars showed a null allele, all those derived from ‘Tremie’ a fragment of 152 bp and those related to Cadenza a fragment of 154 bp (Figure 3).

**Figure 3.**
A. Genetic linkage map of wheat chromosome 5DL constructed using consensus of Tremie populations.
B. Banding pattern of SSR marker GWM469. \(Xgwm469-5DL\) alleles linked to SBCMV resistance are indicated by arrows. Lanes 2, 3 and 5 represent resistant cultivars related to ‘Tremie’, 4 and 6 susceptible cvs., 7 ‘Cadenza’ (Perovic et al. 2008).

Physical mapping using nulli-tetrasomic lines revealed that this gene is located on chromosome 5DL5 which is syntenic to rice chromosome 3. In expression profiling, using a 10K unigene array from barley, about 80 genes differentially expressed between infected and non infected plants were detected. The tests were carried out on parental genotypes and bulks of resistant and susceptible lines after natural SBCMV infection by *Polymyxa graminis*, in growth chamber experiments in which mRNA was extracted from roots, hypocotyl and leaves at the time when the virus was first detectable in these tissues in susceptible genotypes. Out of these 80 genes, only 15 turned out to be differentially
expressed between resistant and susceptible cultivars, i.e. 7 in roots, 4 in hypocotyl and 4 in leaves. Interestingly, 5 of these genes show the highest homology to the region of rice chromosome 3 which is syntenic to wheat chromosome 5DL5. Mapping of these genes is now in progress. Besides the gene on 5DL, a QTL for SBCMV resistance was identified by association studies on chromosome 2BS of T. durum (Maccaferri et al., 2008) and also a major gene on chromosome 2BS of T. aestivum called Sbm2 (Bayles et al., 2007). In this respect it is interesting to note that lines carrying both genes (Sbm1 and Sbm2) showed a significantly lower virus titre than lines carrying either gene alone, indicating that the two resistances act in a complementary way to limit virus spread (Bayles et al., 2007).

Conclusions and future prospects

Molecular markers already facilitate efficient breeding for virus resistance or tolerance in barley and wheat. In the last few years several resistance genes against viruses have been isolated in different plant species. Knowledge of these genes which can help identify candidate genes for virus resistance in cereals, as well as expression profiling techniques and knowledge of the synteny between the sequenced rice genome and barley and wheat, will lead to an enhanced isolation of virus resistance genes in cereals and a deeper understanding of pathosystems in the future. Detection of virus resistance loci and their isolation may also be enhanced by the availability of efficient genome wide SNP-detection methods (DArTs, Illumina Assays) facilitating association genetics based detection of resistance loci. Besides this, isolation of virus resistance genes will be fostered by the ongoing efforts to sequence the barley and wheat genome. The isolation of genes involved in virus resistance will transfer breeding for virus resistance in cereals to the allele level facilitating the identification of novel alleles and their directed use in molecular breeding strategies in order to enhance virus resistance.
References


Genomic dissection of phenotypic variation in barley

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Recent advances in genetic marker technology in barley are providing an opportunity to extend genetic research from experimental populations towards a broader collection of germplasm that is immediately relevant to current plant breeding activities. The improved resolution and efficiency provided by approaches that include association mapping, combined with direct links to putative regional gene content through conservation of synteny with the genomes of rice and \textit{Brachypodium}, are facilitating the isolation of genes controlling a range of barley phenotypes. The DNA sequences of these genes are providing a gateway to basic and applied research that will help capture basic biological understanding and in many cases generate added value in crop improvement. To further improve trait-gene isolation an international roadmap has been developed towards the delivery of a gold-standard reference barley genome sequence. While work towards this objective has been initiated its successful completion will require considerable collaborative international investment.

Introduction

Genetic analysis of all important crop species underwent a fundamental change in the late 1980’s with the development and widespread adoption of molecular marker technologies. Whole genome RFLP-based genetic maps of barley were
published in the early 1990’s (Graner *et al.*, 1991, Kleinhofs *et al.*, 1993) and these provided a general vehicle for genome wide trait analysis. However the RFLP mapping technology lacked portability and was soon supplemented (but not immediately replaced) by PCR-based AFLP (Waugh *et al.*, 1997) SSAP (Waugh *et al.*, 1997) and SSR marker technologies (Liu *et al.*, 1996, Ramsay *et al.*, 1999). Of these, SSRs in particular have been widely applied in genetic studies due to a combination of their in formativeness and ease of application.

The important point is that genetic markers allowed the architecture of many traits to be investigated.

**Genetic linkage mapping**

Since the original RFLP studies, an enormous number of genetic analyses have been conducted for a wide range of traits in many and diverse barley populations. Most often these have been doubled haploid populations comprised of between 100 and 200 independent lines. More recently advanced backcross populations (von Korff *et al.*, 2005, 2006, 2008) and single chromosome substitution lines (Matus *et al.*, 2003) have been developed to search for novel alleles from outside the elite cultivated genepool. Linkages established between molecular markers and traits in these populations are sometimes sufficient to allow the development of diagnostics that can be used to screen germplasm or segregating populations by Marker Assisted Selection or MAS (e.g. markers for resistance to *Barley yellow mosaic virus* conferred by *Rym4/5*, Graner *et al.*, 1999).

Once markers linked to target genes have been detected in small populations, populations developed from the same parental clones including a very large number of meiotic events (*i.e.* lots of individuals) are relatively easily generated. The increased number of recombination events between linked markers in these large populations form the basis for fine-mapping and positional cloning the target gene. Positional cloning has been used to clone several important barley genes including *Hooded* (Müller *et al.*, 1995), *Mlo* (Buschges *et al.*, 1997) and
Once identified, these gene sequences provide a number of opportunities that are not afforded by simply identifying linked molecular markers. These include:

- the information required to start investigations into how the genes actually function in trait biology;
- the molecular template for the discovery and development of perfectly diagnostic markers for application in molecular breeding;
- the basis of targeted molecular screening and classification of the molecular diversity that exists in bio-diverse germplasm allowing prioritisation of potentially superior alleles for introgression into the cultivated gene pool;
- the raw material for GM based approaches to crop improvement;
- an important tool for addressing a range of other more academic questions including those related to population dynamics and domestication.

Consequently, increasing the ability with which geneticists can identify and isolate genes conditioning target phenotypes has been a long term strategic priority for crop plant research communities.

**High ‘–plex’ markers that streamline genetic analysis**

The PCR-based marker assays described above suffer from the need to conduct genetic analysis using serial assays (i.e. one at a time) and rely on marker resolution by gel electrophoresis. Diversity Array Technology (DArT) overcame the multiplexing issue by parallelising marker assays on modified glass slides using a microarray-type format (Wenzl *et al.*, 2004, 2006, Hearnden *et al.*, 2007). However, DArT generates dominant vs. null markers that lack genetic power in many applications (i.e. they do not detect heterozygotes).

More recently, we have developed a high density gene sequence-derived SNP marker technology (Rostoks *et al.*, 2005, 2006) based on the Illumina GoldenGate and Oligo Pool Assays (OPAs). These have enhanced multiplexing and provided robust co-dominant bi-allelic markers that are more genetically
informative than DArTs (Rostoks et al., 2006). Each of 2 barley OPAs (BOPA1 and BOPA2; Close et al., in preparation) scores SNP genotypes at 1536 independent genetic loci in a single assay. The assays are very robust and informative across a wide range of germplasm (Figure 1). We initially used BOPA1 and 2 for genetic analyses of reference bi-parental mapping populations to establish gene order.

The resulting gene based genetic maps, after supplementing with ‘legacy’ information from genic RFLP probes, EST-SSRs (Stein et al., 2007) and new data from mRNA abundance-based transcript derived markers (TDM’s, Potokina et al., 2007, Luo et al., 2007) contain over 5000 uni-genes (Luke Ramsay and Arnis Druka personal communication). Importantly, this gene-map provides a conduit to detailed comparative genetic analysis with the fully sequenced model genome sequences of rice (Goff et al., 2002) and Brachypodium by exploiting the conservation of synteny across cereal genomes. It facilitates gene discovery both directly via the identification of candidate genes and indirectly by providing information for targeted marker development for saturation mapping in positional cloning programmes (Bruggeman et al., 2002, Komatsuda et al., 2007). As a result of this development in barley marker technology, in many organisations large scale genotyping has moved out of the lab into dedicated genotyping centres (e.g. the Southern California Genotyping Centre (http://scgc.genetics.ucla.edu/) and USDA-ARS Biosciences Research Labs in Fargo).
Figure 1. *Illumina* Beadstudio 3 software graphical display of the allele calls at a single SNP locus (of the 1536) assayed on a collection 480 barley genotypes. The axes represent the intensity values of cy3 vs. cy5 fluorescent dyes that are incorporated in the assay in a SNP-allele dependent manner. Red dots have genotype AA, blue dots genotype aa, purple dots are heterozygotes (genotype Aa). Black dots are scored as null alleles or fails as they fall outside the limits of defined allele clusters.

Genetic analysis of phenotypic traits

Major gene and QTL mapping in biparental populations has been successful in identifying regions of plant genomes that carry important alleles for many traits of interest. In barley, hundreds of QTL have been identified that explain variation for disease resistance and also yield, malting quality, and other agronomic traits (see http://wheat.pw.usda.gov/GG2/maps.shtml#barley). However, the majority of these QTL are not in regular use within breeding programmes. The main reasons for this are that QTL mapping populations often use parents that are irrelevant to current breeding, the traits of interest are easier or cheaper to score phenotypically, there is linkage drag between desirable and undesirable trait alleles and the markers do not have predictive value in different genetic backgrounds.
A significant driver behind our efforts to develop BOPA1 and 2 was to enable us to pursue a different type of genetic analysis that would in principle overcome some of these issues and generate results that are relevant to current crop improvement. The approach we adopted, termed either Linkage Disequilibrium (LD) or Association mapping (Jannink et al., 2001), is a population genetics approach that relies on the detection of population-wide marker-phenotype associations. Based on results from high resolution DNA sequence based LD studies (Caldwell et al. 2006) and from analyses of haplotype diversity (Rostoks et al. 2005) we outlined a strategy to exploit association mapping in the elite cultivated barley genepool. While barley is an inbreeder, we considered the cultivated genepool to be an unique artificially constructed pseudo-outbreeding population because of the level of outcrossing induced by breeding. We proposed that the resulting level of genome shuffling would facilitate the association mapping approach (Rostoks et al. 2006). Several current projects worldwide, pioneered by AGOUEB in the UK (http://www.agoueb.org/) BarleyCAP in the USA (http://barleycap.cfans.umn.edu/), and now joined by several others including ExBarDiv in the EU (http://pgrc.ipk-gatersleben.de/barleynet/projects_exbardiv.php) are exploring this approach.

**Figure 2.** Association mapping of a bi-allelic SNP. $R^2$ (a measure of Linkage Disequilibrium) is shown on the y-axis with the order of genes on the barley map on the x-axis (top of the short arm of chromosome 1H to the end of the long arm of chromosome 7H). An unmapped SNP marker that was polymorphic within in a collection of 190 barley cultivars was mapped to Chromosome 5H at position 105.67 cM.
Current evidence (Figure 2) shows that previously unmapped bi-allelic markers and simple traits can be genetically located using whole genome association mapping and that it will be a valuable addition to bi-parental populations for detecting marker-phenotype associations in breeding-relevant gene pools (Kraakman et al., 2004, 2006, Rostoks et al., 2006). The examples followed to date, that have been conducted on relatively limited sample sizes, suggest that the resolution of association mapping is considerably better than that achieved using similarly sized bi-parental populations (our unpublished results). We have used this approach to identify candidate genes underlying morphological and developmental genes in barley, using the synteny with the rice genome sequence as an intermediate (Goff et al., 2002). Though not as advanced we are currently exploiting this approach to investigate resistance to Rhynchosporium secalis and plan to investigate the genetics of Ramularia collo-cygni and Fusarium graminearum with colleagues at the John Innes Centre in the near future.

**Surrogate phenotypes and disease resistance**

There is currently considerable interest in the use of surrogate phenotypes such as mRNA transcript abundance as a means to investigate the genetics of complex traits in segregating populations. Using the Barley1 Affymetrix gene chip (a microarray platform), we assembled a mRNA abundance data set from the Steptoe x Morex (St/Mx) reference barley mapping population which had previously been used to positionally clone Rpg1, a major gene controlling stem rust resistance in barley. Genetic analysis of the data from each of the 22800 genechip probesets using the OPA-derived SNP-map as a framework revealed a genome-wide distribution of 23,738 significant mRNA abundance QTLs (expression-QTL or eQTL). Given the density of this information we explored the potential of these mRNA abundance traits as surrogates for the identification of candidate genes underlying the reaction of barley to Puccinia graminis f. sp. tritici. We used legacy data recording the phenotypic reaction of each of the individuals in the population to the pathogen. Analysis of the quantitative resistance trait data identified six genetic loci associated stem rust
resistance/susceptibility. Two of these coincided with \textit{Rpg1} and \textit{rpg4/Rpg5} (known resistance genes) and the remaining four were novel. Correlation analysis between resistance data and the 23,738 mRNA abundance QTL put \textit{Rpg1} mRNA abundance, in the top 5 candidate genes for the major QTL on Chromosome 7H, the location corresponding to \textit{Rpg1}. Similar analyses identified candidate genes for \textit{rpg4/Rpg5} on chromosome 5H (Druka \textit{et al.}, 2008). We have recently extended this approach to examine leaf rust infected leaf material in the same population and have once again identified several candidates for partial disease resistance.

\textbf{The value of genomic models}

Through the development and application of contemporary genetic technologies and, latterly, genomics tools and resources, we know a great deal about the origins of our major crop plants. Within the monocot lineage, a remarkable conservation of gene sequence, content and order (conserved synteny) has been demonstrated across genomes ranging in size from 400Mb (rice) to 17,000Mb (wheat) and mechanisms have been proposed for their genome evolution from a common proto-ancestor (Moore \textit{et al.}, 1995, Salse \textit{et al.}, 2008). Given that the rice genome has been completely sequenced (and recently joined by \textit{Brachypodium} (355Mb in size)), conserved synteny facilitates the use of genomic models as bridges to detailed genetic analysis of the larger genome crops that include barley (5,300Mb genome) and wheat (17,000Mb genome). Thus, if we know the genetic location of a gene in barley, it is possible to postulate the identity of the adjacent genes in barley by inference from the genes surrounding the orthologous gene in the rice or \textit{Brachypodium}. This has been of considerable value in positional cloning efforts across the larger genome monocots (e.g. Uauy \textit{et al.}, 2006) and will remain key to exploitation of bi-parental and association mapping. However several other experiences also record that using rice or \textit{Brachypodium} as a genomic model will not work for all barley genes and that to achieve success it will be necessary to work directly in the barley genome.
A barley physical map leading to a reference genome sequence

Despite the obvious power of genomic models, it is clear that the availability of a high quality reference genome sequence of barley itself will be required to access any gene of interest. Consequently, an International Barley Genome Sequencing Consortium was formed in 2006 (IBSC, http://barleygenome.org). A white paper developed by this group presents a roadmap towards achieving this objective (http://www.public.iastate.edu/~imagefpc/IBSC%20Webpage/IBSC%20Template-home.html). The strategy is based upon the establishment of a genetically anchored physical map of the barley genome and subsequent sequencing along the minimal tiling path of Bacterial Artificial Chromosome (BAC) clones that comprise the physical map. Work was initiated more than two years ago to address this objective. The bulk of the current effort is spearheaded by Nils Stein and Andreas Graner at IPK Gatersleben in Germany, where they are over half way towards their objective of building a BAC–based physical map. However the strategy developed two years ago is already subject to amendment as recent technical advances provided by next generation sequencing methodologies of Illumina, Roche and Applied Biosystems, mean that the current plan will certainly evolve.

Acknowledgements

The work described above was sponsored by Defra and the Scottish Government Rural and Environment Research and Analysis Directorate (RERAD) through the Sustainable Arable LINK programme with the HGCA as an additional industry sponsor. We also acknowledge BBSRC Exploiting Genomics programme funding to RW, and funding to all of the SCRI-based authors from RERAD Work Package 1.1.

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Strategies for the control of take-all

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Take-all, caused by the soil-borne fungus, Gaeumannomyces graminis var. tritici, is a problem in cereal crops worldwide. Predicting when severe disease is likely to occur is difficult because many inter-connected factors can influence the interaction outcome. The occurrence of severe take-all will largely depend on the amount of inoculum present in the soil at the time of sowing. In this paper some of the factors which can affect the disease and ways of reducing soil inoculum are discussed.

The name take-all was first used in Australia in the 1870s, but it was recognised as early as 1852 as a devastating disease of wheat. Today, take-all occurs worldwide and is regarded as the most damaging root disease of wheat. In the U.K. losses due to take-all have been estimated to be up to £55M p.a. The disease is usually only a problem when two or more susceptible cereal crops (mainly wheat or barley) are grown consecutively. The fungus survives in the absence of a living host as mycelium in or on host root residues, which are the most important sources of inoculum for the following crops. The infectivity of these colonised residues decreases due to decomposition. Smaller root fragments decompose faster and also require warmer soils to infect new hosts, than do larger fragments. The rate of decay of soil-borne inoculum is influenced by many factors but inoculum concentrations decrease to levels that are difficult to detect in less than one year. This explains why a one-year break is normally sufficient to provide effective control of the disease.

Take-all occurrence in the field is patchy and being able to predict when a severe outbreak will occur has always been difficult. However, some factors are known
which can encourage the disease. Therefore, it is important to prepare for crops at risk. The following are examples of factors which can affect the severity of the disease.

**Crop nutrition**

Nitrogen: The interaction between nitrogen form and take-all is complex but in general terms there is little to choose between the forms available. However, in field experiments, where forms of nitrogen (N) were compared, ammonium sulphate consistently gave less disease compared to ammonium nitrate, urea or ammonium chloride fertilisers (Gutteridge *et al.*, 1987).

Perhaps more important is the timing of N applications. Crops grown at risk of take-all should not run short of N as this will impede root production and put the plant under stress conditions which will favour the disease. It is generally accepted that an early application of 40-60KgN/ha in February/March, followed by the main dressing in April, will help reduce take-all severity on the roots.

Nitrogen can reduce the severity of take-all and is most effective in the range of 0-100 KgN/ha. However, manipulation around the commercial rate (200KgN) will have little effect on the disease.

Phosphorous: Phosphate deficient soils (less than 15mg/kg of soil) favour the take-all fungus. In such soils take-all inoculum builds up more rapidly under the first susceptible crop, compared to soils with adequate reserves of P, and consequently a second cereal will be at a higher risk from severe disease.

Phosphate has no direct effect on the take-all fungus, as it has been shown that once inoculum is present in the soil applications of superphosphate to correct the deficiency did not stop severe disease developing in the following crop (Gutteridge *et al.*, 1996). In order to reduce the risk of take-all to second crops, level of phosphate in the soil should be at 20mg/kg (index 2) before the start of a cereal sequence and any deficiency rectified before the break-crop.
Potassium: Information on the effect of potassium on take-all is limited but take-all severity was increased when large dressings of K (120kg/ha) were applied to phosphate deficient soils (Mattingley et al., 1980). In an experiment where soils had a range of K deficiencies and P was not limiting, take-all was more severe where K was deficient than where it was in adequate supply when a second wheat was grown (Gutteridge, unpublished). Indications are that soils should be kept at index 2 to reduce the risk of severe take-all.

Micronutrients: Both manganese and sulphur deficiencies have been linked to an increase in severity of take-all. But there is no strong support that either is a major factor in causing a severe outbreak of the disease in the UK. Symptoms of magnesium deficiency and take-all disease occurred in a second wheat crop grown at Woburn in Bedfordshire. Application of magnesium fertiliser, which cured the deficiency symptoms and increased yields, had no effect on take-all severity (Bolton & Slope, 1971). It is only in the last ten years, as a result of reduced sulphur emission, that sulphur deficiencies have been widespread in crops in the UK. Sulphur is routinely applied to crops but despite this addition severe disease still occurs, therefore, this nutrient is unlikely to affect the disease.

**Crop rotation**

If managed well, all break-crops (i.e. a non-cereal crop) will reduce take-all inoculum to negligible amounts and the risk of severe disease to a following crop. The amount of carry over of inoculum may well depend on how infective the soil is prior to sowing the break-crop. The greater the take-all infectivity of the soil, the more carry over of inoculum, but even so, this poses little risk of severe disease on the first crop. However, in this situation, because the disease will be more widespread on the first wheat, although generally only in the slight disease category, then inoculum build-up is likely to be more rapid and pose a greater risk of severe take-all to the second crop.
The presence of carriers of the take-all fungus (cereal volunteers and grasses) through the break-crop year can undermine the effectiveness of the break. For example, oilseed rape, wheat, wheat has become a common rotation. The infectivity of the soil after the second wheat is usually high and the rape crop is either direct drilled or sown after minimum cultivations. The period between harvest and sowing the rape crop is short and consequently cereal volunteers are common in the rape crop. These volunteers soon become infected with take-all. Experiments have shown that the way these volunteers are managed affects how much take-all is found in the first wheat crop. If the volunteers are prevented from establishing, then disease is the same as a normal clean break. If they establish and are destroyed by a selective herbicide spray applied either early (November) or later (February), then, although more disease occurs it is usually only slight and the break is still effective. However, if volunteers are left untreated patches of severe take-all may be seen in the first crop and so compromise the effectiveness of the break.

*Phialophora graminicola*, an antagonist to the take-all fungus, builds-up under a grass ley and can delay the onset of severe take-all (Slope *et al.*, 1979). A two year ley is preferable as this allows, a) the take-all fungus to decline, and, b) the *Phialophora* populations to establish effectively. A sequence of wheat crops grown in this situation showed that severe take-all was delayed by a year and offers an alternative in take-all management.

A first wheat crop after set-aside is usually not affected by severe take-all, providing that there had been effective removal of all green cover as early as was permitted (Jenkyn *et al.*, 1998). Before set-aside was set to zero, some farmers suggested a wheat / set-aside rotation. The implication of this is unclear, but the likely scenario is that as the number of cycles increases so does the risk of severe take-all occurring in that first crop. A more common approach to managing take-all is by using non host crops such as oats, oilseed rape or potatoes. This offers a better option as there is a longer interval between susceptible crops providing wheat volunteers and carriers of the take-all fungus.
(rhizomatous grasses and *Bromus* spp.) are strictly controlled. Failure could result in severe disease in the first wheat crop, as was the case in some crops in 2008. Severe take-all was confirmed in a first wheat crop after oats, where wheat volunteers were common in the oat crop and after peas or potatoes, where both sterile and barren brome was a problem.

Cereal species differ in their susceptibility / escape mechanism to take-all. Winter wheat is the most susceptible, rye the least and barley and triticale intermediate (Gutteridge *et al.*, 1993). Oats may be used as a break-crop for take-all, providing the oat attacking strain of the fungus is absent, and is the only cereal which can be used in this way. All other cereal species, whether winter or spring sown, are capable of building-up potentially damaging amounts of take-all inoculum.

If successive take-all susceptible crops are grown the disease builds up to a peak and then declines, the phenomenon known as take-all decline (TAD). TAD has been shown to occur in winter-sown wheat, barley, and triticale and spring barley. Although the damaging effect of take-all, at its peak, is most severe on winter wheat, the resulting TAD is robust and can protect the less susceptible cereals should a change of cereal species be introduced. Decline built up on the less susceptible cereals, barley and triticale, can protect either of these species but it is not robust enough to protect winter wheat. Therefore, introducing wheat into a barley or triticale sequence will result in severe take-all developing (Hornby & Gutteridge, 1995). Once TAD is established a one year break-crop or fallow can be introduced into the sequence with little loss of the decline factor. How often this introduction can be made is uncertain, but, when a three year break or three cycles of break, wheat, wheat was introduced in a TAD soil, the decline factor was lost. Decline needs to re-establish itself after the break and perhaps five years is required before another break is taken.
**Soil type**

There is no comprehensive guide to soil types and their take-all characteristics. Even within a particular soil type the series that make up that type can vary in their conduciveness to the disease. For example, in the chalky boulder clay soils, the Ragdale series is more prone to take-all than the Hanslope series (Catt *et al.*, 1986). The number of soil types within a field has increased as fields have been enlarged over the decades and management of these fields is more complex. Assessing a take-all risk factor would be difficult and local knowledge is likely to be more important than which soil type.

However, in general the light soils (sand, sandy loams and loams), where the fungus can spread more easily through the soil, and the chalky soils of the Downs (where rooting is restricted) tend to be more take-all prone than the more well structured soils (chalky boulder clays). These generalisations may be too simplistic as both location and climate may well interact with disease. For example, in fields where there are depressions or at the bottom of a slope, where the soil tends to remain wetter, take-all is often more severe in these locations than in the drier upper parts of the field.

**Risk assessment**

First wheats grown after ‘clean’ break-crops (i.e. where carriers of the take-all fungus are controlled, for example cereal volunteers and a number of wild and cultivated grasses) will usually have fewer than 15 per cent plants infected, with only slight symptoms. A following second wheat crop, can nevertheless, be severely affected by take-all. Therefore, although visual assessments of take-all lesions on plant roots provide a good indication of the damage caused to that crop, they do not provide a reliable prediction of risk to a following crop. Research at Rothamsted, using a soil core bioassay, has shown that infective inoculum starts to build up in fields in the top 10-15 cm layer of soil in April/May and, in favourable conditions, continues to increase through to harvest. The
amount of inoculum in the soil after harvest was shown to be positively, and linearly, related to the severity of disease in the following wheat crop.

The risk of severe take-all occurring is largely dependent on the amount of inoculum in the soil at the time of sowing. Recent results from field experiments within the Wheat Genetic Improvement Network (WGIN, for more information see http://www.WGIN.org.uk) has shown that different winter wheat cultivars can build up different amounts of take-all inoculum in the soil when grown as a first cereal crop (Rothamsted Research Association Newsletter Issue 27, June 2008, for more information http://www.rothra.org). For example, the Hereward plots consistently gave the most root infection in the bioassay, Cadenza gave the least and Riband was intermediate. The ability to manipulate soil inoculum by careful cultivar selection for the first wheat crop is new and may offer another way of reducing the potential take-all risk in short rotations.

The bioassay method for measuring the take-all infectivity of the soil is a useful research tool but is limited and not commercially viable because it is time consuming, labour intensive and requires controlled environment conditions for the assay.
Figure 1 Bioassay method – soil cores taken using an auger, inverted into a plastic drinking cup, sown with wheat and grown in a constant environment room. The seedlings are assessed for disease incidence and severity after five weeks (Slope et al., 1979). Typically five and up to 50 pots are required to assess experimental plots or a single field, respectively.
A molecular method based on real time PCR for detecting take-all DNA in the soil has been developed in Australia by SARDI (http://www.planthealthaustralia.com.au), called PreDicta B. This has recently been successfully tested in New Zealand, where climatic conditions are similar to those in the UK. A preliminary evaluation comparing the take-all DNA and the wheat seedling bioassay was undertaken at Rothamsted and in Suffolk in 2007/08 using HGCA funds. Results have shown a positive relationship between the two methods. The molecular technique has the distinct advantage in that it both quantifies and predicts take-all inoculum levels in the soil. The PreDicta B test is easier and less time consuming than a bioassay and therefore the results can be obtained more quickly and could potentially be available before cropping decisions are made for the following year. The PreDicta B test would allow a more accurate field-by-field risk assessment for take-all. In turn, this may reduce the number of crops at risk from severe take-all by
informing the farmer / farm agronomist earlier so that appropriate changes to
the rotation could be made when high take-all inoculum levels are detected.

Other precautions can be taken to reduce the severity of take-all in crops at risk
from the disease.

**Cultivations**

In long runs of cereals there is little to choose between minimum cultivations and
ploughing as the advantages and disadvantages even themselves out over time.
More important are the problems each system may cause, for example, minimum
cultivations encourage grass weeds. With the emergence of herbicide resistant
blackgrass and ryegrass becoming more common it is increasingly difficult to
control them. In short sequences of cereals, ploughing generally has the
advantage. At harvest the majority of take-all inoculum is in the top 10cm and
ploughing buries this and brings less infective soil to the surface. Sowing into this
less infective soil causes less early infections and gives the plant time to establish
before reaching the more infective soil. Conversely, minimum cultivation of first
wheat stubble leaves the highly infective soil near the surface where infection of
a second wheat crop can be much more rapid.

**Sowing date**

It is now generally accepted that the disease is likely to be less severe the longer
the interval between harvest and the sowing of the next susceptible crop. Sowing
time varies from year to year depending on work load and soil conditions and a
compromise is to be expected. A crop sown in ideal conditions is better than one
going in where soil structure can be damaged. Ideally the order for sowing wheat
is first wheat crop (mid September), continuous wheat and wheat at high take-all
risk last (mid October).

Cereal volunteers between susceptible crops, the ‘green bridge’, should not be
allowed to develop. Soil temperatures in August / September are high and moist
conditions provide an ideal environment for rapid infection of cereal volunteers by
take-all. This extra root infection either maintains the overall inoculum by counter balancing the loss of inoculum due to decay, or can increase soil inoculum and, therefore, increase the risk to the following susceptible crop. In an experiment at Rothamsted, Gutteridge & Hornby (2003) demonstrated that the presence of volunteers between crops partially offsets the expected beneficial effect of decreased disease with a mid-October sown, compared to a mid-September sown, wheat crop.

**Fungicides**

The wheat take-all fungus is, like all soil-borne, root-infecting fungi a difficult target for fungicide control. However, two fungicides, fluquinconazole and silthiofam, which have useful activity against take-all when applied to the seed are now available (Bateman et al., 2008). Both provide only partial control of the disease but, when used in conjunction with other agronomic inputs, can give a respectable decrease in take-all severity and an increase in yield. Two other fungicides, azoxystrobin (Jenkyn et al., 2000) and fluoxastrobin, applied as a spray before / at stem extension, have been shown to decrease the severity of the disease. However, these two foliar applied fungicides have been erratic in their performance. Limited data suggests that moist soils and / or rain shortly after spraying are required for these sprays to be most effective. These conditions would allow the active fungicide to penetrate the soil, perhaps by way of the root channels, and protect the upper portion of the root. When used in conjunction with the seed treatments these spray applications can have an additive effect in controlling the disease.

**The full spectrum of consequences of severe take-all infection**

Take-all causes a severe loss of yield in susceptible crops and can restrict rotational options. However, presently wheat is the most profitable crop and the temptation to grow wheat, despite the take-all risk, is great. Take-all also affects grain quality by reducing thousand grain and hectolitre weights, an increased proportion of small grains and an increase in grain N (Gutteridge et al., 2003).
Severe take-all causes the plant to die prematurely and as a consequence the crop cannot make use of all the available nitrogen. The amount of residual inorganic N (ammonium and nitrate) left in the soil after harvest can be up to three times more in areas where severe take-all has occurred compared to areas relatively free of the disease (Macdonald et al., 1997). Most of this N remained in the upper 0-25cm layer of soil; however, recent studies have shown that in wet summers the N is pushed further down the soil profile and is more readily available for leaching. The avoidance of this potential environmental impact is paramount and precautions need to be taken to minimise the risk.

Acknowledgements

We would like to thank Dr. John Jenkyn and Dr. Geoffrey Bateman who led research topics referred to in this paper. We would also like to thank the staff of Rothamsted farm for maintaining and good husbandry of the experiments. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the United Kingdom. In addition, specific funding has been received from the HGCA, Defra, Syngenta and Bayer CropSciences.

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Managing Rhynchosporium risk in barley

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The barley disease caused by the fungus *Rhynchosporium secalis* (commonly known as Rhynchosporium or leaf scald) is the main disease of barley in the north and west regions of the UK. This paper discusses options open to growers to manage the disease in an integrated way, making use of knowledge about symptomatic and asymptomatic disease epidemics, varietal resistance, fungicide activity and fungicide resistance issues. UK growers’ preference for fungicides to manage disease is at risk from a review of EU pesticide legislation and also threats from fungicide resistance. Future management approaches including the use of molecular diagnostics and risk management tools are discussed including knowledge exchange approaches to best deliver the advice.

Introduction

*Rhynchosporium secalis* remains the most significant economic disease threat to affect barley in the wetter regions of the UK. Fungicides remain the most popular method for growers to control the disease. Other options for control include manipulation of agronomic parameters, the use of resistant varieties and the development of decision support systems which would include all of the above. A review of EU pesticide directive 91/414/EEC will potentially limit the choice of fungicides available to growers within a 5-10 year time scale. Fungicide resistance also has an impact on the fungicide choice available and although the strobilurin fungicides continue to be effective, resistance shifts have occurred to triazole fungicides, which are currently the main fungicide group used to manage Rhynchosporium. Despite the reliance on fungicides as the key method to
manage disease, the Defra-funded winter barley disease survey highlights losses
due to uncontrolled disease amount to 4.5\% of yield, despite fungicide
application to 95\% of the crop, indicating that fungicides are not a sustainable
long term solution to manage disease (Hardwick, Slough & Jones, 2000).

**Rhynchosporium disease epidemics**

Knowledge of the pathogen is essential to understand the best approach to
manage the causal disease. Seed infection was shown to be a major source,
which can lead to widespread development of Rhynchosporium symptoms in
January to February and other authors (Shipton, 1974; Lee, 2002; Zhan, 2008)
concur. The pathogen can develop in the crop asymptomatically during the winter
before causing widespread visual symptoms in January – February.
Understanding the importance of this phase on disease development and yield
remains under investigation, but preliminary studies (Oxley *et al.*, 2008a)
showed that molecular diagnostics were a useful tool to determine disease levels
in high pressure crops by testing leaves and shoots before treatment timings.
Visual assessments were also effective, but a diagnostic test was more sensitive
where disease symptoms had yet to appear.

By testing leaves for *R. secalis* DNA late in the season, it was concluded that a
yield response to fungicide occurred both in crops where visual symptoms were
present and also in crops where *R. secalis* DNA was detected in the absence of
symptoms. Yield responses to fungicide were 2\% where no symptoms were seen
and DNA levels in the upper leaves were<10 pg DNA/g. The yield response
increases to 10\% where *R. secalis* were detectable at 10–40 pg DNA/g inside
symptomless plants (Oxley *et al.*, 2008b).

**Varietal resistance**

Information on the varietal resistance to Rhynchosporium is updated annually
and is available to growers through the HGCA Recommended List (Anonymous,
2008). Varietal resistance is scored on a 1-9 scale where a higher number
represents better varietal resistance. For a particular market segment, growers are advised to choose a variety with the best quality features for the chosen market, the best yield and finally best resistance for the common diseases within their region. Even when a variety has been chosen with the best varietal resistance characteristics, it is important to take account of the responsiveness of the variety to fungicide. A variety susceptible to disease but with a good response to fungicide is likely to be a better choice than a resistant variety with a low yield response.

There has been some debate on the accuracy of the varietal resistance scores for winter barley. Winter barley varieties on the current Recommended List have scores for varietal resistance to Rhynchosporium between 6 and 8. A grower might assume that all varieties show reasonable varietal resistance on these scores, when in fact they can show high levels of disease and good yield responses to fungicide in trials and in practice. Reasons for the discrepancy may include the timing of the assessments for visible disease and the importance of asymptomatic infections. Visible symptoms of Rhynchosporium peak in the winter and early spring (Oxley, 2008b). A second visible peak of disease can occur later in the season, but this is generally lower. Most disease assessments on variety trials occur late in the season when disease levels are likely to be low and not when the disease levels are at their peak. Early disease is associated with the major loss in yield (Bingham et al., 2007), so more accurate varietal resistance ratings could be determined through earlier visual disease assessments. For spring barley, varietal ratings are a more accurate measure of the susceptibility of the variety to Rhynchosporium. The disease epidemic in the spring barley occurs later in the season and corresponds better to the time disease assessments are made.

**Fungicide activity and resistance**

Fungicides remain the key input for managing Rhynchosporium in commercial crops. Information on activity and cost effectiveness is available through
fungicide performance testing (Oxley & Hunter, 2005). The method used tests fungicides at different dose rates under high disease pressure situations. There are concerns that growers may choose the most effective fungicide and use it by itself. In practice, there are many disease threats affecting a crop and using fungicide mixtures is the best approach to widen spectrum of activity. An approach to limit the risk of fungicide resistance is to use fungicides in mixtures and sequences to minimise the threat from fungicide resistance (Oxley et al., 2006). For Rhynchosporium, this has shown to be a practical economic solution since there are five different active fungicide groups available to manage the disease (Oxley et al., 2008b). This research on fungicide mixtures also demonstrated that using a DMI fungicide in mixture with one or two alternative fungicide groups reduced the risk of less sensitive isolates surviving at the end of a season compared to using the DMI fungicide alone.

Risk decision tools

A survey of barley growers carried out by SAC in 2008 showed that new fungicides to control Rhynchosporium and new information on cost-effective disease control are a high priority (personal communication Clare Hall). Decision support system tools were however given a low priority in the survey. To provide growers with the information they require on cost-effective disease management, it is important to develop risk decision tools as a method of summarising and prioritising the range of factors which are involved in determining the severity of a Rhynchosporium epidemic and the likely benefit of any treatment decision. Grower reluctance to use decision support systems shows that such tools either need to be simple paper-based risk assessments, technical notes (Oxley & Burnett, 2008), or more sophisticated web-based tools targeted at consultants, who can provide growers with risk estimates and treatment decisions during the season based on the probability of economically damaging levels of disease occurring (Collett, 2003).
Discussion

A combination of skills from molecular biology and practical plant pathology has led to an increase in our knowledge about *R. secalis* and its impact on barley yield. The challenge for scientists is to make use of the new information and disseminate it in a practical way which helps growers to manage the disease in a cost-effective manner. Changes in varieties, fungicide performance and resistance issues and the economics of crop production need to be taken into account when giving such advice. Seasonal changes play an important part in the disease epidemic within a season. Longer term changes in the climate may lead to unforeseen effects with changes in cropping patterns and cultivations affecting disease epidemics (Davies, 2008). Grower views on managing disease are important, but their preference for fungicide solutions may be limited by developments in fungicide resistance and also changes in EU policy. By working with the industry and policy makers, risk decision tools are one method to bring together diverse and potentially conflicting information on the pathogen, host, environment and economics which can be used to inform both policy makers and also growers on crop disease management.

Acknowledgements

We would like to thank the Scottish Government and HGCA for funding barley disease research.

References


Marker-assisted breeding for improved resistance to disease in wheat

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Wheat breeders continue to produce varieties with improved resistance to disease, but DNA marker technology now offers new opportunities for manipulating disease resistance genes more efficiently in breeding programmes.

Introduction

Breeding wheat for resistance to disease has historically been a trial and error process because the genetics underlying resistance often remains uncharacterised. Despite thorough pathology testing, varieties have frequently followed the familiar ‘boom and bust’ cycle when new virulence factors evolve within the pathogen populations to match the genetic resistance factors being deployed in new varieties. If the breakdown of resistance is dramatic then this has significant financial consequences for both the grower and breeder. In a little over forty years, there have been a number of notable examples of resistance breakdown in the UK including:

Yellow rust (*Puccinia striiformis*) on cvs. Rothwell Perdix, Joss Cambier, Slejpner, and Brigadier

Brown rust (*Puccinia triticina*) on cv. Buster

Mildew (*Blumeria graminis*) on cv. Shamrock
The appearance of other new races has been of less significance to the grower, but has still been important in terms of fine-tuning strategies for resistance breeding.

An understanding of the genes which protect a variety from damaging disease infection can sometimes provide an indication of the risk of breakdown to new physiologic races should the variety be released to growers. Of course, knowledge of the current race situation in Britain is very important, but it is also prudent for breeders and pathologists to monitor race developments outside the UK. The United Kingdom Cereal Pathogen Virulence Survey (UKCPVS) reports annually on the population dynamics of a range of important cereal diseases and also identifies some of the resistance factors in current varieties. Specific resistance genes carried by wheat varieties can sometimes be determined by testing with individual races with known virulence profiles. This can be done in the glasshouse, as seedlings, or in the field, as adult plants. Certain resistances are only expressed in the adult plant as opposed to the overall resistance types which are expressed at all growth stages.

**Disease resistance genes in wheat**

Many fungal disease resistance genes have been identified in wheat and are listed in the catalogue of gene symbols (McIntosh et al., 2008). For example, a total of sixty-one designated \( Lr \) genes for resistance to *Puccinia triticina*, and forty-one \( Yr \) genes for resistance to *Puccinia striiformis* have been named officially. These resistance \( (R) \) genes have been identified in a wide range of genetic backgrounds from all around the world and consequently only a small subset can be found in elite UK varieties (UKCPVS Annual Reports, see Anonymous, 1967-2007). In many cases the chromosome on which the gene resides is known, but unfortunately not all the resistances have been mapped in relation to PCR-based markers such as microsatellites.
Many of these R-genes encode the plant resistance proteins that recognise the pathogen’s avirulence factors, which in turn trigger the signal transduction cascades that mobilise the plant’s defence response (Hammond-Kosack & Parker, 2003). The largest known family of plant R-genes encode proteins with a nucleotide-binding site (NBS) and C-terminal leucine-rich repeat (LRR). By designing PCR primers in these conserved domains it has been possible to amplify so-called resistance gene analogues (RGA’s). Dilbirligi et al. (2004) mapped 310 RGA loci and identified twenty-six R gene clusters in the wheat genome, eighteen of which co-located with phenotypically characterised resistance loci. Unfortunately these NBS-LRR genes are generally short-lived as the pathogen population can rapidly evolve to overcome these race-specific resistances.

In contrast, adult plant resistance (APR) genes tend to be quantitatively inherited and generally race-nonspecific because they can act against the pathogen pre-rather than post-cell invasion (Collins et al., 2008). For example, Lr34 has recently been shown to encode an ABC (ATP-binding cassette) transporter that confers resistance to multiple fungal pathogens (B. Keller. pers. comm.). Combinations of such partial resistances are capable of providing near-immunity. Studies by Singh et al. (2005) have shown that a high level of resistance (approaching immunity) to both yellow (stripe) and brown (leaf) rusts of wheat can be achieved by accumulating 4 or 5 ‘slow rusting’, partial resistance genes.

**Conventional resistance breeding**

The UK environment is ideal for wheat disease development and the pressure from a number of pathogens is frequently high. Therefore the routine pathology screening of wheat breeding programmes is usually highly effective in eliminating lines with inadequate levels of resistance to current disease races. Field disease nurseries are normally grown at a range of locations where conditions are highly favourable for disease development, but when necessary artificial inoculation is also used.
Working with major gene resistances is extremely easy for the wheat breeder due to their high heritability, but the problem is that these genes totally mask any underlying background resistance (i.e. partial resistance). Partial resistances are additive in effect and small improvements in total resistance can be accumulated over many years. Breeding for immunity to disease based on single race-specific genes may mean that the carefully constructed pyramids of partial resistance factors are now being lost.

The original varietal sources for many of these resistance genes can be traced back through the pedigree records, but there are several resistances deployed in elite UK varieties that were originally derived from inter-specific crosses. For example, Pch1 for resistance to eyespot (Oculimacula yallundae and O. acuformis) was introgressed from Triticum ventricosum (Maia, 1967), as were Yr17, Lr37 and Sr38 (Bariana & McIntosh, 1993). The 1RS-1BL rye translocation carries a disease resistance gene cluster comprising Pm8, Lr26, Sr31, and Yr9 (Zeller & Hsam, 1984). Currently, European wheat breeders are exploiting the yield and disease resistance benefits of varieties with introgressions from ‘alien’ species such as Triticum dicoccoides. This material is providing novel sources of major gene, race-specific disease resistance, which are highly effective, at least whilst the variety is in official trials! A future problem will be that combinations of recently failed resistances will provide another layer of potentially short-lived resistance.
Marker-assisted breeding for disease resistance

In order to efficiently manipulate disease resistance using marker-assisted selection (MAS) the various resistances present in elite UK wheat varieties need to be genetically mapped. The problem for all commercial plant breeders is that the map location of very few of these R-genes has been published. The DEFRA Sustainable Arable LINK programme has enabled the initiation of several research projects in the area of wheat disease research (http://defrafarmingandfoodscience.csl.gov.uk/) and has significantly increased our understanding of the genetics of resistance to Septoria tritici, Fusarium head blight and Soil-borne cereal mosaic virus (SBCMV). However each breeding company is also undertaking its own research to map and develop markers for all traits of agronomic interest, including disease resistance.

In Limagrain UK our approach has been to use both pedigree information and results from classical pathology experiments in order to identify durable sources of disease resistance that are currently in the breeding programme. It has then been possible to identify either a double haploid (DH) or single seed descent (SSD) population that can be used for genetic analysis. In general it is relatively easy to identify markers for resistance genes on alien introgressions (Helguera et al., 2003) that are completely diagnostic (i.e. the presence of the resistance allele can be predicted with 100% accuracy in all breeding populations). However, unless the DNA sequence of both the alien and elite are known then often microsatellite markers developed from Triticum aestivum sequences fail to amplify from the introgressed segment, i.e. a null allele (Figure 1). This type of dominant marker can be used for genotyping DH populations, but it cannot be used to identify heterozygous lines in a conventional pedigree breeding scheme and there is also the issue of identifying true nulls from failed PCR reactions.
A major issue in wheat is the low level of DNA polymorphism and the extent of linkage disequilibrium (LD) in the genomic regions harbouring the R-gene clusters. For example, a single major gene can easily be located to a chromosome arm in a mapping population, but it is very rare that a tightly-linked marker (< 5 cM) is found that is polymorphic in every cross. We have mapped _Lr1_ from cv. Glasgow on 5DL and _Yr15_ from cv. Cortez on 1BS using microsatellites and in both cases the alleles at the linked marker loci occur in both resistant and susceptible varieties. R-gene clusters have been shown to rapidly evolve in cereals (Leister _et al._, 1998) and are frequently found in regions of high recombination (e.g. near the telomeres), which accounts for the low level of LD. However, the high level of recombination has enabled the map-based cloning of a number of resistance genes in wheat (Yahiaoui _et al._, 1998; Feuillet _et al._, 2003; Huang _et al._, 2003). By sequencing the alleles of these genes “perfect” DNA markers can be developed, which will allow the unequivocal genotyping of both parental lines and breeding populations.

**Future prospects of MAS for disease resistance loci**

The development of a set of perfect markers for the most important R-genes for the UK will undoubtedly take many years, but there are also other constraints on
using MAS for disease resistance. Firstly, UK breeders are breeding for resistance to four or five different fungal pathogens, in addition to all the other agronomic traits. If each of these resistances is controlled by three or four partial R-genes then very quickly the necessary population size required to pyramid all these effects will become enormous. This is one of the reasons why it is far easier to use major resistance genes despite the fact that they are short-lived. The second reason is that the main focus of the UK official trials system is treated yield and if a resistance gene carries a yield penalty then this will compromise the potential commercialisation of that variety (Brown, 2002). Unless more emphasis is placed on disease resistance, for example due to changes in the legislation governing the use of fungicides, then it is unlikely that MAS for durable disease resistance will be used to its full potential in the UK.

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The final straw: crop archives and the recent history of cereal pathosystems

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The factors influencing the occurrence of disease epidemics in cereal crops are in many cases well known, and have been used as the basis for forecasting systems predicting disease risk. Much less is known, however, about longer term changes in disease incidence, such as why certain diseases become more common while others decline in importance. Historical crop archives provide an opportunity to study long term trends, not only in disease occurrence, but also in the genetic properties of major pathogens of interest. Recent analyses of crop samples collected from wheat and barley crops over a period of more than 150 years using PCR amplification of host and pathogen DNA and gene sequencing are starting to reveal new clues to evolutionary changes in cereal pathosystems over the period coinciding with the development of modern agriculture. Rapid changes in pathogen genes subject to strong selection pressures, such as those encoding fungicide targets, have been detected, along with more gradual shifts in disease incidence linked to environmental change. The opportunity exists to further explore pathogen variation and adaptation over many generations in response to host genotypes and agronomic practice as well as changes in the crop environment such as soil properties, pollution and global warming.

Introduction

The spread and intensification of agriculture over time, along with changes in agronomic practices and the types of crops grown, have been accompanied by corresponding changes in the distribution, incidence and severity of crop pathogens. Some diseases have increased in importance, while others have declined or disappeared. From time-to-time, an apparently new disease may emerge. The pathogens themselves evolve in response to selection pressures
imposed by agriculture, such as the deployment of genetically resistant crop cultivars, or the use of fungicides. In some cases, the factors driving pathogen evolution are well documented, such as host genetic uniformity, or widespread use of single-site inhibitor fungicides with the same mode of action, while in others the reasons for alterations in the relative importance of certain diseases are a subject for speculation. Recently, climate change has been invoked to explain observed shifts in the incidence and severity of some crop pests and diseases. Whatever the underlying reasons, practitioners of crop protection have to deal with a constantly shifting target. The ability to predict, and hence pre-empt, emerging problems is at present limited.

One difficulty in interpreting trends in disease incidence and importance is the relative lack of good information available for most crops, especially over longer periods of time. In the UK, annual survey data on major diseases of cereals and oilseeds have been collected since the 1970s (King, 1977; Polley et al., 1993). This continues to the present day (see http://www.cropmonitor.co.uk). The UK Cereal Pathogen Virulence Survey (Bayles et al., 1997) has added further information by assessing the predominant pathotypes of diseases such as the rusts occurring each year. But such data, while valuable, are only small pieces of a much larger jigsaw. They document the very rapid responses of pathogen populations to intense selection pressures exerted by, for instance, crop monocultures containing one or a few resistance genes, but are unlikely to reveal more subtle changes in pathosystems linked to longer term evolutionary processes. This requires data collected over a timescale that reflects more gradual developments in agricultural practice, such as changes in the types of crops grown, as well as shifts in environmental factors such as climate change. Long-term data might enable us to re-construct pathogen evolution over a sufficient period to detect rare events such as alterations in host range or pathogenicity linked to hybridisation (Brasier et al., 1999) or horizontal gene transfer (Oliver & Solomon, 2008). At present the most convincing evidence for
the occurrence of such events comes from genetic analyses or comparative genomics (Stukenbrock & McDonald, 2008).

**Long-term experiments and crop and soil archives**

Worldwide there are a series of long-running experiments with crops that have set out to study the effects of different management regimes on crop performance and soil properties. The Duke University website (http://ltse.env.duke.edu/db/inventory/ltse) lists more than 250 long-term soil ecosystem studies. Forest Research in the UK also hosts a database of long-term forestry trials (http://noltfox.metla.fi ). Very few of these experiments, however, include retention and archiving of crop samples. The Rothamsted classical experiments, established by Lawes and Gilbert between 1843 and 1856, are unique not only in duration of the studies, but also because annual samples of crops (seeds, and chopped leaves and stems) and soil were retained and catalogued in an archive that now comprises more than 300,000 samples (Lawes Trust, 2006). Importantly, meteorological records and information on the crop varieties grown were also kept. This resource potentially preserves biological material reflecting agricultural and environmental changes occurring over a period of more than 160 years.

The main long-term experiments at Rothamsted being used for microbiological analyses are listed in Table 1. The most important in the context of the current review are Broadbalk Winter Wheat started in 1843, and Hoosfield Spring Barley started in 1852. Both have continued largely unchanged to the present day. Until recently the main uses of the archives associated with these experiments have been to analyse crop nutrition and nutrient status in soil related to the different fertiliser treatments applied to the plots. Soil analysis has also revealed trends in pollutants derived from industrial sources as well as fallout from the nuclear bomb tests in the 1950s. The range of potential uses was dramatically extended with the discovery of the polymerase chain reaction (PCR) and the revelation that DNA in historical samples could not only be detected but also analysed to provide
information about microorganisms of interest preserved in soil, seeds, or crop residues (Ristaino et al., 2001). These advances suggested that the unique historical resource at Rothamsted might be used to reveal information about the pathogens associated with cereal crops since the nineteenth century, and, more intriguingly, to study the co-evolution of such pathogens with their host crop during the development of modern agriculture.

Table 1. Long-term experiments at Rothamsted currently being used for microbiological analyses. For more details see Lawes Trust (2006).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Crop</th>
<th>Duration</th>
<th>Material archived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broadbalk</td>
<td>Winter wheat</td>
<td>1843-present</td>
<td>Soil, seeds, dried stem and leaf</td>
</tr>
<tr>
<td>Hoosfield</td>
<td>Spring barley</td>
<td>1852-present</td>
<td>Soil, seeds, dried stem and leaf</td>
</tr>
<tr>
<td>Park Grass</td>
<td>Pasture</td>
<td>1856-present</td>
<td>Soil and herbage</td>
</tr>
<tr>
<td>Exhaustion land</td>
<td>Winter wheat, Potatoes, Cereals</td>
<td>1856-1875 1876-1901 1902-present</td>
<td>Soil and crop samples Dried tubers Soil and crop samples</td>
</tr>
<tr>
<td>Highfield</td>
<td>Cereal rotation, Grass, Fallow</td>
<td>1949-present</td>
<td>No systematic archiving of samples</td>
</tr>
</tbody>
</table>

To test this possibility, several technical issues first had to be resolved. Is pathogen DNA of sufficient quality preserved in the stored crop samples? This was a particular concern as samples in the archive were oven dried, usually at 80°C, which is likely to cause some degradation. Secondly, is it possible to quantify the DNA and relate this to the incidence of particular diseases over time? More crucially, can particular pathogen genes or genomic regions likely to be under selection by agriculture or environmental factors be detected and sequence information obtained to show how the pathogens themselves have
evolved? There was also the spatial issue of whether samples collected from a single field in one geographic region could be representative of national, continental or global trends in pathogen populations.

**Pathogen abundance**

The first study to attempt to answer some of the questions concerned the two main leaf blotch pathogens of wheat in NW Europe, *Mycosphaerella graminicola* (Septoria leaf blotch), and *Phaeosphaeria nodorum* (leaf and glume blotch). It was already known from annual surveys that the incidence of these two diseases had changed over the past thirty years with Septoria tritici blotch becoming the more abundant and damaging disease (Cook et al., 1991; Hardwick et al., 2001). To deal with the potential problem of DNA degradation, the quantitative PCR assay developed used minor groove binder-conjugated probes targeting small DNA fragments. Pathogen-specific polymorphisms in β-tubulin were used to detect *M. graminicola* and *P. nodorum*, while wheat DNA was amplified from the ATP synthase α-subunit (Bearchell et al., 2005). Figure 1 shows that larger amplicons for each target were also recovered from many samples throughout the archive, irrespective of sample age using conventional PCR as described by Bearchell (2003).
Changes in the amounts of DNA of the two pathogens (Bearchell et al., 2005) showed that *M. graminicola*, the currently predominant pathogen, was also abundant in the mid-19th century (Figure 2). *P. nodorum* DNA could be detected at intervals throughout the archive, and increased during the 20th century, with a peak around 1970. The ratio of DNA of the two pathogens was shown to correlate well with the ratio of severity of the two diseases derived from UK survey data between 1970 and 2003 (Figure 3). This suggests that results from PCR analysis of the Broadbalk crop samples were broadly representative of disease trends across England and Wales.
When correlations were made between the ratio of pathogen abundance and a range of agronomic and weather factors, including environmental pollutants, the closest relationship was found with atmospheric SO$_2$ emissions. Subsequent analyses of stored grain samples showed that amounts of *P. nodorum* DNA were related to changes in spring rainfall, summer temperature and SO$_2$ emission (Shaw et al., 2008). No *M. graminicola* DNA was detected in grain, confirming that this pathogen is rarely seedborne (Brokenshire, 1975). Long-term variation in the two pathogens in leaf and grain was again dominated by factors related to atmospheric pollution by SO$_2$. This unexpected conclusion shows that the factors most strongly influencing long term trends in the incidence and abundance of plant diseases may not be those that have been assumed to have the greatest effect.
Figure 3. Relationship between ratios of pathogen DNA of *P. nodorum* and *M. graminicola* recovered from Broadbalk crop samples and survey data on visual severity of the two diseases in England and Wales 1970-2003. For more information see Bearchell *et al.* (2005).

The successful detection and quantification of historical fungal DNA in crop samples from Broadbalk encouraged a search for other crop pathogens. The pathogens detected to date are listed in Table 2, and include several barley diseases as well as *Phytophthora infestans* from dried potato tubers stored from the 1870s (Ristaino *et al.*, 2008). Amongst the barley pathogens detected, one interesting finding is that *Ramularia collo-cygni*, a recently emerging disease problem, is also present in 19th century crop samples (Fountaine & Fraaije, unpublished).
Table 2. Pathogens detected to date in the Rothamsted crop archives.

<table>
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<tr>
<th>Pathogen</th>
<th>Host crop</th>
<th>Detected in</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycosphaerella graminicola</em></td>
<td>Wheat</td>
<td>Stem/leaf</td>
</tr>
<tr>
<td><em>Phaeosphaeria nodorum</em></td>
<td>Wheat</td>
<td>Stem/leaf and seed</td>
</tr>
<tr>
<td><em>Rhynchosporium secalis</em></td>
<td>Barley</td>
<td>Stem/leaf and seed</td>
</tr>
<tr>
<td><em>Pyrenophora graminea</em></td>
<td>Barley</td>
<td>Stem/leaf and seed</td>
</tr>
<tr>
<td><em>Ramularia collo-cygni</em></td>
<td>Barley</td>
<td>Stem/leaf and seed</td>
</tr>
<tr>
<td><em>Phytophthora infestans</em></td>
<td>Potato</td>
<td>Tubers</td>
</tr>
</tbody>
</table>

**Detection of pathogen genes**

The next question to address was whether accurate sequence data for genes likely to be under selection by agriculture could be recovered from the extracted DNA. Pathogen genes of potential interest include those encoding effectors or determining virulence/avirulence to particular crop genotypes, genes involved in the biosynthesis of mycotoxins, mitochondrial genes (diversity of haplotypes) and neutral markers not expected to be under selection, such as microsatellites. One obvious class of genes to study were those encoding protein targets of site-specific fungicides where particular mutations have compromised fungicide efficacy and hence control of the disease.

One obvious class of genes to study were those encoding protein targets of site-specific fungicides where specific mutations have compromised fungicide efficacy and hence control of the disease.

An early example is the methyl benzimidazole (MBC) fungicides that interfere with the function of the cytoskeletal protein β-tubulin. These were introduced in the 1970s and quickly encountered problems of resistance in some initially sensitive target pathogens. In most cases the occurrence of resistance was
correlated with a specific mutation in the β-tubulin gene at codon 198 (E198A). Primers were designed to amplify the relevant region of the *M. graminicola* β-tubulin gene. Using a triple probe allele-specific assay, the incidence of the wild-type β-tubulin gene and the mutated form (E198A) from *M. graminicola* were determined in archive samples from 1847 to the present using a nested-PCR approach (Fraaije, unpublished). This procedure was first described for detecting quinone outside inhibitor-sensitive (QoI-sensitive) and QoI–resistant alleles in field populations of *M. graminicola* (Fraaije et al., 2005). Figure 4 shows some results of this study. The *M. graminicola* population in Broadbalk was converted from a uniformly wild-type to MBC resistant population within a single season (1984), coinciding with the first use of these fungicides on the sampled plots.

**Figure 4.** Readout from realtime PCR simultaneously detecting MBC-sensitive (E198) and resistant (A198) alleles in archive samples using three different probes. One detects both alleles (R+S) giving a measure of total pathogen β-tubulin. The others (R or S) measure the proportion of resistant (R) or sensitive (S) alleles present. From 1849 to 1984 only the MBC sensitive form is detected, whereas only the R form is detected thereafter.

This dramatic switch from an MBC-sensitive to resistant population probably reflects not only the extreme selection pressure exerted by these highly active chemicals, but also selection operating on a population that had been previously exposed to MBC fungicides in crops outside Rothamsted, where MBCs had been in use for several seasons. It is likely that a low level of the resistant allele, not
reliably detected by the assay used below 3\% was already present in the pathogen population and increased immediately after MBC fungicides were applied to most of the Broadbalk plots. The analysis also showed that the A198 allele has persisted to the present at a high level, in the absence of any use of MBC fungicides since 1998, confirming that the mutation incurs little or no fitness cost to resistant isolates. Work has now started on target genes of other fungicide groups including the QoIs and inhibitors of sterol biosynthesis such as the triazoles.

**Conclusion**

Why did Lawes and Gilbert store crop and soil samples from the experimental plots every season? Was this really inspired foresight or simply the Victorian obsession with collections? Whatever the answer (and it might be a mixture of the two) it is unlikely that they could have anticipated the diverse uses the archive they started has now been put to, or the increasing scientific relevance of their long-term experiments. To an evolutionary biologist the 165 years spanned by the Broadbalk samples might seem insignificant measured against the timescales of adaptation and speciation. However, if one makes a conservative estimate of the number of infection cycles that a polycyclic pathogen such as *M. graminicola* completes in one season (c. 5), the archive preserves at least 800 asexual generations as well as more than 160 cycles of sexual recombination. A succession of around 15 varieties of wheat and 12 of barley are represented. Furthermore, the timespan of the archive covers the whole period of development of modern intensive agriculture, such as cultivar improvement through scientific breeding, and the introduction and application of synthetic fertilisers and pesticides. The influence of environmental changes linked to the industrial revolution and global warming are also preserved. Molecular detection and quantification of specific pathogens, as well as sequencing of particular genes or genomic regions in historical DNA, has already demonstrated the potential value of the archives for reconstructing the recent evolution of cereal pathosystems. With the growing realisation that reconstructing past trends may...
in fact be of value in anticipating the future, it seems certain that crop archives will continue to reveal important clues to the evolutionary forces and factors operating over time in crop ecosystems.

Acknowledgements

We thank Sarah Bearchell, James Fountaine, Simon Atkins, Mike Shaw, Bruce Fitt, and Paul Poulton for provision of information, including on work in progress. The Rothamsted long-term experiments are supported by the Lawes Agricultural Trust. Rothamsted Research receives grant aided support from the Biotechnology and Biological Sciences Research Council of the UK.

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<td>69, 110, 111</td>
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<td>69, 99</td>
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