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Role of inoculum sources in *Rhynchosporium* population dynamics and epidemics on barley

by

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CONTENTS

1. ABSTRACT

- Rhynchosporium leaf blotch of barley, caused by the fungus *Rhynchosporium secalis*, is of increasing importance in world agriculture. It is the most serious disease of winter and spring barley in the UK, causing substantial losses nationally, despite expenditure of £50M per year on fungicides. The disease is difficult to control with fungicides and severe epidemics may appear suddenly. The sources of inoculum responsible for starting such epidemics are not well understood.

- This project aimed to clarify the origin and early dynamics of epidemics using molecular techniques (quantitative PCR) that can detect and quantify the DNA of the pathogen in barley plants before symptoms occur. The same techniques can also detect genetic characteristics of the fungus, such as mating type, virulence, and genes responsible for resistance to fungicides. Each season, epidemics were monitored on both winter (October-sown) and spring (March-sown) barley on samples from current crops from sites in England and Scotland. Work was also done on historical spring barley samples archived at Rothamsted over the last 150 years. Thus short-term and long-term changes in the pathogen population were studied.

- Seed-borne inoculum was identified as a significant source for early infection of barley crops, with substantial amounts of *R. secalis* DNA found in seedlings of crops grown from infected seed. However, there was little evidence that severity of seed infection influenced amounts of pathogen DNA in leaves, disease severity (leaves) or yield loss later in the cropping season.

- Whilst small amounts of airborne *R. secalis* inoculum were collected in different seasons at different sites, this did not provide evidence that airborne inoculum played an important role in the development of epidemics.

- The discovery that *R. secalis* can colonise barley crops extensively throughout the cropping season (from seed to seed) in the absence of visual symptoms has completely changed the understanding of the disease by the industry (with implications for use of fungicides, breeding programmes and the HGCA Recommended List of barley cultivars).

- Substantial early symptomless infection was identified in winter barley crops but epidemic severity late in the season was largely dependent on the amount of spring rainfall, which encouraged secondary disease spread by splash dispersal of pathogen spores. Therefore the PCR quantification of early *R. secalis* infection could not be accurately used to predict epidemic severity late in the season.

- Use of quantitative PCR provided new insights into the operation of host resistance against *R. secalis*, especially in work with a barley mapping population. However, the early season PCR assessments of cultivar resistance could not accurately be used to predict resistance ratings based on late season disease assessments.

- Markers associated with new sources of resistance to *R. secalis* were identified in a barley mapping population and methods to screen material for resistance were improved.

- Work with samples from the 150-year barley archive at Rothamsted provided unique insights into the long-term dynamics of *R. secalis* on barley crops. Temporal patterns in the amounts of *R. secalis* DNA over the past 40 years were found to be consistent with those recorded in the Defra/HGCA barley disease surveys since the 1970s. Analysis over the entire 150-year period, using quantitative species-specific PCR, confirmed the increase in severity of rhynchosporium epidemics observed in national surveys. Of the factors investigated, the most likely explanation was the change in height of barley cultivars through the introduction of new short-strawed cultivars. This work also provided information about the long-term dynamics of other barley pathogens, such as *Pyrenophora*.

- Knowledge from this project is being combined with new information from related projects being funded by BBSRC LINK, Defra, HGCA and RERAD (at ADAS, and in Scotland, SAC and SCRI) to develop guidelines for crop husbandry and agronomic practices to reduce *Rhynchosporium secalis* population size and genetic variation to achieve sustainable control of rhynchosporium disease of barley.

2. REPORT

2.1. Introduction/Background and aims

Rhynchosporium secalis is the causative agent of rhynchosporium leaf blotch on barley and has recently been the most damaging foliar pathogen (Figure 1). As the most important disease of winter and spring barley in the UK, it can cause yield losses of 30-40% and decrease grain quality, thus discounting malting barley prices. To optimise control of this disease, it is essential to understand the role of different inoculum sources and their influence on the dynamics of *R. secalis* populations and epidemic development during the growing season.

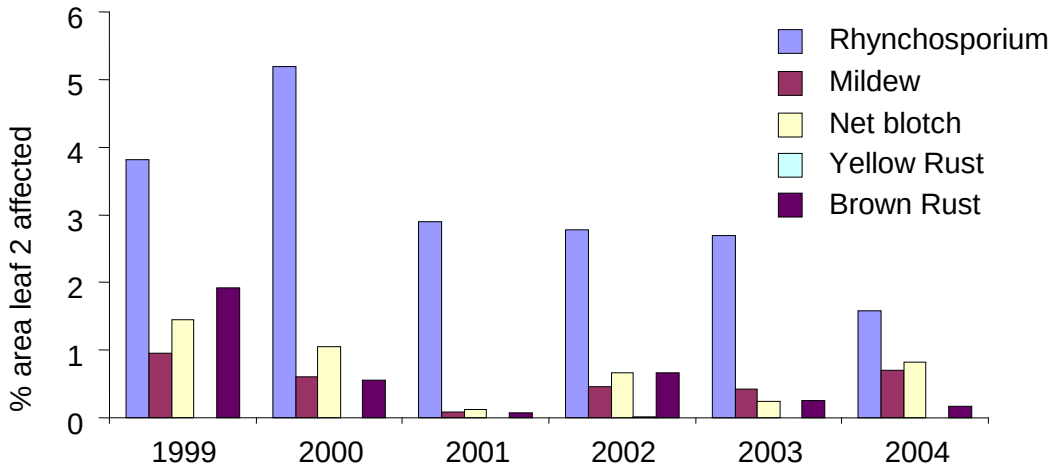


Figure 1. Results from the CropMonitor survey of winter barley crops in England and Wales (1999 to 2004, www.cropmonitor.co.uk) showing severity (percentage area of leaf two affected) of diseases of winter barley at GS71-73.

Field experiments at Harpenden suggested that seed-borne *R. secalis* was the main source of primary inoculum during recent growing seasons (Figure 2; Fountaine *et al*., 2007) and that its importance may have been underestimated. 'Volunteer' plants growing in fields after barley cultivation can act as a 'green bridge' for the pathogen, providing inoculum after a break in cropping (Figure 3). Infected grain or stubble in the field after harvest can be a source of inoculum for such volunteers. The cloning of the *R. secalis* mating type genes (Foster & Fitt, 2003) and the discovery that both mating types occur in populations of *R. secalis* in the UK and throughout the world (Linde *et al*., 2003) suggested that *Rhynchosporium* may produce sexual ascospores that would be expected to be airborne (as yet undiscovered).

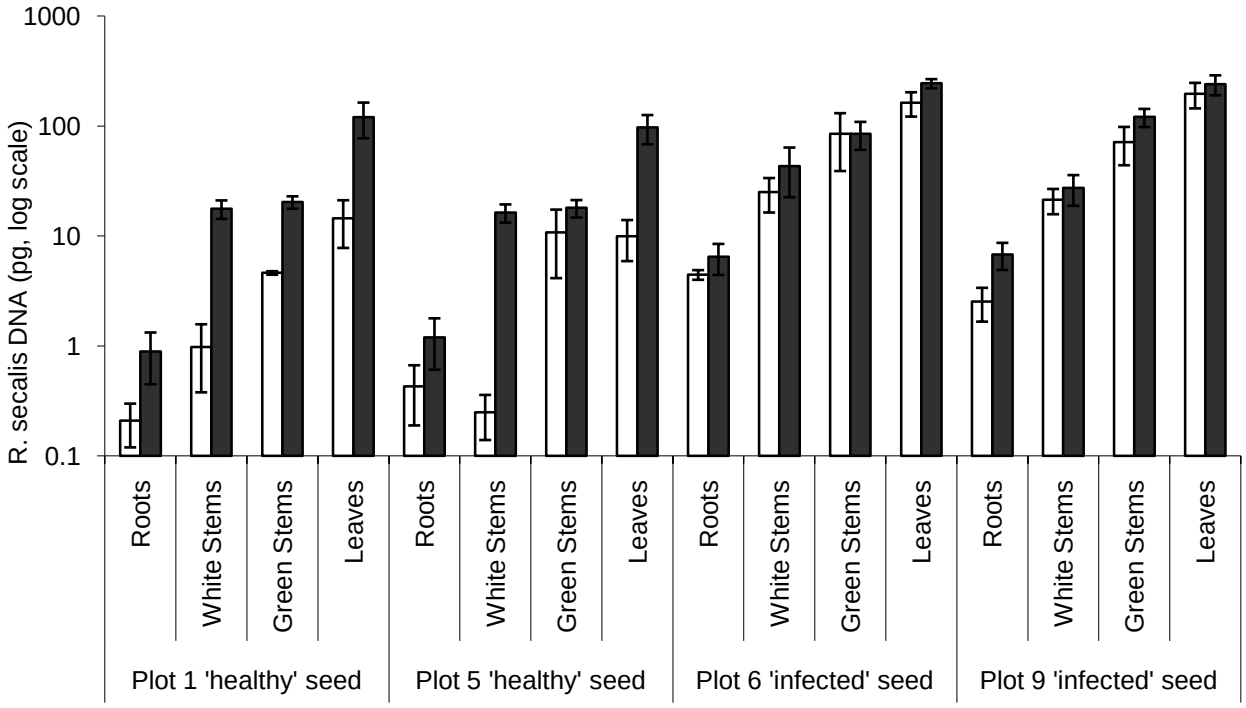


Figure 2. Amount of *Rhynchosporium secalis* DNA (pg in 50ng total DNA) detected by quantitative PCR in different parts of winter barley plants from plots sown with 'healthy' commercial seed or farm-saved seed 'infected' by *R. secalis*. Samples were taken on the 17 Feb 2004 (□) or 24 April 2004 (■) (Fountaine *et al*., 2007).

Figure 3. Barley volunteer showing symptoms of rhynchosporium leaf blotch, growing in a field of wheat at Rothamsted (Harpenden).

The use of PCR on Burkard spore trap tapes has confirmed the presence of airborne *R. secalis* inoculum at Harpenden but the spore type involved is not yet known (Figure 4; Fountaine *et al*., 2005). Quantitative PCR assays for *R. secalis* have been developed and used to quantify symptomless infection in seeds and seedlings (Fountaine *et al*., 2007), and can be combined with airborne spore trapping and genetic fingerprinting methods (Goodwin *et al*., 1994; Von Korff *et al*., 2004, West *et al.*, 2008, 2009) to examine the potential role of ascospores and seed-borne inoculum in *R. secalis* population dynamics and epidemics.

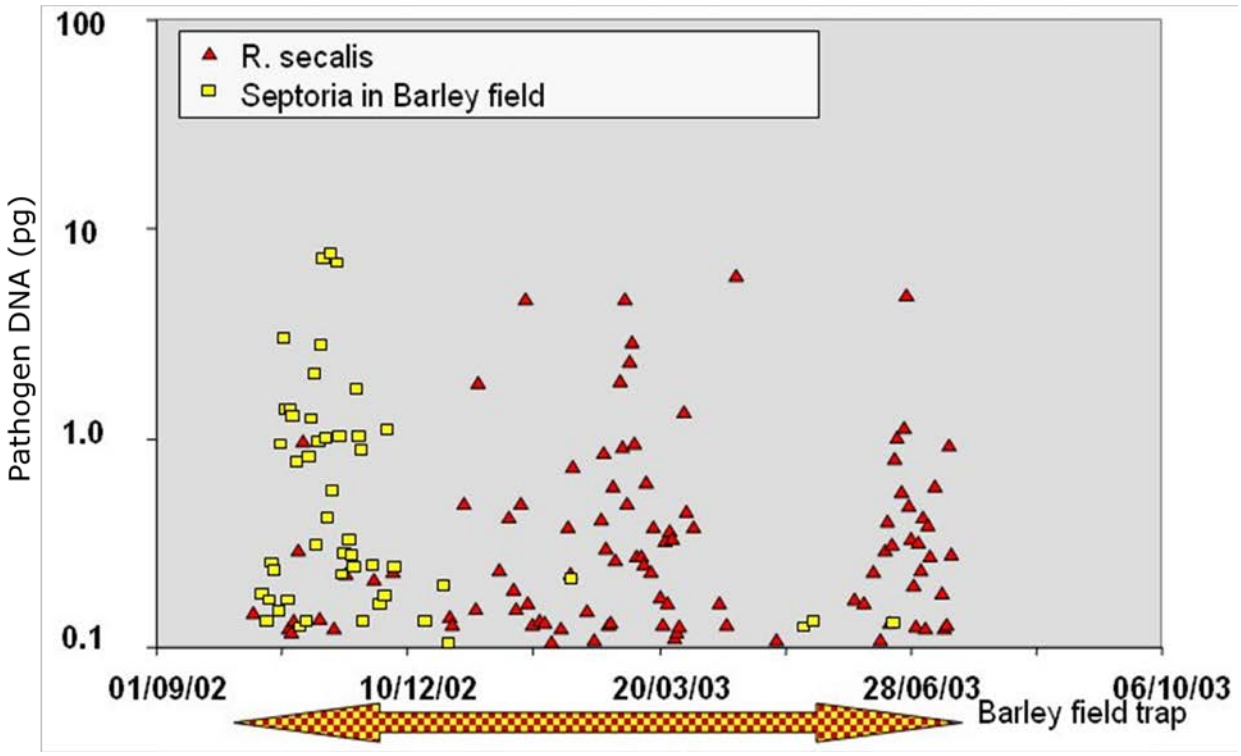


Figure 4. DNA (pg) of *R. secalis* and *Mycosphaerella graminicola* (*Septoria tritici*) in air samples collected from above a barley crop by a Burkard spore trap operating between September and March (shown by arrow) in the 2002-2003 growing season at Rothamsted (Harpenden). DNA was quantified using quantitative PCR.

Whilst ideally rhynchosporium control would rely on deployment of resistance genes in spring and winter barley, the genetically variable *R. secalis* (Newton *et al*., 2001) has developed virulence to most major resistance genes in UK cultivars (UK Cereal Pathogen Virulence Survey, e.g. Jones & Newton, 1998). Current methods to assess resistance of barley to *R. secalis* frequently over-estimate early season resistance (Oxley *et al*., 2003). Resistance in winter barley is poorly characterised, although sources of polygenic resistance may be available (Newton *et al.*, 1997).

Events early in the growing season, when the disease is symptomless, greatly influence subsequent severity of epidemics (Henman *et al*., 2002) and different phases in epidemics have been identified (Oxley *et al.*, 2003). Quantitative PCR assays now provide a means to accurately measure early infection of tissues, even without visible symptoms, during the long latent period of this polycyclic disease (Davis & Fitt, 1990, 1992, 1994) and suggest that differences in cultivar resistance can be identified at this symptomless stage (Fountaine *et al*., 2007). Whilst high rainfall and leaf wetness are known to favour spread of *R. secalis* (Fitt *et al.*, 1986, 1989), there is little information on how interactions between climate, host cultivar and pathogen populations influence asymptomatic infection and epidemic dynamics.

The network of linked experiments done by the consortium members over a wide range of climates provides the opportunity to test the hypotheses that rhynchosporium epidemic severity and barley cultivar resistance can be predicted early in the growing season using quantitative PCR on samples taken during the symptomless phase in epidemics. It is not clear why there has been a long-term trend, starting in the mid-20th century, towards increased severity of rhynchosporium on UK barley crops. The availability of archived samples from the Rothamsted Hoosfield long-term spring barley experiment (started 1852) and quantitative PCR assays for *R. secalis* (Fountaine *et al.*, 2007) and other barley pathogens provides an opportunity to test hypotheses about long-term changes in their importance. DNA from the 150-year series of Hoosfield barley samples has already been extracted and amplified with *Rhynchosporium*-specific primers; the results have shown different amounts of pathogen DNA were present in different years (Figure 5). It is now possible not only to test hypotheses about changes in the incidence and severity of this pathogen but also to assess changes in genomic regions or genes encoding traits of agronomic interest (e.g. fungicide sensitivity).

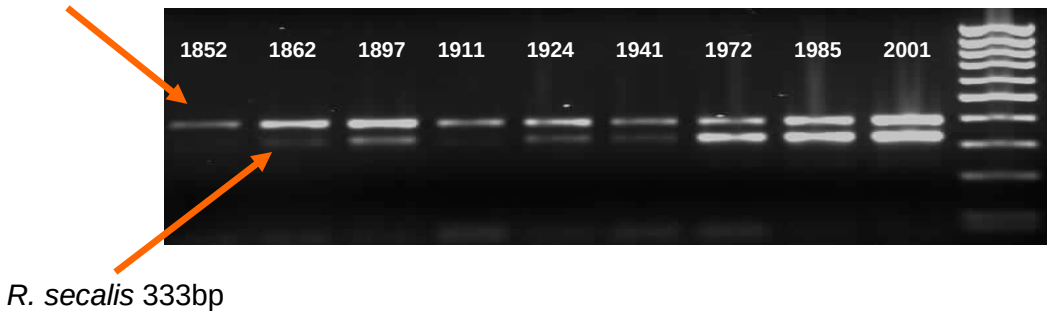


Figure 5. Presence of *Rhynchosporium secalis* DNA in spring barley leaf samples selected from the Rothamsted (Hoosfield, experiment started 1852) archive, demonstrated by end-point PCR. Bands associated with *R. secalis* DNA were compared with bands associated with barley DNA to demonstrate that the PCR was working correctly.

Epidemics of rhynchosporium disease in barley have become increasingly difficult to control in the UK. Continuous cultivation of spring and winter barley, large-scale deployment of cultivars without durable resistance and over-reliance on fungicides for disease control have exacerbated this problem (Oxley *et al.,* 2003; Zhan *et al*., 2008). The few major resistance genes in spring barley are difficult to combine with high yield and quality (Thomas, 2003). Genetic changes in *R. secalis* populations (Newton *et al*., 2001) have meant that some major resistance genes were rendered ineffective and efficacy of several fungicides was eroded. Few active ingredients are now available to control the disease and it is anticipated that QoI resistance may develop in populations of *R. secalis*.

Lower grain prices, reduced profit margins and the potential removal of effective fungicides as a result of EU legislation (91/414) (Mahmuti *et al*., 2009) have all increased the importance of effective, integrated strategies to manage rhynchosporium. Through links of consortium members to complementary work funded by BBSRC LINK, RERAD, Defra LINK and HGCA, there is now the opportunity to achieve more sustainable control of rhynchosporium on barley by integrating good crop husbandry, durable resistance and well targeted chemical control. The project aimed to investigate the source(s) and early dynamics of epidemics of *R. secalis* on barley. The following questions were investigated:

1. Is seed-borne inoculum a significant source of early infection in the crop (Task 1)?
2. Do sexually-produced ascospores contribute to epidemic development and pathogen population structure (Task 1)?
3. Can quantification of early symptomless *R. secalis* (by PCR) be used to predict rhynchosporium epidemic severity (Task 2)?
4. Can quantification of early symptomless *R. secalis* (by PCR) be used to understand barley cultivar resistance (Task 2)?
5. Why did severity of rhynchosporium epidemics increase in the mid-20th century in the UK (Task 3)?

Results from the project are being used to develop guidelines for the sustainable control of rhynchosporium by optimal husbandry and improved deployment of barley cultivar resistance and fungicides (Task 4).

2.2. Materials and methods

There were winter (October-sown) and spring (March-sown) barley experiments over three or four cropping seasons, respectively (winter barley, 2006/7, 2007/8, 2008/9; spring barley, 2006, 2007, 2008, 2009). Cultivars with contrasting HGCA Recommended List ratings for resistance (in parentheses) to rhynchosporium (winter barley: Sumo (5), Saffron (6), Flagon (8), Manitou (9); spring barley: Optic (4), Troon (4) Oxbridge (7), Doyen (8)) were grown in these experiments. Each season, there were up to nine winter barley and up to nine spring barley trials sown with commercial seed at sites in England and Scotland (Figure 6). Each trial had three replicates and most were taken to yield. In trials taken to yield, 12 plots received HGCA RL protocol fungicide mixture treatment programmes and 12 plots did not (Task 2). In unsprayed plots of winter barley experiments at Harpenden, England and Dundee, Scotland, detailed quantitative PCR and visual assessments of epidemic dynamics were done (Task 1). Rhynchosporium long-term population dynamics were studied with samples from the Rothamsted Hoosfield spring barley experiment, starting in the 1850s (Task 3). Data from all sites and additional data from industry partners were collated at Rothamsted. The consortium used these data to develop new guidelines to control rhynchosporium (Task 4).

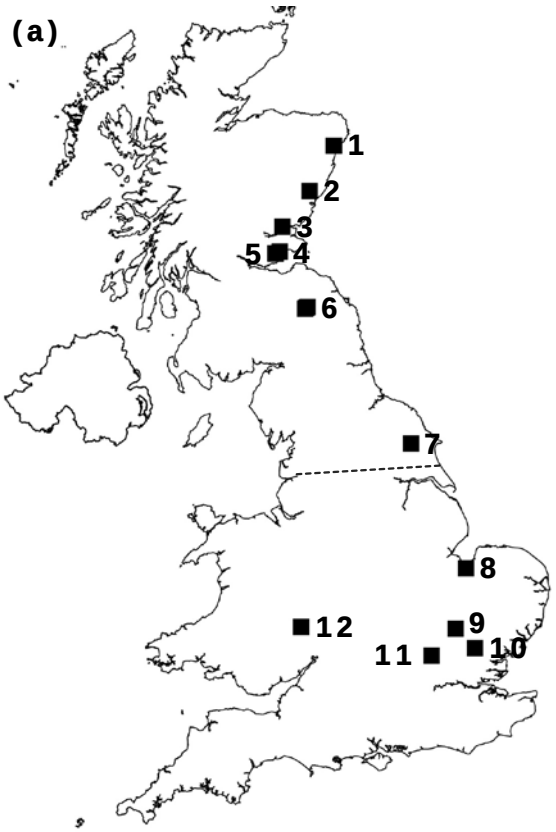
2.2.1. Task 1: Role of different inoculum sources in establishment of epidemics of *Rhynchosporium secalis* on barley

To investigate the relative importance of different potential sources of primary inoculum (e.g. infected seed, airborne ascospores), work was done with unsprayed plots of winter barley and spore samplers.

Seed-borne inoculum

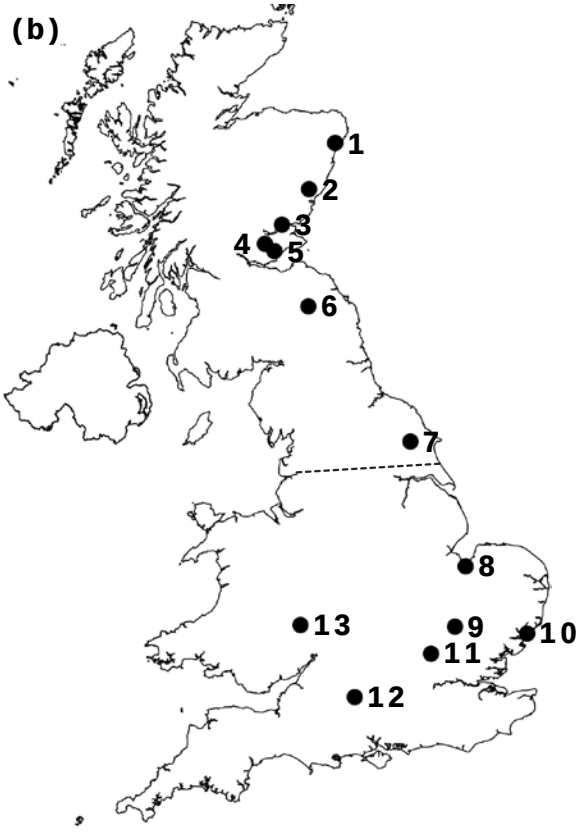
To identify the role of seed-borne infection, work was done in the unsprayed plots of winter barley experiments sown with commercial seed, together with additional plots sown with ‘farm-saved’ seeds, at Dundee in the first two seasons (2006/7, 2007/8). Plots of the winter barley cultivars (Flagon, Saffron, Manitou and Sumo) derived from batches of either farm-saved or commercial seed were sown at each location.

The amount of *R. secalis* DNA was measured in the upper three leaf layers at Growth Stage (GS) 26, GS39 and in the seed at harvest. Visual observations of disease were also made throughout the season and yield was determined. The amount of *R. secalis* DNA in individual seeds was measured in winter and spring barley. To find the tissues which contained the greatest amounts of *R. secalis* DNA, individual seeds were dissected and the amounts of *R. secalis* DNA in the endosperm of the seed and in the seed coating were compared to the amounts of *R. secalis* DNA detected in whole seeds.



Key to Winter Barley sites:

1. Tipperty (North) – Ellon
2. Tipperty (North) – Coldstream
3. SCRI - Dundee
4. Central – Glenrothes
5. Central – Skeddoway
6. South Borders – Whitehouse, Ploughlands & Broomhall
7. ADAS – High Mowthorpe
8. ADAS – Terrington
9. Masstock – Fowlmere
10. Masstock – Throws Farm
11. Rothamsted – Harpenden
12. ADAS - Rosemaund



Key to Spring Barley sites:

1. Tipperty (North) – Ellon
2. Tipperty (North) – Coldstream
3. SCRI - Dundee
4. Blairnathort - Kinross
5. Central – Skeddoway
6. South Borders – Manorhill
7. ADAS – High Mowthorpe
8. ADAS – Terrington
9. Masstock – Fowlmere
10. KWS Suffolk – Trimley St. Martin
11. Rothamsted – Harpenden
12. Masstock – Marlborough
13. ADAS - Rosemaund

Figure 6. Location of winter (a) and spring (b) barley field sites in UK. Dashed line represents theoretical North/South divide for assessing resistance against *Rhynchosporium secalis* according to HGCA Recommended List protocols.

To determine if leaves late in the season contained viable inoculum that could spread to the grain, extra samples were taken from all the LINK trial sites at GS75. These leaves showed visible symptoms of *R. secalis* infection but had senesced. Spores were washed from the leaves and used to inoculate cultivar Sumo in the glasshouse.

Airborne inoculum

To test the hypothesis that a sexual stage of *Rhynchosporium secalis* producing airborne ascospores might have a role in early epidemic establishment, continuous samples of airborne spores were collected with Burkard spore samplers near infected debris and crops at Harpenden, England and Kinross, Scotland sites. Samples were sent to Rothamsted and tested for the presence of *R. secalis*. The tapes from the spore samplers were divided into daily sections and the DNA extracted according to the protocol outlined in Lacey and West (2006). The amount of *R. secalis* DNA was compared with the amount of DNA from the oilseed rape pathogen *Leptosphaeria maculans*, ascospores of which are known to be produced on crop residues in autumn and are air-dispersed to initiate disease epidemics (Fitt *et al*., 2006).

Races of Rhynchosporium secalis

Further information about potential sources of inoculum over a larger area was obtained by analysing frequencies of isolate virulence phenotypes using the UK Cereal Pathogen Virulence Survey (UKCPVS). Before harvest, flag leaves with rhynchosporium symptoms were sent from unsprayed plots at the 9 sites in England and Scotland (see Task 2) (samples from several cultivar/site combinations each season) to the UKCPVS so that the virulence phenotypes of the populations could be analysed using a differential set of barley genotypes in seedling and small plot assays (Jones & Newton, 1998). This provided information from this series of experiments on the distribution of *R. secalis* pathotypes with different virulence factors present at the end of each season, as well as season to season variation. The analysis complemented UKCPVS work with samples from other barley crops at a range of locations in England and Scotland in the HGCA-funded component of the UKCPVS (testing done at NIAB, Cambridge). Isolations were made from thirty samples representing all four winter barley cultivars (Flagon, Saffron, Manitou and Sumo) and five of the sites in 2007 for virulence (pathotype) and molecular genotype analysis. Single spore-derived isolates were tested by NIAB on whole seedlings as part of the United Kingdom Cereal Pathogen Virulence Survey (http://www.hgca.com/content.output/56/56/Crop%20Research/Crop%20Research/UK%20Cereal%20Pathogen%20Virulence%20Survey.mspx), and by SCRI on detached leaves using established methods (Newton *et al*., 2001). In addition, DNA was extracted and characterised using a set of 14 microsatellite markers (simple-sequence repeats or SSRs) at SCRI.

In 2006 and 2007, 87 single-spore isolates of *R. secalis* were obtained by isolation from the spring barley genotypes and these isolates were characterised using SSR markers. In 2007, 2008 and 2009, 23, 205 and 289 single-spore isolates, respectively, were obtained and characterised from winter barley. These were obtained on one, two or three sample dates, respectively, to sample the epidemic more often as it progressed in 2008 and 2009. Isolations were also made from the progeny of the Leonie-Pearl-Cocktail mapping population expressing different levels of resistance. Detailed population analysis is still in progress.

2.2.2. Task 2: Short-term (within season) dynamics of rhynchosporium epidemics

Development of rhynchosporium epidemics in plots with or without fungicide treatment

Relationships between amounts of asymptomatic *R. secalis* in tissues early in the season and subsequent severity of rhynchosporium disease were studied on the susceptible and resistant winter and spring barley cultivars in replicated trials (three replicates) with and without fungicides in England and Scotland (total of up to nine locations each for winter barley and spring barley), sown with commercial seed (Figure 6). Two resistant cultivars (winter barley Flagon and Manitou; spring barley Oxbridge and Doyen) and two susceptible cultivars (winter barley Sumo and Saffron; spring barley Optic and Troon) were planted in randomised plots. In total, there were three replicates per treatment and 24 plots. Plots were visually monitored for disease symptoms throughout the season; leaf samples were taken at growth stages (GS) 26 and 39 and harvest grain samples were taken for DNA extraction and quantification of *R. secalis* DNA. To investigate symptomless infection early in the season on winter barley, asymptomatic leaves and leaves with lesions were taken from a Sumo plot at Rothamsted and the DNA was extracted. The number of spring barley trials was decreased in 2008 and 2009 because little disease developed in them in 2006 and 2007.

Early, asymptomatic, infection by *R. secalis* was quantified using quantitative PCR (Fountaine *et al.*, 2007) on seeds (50 samples) and whole plants sent to Rothamsted from all the experiments in England and Scotland between crop emergence and stem extension (December/January for winter barley; April/May for spring barley) (i.e. before any fungicide treatments were applied; bulk samples of 10 plants from a total of about 150 plots each season). Final rhynchosporium assessments on the upper leaves were done at the end of the season using current HGCA Recommended List protocols for deriving resistance ratings (June/July) in both unsprayed and fungicide-treated plots. Weather data for all sites were obtained from the nearest synoptic weather station. Yield data were obtained from these replicated experiments with/without the HGCA recommended list fungicide programme (HGCA, 2009) (Talius was applied across all replicates to control powdery mildew). Relationships between asymptomatic infection and late season disease severity/yield loss were examined by regression.

More detailed work was done at Harpenden (England) and Dundee (Scotland) in unsprayed plots of these winter barley and spring barley experiments. Dynamics of *R. secalis* populations were assessed from sowing (seed testing) until harvest using quantitative PCR assays. The PCR technique was used on seed lots and to detect asymptomatic infection before disease lesions were apparent. Twenty random plants from each unsprayed plot at both locations were sampled during the season at GS12 (2 leaves unfolded), GS30 (ear at 1cm) and GS59 (emergence of inflorescence complete). Bulk samples of roots, stems and leaves from each plot were tested to quantify the amounts of *R. secalis* DNA (total of 180 samples per season). For these crops, to monitor the development of rhynchosporium epidemics, there were also visual assessments of disease symptoms during the season at primary leaf infection, secondary spread and ear phases of epidemics. These visual assessments were accompanied by records of crop growth stages.

Effects of cultivar resistance on development of rhynchosporium epidemics.

To assess the importance of resistance expressed during the symptomless phase of epidemics, a mapping population of doubled haploid lines from a cross between a resistant winter cultivar, Leonie, and a susceptible spring cultivar, Cocktail, (Cocktail x (Leonie x (Leonie x Pearl))) was grown at two sites (KWS, Cambridgeshire; Dundee, Scotland) in rows and characterised for resistance in more detail.

Both severity (% leaf area affected) of visual disease symptoms (GS26 and GS50) and amounts of *R.* secalis DNA (GS50) were recorded for the mapping population. Relative amounts of symptom expression for each line were derived from these two measurements. Rhynchosporium disease was present in both years with significantly greater severity of disease in the second year. Multi-environment QTL mapping was done on the three resistance characters.

To investigate the usefulness of qPCR for screening cultivars for resistance against *R. secalis*, an agreement was drawn up with the AGOUEB consortium (Association Genetics of UK Elite Barley, www.agoueb.org) to sample its winter and spring barley cultivar trials. Winter barley was grown at Dundee and spring barley at Tipperty, Scotland and KWS, Cambridgeshire. AGOUEB provided the phenotype data for 64 cultivars in these trials (visual disease, crop height, crop habit etc.) and SCRI provided the genotype data. To measure symptomless *R. secalis* in these cultivars, leaf samples were sent to Rothamsted for qPCR at GS50.

2.2.3. Task 3: Long-term dynamics of rhynchosporium epidemics (since 1852)

The hypothesis that rhynchosporium is a relatively recent and emerging threat to the UK barley crop was examined by studying the long-term dynamics of *R. secalis* populations using on an archive of samples from the Hoosfield spring barley experiment, using qPCR and methods applied in work on the long-term Broadbalk winter wheat experiment (Bearchell *et al.*, 2005). The Hoosfield barley experiment, run continuously at Rothamsted (Harpenden, England) since 1852, contains a series of plots, each receiving distinct fertiliser inputs. Samples of barley straw (chopped/crushed leaves and stems) and grain have been archived at harvest in almost all years since the start of the experiment (Johnston, 1994). Samples were oven-dried, stored in sealed containers and maintained above 5°C. Symptoms of fungal disease are visible on archived leaf specimens; extensive data on cultural practices and weather were recorded and are therefore available. Leaf/stem and grain samples from the barley archive were tested for the incidence and relative abundance of *R. secalis* and other pathogens each year using quantitative PCR measurements to reveal long-term trends in rhynchosporium severity.

For this work, leaf/straw samples were obtained from the Rothamsted archive of samples, for a single plot (4A) which had received the same treatment over the period from 1886 to the present day, and DNA was extracted from them (i.e. 1886-2006, 116 samples); a similar number of grain samples were obtained. Preliminary results showed that *R. secalis* DNA could be amplified from samples at different time points throughout the period covered by the archive, and that the relative amount of DNA in proportion to a barley reference amplicon (chloroplast ATPase b subunit) varied between seasons (Figure 5).

2.2.4. Task 4: Guidelines for sustainable control of rhynchosporium

There was a focus on exploitation of results throughout this LINK project. Involvement of HGCA, DuPont, Masstock Arable and KWS-UK ensured constant dialogue about exploitation of results by the industry. Information was integrated with agronomic advice on barley cultivation from other sources, including fungicide use, by DuPont, Scottish Agronomy and HGCA, to provide improved advice on cultivar resistance ratings, sowing dates and risk of severe epidemics to growers and advisers. HGCA organised a barley disease workshop to bring together members of all teams working on different barley disease projects (e.g. fungicide spray timing, use of mixtures, canopy management) including projects funded by HGCA, Defra and RERAD (at ADAS, SAC and SCRI) to improve strategies for managing the disease. This information was incorporated into an HGCA Barley Disease Management Guide.

2.3. Results

2.3.1. Task 1: Role of different inoculum sources in establishment of epidemics of *Rhynchosporium secalis* on barley

Seed-borne inoculum

The experiment at Dundee comparing farm-saved seed and commercial seed showed that, although amounts of *R. secalis* DNA were often greater (by 10- to 100- fold in some cases) in the farm-saved seed lots compared to the commercial seed, the amount of *R. secalis* DNA in the leaf samples taken at GS26 and later in the season were not significantly different between the two sources of seed in the winter barley trials (Figure 7). The visual symptoms of rhynchosporium disease within the crop also did not differ significantly between the two sources of seed (Table 1) but there was a smaller yield of plots grown from farm-saved seed compared to those grown from commercial seed (Figure 8). This difference was not significant in these small scale plots. Amounts of *R. secalis* DNA in the spring barley trials were very small and therefore results from these trials are not shown.

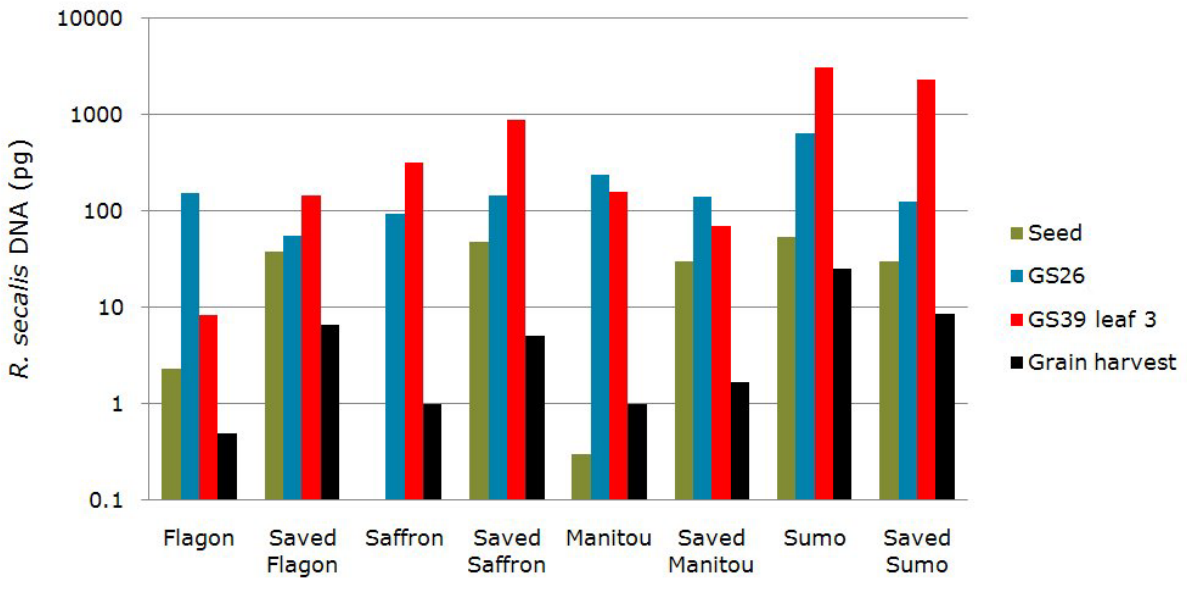


Figure 7. Comparison of amounts of *Rhynchosporium secalis* DNA in farm-saved seed (contaminated) and commercial seeds of four winter barley cultivars, and amounts of *R. secalis* DNA at GS26, GS39 (leaf 3) and in grain at harvest in winter barley grown in trials at Dundee from these two sources of seed.

Table 1. Comparison of visual symptoms in winter barley plots grown from farm-saved (contaminated) or commercial seed at Dundee using winter barley cultivars (Flagon, HGCA RL resistance rating 8; Manitou, 9; Sumo, 5; Saffron, 6) at GS39 and GS75. Figures shown are % leaf area affected by rhynchosporium leaf blotch symptoms.

	% leaf area affected			
	Commercial seed		Farm saved seed	
	GS39	GS75	GS39	GS75
Flagon	0	0.2	0.03	0
Manitou	0.1	0	0.1	0.1
Sumo	1.4	0.23	1.2	1.0
Saffron	0.05	0.2	0.5	0.4

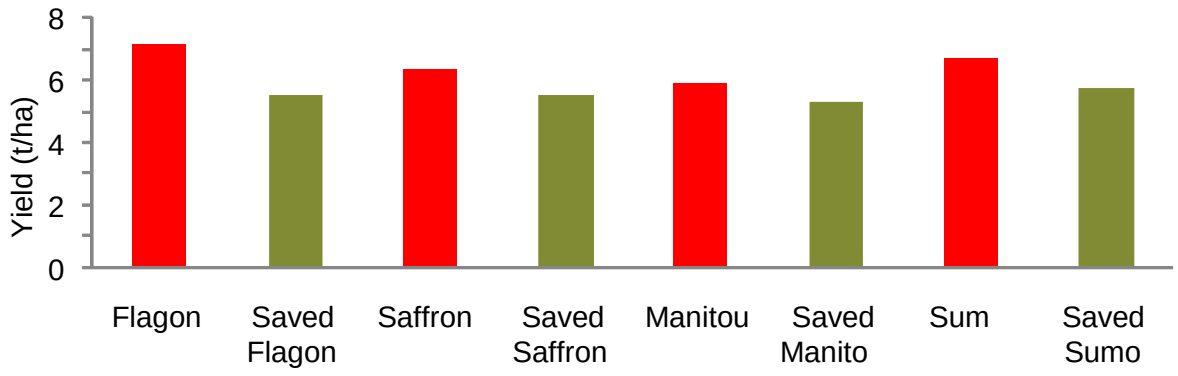


Figure 8. Yield of winter barley (cv. Flagon, Saffron, Manitou, Sumo) in plots sown with farm-saved (contaminated) or commercial seed trials at Dundee.

R. secalis DNA was detected in all individual seeds of winter or spring barley (Figure 9). Following seed dissection, *R. secalis* DNA was detected in both the seed endosperm and the seed coat, with more in the latter but this difference was not significant (Figure 10). *R. secalis* was present in all parts of seedlings (i.e. the seed, roots and shoots, Figure 11). In the winter barley field experiments, *R. secalis* DNA was routinely observed in DNA extracted from the roots from samples taken at GS26 (data not shown).

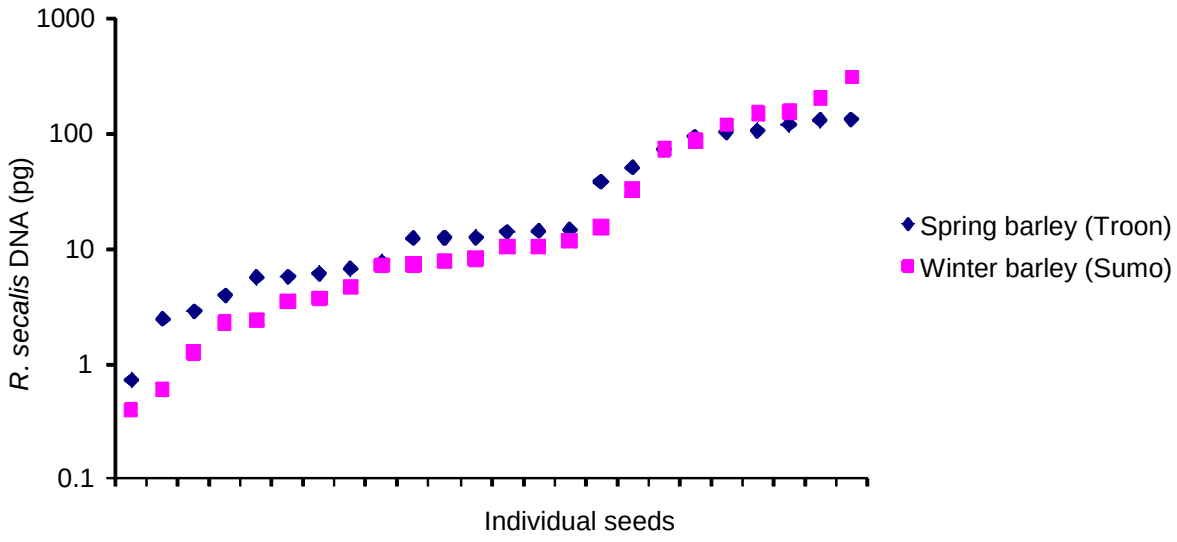


Figure 9. Amounts of *Rhynchosporium secalis* DNA (pg DNA in 50ng total DNA) detected in individual seeds of winter barley or spring barley.

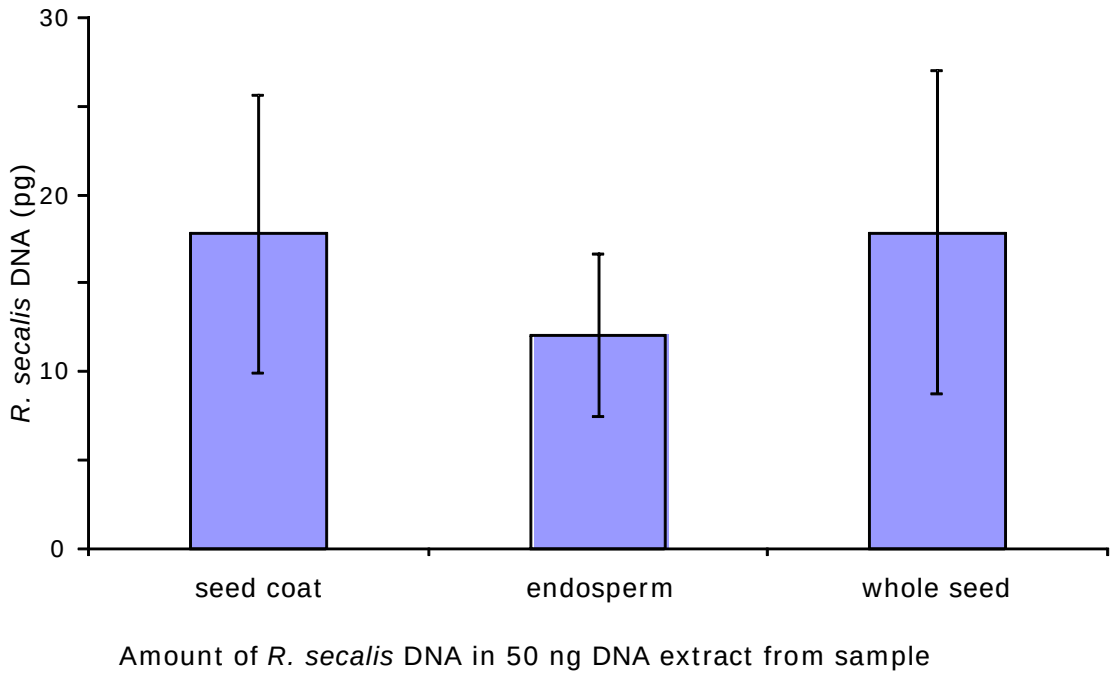


Figure 10. Amounts of *Rhynchosporium secalis* DNA in the seed endosperm and seed coating of individual barley seeds. Amounts shown are the mean values for of 10 dissected seeds and compared to the amount detected in 10 whole seeds. Vertical error bars shown are standard errors of the mean.

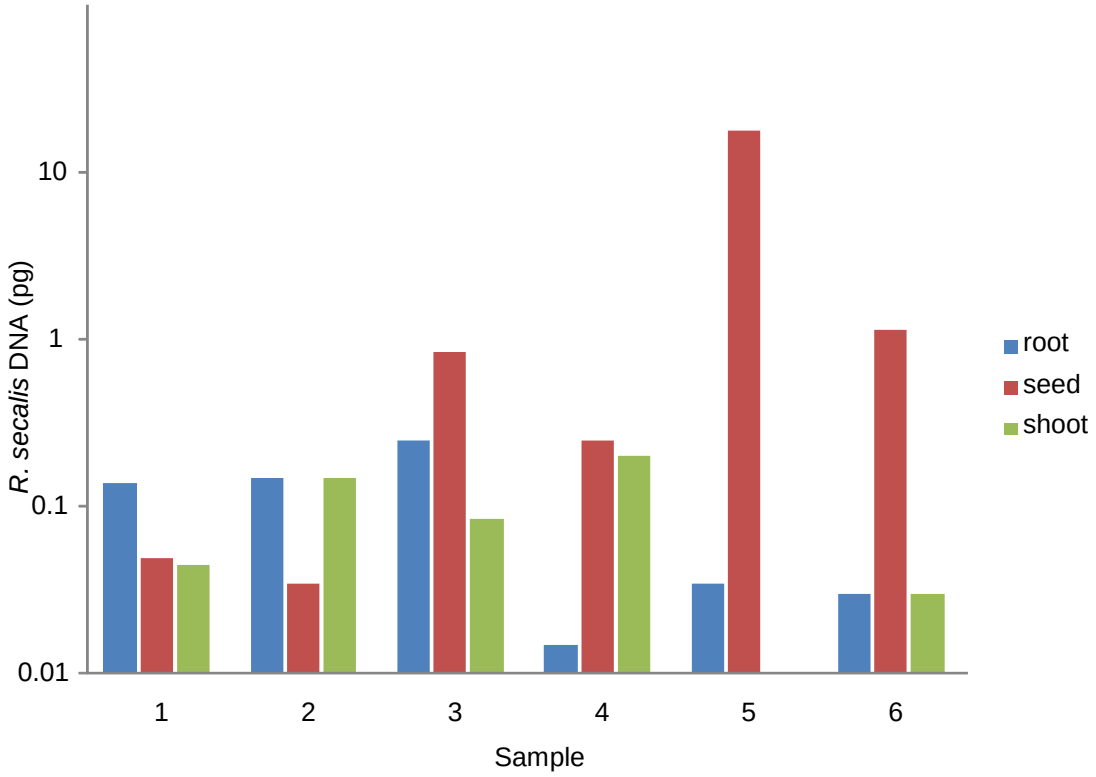


Figure 11. Amounts of *Rhynchosporium secalis* DNA (pg DNA in 50ng total DNA) found in root, seed and shoot tissues of 1 week old barley seedlings. Data from six seedlings shown.

In samples taken from winter barley experiments late in the growing season at GS75, *R. secalis* DNA was detected on senesced leaves and on leaves of fungicide-treated resistant cultivars that had remained symptomless throughout the season (Figure 12). Following inoculation of seedlings of cv. Sumo with spore washings from infected leaves taken from all LINK trial sites, visible symptoms developed after 14 days, indicating that the inoculum taken from these leaves was viable.

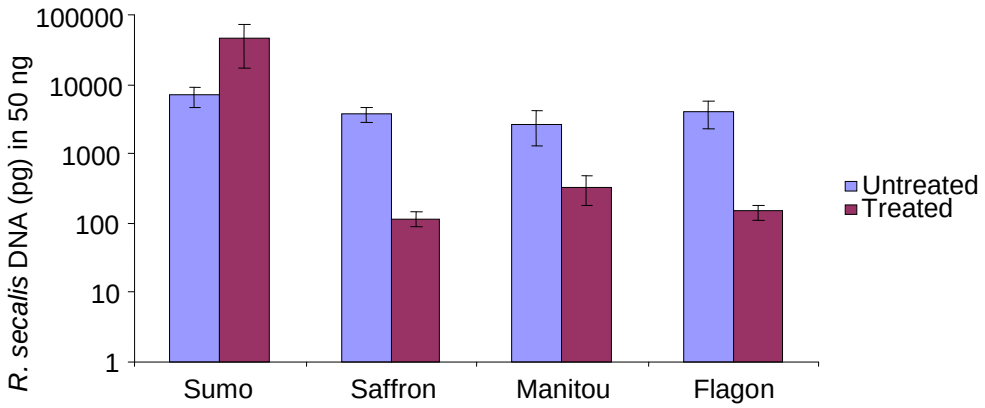


Figure 12. Detection of *Rhynchosporium secalis* DNA in leaf samples taken from fungicide-treated and untreated plots at the Rothamsted winter barley trial at GS 75. Amounts were similar at other trials sites. Visual symptoms were as follows Sumo, Saffron, Manitou and Flagon untreated (23.3%, 3.3, 0 and 0 leaf area affected) and Sumo, Saffron, Manitou and Flagon treated (3.3%, 0, 0 and 0 leaf area affected).

Airborne inoculum

The amount of *R. secalis* DNA detected on spore tapes from Burkard spore traps was very small at the sites in England and Scotland (Figure 13). When compared to the amount of *L. maculans* DNA (a pathogen with a known sexual stage releasing ascospores throughout autumn), there was no evidence for a widespread airborne source of *R. secalis* inoculum that is important in initiating epidemics (Figure 14).

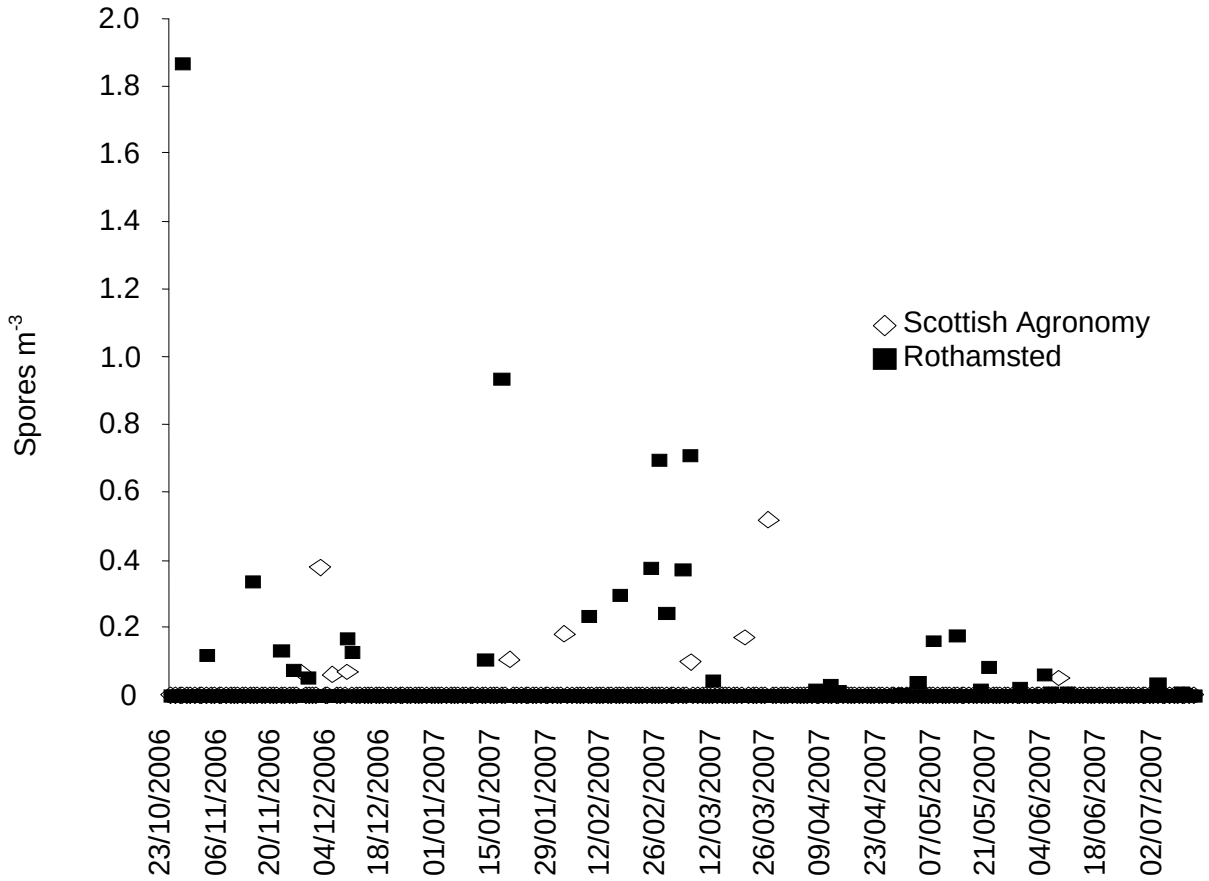


Figure 13. Estimated number of *Rhynchosporium secalis* spores (spores m^{-3}) detected by Burkard spore samplers in air above a winter barley crop at Rothamsted (Harpenden, England) and above grass at a Scottish site (Kinross) between October 2006 and July 2007. Amounts of pathogen DNA were estimated using quantitative PCR. Numbers of *R. secalis* spores were estimated from amount of *R. secalis* DNA and a calibration curve (obtained using *R. secalis* conidia).

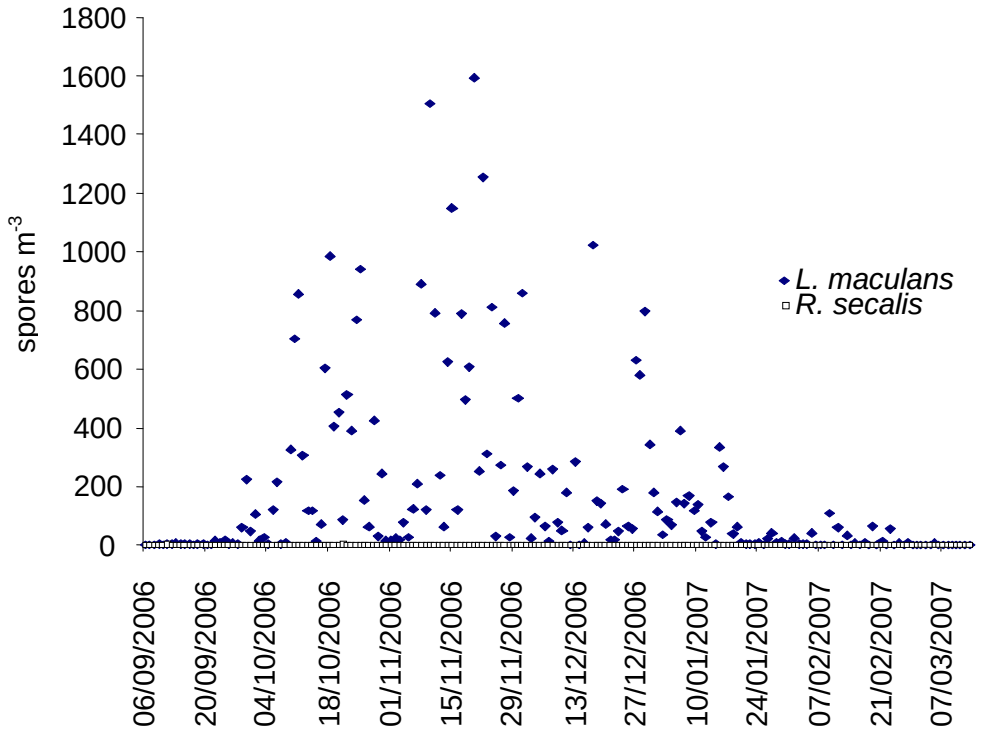


Figure 14. Comparison of estimated numbers of air-borne spores of *Leptosphaeria maculans* and *Rhynchosporium secalis* collected by a Burkard spore sampler in air above a winter barley field at Rothamsted (Harpenden, England) from September 2006 to March 2007. Numbers of *L. maculans* spores were estimated from amount of *L. maculans* DNA (Huang *et al*., 2011)

Races of Rhynchosporium secalis

DNA extracted from *R. secalis* isolates made from lesions on winter barley cultivars (Sumo, Saffron, Manitou and Flagon) was characterised using 14 microsatellite markers (SSRs). Most SSR genotypes were unique, showing more relatedness within cultivar plots and little relatedness between plots or sites. There was little correlation between seedling tests and detached leaf tests in identification of pathotypes for *R. secalis* isolates tested; however, there was a batch effect in seedling tests thus limiting their value. There were more similar pathotypes within plots than between sites or cultivars. SSR genotype was not related to pathotype.

The isolates in Figure 15 represent 30 out of 77 isolates sampled in 2007 from the winter barley trials. The single-spore isolates made in 2007, 2008 and 2009 that were SSR genotyped from the spring barley genotypes showed no good associations with site or cultivar. However, within a site isolates were more closely related amongst those from resistant cultivars than those from susceptible cultivars, implying more selection by resistant cultivars. For isolations made from the Leonie-Pearl-Cocktail population progeny expressing different levels of resistance, the dominant factor in isolate relatedness was spatial proximity of sampled plants to each other in the trial. These data are contributing to further population genetic analysis being done to define the relative strengths of selection by different factors on *R. secalis* populations leading to improved risk assessment.

Location	Isolate Name	Leaves Collected	Date Isolated	Seedling Octal Rating	Seedling NIAB UKCPVS	DLT Octal Rating	DLT BRR Factor	Cultivar	Marker														
									1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Dupont	L2A	May-07	Sep-07	7	1,2,3	337	1,2,3,4,5,7,8	Sumo	100	229	126	217	138	228	193	177	167	172	245	203	196	318	I3
High Mowthorpe	L43A	Apr-07	Sep-07	217	1,2,3,4,8	316	3,4,7,8	Saffron	118	229	126	232	134	232	193	200	147	141	199	220	216	314	B
High Mowthorpe	L43B	Apr-07	Sep-07	5	1,3	334	3,4,5,7,8	Saffron	118	229	126	232	134	230	193	200	147	141	199	220	216	314	B2
High Mowthorpe	L43C	Apr-07	Sep-07	5	1,3	34	3,4,5	Saffron	118	229	126	232	134	232	193	200	147	141	199	220	216	314	B
High Mowthorpe	L43D	Apr-07	Sep-07	5	1,3	4	6	Saffron	118	229	126	232	134	232	193	200	147	141	199	220	216	314	B
High Mowthorpe	L47A	Apr-07	Sep-07	15	1,3,4	374	3,4,5,6,7,8	Sumo	103	229	126	217	141	226	232	177	167	166	228	201	196		X1
High Mowthorpe	L47B	Apr-07	Sep-07	15	1,3,4	375	1,3,4,5,6,7,8	Sumo	140	231	129	246	138	209	220	177	147	166	262	214	160	316	D
High Mowthorpe	L47C	Apr-07	Sep-07	5	1,3	124	3,5,7	Sumo	140	231	129	246	138	209	220	177	147	166	262	214	160	316	D
Scottish Agronomy	L73A	May-07	Sep-07	1	1,	27	2,5	Flagon	103	229	126		140	226	216	165	176	169	268	195	198	318	E
Scottish Agronomy	L74B	May-07	Sep-07	5	1,3	136	2,3,4,5,7	Flagon	103	229	126	217	140	226	216	165	176	165	268	195	198	318	E2
Scottish Agronomy	L90B	May-07	Sep-07	7	1,2,3	137	1,2,3,4,5,7	Sumo	103	229	126	217	140	213	232	188	147	166	268	195	196	318	R3
Scottish Agronomy	L90C	May-07	Sep-07	15	1,3,4	124	3,5,7	Sumo	103	239	126		138	226	232	200	147	166	200	201	196	318	R4
Scottish Agronomy	L101B	May-07	Sep-07	15	1,3,4	237	1,2,3,4,5,8	Sumo	103	229	126	217	141	228	232	188	176	166	257	201	196	318	R5
Scottish Agronomy	L102B	May-07	Sep-07	15	1,3,4	234	3,4,5,8	Saffron	100	229	126	217	138	228	193	177	157	172	239	203	196	318	I1
Scottish Agronomy	L104B	May-07	Sep-07	17	1,2,3,4	137	1,2,3,4,5,7	Saffron	100	229	126	217	138	228	193	177	167	172	245	203	196	318	I2
Dupont	L1A	May-07	Nov-07	314	3,4,7,8	376	2,3,4,5,6,7,8	Sumo	103	240	126	217	138	226	232	200	147	166	257	201	196	318	J
Dupont	L1B	May-07	Nov-07	214	3,4,8	174	3,4,5,6,7	Sumo	100	266	139		138	213	206	200	147	172	292	201	195	318	K
Dalgety	L6A	May-07	Nov-07	314	3,4,7,8	137	1,2,3,4,5,7	Saffron	103	229	126	217	141	226	216	165	176	169	257	201	198	318	L1
Dalgety	L6B	May-07	Nov-07	214	3,4,8	115	1,3,4,7	Saffron	118	233	129	217	134	209	240	200	147	172	274	201	160		B3
Dalgety	L12A	May-07	Nov-07	214	3,4,8	137	1,2,3,4,5,7	Sumo	103	229	117		138	215	234	177	162	166	233	201	191	320	N
Dalgety	L12B	May-07	Nov-07	314	3,4,7,8	174	3,4,5,6,7	Sumo	121	245	126		134	209	226	188	147	166	268	222	198	318	O
Dalgety	L18	May-07	Nov-07	355	1,3,4,6,7,8	134	3,4,5,7	Saffron	118	238	129		134	209	232	200	147	141	220	220	200	312	B4
Rothamsted	L32B	May-07	Nov-07	255	1,3,4,6,8	176	2,3,4,5,6,7	Saffron	118	231	129	239	135	209	238	211	157	172	210	218	160	354	Q1
Rothamsted	L32C	May-07	Nov-07	314	3,4,7,8	176	2,3,4,5,6,7	Saffron	118	231	129	239	136	209		211	157	172	210	220	160	354	Q2
Rothamsted	L38A	May-07	Nov-07	214	3,4,8	137	1,2,3,4,5,7	Sumo	103	229	117	217	140	213	238	188	147	141	286	201	196	316	R1
Rothamsted	L38B	May-07	Nov-07	14	3,4	137	1,2,3,4,5,7	Sumo	103	229	117		140	215		188	147	141		222			R2
High Mowthorpe	L46	May-07	Nov-07	14	3,4	174	3,4,5,6,7	Sumo	103	229	126	217	140	226	216	165	176	169	268	201	198	318	L2
Scottish Agronomy	L60A	May-07	Nov-07	314	3,4,7,8	136	2,3,4,5,7	Saffron	103	229	126	217	140	226	232	177	167	166	245	201	196	320	X2
Scottish Agronomy	L60B	May-07	Nov-07	314	3,4,7,8	174	3,4,5,6,7	Saffron	118	231	129	239	134	209	240	211	157	172	210	220	160	354	Q3
Scottish Agronomy	L77	May-07	Nov-07	314	3,4,7,8	356	2,3,4,6,7,8	Manitou	103	229	126	217	142	213	232	165	171	166	228	199	195	316	X3

Figure 15. SSR and virulence profiles of 30 single spore derived-isolates of *R. secalis* from five locations from winter barley in 2007. 'Octal race' designation is based on the 'BRR' (Barley Rhynchosporium Resistance) factor, i.e. whether it forms symptoms on the differential cultivar numbered from 1 to 8. The same differential cultivars were used in the 'seedling NIAB UKCPVS' and 'DLT' (Detached Leaf Test) assessments. Markers 1-14 are the SSR sequences published by Linde *et al*. (2005).

2.3.2. Task 2: Short-term (within season) dynamics of rhynchosporium epidemics

Development of rhynchosporium epidemics in plots with/without fungicide

Few severe rhynchosporium epidemics were recorded in the UK field trials during the project. Severity of visual rhynchosporium symptoms varied, with more severe symptoms recorded in sites in Scotland than in England and differences between years. In winter barley, *R. secalis* DNA was detected very early in the cropping season in large amounts. At Harpenden in 2007, there was a steady increase in asymptomatic *R. secalis* colonisation in untreated Sumo throughout the cropping season (Figure 16). The same early infection was not observed in spring barley.

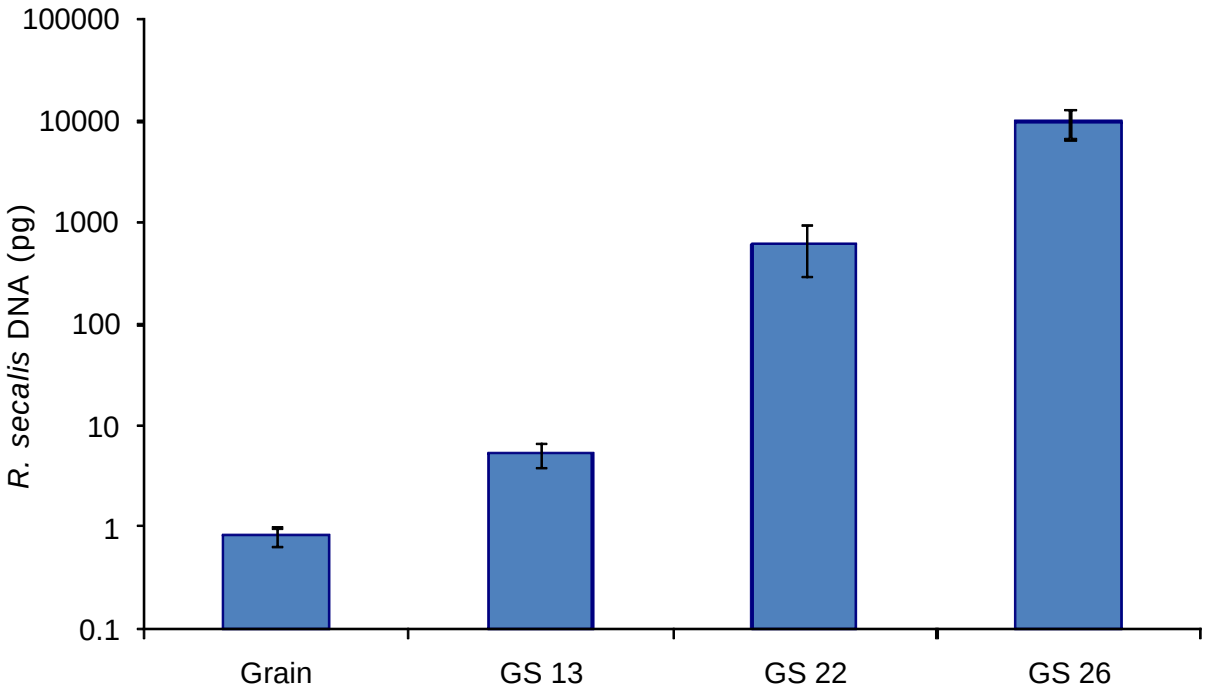


Figure 16. Detection of *Rhynchosporium secalis* DNA (pg DNA in 50ng total DNA) on cv. Sumo at Rothamsted (Harpenden, England) in 2007. Visual symptoms of disease were assessed on leaves at GS 13, 22 and 26 as 0%, 0% and 1.3%, respectively.

The amount of *R.secalis* DNA in samples of untreated Sumo taken at GS26 from sites in 2009 was significantly less than the amount of *R. secalis* DNA in samples taken in 2007 and 2008 (Figure 17). However, due to a subsequent period with rainfall and thus favourable weather conditions for spread of the disease, a larger amount of *Rhynchosporium secalis* DNA was measured for some sites later in the season at GS39 in 2009 (Figure 18). Asymptomatic leaves and leaves with lesions were taken from a Sumo plot at Rothamsted early in spring. Most leaves with symptoms had large amounts of *R. secalis* DNA but several asymptomatic leaves also had similar amounts of DNA (Figure 19). Microscopic examination of these asymptomatic leaves with large amounts of *R. secalis* DNA showed asymptomatic colonisation and sporulation by *R. secalis* (Figure 20).

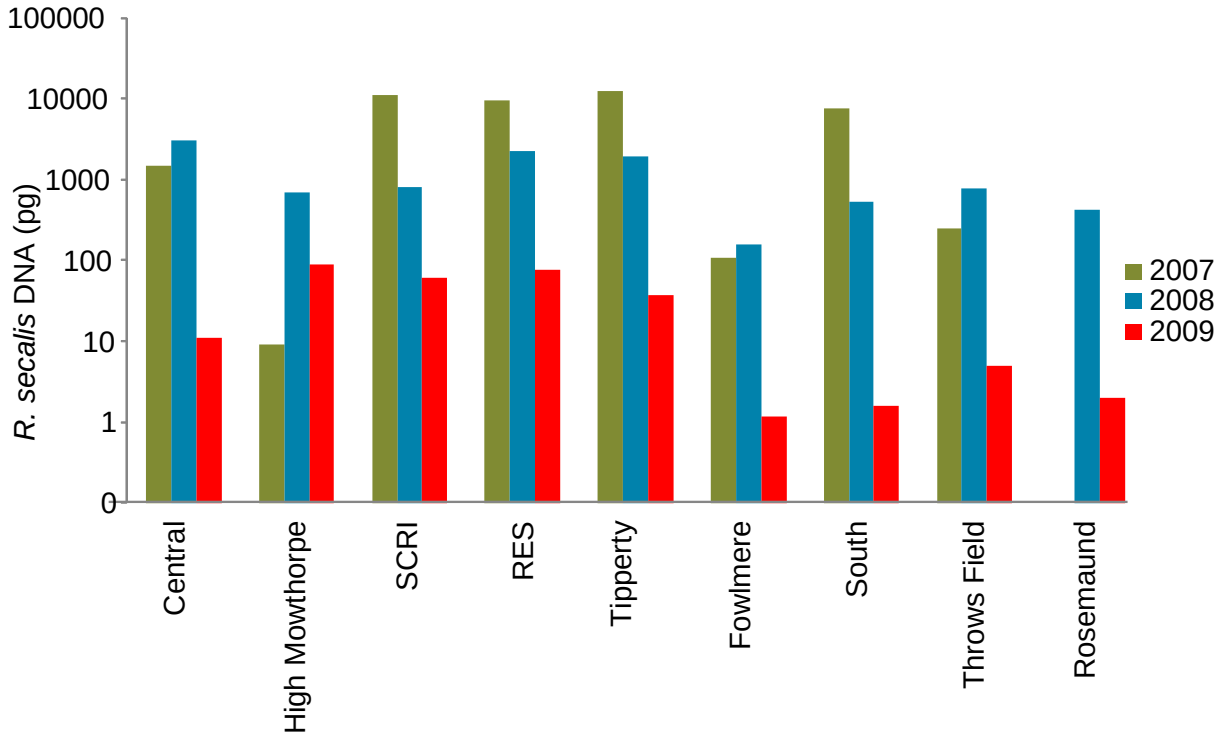


Figure 17. Amounts of *Rhynchosporium secalis* DNA (pg in 50ng total DNA) in untreated winter barley cv. Sumo at GS 26 from all trial sites in 2007, 2008 and 2009.

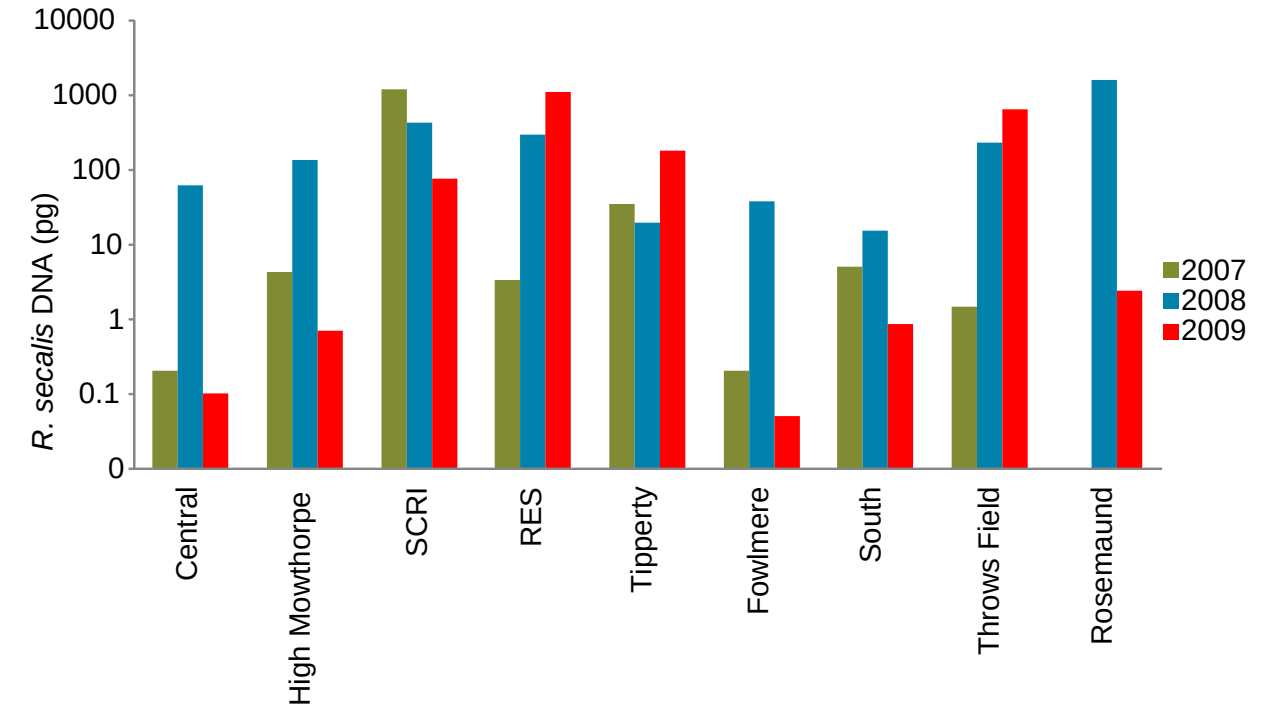


Figure 18. Amounts of *Rhynchosporium secalis* DNA (pg in 50ng total DNA) in untreated winter barley cv. Sumo leaf 2 at GS 39 at all trial sites in 2007, 2008 and 2009.

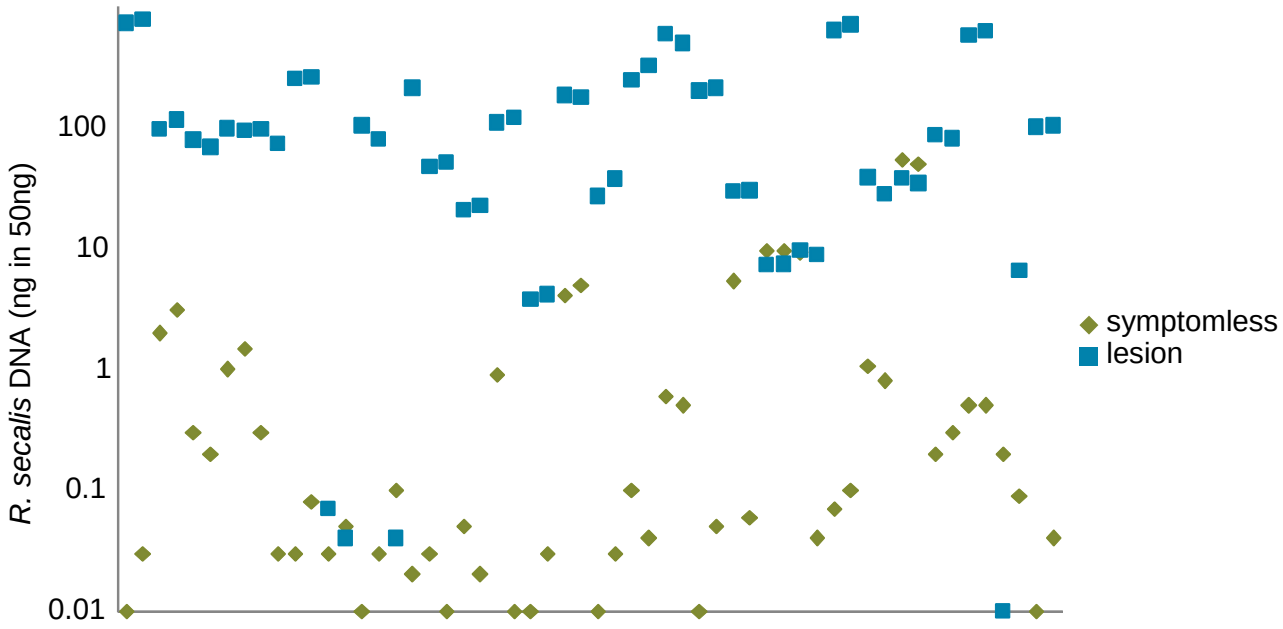


Figure 19. Amount of *Rhynchosporium secalis* DNA in barley leaves with or without rhynchosporium leaf blotch lesions, sampled from cv. Sumo in early spring. Each point represents a single leaf.

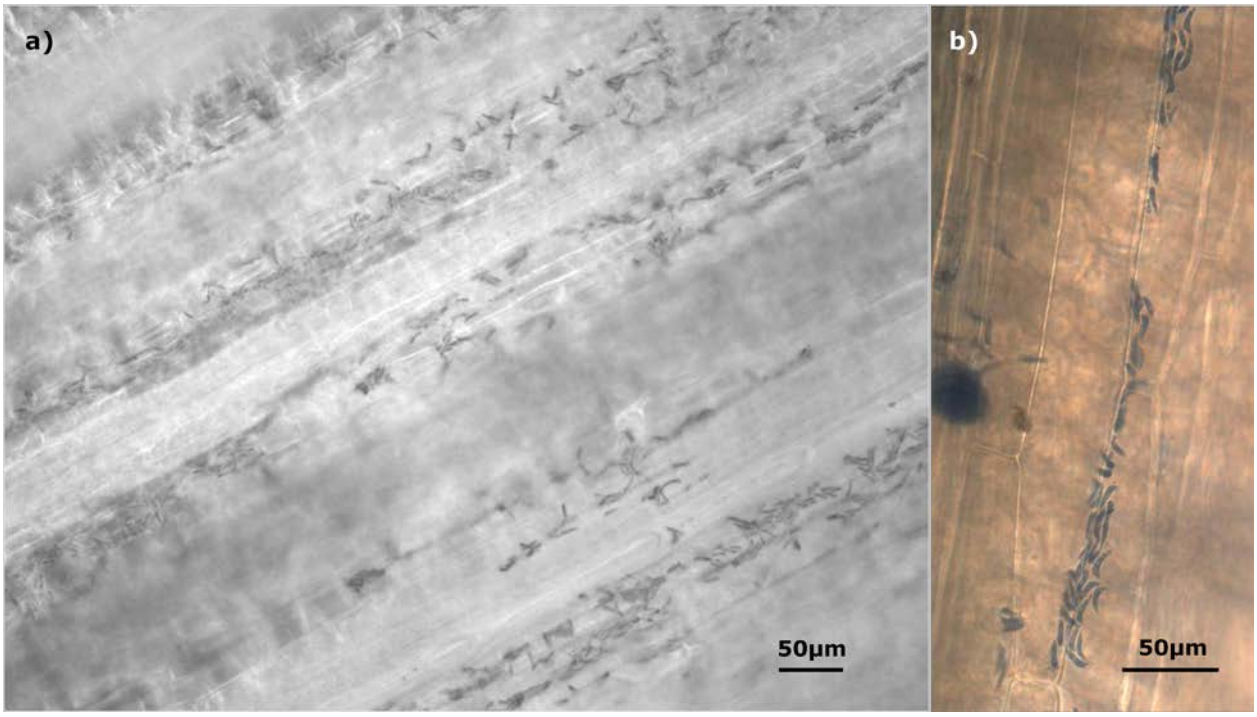


Figure 20. a) Light micrograph of an asymptomatic winter barley leaf (cv Sumo) showing abundant *Rhynchosporium secalis* sporulation b) stained with trypan blue. Leaf sampled at Harpenden in early spring.

Large amounts of *R. secalis* DNA were recorded at GS26 at one site (Central Scotland, Figure 21). Rainfall during April 2007 was unusually low. When rainfall increased after April 2007, no significant amounts of *R. secalis* DNA were recorded. Total rainfall in April 2008 was greater than in April 2007 or April 2009 and the amounts of *R. secalis* DNA recorded at GS39 were also significantly greater than those in 2007 or 2009. There were similar associations between the amount of rainfall in April and subsequent development of epidemics at other sites (data not presented)

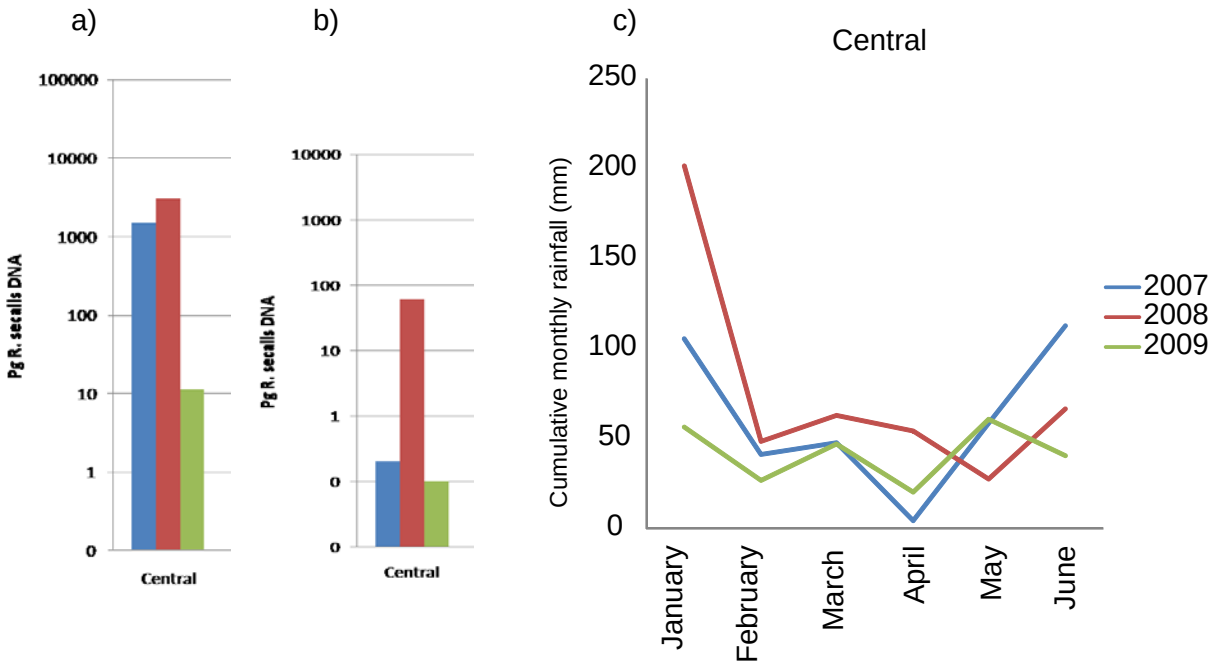


Figure 21. (a) *Rhynchosporium secalis* DNA from untreated winter barley cv. Sumo at GS 26 from the Scottish Central site in 2007 (blue bars), 2008 (red) and 2009 (green). (b) *R. secalis* DNA from untreated Sumo at GS 39 from the Central site in 2007 (blue bars), 2008 (red) and 2009 (green). (c) Rainfall (mm) from January to June at the Central site in 2007 (blue line), 2008 (red) and 2009 (green).

Fera provided disease data from winter barley surveys from 1981 to 2005, (http://www.cropmonitor.co.uk/ ; Figure 22) Weather data from several sites in the UK were combined to provide a summary of the weather over the whole of the UK to compare with the visual disease observations from Fera. Weather factors were taken individually (i.e. rainfall in April) or together (i.e. total rainfall during spring, or temperature during winter etc.). In the regression analyses, the only weather factor that had a significant effect on rhynchosporium disease at GS75 was rainfall in April.

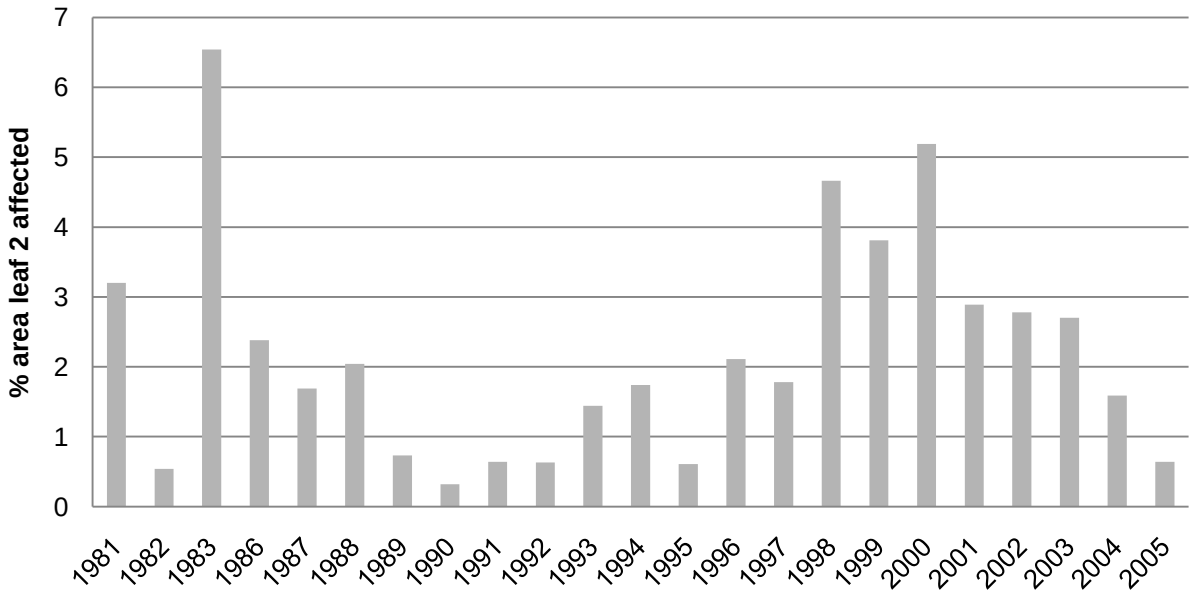


Figure 22. Data provided from CropMonitor (Fera) showing the average % visual area affected by rhynchosporium on leaf 2 of winter barley crops sampled in England and Wales at GS75 from 1981 to 2005 (http://www.cropmonitor.co.uk/).

In winter barley trials, all crops showed a significant yield response to fungicide, with the greatest response in cv. Manitou in 2007 (due to a severe brown rust epidemic). Fungicide significantly decreased amount of *R. secalis* DNA in susceptible cultivars (e.g. Sumo, Figure 23) but also significantly decreased it in more resistant cultivars. Fungicide treatments greatly increased yield of both susceptible and resistant cultivars, suggesting that some yield responses were due to effects of fungicides on other diseases. Fungicides did not prevent spread of *R. secalis* onto grain. More *R. secalis* DNA was recorded in trials in Scotland than England. Spraying with fungicide at GS26 decreased visual symptoms and amount of *R. secalis* DNA in the next leaf layers. In spring barley trials amount of *R. secalis* DNA was affected by cultivar or fungicide, although the yield response to fungicide was not significant.

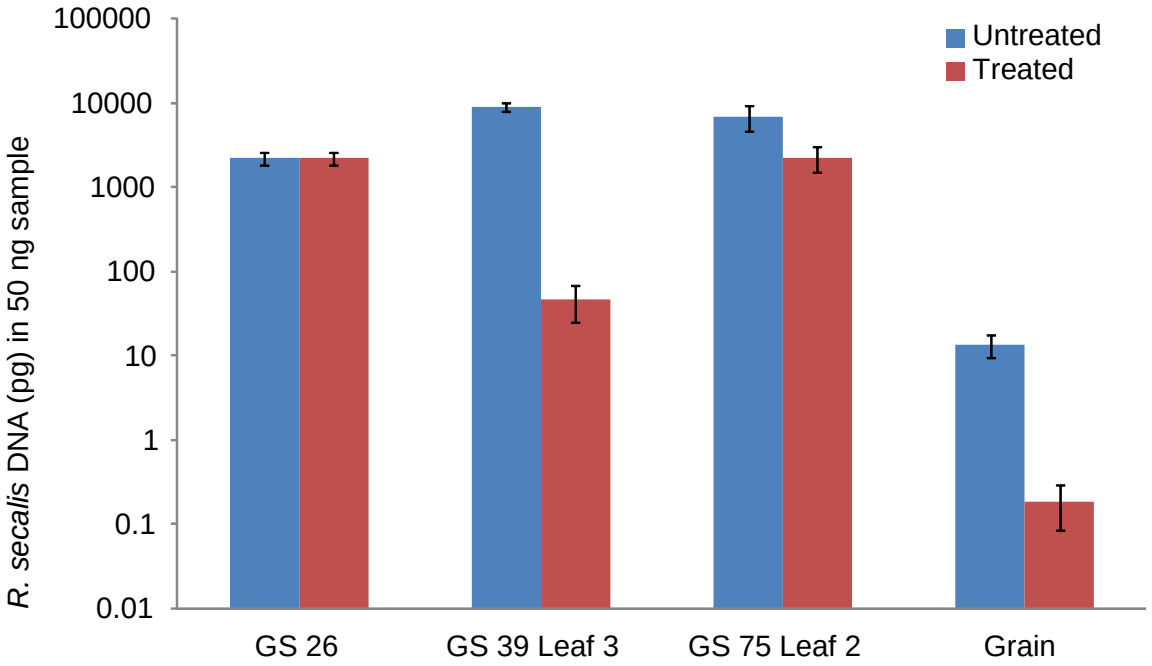


Figure 23. Impact of fungicide treatment on the amount of *Rhynchosporium secalis* DNA. Treatment of winter barley cultivar Sumo at Rothamsted 2008 with T1 spray at GS 26 [Acanto (picoxystrobin) plus Proline (prothioconazole)] and T2 spray at GS 39. Samples at GS26 and GS39 were taken before spray treatments were applied.

Effects of cultivar resistance on development of rhynchosporium epidemics.

The amount of *R. secalis* DNA recorded in winter barley field trials was inversely related to the HGCA RL cultivar resistance rating (74% variance accounted for, Figure 24). There was clear evidence that cultivar resistance decreased the amount of *R. secalis* DNA recorded in leaves from GS22 to GS39 (Figure 25), even in the absence of visible symptoms.

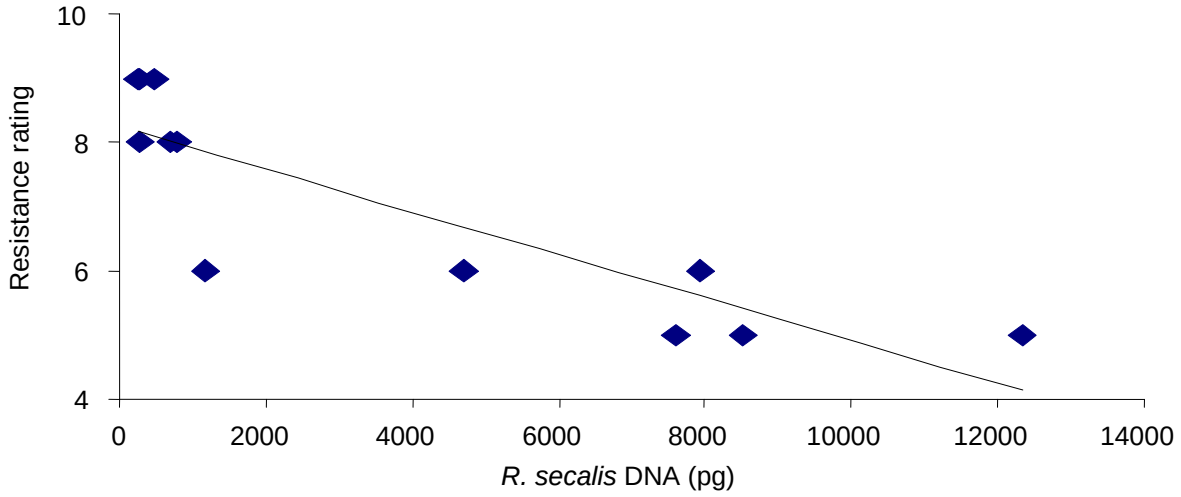


Figure 24. Relationship between the resistance rating and amount of *Rhynchosporium secalis* DNA for winter barley at three Scottish trials sites (Tipperty, Central and South). Line of best fit equation: y=-0.0003x+8.24, R^2=0.74

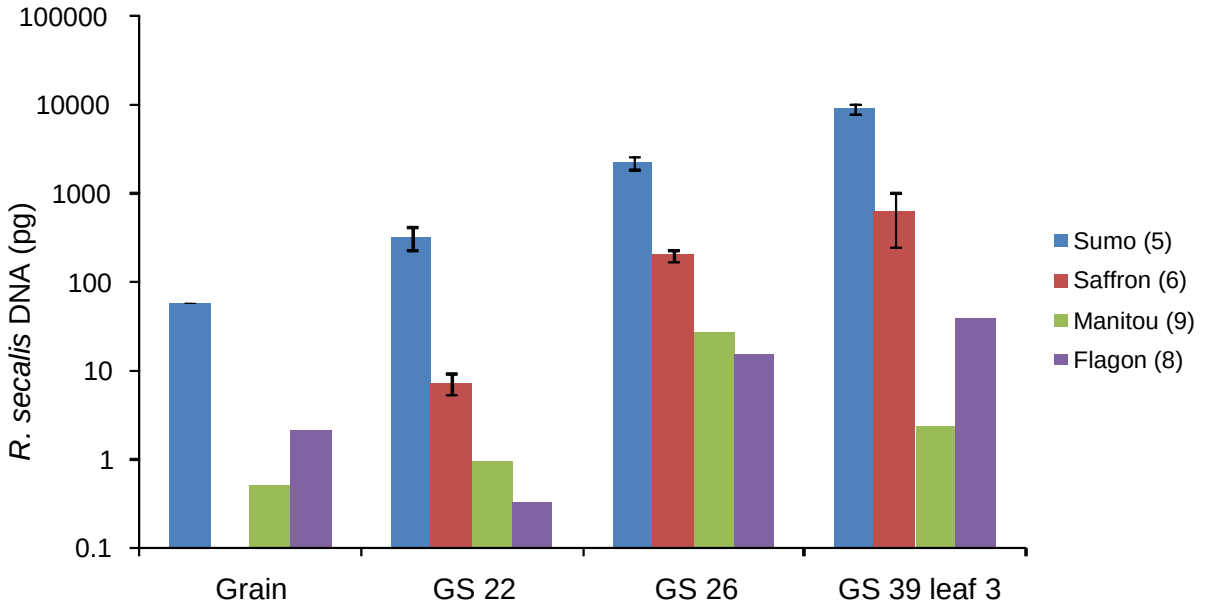


Figure 25. Impact of cultivar resistance on the amount of *Rhynchosporium secalis* DNA (pg in 50 ng total DNA). Figures in brackets represent the HGCA resistance rating for the cultivar (1, susceptible; 9, resistant).

When 190 lines from a doubled haploid population of Cocktail x (Leonie x (Leonie x Pearl)) were grown at Dundee in the winter,genetic analysis of these lines demonstrated a good genetic correlation (r_A=0.87) between visible symptoms (% area affected) and amount of *R. secalis* DNA. This was reflected in the Quantitative Trait Locus (QTL) analysis. Three significant QTL were identified on chromosomes 2H, 3H and 7H. Each of these QTL was significantly associated with both visual symptoms and amount of *R. secalis* DNA. A single significant QTL for relative expression of symptoms (i.e. the severity of visual symptoms relative to the total amount of *R. secalis* DNA) was identified (Figure 26). Greater percentages of leaf area affected and larger amounts of *R. secalis* DNA were observed in those lines with spring barley characteristics, suggesting that they were more susceptible.

Although there was a significant relationship between the amount of disease assessed by qPCR and the amount assessed by visual observation for these trials with the mapping population, there was a very poor relationship between the amount of *R. secalis* DNA and the resistance rating based on visual assessment of disease symptoms for the 64 winter barley cultivars assessed in the AGOUEB (http://barleygenetics.net/lukesite/html/agoueb/) trial (Figure 27).

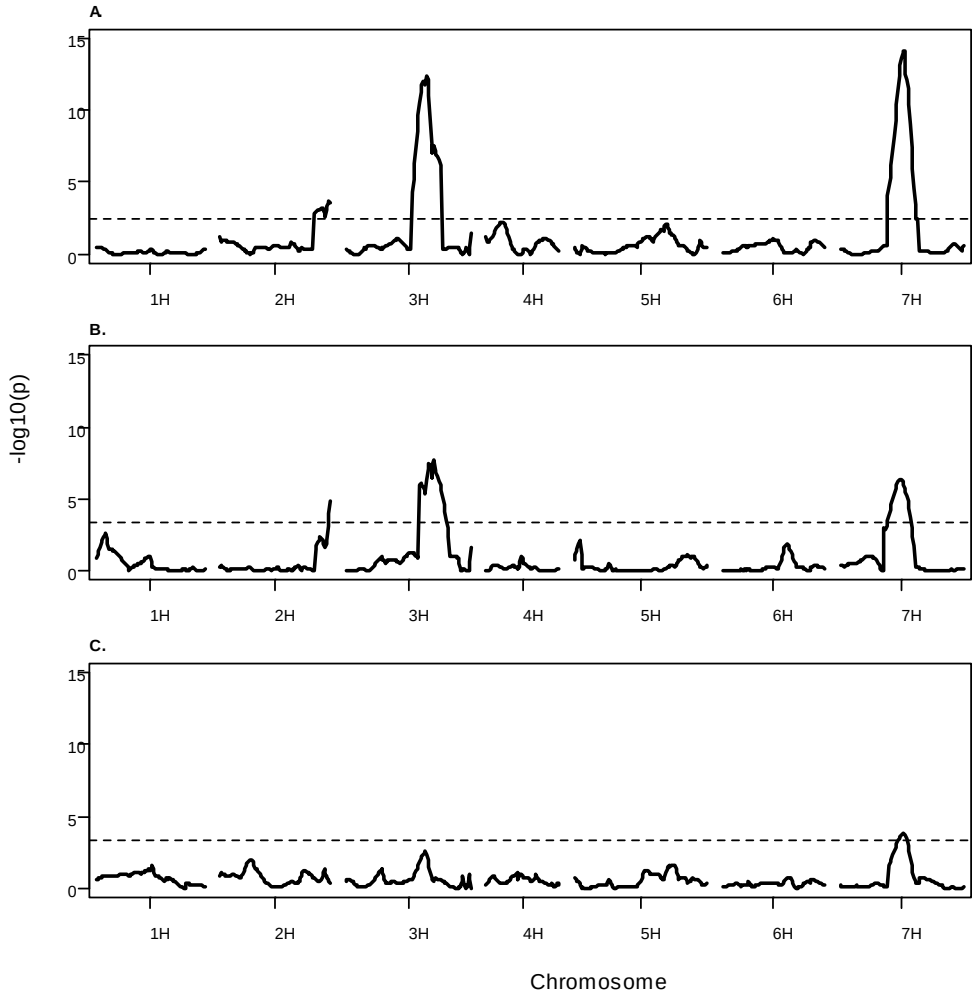


Figure 26: Results from a multi-environment QTL genome scan for three different aspects of barley resistance to *Rhynchosporium*. A: plot disease score; B: total *Rhynchosporium* DNA; C: Relative disease expression (i.e. the severity of visual symptoms relative to the total amount of *R. secalis* DNA). For each plot, solid lines show the significance of the test statistic across each barley chromosome, the dotted line indicates the value of a test statistic equivalent to a genome wide significance threshold of 0.05.

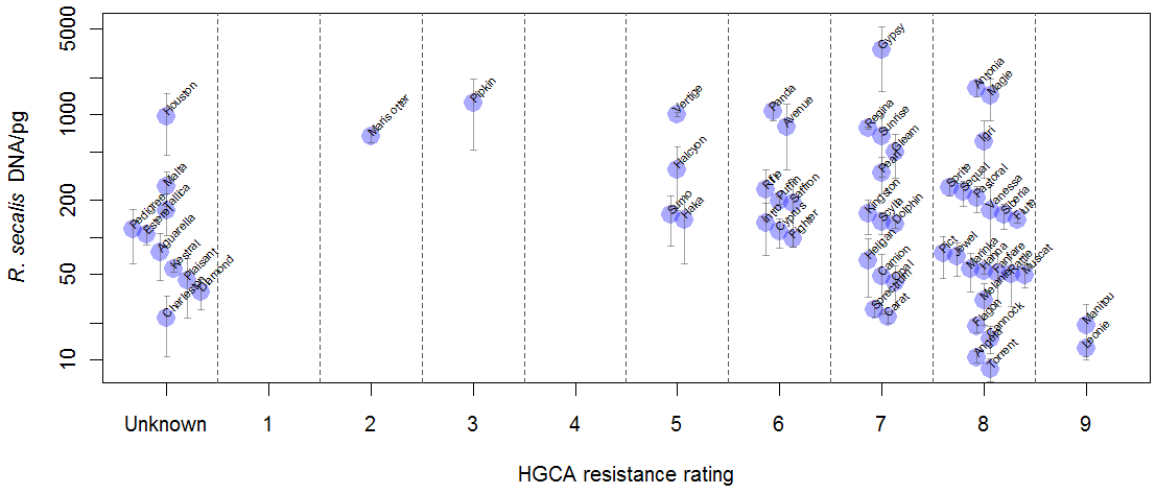


Figure 27. Amounts of *Rhynchosporium secalis* DNA in 64 winter barley cultivars from the AGOUEB trials. Figures by the cultivar names represent the historical HGCA resistance rating. When cultivar resistance rating was regressed against amount of *R. secalis* DNA, R^2=0.32.

2.3.3. Task 3: Long-term dynamics of rhynchosporium epidemics (since 1852)

When changes in the amounts of *R. secalis* DNA found in grain and leaf/straw samples from the Rothamsted Hoosfield experiment over the period 1888 to 2006 were compared with the cultivar grown (Figure 28), very small amounts of *R. secalis* DNA were found in straw samples from 1917 to 1964 when the cultivar Plumage Archer was being grown. An increase in the amount of *R. secalis* DNA in the samples began to occur in the early 1960s. This coincided with the introduction of short-strawed cultivars (the first being Maris Badger). The amounts *R. secalis* DNA in grain were much smaller but followed a similar trend. Large amounts of *Pyrenophora graminea* (barley leaf stripe) DNA in grain were detected from 1886 until the introduction of Plumage Archer in 1917, with much smaller amounts detected throughout the archive since that date.

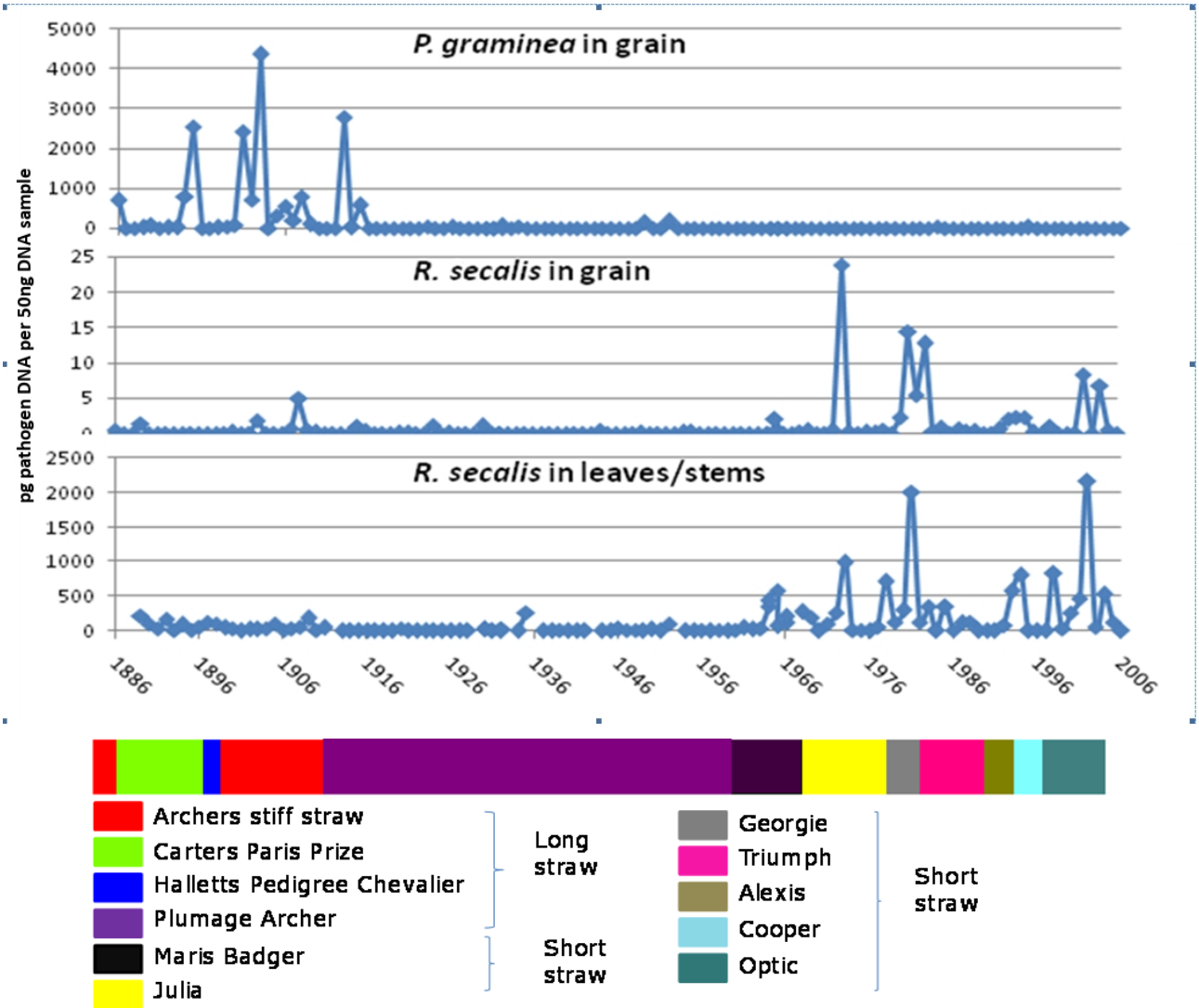


Figure 28. Amounts of *Rhynchosporium secalis and Pyrenophora graminea* DNA measured in archived spring barley grain and leaf/straw sampled from 1886 to 2006. The cultivars grown over that period are shown in the coloured blocks. Note different units on the Y-axes for the three graphs (scale is pg of pathogen DNA per 50 ng of DNA sample).

2.3.4. Task 4. Guidelines for sustainable control of rhynchosporium.

The key messages from this project to the agricultural industry (growers, breeders, HGCA for Recommended List trials, agrochemical companies) are:

- There is widespread symptomless infection by *Rhynchosporium secalis* in UK barley crops. This explains why symptoms can suddenly appear extensively across untreated barley crops. An example of this occurred in spring 2010 when many growers did not apply fungicides to winter barley because of the dry spring weather, but the crops had already been infected during earlier wet weather.

- Control of symptomless *Rhynchosporium secalis* infection can contribute to yield responses to fungicides applied to apparently 'healthy' winter barley crops (such yield responses, e.g. to autumn fungicides, have been observed in parallel projects). However, it is difficult to directly relate these yield responses to control of symptomless *Rhynchosporium secalis* because fungicides often control other diseases present as well. If growers wait until rhynchosporium symptoms are present on upper leaves of their crops, then maximum yield responses will not be obtained.

- *Rhynchosporium secalis* sporulates profusely on symptomless leaves of barley crops; these spores are spread to new leaves and new plants by rain-splash. Thus *Rhynchosporium secalis* can contin*ue* to spread in barley crops throughout the growing season (from seed to seed) in the absence of visual symptoms.

- Use of molecular diagnostics to detect symptomless infection by *Rhynchosporium secalis* early in the growing season (autumn/winter) can provide an indication of the risk of severe epidemics in winter barley crops but does not give an accurate prediction of epidemic severity in spring, which is greatly influenced by April rainfall.

- Whilst spring barley cultivars are intrinsically more susceptible to *Rhynchosporium secalis* (see results with mapping population) if they are grown together with winter barley at the same time, the amounts of symptomless *Rhynchosporium secalis* DNA observed in winter (autumn-sown) barley crops in this work were generally far greater than those observed in spring (spring-sown) barley crops.

- There is widespread infection of barley seed by *Rhynchosporium secalis* which can serve as a source of inoculum to infect crops. However, secondary spread of *Rhynchosporium secalis* by splash dispersal is more important in determining the ultimate severity of epidemics. Nevertheless, it may be of value to protect seed to minimise transmission of infection to new crops or regions.

- There is a poor relationship between the amount of *Rhynchosporium secalis* DNA present in leaves and the severity of visual symptoms on those leaves.

- There is generally less *Rhynchosporium secalis* DNA present in leaves of more 'resistant' cultivars than leaves of more 'susceptible' cultivars (resistance/susceptibility defined according to HGCA Recommended List rating).

- The QTL for resistance identified by assessing amount of *Rhynchosporium secalis* DNA in symptomless leaves of lines in a mapping population were the same as those identified by assessing visual symptoms in an inoculated disease nursery that received regular irrigation. Thus, there are possibilities to use molecular diagnostics to assess resistance in trials where irrigation is not available or to provide early indications of likely differences in resistance between breeding lines.

- Furthermore, use of molecular diagnostics may help to improve the accuracy of resistance ratings in HGCA Recommended List trials.

2.4. Discussion

This discussion is structured around the five questions listed in the Introduction, with an additional section summarizing the benefits of this project.

2.4.1. Question1. Is seed-borne inoculum a significant source of early infection in the crop (Task 1)?

Seed-borne inoculum was identified as a significant source for early infection of barley crops, with substantial amounts of *R. secalis* DNA found in seedlings of crops grown from infected seed. As *R. secalis* DNA was detected in all seed batches tested, it appears that seed infection is widespread. From the results shown, it is clear that many seed lots have some level of contamination independent of whether the cultivar was resistant or had been treated with fungicide. The presence of *R. secalis* DNA was detected by PCR in both the endosperm and the seed coating. Further investigation into the exact location of the pathogen within the seed is necessary and may be done using the transgenic *R. secalis* isolates expressing fluorescent reporter genes produced as part of the PhD work that developed from this project (Thirugnanasambandam *et al*., 2011). It is also important to determine how the fungus grows from the seed to colonise the seedling, whether this is by endophytic growth or spread from the seed coat to the seedling. This work shows that *R. secalis* can be found in all tissues of seedlings from the grain, roots and shoot. DNA was extracted from the roots from the GS26 samples in these field experiments and *R. secalis* was routinely found. This suggests that the pathogen spreads from the grain into roots and shoots and from there may spread throughout the plant. Seed may also be an important route for spreading *R. secalis* isolates with new virulence alleles or fungicide resistance to new areas and further investigation of this possibility is necessary. Methods for treating seed to limit viable pathogen inoculum are needed.

There was little evidence that severity of seed infection influenced amounts of pathogen DNA (leaves), disease severity (leaves) or yield loss later in the cropping season. However, detection of *R. secalis* DNA in senesced leaves and in leaves of fungicide treated resistant cultivars that had remained symptomless throughout the season demonstrates that even treated resistant cultivars that appeared to be free from visual rhynchosporium symptoms throughout the season could still produce contaminated seed, and screening of seed lots may therefore be necessary.

2.4.2. Question 2. Do sexually-produced ascospores contribute to epidemic development and pathogen population structure (Task 1)?

Whilst small amounts of airborne *R. secalis* inoculum were collected in different seasons at different sites, there was no evidence that airborne inoculum played an important role in development of epidemics. No *R. secalis* DNA was detected in soil taken from the severely diseased rhynchosporium nursery site at SCRI (data not shown) demonstrating that soil is unlikely to be a source of inoculum.

2.4.3. Question 3. Can quantification of early symptomless *R. secalis* (by PCR) be used to predict rhynchosporium epidemic severity (Task 2)?

Substantial early symptomless infection was identified in winter barley crops but epidemic severity late in the season was largely dependent on amount of spring rainfall (which encouraged secondary disease spread by splash dispersal of pathogen spores). Therefore the PCR quantification of early *R. secalis* infection could not be used to predict epidemic severity late in the season accurately. A mild wet winter can spread the pathogen to adjacent plants and quickly disperse the pathogen throughout the crop. If infected seeds act as inoculum sources and mild winters allow rapid colonisation, wet periods could spread the pathogen throughout the crop. The mild wet period in 2007 allowed rapid colonisation and spread throughout the early part of the season so that when samples were taken at GS26 the amount of *R. secalis* DNA in plants grown from commercial seed and farmed saved seed was similar. For *R. secalis* to spread to upper leaves, the spores need to be dispersed by raindrop splashes. Therefore, if rain was a limiting factor, spread of the pathogen from lower leaves would not occur and the plant would escape infection as it grew away from the inoculum source. For example, in April 2007 there was little rainfall and thus limited spread of the inoculum, even though large amounts of *R. secalis* DNA had been recorded in leaves at GS26. When rainfall increased after April in 2007, no significant amounts of *R. secalis* DNA were recorded. Rainfall in April was greater in 2008 than in 2007 and 2009 and the amounts of *R. secalis* DNA recorded at GS39 were also significantly greater than those in 2007 and 2009. This indicates that rainfall is important in spreading the disease up the plant.

This work suggests that an epidemic of *R. secalis* on winter barley follows a bi-phasic pattern. Seed, volunteers and stubble can act as foci of early season infection in autumn/winter (Task 1). Colonisation of seedlings can occur quickly and this is often asymptomatic. Sporulation occurs even in the absence of visual disease symptoms and spores can be splash-dispersed to adjacent plants so that the disease quickly spreads. Mild, wet winters can increase the risk of colonisation and spread. Secondary spread from the lower leaves up the plant is dependent upon rainfall during

April. If there is little rainfall in April, severe late season epidemics will not occur irrespective of initial amounts of inoculum present (Figure 29).

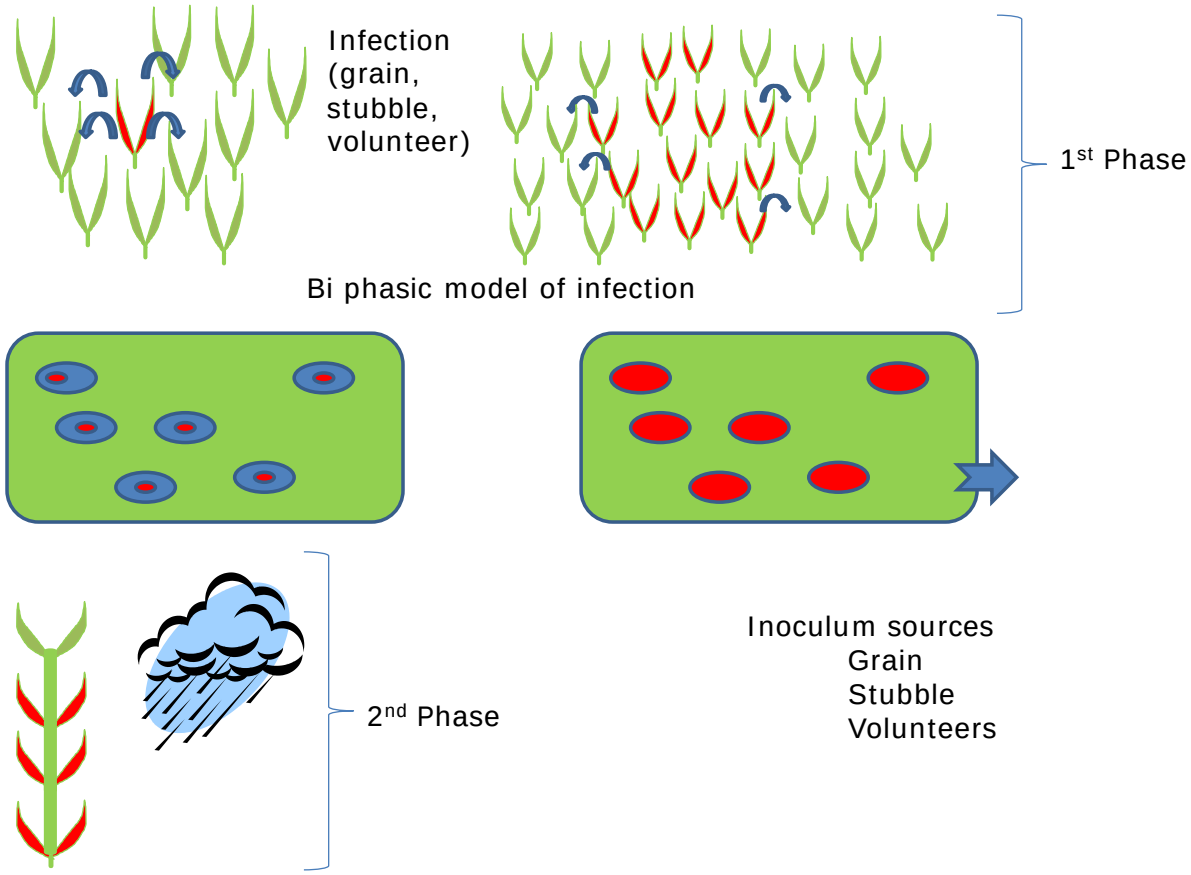


Figure 29. Diagrammatic representation of the bi-phasic pattern of a rhynchosporium epidemic in winter barley. The first phase is the increase of the foci of infection from an inoculum source of seed, stubble or a volunteer. The foci are increased by wet, mild winters. Spread outside the field is limited as there is no airborne dispersal. The second phase is rain-splash dispersal of spores up the plant from the lower source of inoculum. Rainfall in April is significant in determining if an epidemic will occur. Once the pathogen has spread then it can contaminate grain and continue the cycle. Stubble and volunteers can also continue the cycle.

The data discussed in this section demonstrate that molecular techniques can be used to indicate initial risk of an epidemic but cannot be used in isolation. Data on early infection need to be combined with other parameters, especially weather data in spring, to provide an overall prediction of late season epidemic risk. The molecular techniques provide useful tools to assess the asymptomatic colonisation that occurs early in the season and to screen seed batches to determine the amount of *R. secalis* DNA present. However, PCR measurements do not provide information about the viability of inoculum in any particular tissue and presence of large amounts of *R. secalis* DNA does not always mean that it poses a risk.

The data presented suggest that application of fungicide treatments could be better timed, particularly in high risk areas, both to prevent early asymptomatic colonisation and possibly to inhibit spread onto grain late in season. Large amounts of *R. secalis* DNA at GS39 in winter barley can lead to a decrease in yield. Spraying to limit the pathogen at this stage is important, especially if the months of April and May are wet. A dry period in April and May could mean that less spraying is needed to control the disease. It is difficult to determine whether control of symptomless *R. secalis* in winter barley with fungicides is associated with yield response, since fungicides affect other diseases. There is a need for a field experiment with a greater range of fungicide timings and combinations of timings and treatments in which the development of symptomless rhynchosporium and other diseases is followed in detail. The discovery that *R. secalis* can colonise barley crops extensively throughout the cropping season (from seed to seed) in the absence of visual symptoms has completely changed the understanding of the disease by industry (with implications for use of fungicides, breeding programmes and HGCA's barley cultivar Recommended List).

2.4.4. Question 4. Can quantification of early symptomless *R. secalis* (by PCR) can be used to understand barley cultivar resistance (Task 2)?

Use of quantitative PCR provided new insights into the operation of host resistance against *R. secalis*, especially in work with the barley mapping population. Markers associated with new sources of resistance to *R. secalis* have been identified in a barley mapping population, and methods to screen material for resistance have been improved. 190 lines from a doubled haploid population were grown and visual symptom data were taken at GS26 and GS50 and DNA samples taken at GS50. Mapping of these resistance traits revealed that the genetic basis of resistance to development of visual symptoms was closely related to that to accumulation of *R. secalis* DNA. The genetic data on components of resistance validated by knowledge of the presence and quantity of inoculum were of value not only in assessing cultivars for use in different deployment strategies but also for development of markers for use in breeding cultivars with improved resistance. These findings will be exploited in a new Technology Strategy Board project, led by a breeding company (KWS-UK). This information may be combined with information about the distribution of virulence factors in UK *R. secalis* populations to develop strategies for deployment of cultivars with different resistance genes.

2.4.5. Question 5. Why did severity of rhynchosporium epidemics increase in the mid-20th century in the UK (Task 3)?

Work with samples from the 150-year barley archive at Rothamsted provided unique insights into the long-term dynamics of *R. secalis* on barley crops. Temporal patterns in the amounts of *R. secalis* DNA over the past 40 years were found to be consistent with those recorded in the Defra/HGCA barley disease surveys since the 1970s. Analysis over the entire 150-year period, using quantitative species-specific PCR, confirmed the increase in severity of rhynchosporium epidemics observed in national surveys. An increase in the amount of *R. secalis* DNA found in the samples began to occur in the early 1960's. This coincided with the introduction of short-strawed cultivars (the first being Maris Badger). This increase may be the result of genetic susceptibility or the physical structure of the plant (i.e. the distance between the first leaf and the soil and the distance between leaves) meaning that splash dispersed soil-borne inoculum has a shorter distance to travel to reach the leaves and once on the lower leaves has shorter distances to travel to reach the next leaves higher up the plant. This work also provided information about the long-term dynamics of other barley pathogens, such as *Pyrenophora graminea*. Amounts of DNA of *P. graminea* were not great after the introduction of cv. Plumage Archer in the early 20th century; it might be that host resistance and/or the introduction of seed treatments from the mid 1920s onwards have been successful in maintaining a low incidence of this pathogen in seed.

2.4.6. Benefits to industry from this project

This project involved close collaboration with industry partners, who gained the following benefits from it:

- Scottish Agronomy. As one of the largest advisory/trials groups in the UK, it is important to us that any information that sheds new light on the disease is disseminated through advisors, allowing them to implement any changes to disease management that may benefit growers. Outputs from this project, such as identification of seed-borne transfer of the disease as significant and new diagnostic tools to track movement of the disease, will benefit the industry. For example, it will be easier and quicker to screen for new, more resistant barley cultivars and this will potentially reduce the requirement for fungicides.

- HGCA. The project has provided new information on the symptomless infection and colonisation of barley by *Rhynchosporium*. HGCA is currently funding several research and development projects on *Rhynchosporium* that will benefit from the knowledge gained and methods developed during this project. The results of the project have been communicated to growers through updating The Barley Disease Management Guide and through events such as Cereals.

- DuPont. DuPont have been delighted at the outcome of this project and the valuable insight it has given into the life cycle of the rhynchosporium pathogen. This better understanding of the sources of inoculum and development of the disease has re-ignited discussion on control of the disease and has led to a number of theories as to how existing and future chemicals might be better used. This has led DuPont to become part of the new TSB project to build upon this research to explore some of the theories relating to chemical usage whilst others further the genetic research.

- Masstock Arable. The barley crop is an important part of our customers' rotations, allowing early autumn sowing for winter oilseed rape which is the most effective break crop. However it tends to be treated less carefully than winter wheat and can therefore deliver variable yields, decreasing overall profitability. Masstock's involvement in this project has allowed us to better understand the most challenging part of barley crop management (i.e. reliable disease control) and to enable us to advise our customers how to achieve better consistency in this crop's performance.

- KWS. KWS UK Ltd is leading a new TSB project entitled 'Symptomless Infection of BarLey: resIstance breediNG and integrated crop protection Strategies (SIBLINGS)'. The project consortium includes SCRI and Rothamsted. This project aims to deliver more effective control of rhynchosporium of barley through understanding its life cycle. The project uses

new knowledge about the symptomless infection of barley obtained in this BBSRC LINK project to develop new cultivars that are more durably resistant to *Rhynchosporium* and therefore protected against yield loss. Novel molecular and microscopy methods using GFP-tagged pathogen isolates will be used to generate knowledge of resistance mechanisms. The project consortium includes DuPont and Masstock Arable, who will develop crop protection strategies that are strategically well directed for increased yield response and risk reduction. The proposal therefore represents a novel approach to both breeding for durable resistance and more effective crop protection with existing chemicals, whilst informing discovery strategies.

- As a result of work in this BBSRC project, two spin-out PhD projects have started. Both involve collaboration between SCRI and Rothamsted. One, based at SCRI and funded by HGCA, is examining symptomless growth of *Rhynchosporium* in plant tissues, using GFP-labelled isolates (PhD student A. Thirugnanasambandam). The other, funded by the Perry Foundation and based at Rothamsted, is examining the role of grass species in providing a source of inoculum to initiate epidemics (PhD student K. King).

2.4.7. Benefits to science from this project

Resources generated.

- New methods for quantifying amounts of *Rhynchosporium* DNA in infected barley leaf and grain samples were developed for use in this project and applied to the samples sent to Rothamsted by other members of the project consortium.

- New research from Switzerland is suggesting that the *Rhynchosporium* populations that affect barley, rye and some related grasses should be considered as three separate species, namely *Rhynchosporium commune* (barley), *R. secalis* (rye) and *R. agropyri* (couch grass); these three species are in addition to *R. orthosporum* that affects the grass cocksfoot. New end-point PCR primers were developed during this project to distinguish the DNA of these 4 species. These primers are being used in a spin-off PhD project (Perry Foundation project, Kevin King, based at Rothamsted).

- A unique time-series of barley/pathogen DNA from barley leaf/stem and grain samples (1852-2010) from the Hoosfield spring barley experiment at Rothamsted was produced. Work with the barley archive has provided unique insights into the long-term dynamics of *R. secalis* on barley crops (temporal patterns in the amounts of *R. secalis* DNA are similar to those recorded in the Defra/HGCA CropMonitor barley disease survey since the 1970s). It has also provided information about the long-term dynamics of other barley pathogens. These archive samples are also being exploited in other work, for example on the evolution

of specific fungicide resistance mutations in *Rhynchosporium* populations (BBSRC Syngenta CASE PhD project, Nichola Hawkins, based at Rothamsted).

- This project generated genotypes and a genetic map in a winter x spring mapping population that is available for further mapping of disease resistance and other traits that vary between spring and winter germplasm. Markers associated with new sources of resistance to *R. secalis* have been identified in a mapping population, and methods to screen barley material for resistance to *Rhynchosporium* have been improved. These findings will be exploited in a new Technology Strategy Board project, led by a breeding company (KWS-UK).

- A method was established for estimating the relative expression of disease symptoms (i.e. the severity of visual symptoms relative to the total amount of *R. secalis* DNA) from field trial data with qPCR (amount of pathogen biomass in host tissue, whether or not symptoms are present) and visual scores (area of leaves with lesions). This will be used in the new TSB project and is being used in other work.

- Plasmids used at Rothamsted in other BBSRC-funded work were provided to SCRI, who successfully used them to develop Green Fluorescent Protein (GFP) labelled strains of *Rhynchosporium secalis* and *Ramularia collo-cygni* for use in an HGCA funded PhD project that was a spin-out from this BBSRC LINK project (Amar Thirugnanasambandam).

2.4.8. Acknowledgements

We thank the Biotechnology & Biological Sciences Research Council for funding this Sustainable Arable LINK project, which had ‘in kind’ funding from HGCA, DuPont, Masstock Arable, KWS-UK and Scottish Agronomy and support from RERAD-funded staff at SCRI. We are grateful to Peter Street, SA LINK, Neil Paveley, LINK PMC Monitor and Amy Tayler, BBSRC, for attendance at and contribution to consortium meeting discussions on the direction of the project. We thank the many others who contributed to work described in this project, including Sandra Harvey, Rodger White, Teresa Godfrey, Rothamsted; James Lynott, Christine Hackett, JiaSui Zhan, SCRI; Mike Jeffes, Masstock; and David Ogunlana, DuPont. We also thank Judith Turner and her Fera colleagues for CropMonitor data and James Townsend for help with the report.

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