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Impact and interactions of *Ramularia collo-cygni* and oxidative stress in barley

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Abstract

Ramularia leaf spot, caused by the fungus *Ramularia collo-cygni,* has become an established disease in the north of the UK. This research shows the average yield loss associated with ramularia in spring barley ranged from 0.2 – 0.6 t/ha. The estimated cost to the malting barley industry was £10.68m at a grain price of £125/tonne.

The research shows that Recommended List (RL) varieties differ in susceptibility. In winter barley differences in leaf spot levels were not significant and no variety had effective resistance. In spring barley, Quench, Decanter, Oxbridge, Riviera, Rebecca and Jolika showed lowest disease levels. Cocktail, Doyen, Publican and Belgravia exhibited more symptoms, but all RL varieties showed better resistance compared to older varieties Pewter and Prestige. The greatest benefit to fungicide control of ramularia (1.3 t/ha) was with the susceptible spring barley variety Cocktail whilst there was no response with the resistant variety Decanter.

Airborne spores were released two days after a period of leaf wetness. Spore release from winter barley occurred too late to have a major impact on spring barley in the two high disease pressure years, 2005 and 2007. Seed infection and weather conditions played a greater role in the disease epidemic than airborne spores, which potentially play a role in seed infection late in the season.

- Quinone outside Inhibitor (QoI or strobilurin) fungicides no longer achieved control of ramularia and only contributed to 0.1 t/ha in yield with no benefits in green leaf area retention. This resistance mutation first occurred in the UK around 2001-2002, corresponding to the decline in field performance since then.
- Prothioconazole achieved effective control and the best yield response. Chlorothalonil achieved good control of symptoms, but no yield response and may have a role in situations where infection is predominately systemic.
- Morpholine fungicides showed no activity against ramularia. Yield losses from using this type of fungicide seen in 2002 were not observed in 2005-07.

The role of mlo mildew resistance gene on varietal susceptibility to ramularia is complex. Presence of mlo5 increases the susceptibility of the variety to ramularia particularly where the variety is stressed by light. Breeding for resistance should be an important aim for plant breeders if reliance on fungicides is to be reduced.

Project Summary

Introduction

Ramularia leaf spot caused by the fungus *Ramularia collo-cygni* is a relatively new disease of winter and spring barley in the UK. It has become a major disease in the north and is becoming increasingly common further south (Walters *et al.,* 2008 Appendix 3).

In 1999, when the disease started to cause economic damage to spring barley, the only solution available to growers was to use fungicides. Previous HGCA-funded research showed a fungicide treatment applied before symptoms developed at boot stage to ear emergence (GS49-59) was the best approach and that a strobilurin fungicide was an important component of the treatment (Oxley *et al* 2002). In the absence of information to determine disease risk or on varietal susceptibility this fungicide treatment became a routine treatment for barley in the north of the UK.

The HGCA Fungicide Performance research demonstrated poor control from strobilurin fungicides since the work started in 2003 (Oxley & Hunter 2005). The most effective fungicides in 2007 were epoxiconazole (Opus), chlorothalonil (Bravo), prothioconazole (Proline) and boscalid (in Tracker). No activity against ramularia is now seen with strobilurin fungicides, in contrast to earlier work where these fungicides were essential to control the disease (Oxley *et al.,* 2002). Some fungicides can increase symptoms and in 2002, use of fenpropimorph late in the season contributed to rapid leaf death in commercial crops. Current recommendations are to apply a fungicide mixture comprising a triazole (prothioconazole or epoxiconazole) with chlorothalonil +/- boscalid. A strobilurin is also recommended for activity against other diseases and potential green leaf area retention (Oxley & Burnett, 2008).

The aims of this research were: 1) to produce data required for a risk predictor and to test control measures using more resistant and susceptible varieties in UK field trials and 2) to investigate the role of *mlo* mildew resistance on barley leaf spots.

Importance of ramularia on yield

Spring barley

Determining yield loss associated with a single disease is a challenge since no fungicide is specific to a single disease and fungicides can increase yield in the absence of disease symptoms. Early work suggested early fungicide treatment at GS25-30 (T1) in spring barley gave poor control of ramularia (Oxley 2005).

This new research based on six varieties sown at different sites between 2005-07 compared the disease control and yield benefit from fungicides applied to the upper leaves early at flag leaf emergence GS37 (poor timing) or at awns peeping (well timed) at GS49 in resistant and susceptible barley varieties. The aim was to apply the same amount of fungicide to a crop but manipulate the level of ramularia symptoms. This approach was partially successful (Figure 1), but the earlier GS25-30 treatment was also shown to influence the development of ramularia. New fungicide advice will take account of this early contribution in high risk situations. The average yield loss from ramularia ranged between 0.2 – 0.6 t/ha. Yield losses on a susceptible variety can be higher and the largest was seen with Cocktail at 1.3 t/ha. The resistant variety Decanter did not respond to the fungicide treatment. This suggests that breeding for ramularia resistance is an important goal for plant breeders.

The GS45-49 timing continues to be a key timing to target ramularia, but timing of this later fungicide is important to ensure the best margin. If this treatment is applied too early at GS37, a grower may fail to realise an increase in the margin over the single early fungicide after taking account of fungicide costs (Figure 1).

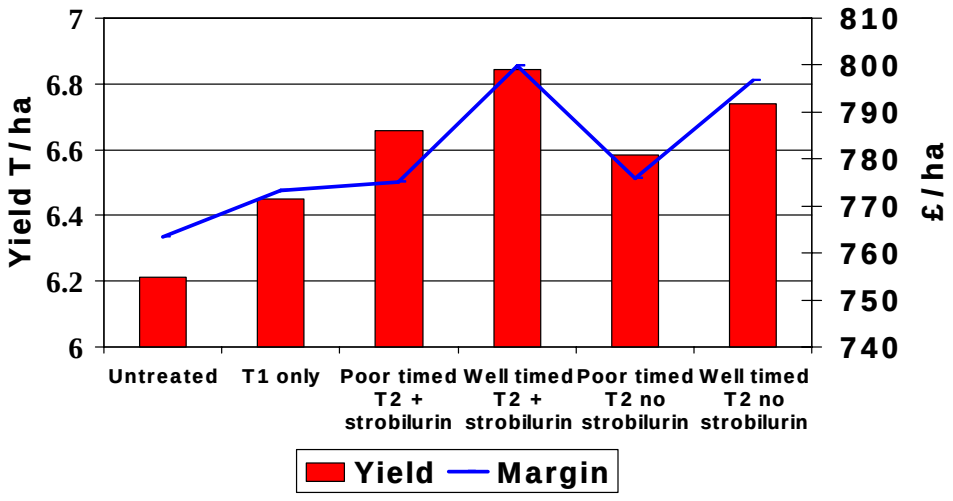

Figure 1 Crop yields and margin over fungicide in spring barley £125/t 05-07

The contribution of a strobilurin fungicide in a mixture has diminished in spring barley where ramularia is known to be resistant to this group of fungicides. This research showed a 0.1 t/ha response from 0.4 l/ha azoxystrobin (Amistar). This is not a significant increase in yield and at grain prices of £125/t this treatment will cost £10/ha for a return of £12.50/ha. There will be other diseases to consider which make the addition of the strobilurin fungicide important at boot stage however. If brown rust, for example, is a potential risk, the strobilurin component would be an important contribution to managing this disease. Although the contribution from azoxystrobin on net blotch has diminished, other strobilurin fungicides (e.g. pyraclostrobin. picoxystrobin or fluoxastrobin) will contribute to net blotch control at this timing.

Focussing on the individual fungicide components, prothioconazole (Proline) achieved the best control of ramularia, the highest yield and lowest screenings. Chlorothalonil (Bravo) also achieved good control of ramularia symptoms and green leaf area retention, but yield and quality were not as high as would be expected for the level of control. Proline therefore either achieved a yield above a level expected from disease control, or Bravo had the opposite effect. New research will focus on the amount of ramularia inside the leaf following treatment with these fungicides. It is suggested chlorothalonil may have little impact on systemic infection, but may be suppressing symptom development as well as protecting against secondary infections from airborne spores. Both fenpropimorph (Corbel) and azoxystrobin achieved poor control of ramularia and green leaf area retention. Both fungicides achieved a similar yield to the untreated. This suggests that beneficial physiological effects from a strobilurin fungicide in absence of disease control were not observed and the detrimental effects from a morpholine fungicide, which were observed in 2002, were not seen in these trials. Care should, however, be taken if a morpholine is applied late to a crop, since this treatment can increase levels of abiotic leaf spots.

Winter barley

Determining yield losses from ramularia in winter barley was a greater challenge. The assumption made was that ramularia is of less importance in winter barley in the UK, since leaf spots occur late in the season. Figure 2 shows the yields and margins from an early fungicide at T1 (GS31-32) and the "poor timing" at GS37 and "well timed" at GS45. Best yields and margins were observed with the "well timed no strobilurin"

treatment. The average benefit from the strobilurin fungicide was negligible, so where ramularia is the main target disease, there is no benefit from using this group of fungicides, but they may contribute towards managing other diseases including net blotch, brown rust and potentially head diseases. The yield benefit from the GS31-32 fungicide was 0.6 t/ha - an additional 0.5 t/ha from the GS49 fungicide (well timed) or 0.4 t/ha from the GS37 treatment (GS37).

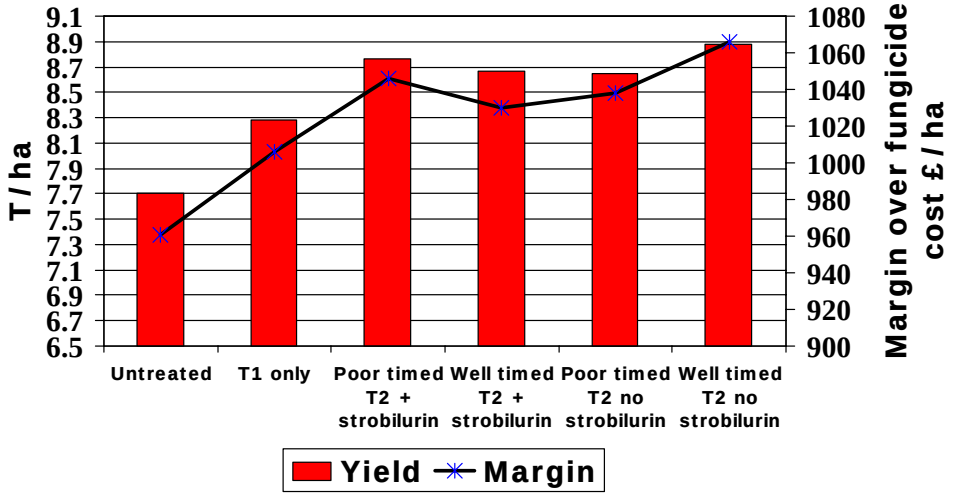

Figure 2 Crop yields and margin over fungicide cost in winter barley £125/t 2005-07

Fungicide resistance

Ramularia resistance to strobilurin fungicides was first suspected to be widespread in 2002 since disease control in the field was minimal compared to previous years. Previous research demonstrated azoxystrobin to be the most effective fungicide in 2000. Since chlorothalonil, prothioconazole and boscalid did not have an approval for barley at that time, azoxystrobin + epoxiconazole (Amistar + Opus) was a popular choice to manage ramularia. Another option used at boot stage was azoxystrobin + fenpropimorph (Amistar pro), particularly where the risk of powdery mildew was high. Use of this latter treatment resulted in crops dying back extensively in 2002. Possible reasons for this die-back are poor control of ramularia from either fungicide, and potential oxidative stress from the fenpropimorph formulation.

A molecular diagnostic test was developed to identify ramularia DNA within a Scottish Government-funded collaborative research. This test was developed further to look for

the single step mutation in the cytochrome b gene known to be linked to total fungicide resistance in other cereal pathogens, namely powdery mildew in wheat and barley and septoria tritici in wheat. This mutation is known as G143A and occurs at codon 143 in the cytochrome *b* gene.

The results show that resistance to strobilurin fungicides developed in populations of ramularia in both Scotland and Denmark. This mutation occurred around 2001-2002 in the UK, corresponding to the decline in field performance during this period. However, we do not know the extent of the distribution of these resistant alleles in the wider population. Levels may have increased year on year since the initial development as seen in other cereal pathogens such as septoria tritici in wheat.

The molecular diagnostic was developed to ensure it was specific to the mutation in ramularia and did not cross react with the same mutation in other pathogens. This test will enable leaf and seed material to be taken, the DNA extracted and the ratio of strobilurin sensitive to resistant ramularia DNA determined.

Future work will be performed using a range of different molecular techniques (real-time PCR and pyrosequencing) to measure the ratio of both the resistance and sensitive alleles conferring QoI resistance in populations of ramularia infected crops. This information will enable us to understand the dynamics of resistance development and the possible role of spore and seed infection in the ramularia life cycle. It is hoped that by gaining a greater understanding of the basic epidemiology and the ability of the fungus to develop fungicide resistance it may help in the development of effective control and anti-resistance strategies.

Spore dispersal and disease development

Information on airborne spores was collected at trial sites over three cropping seasons. Using a molecular diagnostic test, it was possible to measure the amount of ramularia DNA released on a daily basis at one of the trial sites. Ramularia spore release events were compared with the weather (rainfall, temperature, sunlight, humidity, leaf wetness) in an attempt to link spore movement with specific weather patterns. Air spore movement was also linked to visual disease symptoms to determine the importance of the spores as a source of infection. The assumption (or

hypothesis) made at the start of the research was that ramularia spores from winter barley could act as a key source of infection for spring barley.

Spore release events for *R. collo-cygni* were detected in mid to late summer and were very different to the results obtained for the barley pathogen rhynchosporium and the closely related cereal pathogen *Mycosphaerella graminicola* which causes septoria tritici in wheat. These other pathogens exhibited a number of spore release events; however, as they are both splash-borne diseases there is greater potential for spore movement. Results for *Ramularia* DNA indicate that there no movement via this method. Ramularia spore dispersal did correlate with leaf wetness and in each of the three years, spore dispersal occurred 48 hours after a prolonged period of leaf wetness. In two of the three years (2005 and 2007), spore dispersal occurred too late to play an important part in infection of winter or spring barley. See Figure 3 which shows spore dispersal and disease development for 2005. In 2006, where spore dispersal coincided prior to symptom development in the spring barley, visual symptoms in the crop were low. Ongoing research on seed infection suggests seed is a key method of infection for ramularia and not airborne spores. This research shows airborne spores play little part in the epidemic in the current season. They may however, play an important part in the infection of seed for the following season.

Early proposed life cycles for *R. collo-cygni* focused on the overwintering of the pathogen on winter crops and alternative hosts which produced inoculum to infect spring crops (Huss, 2006).

The results from the spore sampler work show that disease symptoms may be due more to seed borne transmission than external infection via spores. Recent work from Germany proposed that symptom expression in winter barley is independent of spore numbers or environmental conditions (Schützendübel *et al.,* 2008). This would imply that latent infections may be the major contributor to disease expression in Germany. Indeed study of disease development and ramularia DNA detection indicated symptom development begins in winter barley prior to spore release events.

More work is now required into potential seed treatments to control or reduce ramularia infections. In addition late season fungicide applications to protect seed crops may have to be considered.

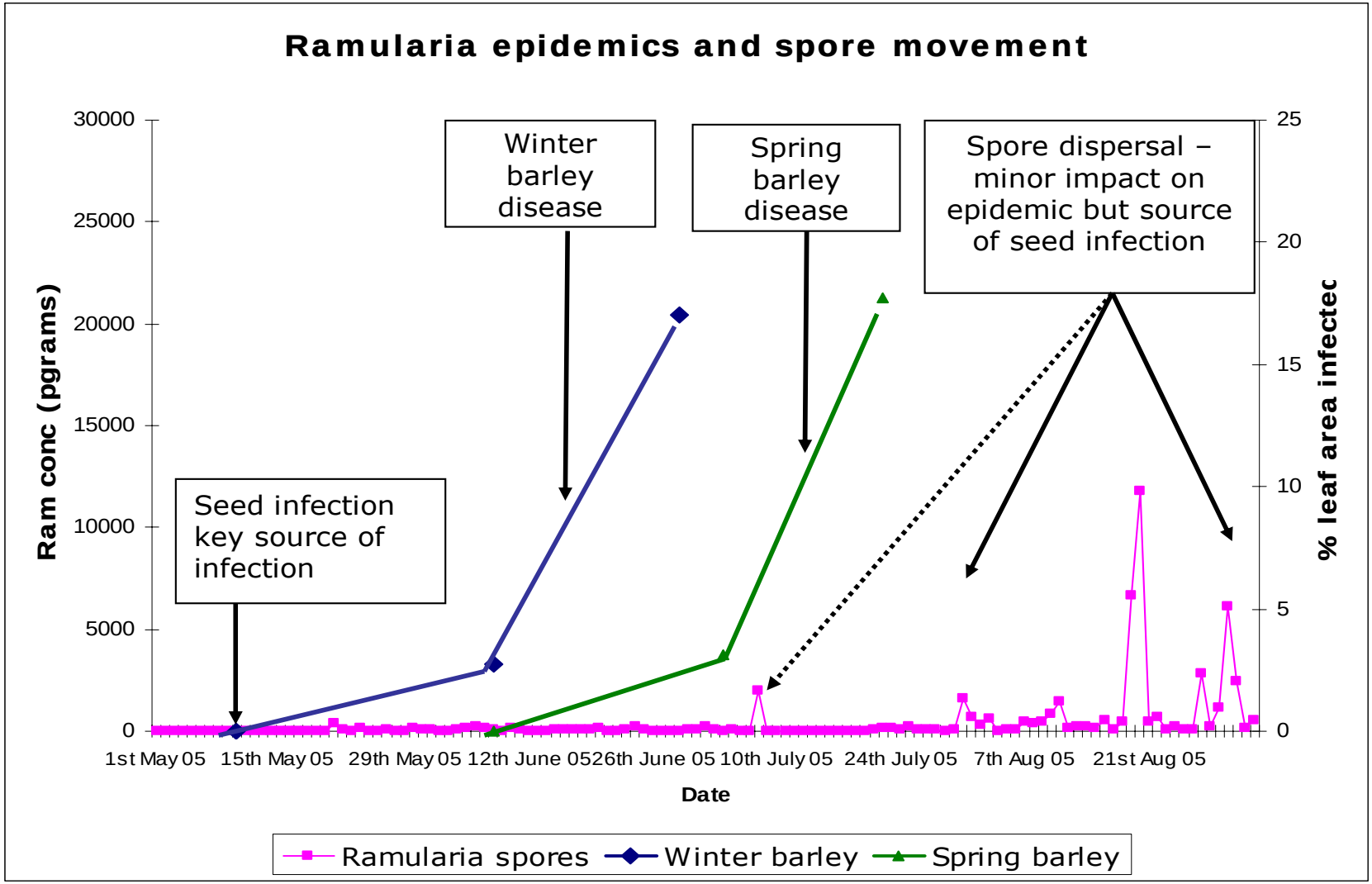

Figure 3 spore dispersal and disease development for 2005

Varietal resistance

Accurate information on varietal resistance to ramularia is challenging since the disease appears late in the season when most disease assessments have been completed. Identification of symptoms can also be a challenge. Expression of ramularia symptoms can also be affected by early disease. Where rhynchosporium, brown rust or powdery mildew infections are high, the upper leaves may be dead before the onset of ramularia. New research also suggests seed-borne infection is a key source of disease. Contamination of seed stocks with ramularia may therefore influence subsequent levels of disease. There may be an assumption that a variety susceptible to seed infection would also be susceptible to the disease. Assessments of the untreated Recommended List varieties were done over a number of seasons. This research was an opportunity to analyse the results to determine the accuracy of categorising varieties as susceptible or resistant to ramularia.

The results show there were varietal differences in susceptibility to ramularia, abiotic leaf spots and also green leaf area retention associated with the leaf spots. Figure 4 shows levels of ramularia in the recommended List spring barley varieties. Decanter was a consistently good variety for showing lower levels of ramularia leaf spots. The same variety also gave a low response to late fungicide. Cocktail was more susceptible to the disease and response to fungicide was greater. Care is required for varieties where little field data is available. Doyen, for example, showed little disease in the first year of testing, but later assessments showed greater levels of disease. Publican and Quench also showed similar levels of disease in the low disease pressure year, but greater differentiation occurred in a high disease pressure year (2007).

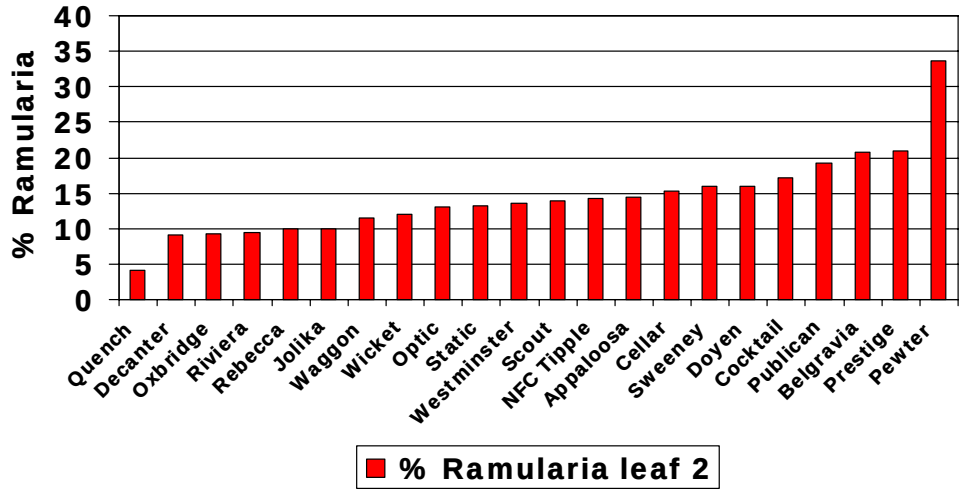

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Figure 4 % Ramularia on spring barley recommended List varieties 2008 and two susceptible varieties

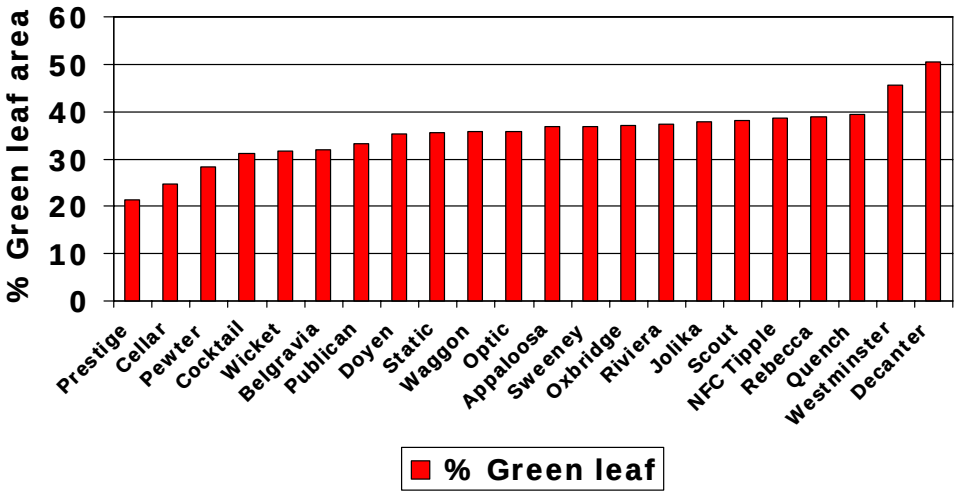


Figure 5 % Green leaf on spring barley recommended List varieties 2008 and two susceptible varieties

Assessing ramularia susceptibility in winter barley remains a greater challenge (Figure 6). The chance of early diseases including rhynchosporium affecting the upper leaves before ramularia develops is greater. Differences between the varieties were not significant, suggesting there is no major varietal resistance to ramularia in winter

barley. Green leaf scores may provide a good indicator of disease risk (Figure 7). Untreated Retriever, for example, can lose green leaf prematurely and field observations suggest this is due to the presence of ramularia. Overall ramularia levels were not, however, high based on disease scoring of Recommended List trials.

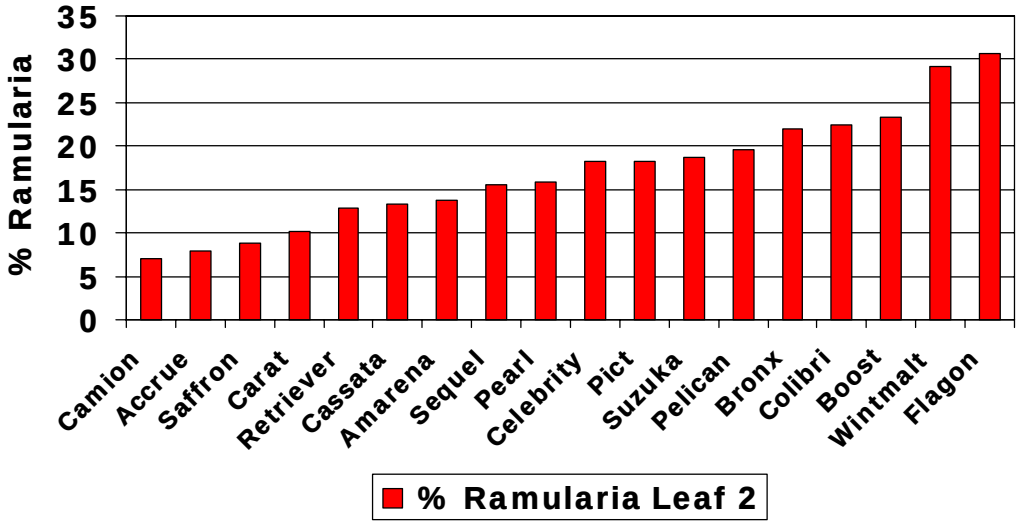


Figure 6 % Ramularia on winter barley recommended List varieties 2008

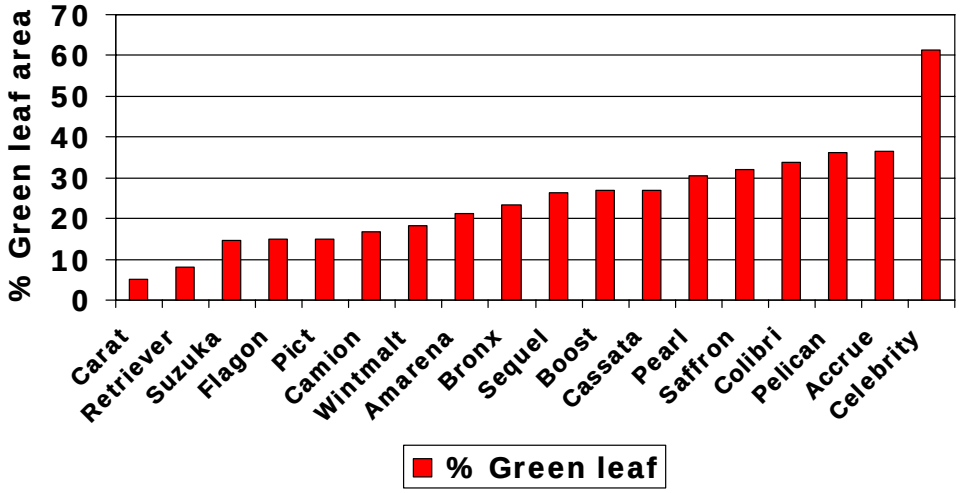


Figure 7 %Green leaf on winter barley recommended List varieties 2008

Role of mlo mildew resistance on ramularia

The role of the mildew resistance gene (mlo gene) on the susceptibility of varieties to ramularia was investigated. Ramularia first appeared to be a commercial problem at a time when many susceptible spring barley varieties had this type of mildew resistance (e.g. Chariot). The results from the field studies showed that mlo resistance alone was not the cause of the increase in ramularia. In fact varieties with mlo resistance may partially contribute to varietal resistance. Varieties with mlo resistance were, however, more susceptible to abiotic leaf spotting when placed under a high light stress prior to infection. As such, use of this resistance may initially help reduce infections by ramularia, but stressed mlo varieties may exhibit greater symptom expression. This was an important discovery which requires more detailed study to determine if damage caused by ramularia occurs in its pre-symptomatic phase, or only where symptoms have been expressed.

Later laboratory studies showed the presence of mlo5 gene was consistently associated with greater varietal susceptibility to ramularia. This was most pronounced where crops were stressed with moderate or high light prior to inoculation. The conclusion therefore was there is a three way interaction between mlo genes in the plant, the pathogen and the environment.

A laboratory method was developed which enabled symptoms of ramularia to be induced by inoculating seedlings with ramularia and inducing stress on the plants before and after inoculation. It was important to give plants a high light stress before inoculation. The importance of light stress after inoculation was of lower importance. The results confirmed that *Ramularia collo-cygni* is responsible for causing the typical symptoms associated with the disease known as ramularia leaf spot. Results on the importance of seed infection were known at the time these experiments were run, and seed stocks used were tested and found to be free from ramularia at the start of the experiments.

Decision tool

On the basis of this research in collaboration with research funded by the Scottish Government on barley pathology, several important pieces of information were identified which are key to developing a decision tool to determine the risk of disease. Others considered to be important may take lower priority in the decision to determine risk of crop loss. A revised lifecycle (Figure 8) was developed on the basis of the results.

Factor	Importance	Action
Seed infection	**High.** Ramularia in seed is major source of infection	Focus on seed health late in season for seed crops Look at potential seed treatment options Test seed to determine risk
Varietal resistance	**High.** Susceptible spring barley varieties respond more to fungicide) Mlo gene can increase expression of symptoms under high light, but may reduce risk of initial infection	Continue to assess varieties for Ramularia resistance. Take account of seed infection as part of the assessment
Fungicide choice	**High**. Strobilurins are resistant and contribute little to disease. DMI fungicides are essential to current control and subsequent yield response. Chlorothalonil contributes to disease control, but less on yield response. Contribution of late foliar fungicides to seed infection is unknown	Fungicide timings and choice can be tailored to high risk varieties and regions.
Spores	**Medium to High**. Spores may play a limited role in any season. They may play an important role in seed infection	Measuring spore release is useful for research and potential seed infection.
Seasonal weather	**High**. Weather and soil conditions which induce stress in glasshouse situations can be extrapolated to field	Waterlogged soils, wet weather and sunshine periods can be used to determine risk of later infection

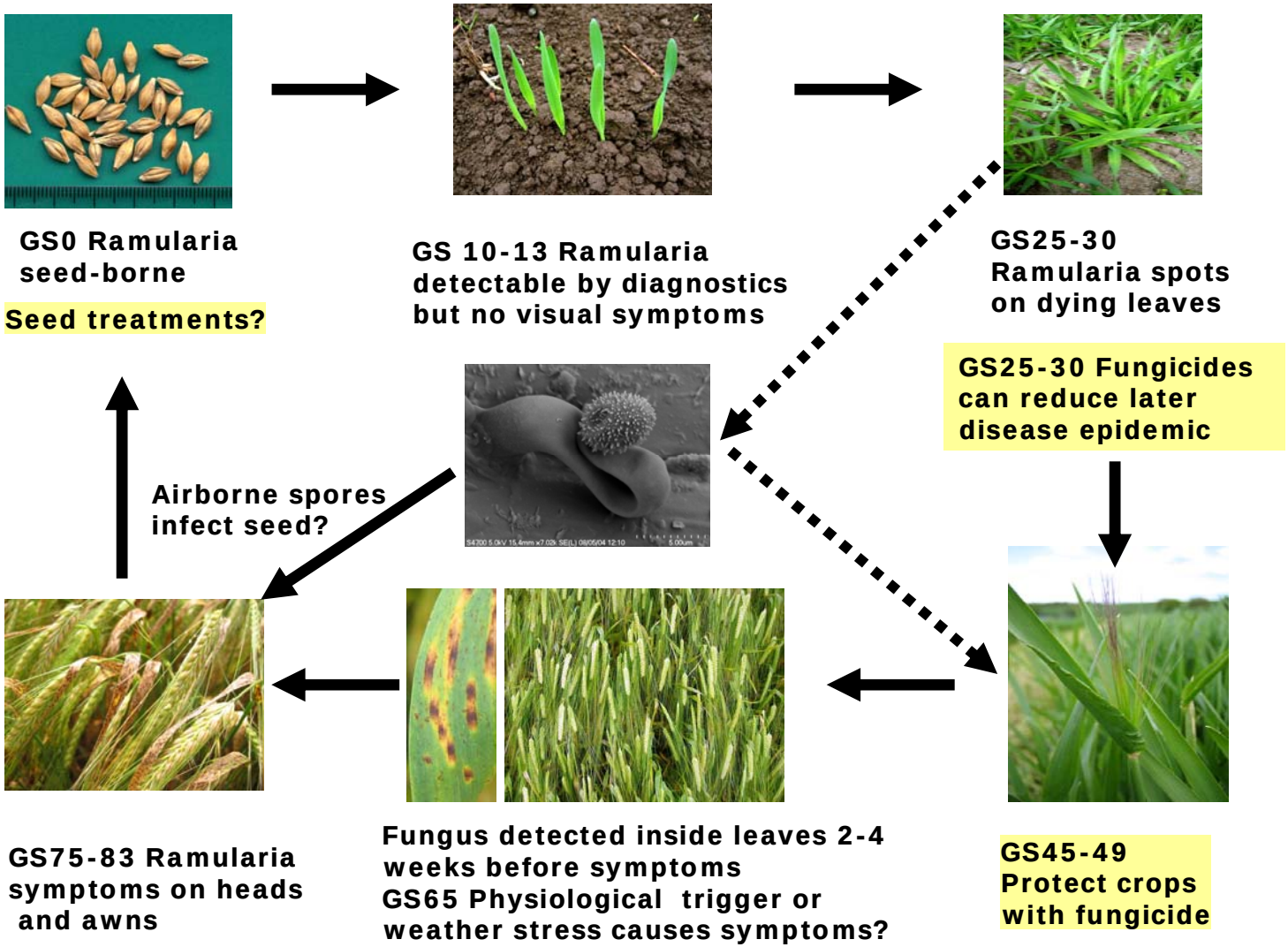


Figure 8 Ramularia lifecycle in barley

Full Project Report

General introduction

Ramularia collo-cygni is now recognised as an important pathogen of barley in Northern Europe and New Zealand. It induces necrotic spotting and premature leaf senescence, leading to loss of green leaf area in crops, and can result in yield losses of up to 25 %. The fungus produces a number of anthraquinone toxins called rubellins, which act as host non-specific toxins with photodynamic activity. These toxins induce lipid peroxidation and are possibly the cause of the chlorosis and necrosis observed in leaves infected with *R. collo-cygni.* The fungus can remain latent in barley plants until flowering, coupled with its very slow growth *in vitro,* makes it difficult to detect in crops. As a result, the epidemiology of this pathogen remains poorly understood. However, the recent development of rapid and reliable PCR methods for specific detection of *R. collo-cygni* offers the prospect of increased understanding of its epidemiology and improved disease control. (Dale et al. 2007).

The overall aim of this research was to determine the conditions in which different barley varieties are at greatest risk from ramularia and abiotic leaf spots and to formulate advice on ways to manage the risk.

To achieve this aim, this report comprises two sections to address two specific objectives:-
a) To produce data required for a risk predictor and test control measures using more resistant and susceptible varieties in UK field trials
b) Investigate the role of *mlo* mildew resistance on barley leaf spots. The following two sections address these two objectives.

a) Produce data required for a risk predictor and test control measures using more resistant and susceptible varieties in UK field trials

Introduction

Ramularia collo-cygni (ramularia) has become a major barley disease in the UK and Eire. It became a major economic disease in 1998 (Oxley *et al* 2002), when many crops of the variety Chariot died back prematurely, leading to losses in yield and quality. The disease has been present every year since 1998, but the severity of the disease varies, depending upon the variety and the weather conditions between tillering and ear emergence. Dull wet weather during this period can lead to a higher incidence of the disease.

Current control measures rely on foliar fungicides applied at booting growth stage (GS45-49), before leaf spots appear. Not all fungicides however are effective, and some, including fenpropimorph can be detrimental, leading to rapid loss in green leaf (Oxley *et al* 2002). Strobilurin fungicides were initially one of the best fungicide solutions to manage this disease (Havis et al 2002). Performance in HGCA funded appropriate fungicide dose trials in recent years (Oxley et al 2006) shows they no longer give good control.

Longer-term solutions to ramularia leaf spots will rely upon varietal resistance. Yield and quality however remain high priorities for plant breeders. Before investing in a programme for ramularia resistance, breeders require more information that Ramularia is a disease worth investment. Information was also missing on the potential loss in yield and quality associated with Ramularia and varietal resistance.

In the first section of this report, the aim of the trials will be to determine the yield loss associated with ramularia leaf spots in barley varieties using varieties with differing susceptibilities to ramularia leaf spot. The results from this will assist breeders to determine the benefits associated with varietal resistance to this disease complex.

The role and importance of the strobilurin fungicide will also be measured in these trials. Although there had been a decline in their activity against ramularia leaf spots,

they may have other benefits in maintaining green leaf area and a reduction in abiotic leaf spots leading to a benefit in yield. As part of the complementary funding by the Scottish Government, the cause of this decline in activity of the strobilurin fungicides to ramularia will be investigated. The hypothesis to be tested is that resistance to strobilurin fungicides has occurred as a result of a single mutation in the mitochondrial DNA in the cytochrome b gene.

Potential negative effects of other fungicides were also investigated. The negative impact of fenpropimorph was documented in previous studies. Field studies were done to measure this impact over two seasons on different varieties.

The impact of weather on spore dispersal is another focus of the complementary research funded by the Scottish Government. All these different aspects studies in this project will form the basis of a ramularia risk forecast which will form part of the conclusions in this section.

Materials and Methods

Development of *Ramularia collo-cygni* in barley varieties and its impact on yield

Spring barley yield loss trials were carried out at two sites in Scotland in 2005 and 2006 and at three sites in 2007. A winter barley trial was sown at one site in each of the three years.

The spring barley varieties used in the trials are listed in Table 1. They were selected to cover a spread of disease susceptibility to Ramularia leaf spots. Poker, Decanter and Oxbridge were the most resistant. Prestige and Cocktail, Cellar and Optic were the most susceptible. Other varieties (Troon and Westminster) were classified as intermediate in susceptibility. Optic, Poker and Prestige were sown in each of the three years of trials. Trials were fully randomised in complete blocks with three replicates.

Table 1 Spring barley varieties in yield loss trials 2005-07

Variety	2005	2006	2007
1	Optic	Optic	Optic
2	Poker	Poker	Poker
3	Prestige	Prestige	Prestige
4	Cellar	Cocktail	Cocktail
5	Westminster	Oxbridge	Oxbridge
6	Troon	Decanter	Decanter

The winter barley varieties are listed in Table 2. Little was known of the varietal susceptibility in winter barley, so a range of two and six row varieties were chosen in 2005. As new varieties entered the Recommended List, and others were removed, the varieties used changed in 2006 and 2007 to provide information on their susceptibility to ramularia leaf spots. Pearl and Colossus were common to all three years trials. Trials were fully randomised in complete blocks with three replicates.

Table 2 Winter barley varieties in yield loss trials 2005-07

Variety	Name 2005	Name 2006	Name 2007
1	Sumo	Saffron	Saffron
2	Sequel	Sequel	Retriever
3	Pearl	Pearl	Pearl
4	Regina	Amarena	Amarena
5	Pict	Camion	Camion
6	Colossus	Colossus	Colossus

Fungicide treatments for the winter and spring barley trials are described in Table 3. previous research showed the later fungicide treatments gave the best disease control. The untreated control (Treatment 1) will be compared to the early fungicide treatment (Treatment 2) to determine the yield and disease impact of the early fungicide. Treatments 3 and 4 comprised the same fungicides used either at the optimum (GS49) timing or sub optimum timing (GS37). In both these treatments, the same fungicides were applied to the upper leaves.

Treatments 5 and 6 followed the same fungicide timings at treatments 3 and 4, but the strobilurin fungicide component was omitted. These treatments will help determine the importance of the strobilurin fungicide on ramularia leaf spots.

Table 3 Fungicide treatments in yield loss trials (Dose rates in l/ha)

	GS25 (T1)	GS37 (T2 poor timing)	GS45-49 (T2 well timed)	Comment
1	Nil	Nil	Nil	Untreated
2	Proline 0.4 + Acanto 0.5	Nil	Nil	T1 only
3	Proline 0.4 + Acanto 0.5	Proline 0.4+ Bravo 1.0 + Amistar 0.5	Nil	Poor timed T2 + strobilurin
4	Proline 0.4 + Acanto 0.5	Nil	Proline 0.4 + Bravo 1.0 + Amistar 0.5	Well timed T2 + strobilurin
5	Proline 0.4 + Acanto 0.5	Proline 0.4+ Bravo 1.0	Nil	Poor timed T2 no strobilurin
6	Proline 0.4 + Acanto 0.5	Nil	Proline 0.4 + Bravo 1.0	Well timed T2 no strobilurin

Amistar – azoxystrobin 200 g/l
Acanto – picoxystrobin 250 g/l
Proline – prothioconazole 250 g/l
Bravo – chlorothalonil 500 g/l

Leaf wax - Impact of fungicides on ramularia

Two spring barley trials were sown in 2005 and 2006. The varieties used were the same as used in the yield loss trials and are listed in Table 4.

Table 4 Spring barley varieties in leaf wax trials

Variety	2005	2006
1	Optic	Optic
2	Poker	Poker
3	Prestige	Prestige
4	Cellar	Cocktail
5	Westminster	Oxbridge
6	Troon	Decanter

The treatments applied are listed in Table 5. They all comprised a standard early fungicide treatment which was common to the yield loss trials. At boot stage (GS45-49), a half dose of each fungicide was applied. The fungicides Bravo, Proline and Amistar were the single components used in the yield loss trials. This trial would determine the benefit of each single fungicide component on disease control and yield.

Table 5 Fungicide treatments in leaf wax trials

	GS25 (T1)	GS37	GS45-49 (T2 well timed)	Comment on impact on leaf spots
1	Proline 0.4 + Acanto 0.5	Nil	Nil	No T2
2	Proline 0.4 + Acanto 0.5	Nil	Bravo 1.0	"good product"
3	Proline 0.4 + Acanto 0.5	Nil	Proline 0.4	"good product"
4	Proline 0.4 + Acanto 0.5	Nil	Corbel 0.5	"Poor product"
5	Proline 0.4 + Acanto 0.5	Nil	Amistar 0.5	"Poor resistant product"
6	Proline 0.4 + Acanto 0.5	Nil	Tracker 0.75	"new good product"

Amistar – azoxystrobin 200 g/l
Acanto – picoxystrobin 250 g/l
Proline – prothioconazole 250 g/l
Bravo – chlorothalonil 500 g/l
Tracker – epoxiconazole 67g/l + Boscalid 233g/l
Corbel – fenpropimorph 750 g/l

Results

Development of Ramularia collo-cygni in barley varieties and its impact on yield

The average results from all trials, varieties and sites over three cropping seasons are presented in the following tables. These provide information on the impact Ramularia has on yield, margin, disease and quality over a wide range of environmental situations. Later results focus on the impact of ramularia in low and high disease pressure situations categorising varieties on their susceptibility to the disease.

Where data was transformed for the analysis, a second table shows the means as %. The Figures provide a visual description of the untransformed data.

Spring Barley

Yields

The average yields for the fungicide treatments from all the spring barley varieties in all three years are presented in Table 6 and Figure 9.

Table 6 Yields (T/ha) for spring barley varieties 2005-07 trials

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	5.466	5.619	5.899	5.919	5.756	5.891
2005 Perth	6.385	6.609	6.815	6.921	6.704	6.761
2006 Bush	7.064	7.333	7.539	7.777	7.338	7.703
2007 Bush	5.666	5.776	6.116	6.315	6.061	6.139
2007 Lanark	6.045	6.362	6.575	6.941	6.605	6.822
2007 Perth	6.638	7.01	7.009	7.198	7.036	7.12
Average	6.211	6.452	6.659	6.845	6.583	6.739
Mean sed for comparison of treatments within trial				0.1673		
LSD				0.3346		

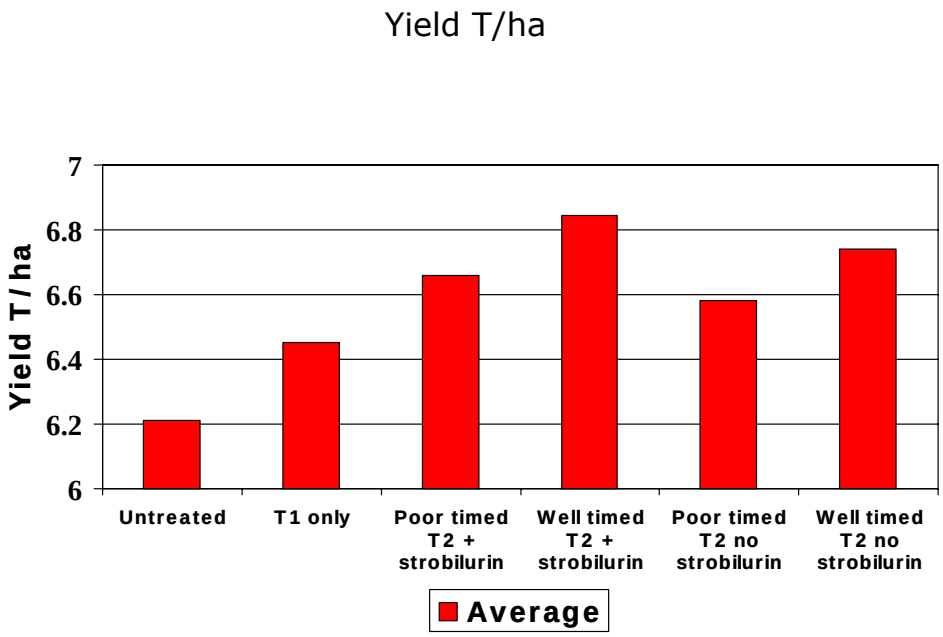


Figure 9 Average Yields based on all varieties, sites and years

The order of yield increase follows a pattern with the lowest yield from the untreated control. Next the early fungicide alone, followed by the poorly times T2 with the best yield from the well timed T2. This was the order of yield response which was expected from the experimental hypothesis. Although there was a trend towards the strobilurin fungicide increasing yield when applied at the T2 timing, the increase was small at 0.106 T/ha

Margin over fungicide cost

Table 7 and Figure 10 show the margin over fungicide using a grain value of £125/tonne.

Table 7 Margin over fungicide cost £125/tonne

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	662.8	658.8	677.6	670.1	666.8	678.4
2005 Perth	796.1	800.4	800.3	810.3	794.2	797.6
2006 Bush	879.8	892.4	891.9	925.1	872	927.4
2007 Bush	702	689.6	712.4	740.1	716.5	725
2007 Lanark	711	741	745.5	805.7	765.4	803.2
2007 Perth	828.2	857.5	822.5	847.7	840.8	849.1
Average	763.3	773.3	775.0	799.8	776.0	796.7
Mean sed for comparison of treatments within trial				23.32		
LSD				46.64		

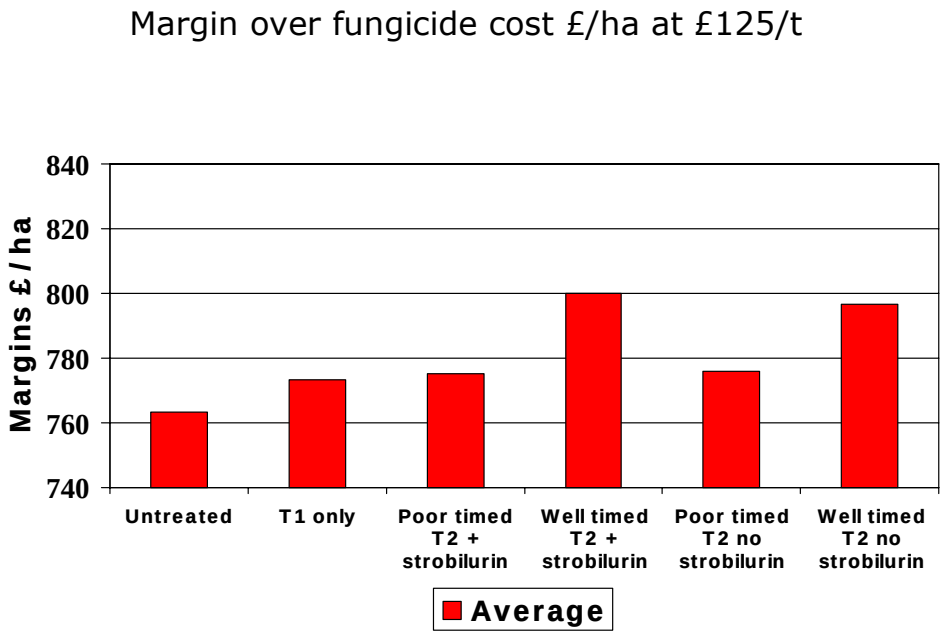


Figure 10 Margin over fungicide costs at £125/tonne 2005-07

The best margin over fungicide cost was obtained from the two spray treatment where the second fungicide was applied at GS49 using a strobilurin fungicide. This shows that despite a minimal yield benefit from the strobilurin, it did pay dividends at £125/tonne. The margin from the two spray programme where the second treatment was applied early was, however, similar to the single treatment. This shows the importance of timing of the second fungicide treatment to achieve the best return.

Ramularia development

Ramularia levels on final leaf 2 in July are shown in Tables 8 and 9 and in Figure 11.

Table 8 Log Late Ramularia on Leaf 2

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	3.408	3.436	1.251	0.729	1.422	0.874
2005 Perth	2.536	0.286	0.14	0.139	0.038	0.115
2006 Bush	2.902	2.95	1.045	0.261	0.99	0.487
2007 Bush	3.026	2.888	1.799	0.223	1.356	0.122
2007 Lanark	2.526	2.477	2.429	1.961	2.458	1.996
2007 Perth	2.312	1.86	2.159	1.515	1.731	1.48
Average	2.785	2.316	1.471	0.805	1.333	0.846
Mean sed for comparison of treatments within trial				0.2069		
LSD				0.4138		

Table 9 % Late Ramularia Leaf 2

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	29.20	30.06	2.49	1.07	3.15	1.40
2005 Perth	11.63	0.33	0.15	0.15	0.04	0.12
2006 Bush	17.21	18.11	1.84	0.30	1.69	0.63
2007 Bush	19.61	16.96	5.04	0.25	2.88	0.13
2007 Lanark	11.50	10.91	10.35	6.11	10.68	6.36
2007 Perth	9.09	5.42	7.66	3.55	4.65	3.39
Average	15.20	9.14	3.35	1.24	2.79	1.33

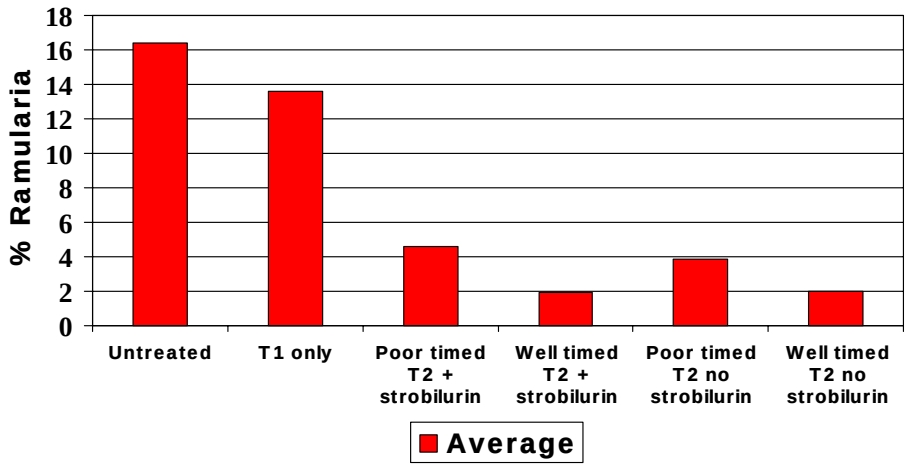


Figure 11 Ramularia levels in July on leaf 2

The well timed treatment achieved the best control. Note the strobilurin component in the fungicide mixture did not contribute to disease control. Levels of disease were higher in the poor timed fungicide treatment, but this treatment also achieved good control. A low level of disease control was achieved from the early treatment alone.

Tables 10, 11 and Figure 12 show ramularia levels presented as area under disease progress curve. This is a better representation of the disease epidemic over the season.

Table 10 Log Area Under Disease Progress Curve (AUDPC)

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	5.254	5.356	2.781	2.643	2.902	2.637
2005 Perth	4.571	1.938	1.453	1.836	1.645	1.766
2006 Bush	4.854	4.43	3.145	3.119	3.69	3.219
2007 Bush	5.481	4.997	3.903	2.826	3.735	2.49
2007 Lanark	5.357	4.976	4.906	4.305	4.94	4.355
2007 Perth	4.816	4.068	4.369	3.809	4.032	3.703
Average	5.056	4.294	3.426	3.090	3.491	3.028
Mean sed for comparison of treatments within trial				0.3053		
LSD				0.6106		

Table 11 Area Under Disease Progress Curve (AUDPC)

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	190.33	210.88	15.14	13.06	17.21	12.97
2005 Perth	95.64	5.94	3.28	5.27	4.18	4.85
2006 Bush	127.25	82.93	22.22	21.62	39.04	24.00
2007 Bush	239.09	146.97	48.55	15.88	40.89	11.06
2007 Lanark	211.09	143.89	134.10	73.07	138.77	76.87
2007 Perth	122.47	57.44	77.96	44.11	55.37	39.57
Average	155.88	72.27	29.76	20.97	31.81	19.66

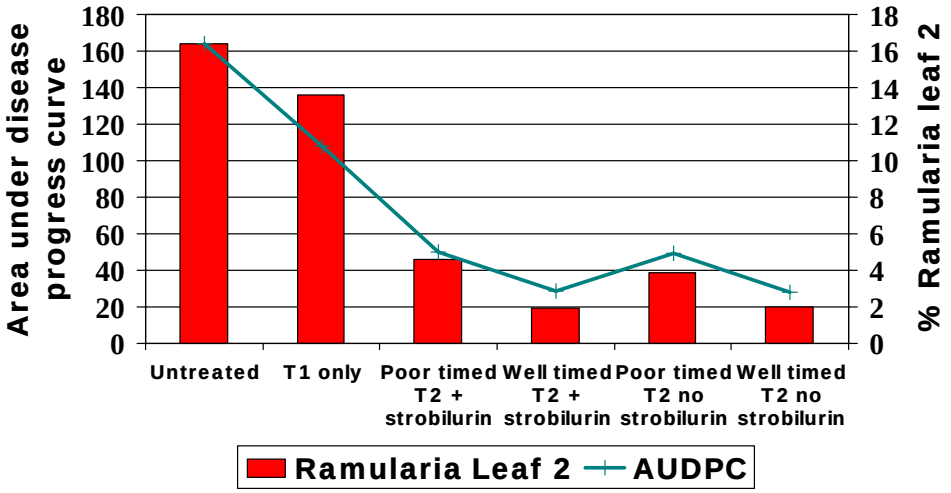


Figure 12 Comparison of late ramularia assessment in July with area under disease progress curve

The area under disease progress curve shows the T1 fungicide has had a greater impact on disease development than the single late assessment suggested. This new information shows the T1 has a greater influence on the ramularia epidemic than previous work shows. Part of the yield benefit from the T1 fungicide may be due to ramularia control, but the majority of the yield benefit associated with the control of ramularia comes from a well timed T2 treatment. The addition of a strobilurin has had no impact on the disease.

Quality assessment

Table 12 and 13 and Figure 13 show how the treatments impact on the screenings through a 2.5 mm sieve. This is a good measure of quality for the malting barley market. Penalties in grain price will be imposed for every 1% increase in screenings above 10%.

Table 12 Log Screenings (<2.5 mm sieve) for spring barley varieties in response to a range of fungicide programmes.

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	2.708	2.532	2.338	2.347	2.33	2.446
2005 Perth	0.458	0.366	0.255	0.198	0.251	0.264
2006 Bush	1.046	0.917	0.806	0.754	0.754	0.832
2007 Bush	2.265	2.172	2.047	1.97	2.055	1.973
2007 Lanark	2.853	2.63	2.497	2.419	2.499	2.415
2007 Perth	1.661	1.557	1.476	1.425	1.462	1.487
Average	1.832	1.696	1.570	1.519	1.559	1.570
Mean sed for comparison of treatments within trial				0.07033		
LSD				0.14066		

Table 13 % Screenings (2.5 mm sieve)

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	15.00	12.58	10.36	10.45	10.28	11.54
2005 Perth	1.58	1.44	1.29	1.22	1.29	1.30
2006 Bush	2.85	2.50	2.24	2.13	2.13	2.30
2007 Bush	9.63	8.78	7.74	7.17	7.81	7.19
2007 Lanark	17.34	13.87	12.15	11.23	12.17	11.19
2007 Perth	5.26	4.74	4.38	4.16	4.31	4.42
Average	6.25	5.45	4.81	4.57	4.75	4.80

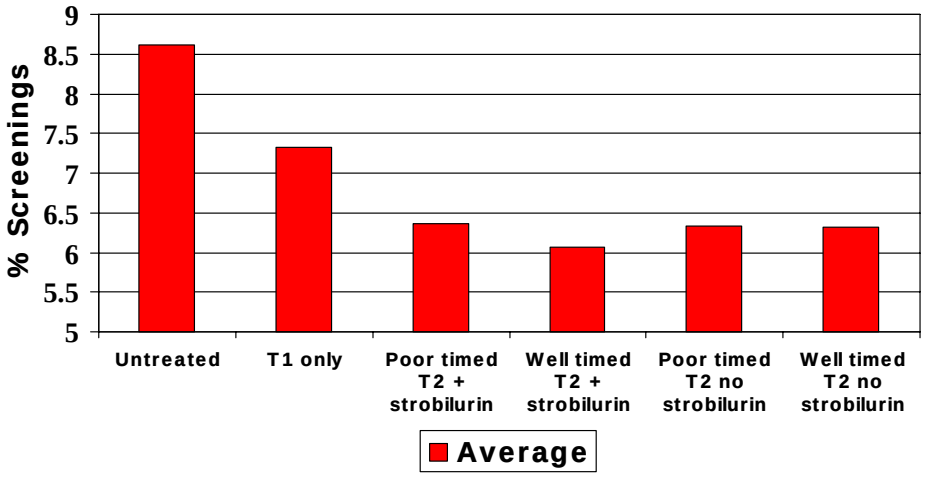


Figure 13 % Screenings (2.5 mm sieve) in field trials 2005-07

In the average data, all treatments were below 10%. Individual trials show how there was major variations between the sites. Quality increased with an increase in yield, with the lowest screenings seen in the well timed T2 + strobilurin. The T1 treatment did however improve quality over the untreated.

Green leaf area retention

Tables 14, 15 and Figure 14 show the impact of the treatments on late green leaf area on final leaf 2.

.Table 14 Angular Green leaf area in spring barley varieties 2005-07

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	16.81	21.96	52.01	54.64	49.67	55.51
2005 Perth	41.65	59.11	59.46	62.33	60.64	61.46
2006 Bush	44.43	50.1	61.96	73.15	64.62	64.7
2007 Bush	45.11	50.85	66.28	75.03	66.3	72.9
2007 Lanark	30.07	41.86	46.03	56.56	46.26	51.49
2007 Perth	42.1	50.48	46.37	55.4	52.2	51.76
Average	36.695	45.73	55.35	62.85	56.62	59.63
Mean sed for comparison of treatments within trial				3.709		
LSD				7.418		

Table 15 % Green leaf area in spring barley varieties 2005-07

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	8.36	13.98	62.11	66.51	58.11	67.93
2005 Perth	44.17	73.64	74.18	78.44	75.96	77.17
2006 Bush	49.01	58.85	77.90	91.60	81.63	81.74
2007 Bush	50.19	60.14	83.82	93.33	83.84	91.35
2007 Lanark	25.11	44.53	51.80	69.63	52.20	61.23
2007 Perth	44.95	59.51	52.39	67.76	62.43	61.69
Average	35.71	51.27	67.68	79.18	69.72	74.45

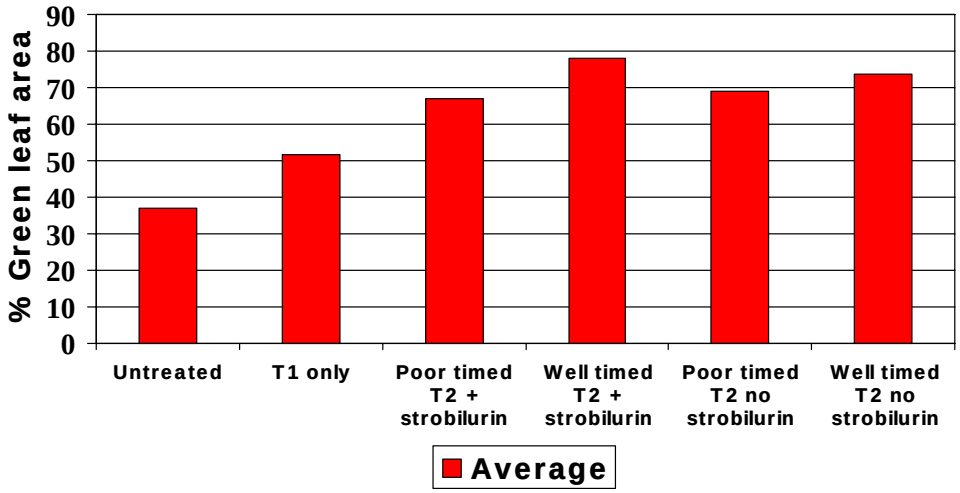


Figure 14 % Green leaf late in season 2005-07 trials

The best yielding treatments also achieved the best green leaf area retention. The T1 only treatment also had an influence on late green leaf area, despite the fact that no fungicide had been applied to the upper leaves assessed.

Winter Barley

Yields

Table 16 and Figure 15 shows the yields from three winter barley trials.

Table 16 Crop yields in winter barley trials 2005-2007

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	8.25	8.82	9.32	9.226	9.202	9.428
2006 Bush	8.433	8.986	9.487	9.363	9.366	9.595
2007 Bush	6.435	7.027	7.491	7.398	7.386	7.608
Average	7.71	8.28	8.77	8.66	8.65	8.88
Mean sed for comparison of treatments within trial				0.16		
LSD				0.32		

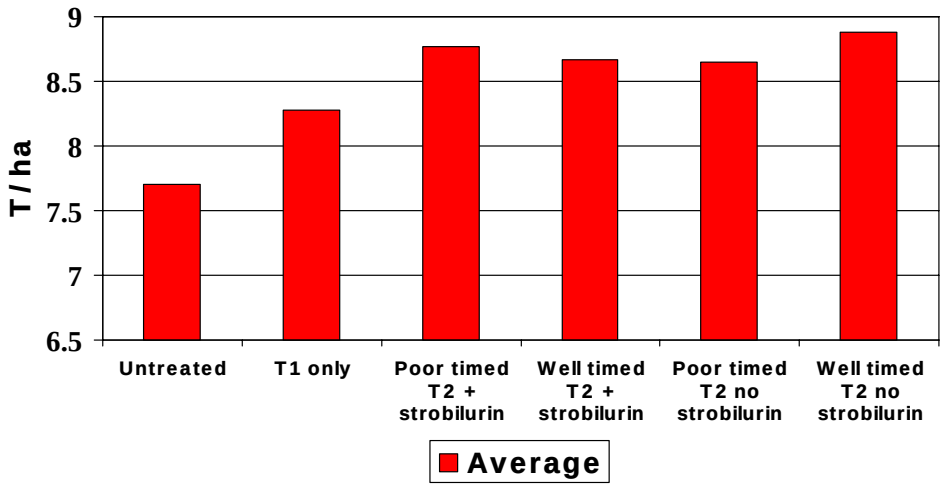


Figure 15 Crop Yields in winter barley 2005-07

Yield differences between the well timed and poor timed T2 and between the plus and minus strobilurin were not significant. The yield response from the early treatment was similar to the later yield response. The difference between the untreated at the T1 (0.57 T/ha) and the difference between the T1 and the well timed T2 with no strobilurin was 0.6 t/ha. Timing of the late fungicide was however less critical in winter barley compared to spring barley.

Margin over fungicide cost

Table 17 and Figure 16 show the margin over fungicide cost.

Table 17 Margin over fungicide cost £125/tonne in winter barley trials 2005-2007

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	1024	1067	1117	1104	1105	1133
2006 Bush	1057	1094	1138	1113	1128	1157
2007 Bush	802	856	884	873	881	907
Average	961	1005	1046	1030	1038	1065
Mean sed for comparison of treatments within trial				22.58		
LSD				45.16		

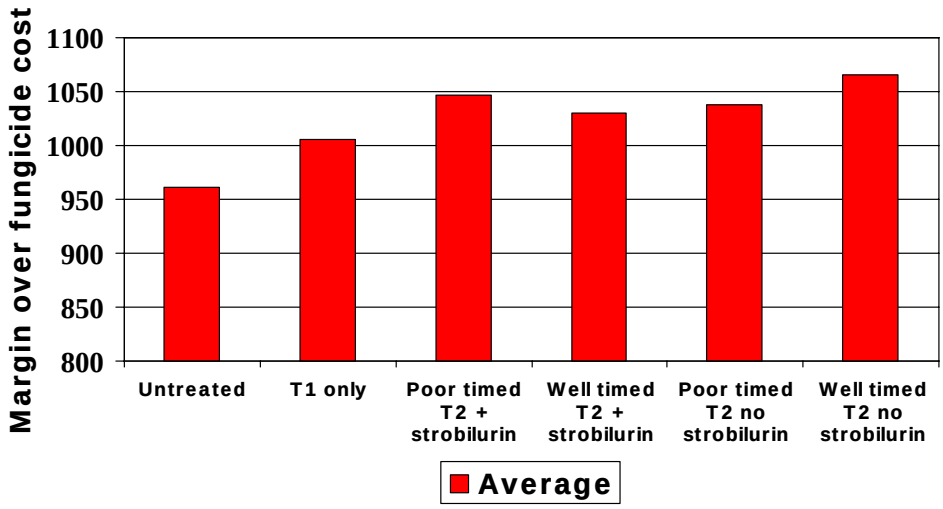


Figure 16 Margin over fungicide cost £125/ha in winter barley 2005-07

The highest yielding treatment achieved the best margin well timed treatment with no strobilurin followed by the poor timed T2 with a strobilurin.

Ramularia

Tables 18, 19 and Figure 17 show ramularia levels late in the season on leaf 2.

Table 18 Transformed Ramularia winter barley trials 2005-2007

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	2.861	2.76	1.988	1.864	2.315	2.089
2006 Bush	2.5	2.626	2.231	1.861	2.308	2.072
2007 Bush	2.743	2.779	2.514	2.106	2.385	2.168
Average	2.7	2.7	2.2	1.9	2.3	2.10
Mean sed for comparison of treatments within trial				0.1429		
LSD				0.2984		

Table 19 % Ramularia on leaf 2 in winter barley trials 2005-2007

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	16.48	14.80	6.30	5.45	9.12	7.08
2006 Bush	11.18	12.82	8.31	5.43	9.05	6.94
2007 Bush	14.53	15.10	11.35	7.22	9.86	7.74
Average	13.90	14.21	8.43	5.98	9.34	7.25

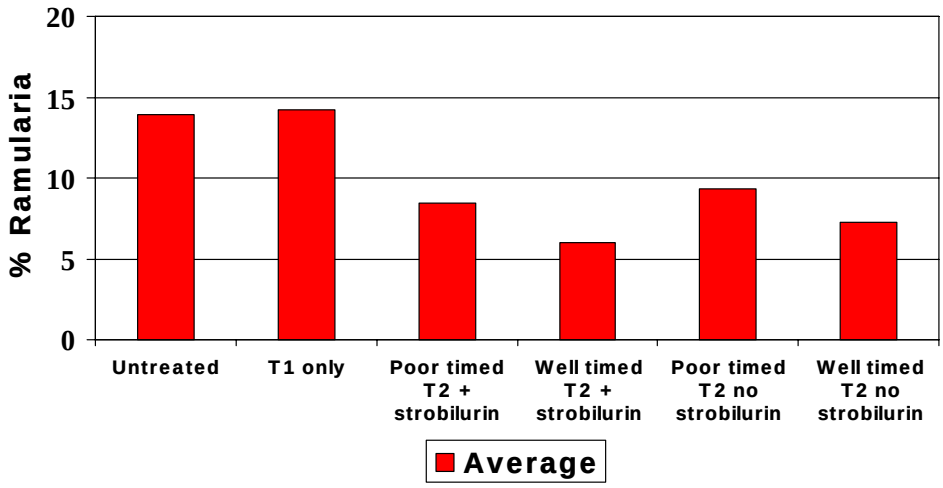


Figure 17 % Ramularia on leaf 2 late in season on winter barley 2005-07

The disease levels followed the pattern expected; with best control form the well timed treatments. Disease levels were on average lower than seen in the spring barley

Area under disease progress curve

Tables 20 and 21 and Figure 18 shows the disease development through the season.

Table 20 Area Under disease progress Curve (transformed) in winter barley varieties 2005-07

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	5.455	5.328	4.702	4.435	4.88	4.661
2006 Bush	4.775	4.839	4.436	3.998	4.471	4.242
2007 Bush	5.353	5.265	4.976	4.502	4.881	4.674
Average	5.194	5.144	4.704	4.311	4.744	4.525
Mean sed for comparison of treatments within trial				0.1355		
LSD				0.271		

Table 21 Area Under disease progress Curve in winter barley varieties 2005-07

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	232.92	205.03	109.17	83.35	130.63	104.74
2006 Bush	117.51	125.34	83.44	53.49	86.44	68.55
2007 Bush	210.24	192.45	143.89	89.20	130.76	106.13
Average	179.25	170.40	109.46	73.56	113.89	91.36
Mean sed for comparison of treatments within trial						
LSD						

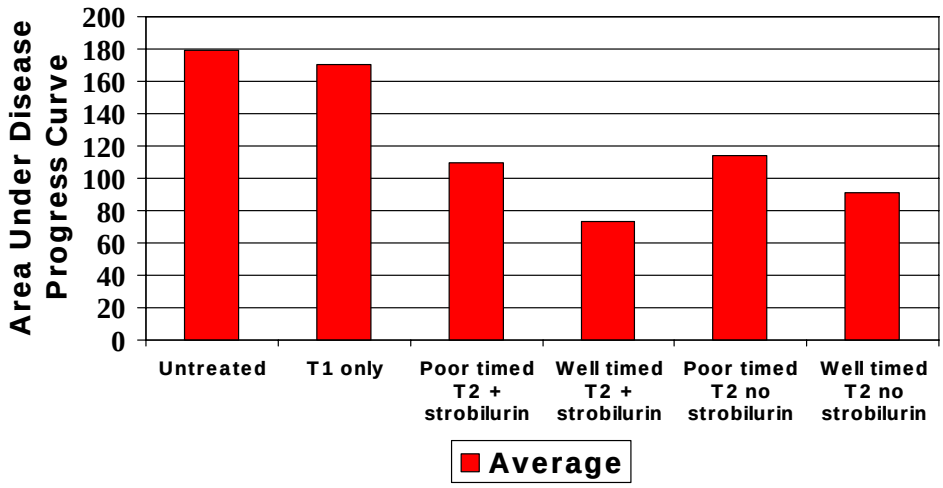


Figure 18 Area under disease progress curve in winter barley 2005-07

The results show little control of ramularia from the T1 fungicide and best control from the well timed treatment plus strobilurin.

Green leaf area

Green leaf area results are shown in tables 22, 23 and Figure 19.

Table 22 Angular Green leaf area in winter barley varieties 2005-07

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	28.22	31.23	44.6	46.46	39.7	48.85
2006 Bush	21.2	23.9	38.22	40.27	32	40.33
2007 Bush	13.39	27.37	45.43	52.2	40.59	50.58
Average	20.93	27.5	42.75	46.31	37.43	46.58
Mean sed for comparison of treatments within trial				3.79		
LSD				7.59		

Table 23 % Green leaf area in winter barley varieties 2005-07

Trial	Untreated	T1 only	Poor timed T2 + strobilurin	Well timed T2 + strobilurin	Poor timed T2 no strobilurin	Well timed T2 no strobilurin
2005 Bush	22.36	26.88	49.30	52.55	40.80	56.70
2006 Bush	13.08	16.41	38.28	41.78	28.08	41.89
2007 Bush	5.36	21.14	50.75	62.43	42.33	59.68
Average	12.77	21.32	46.08	52.29	36.94	52.77
Mean sed for comparison of treatments within trial						
LSD						

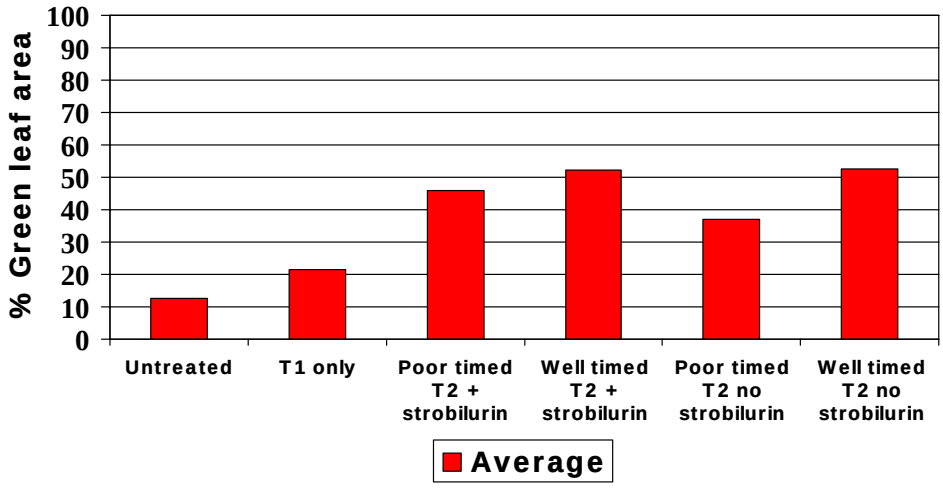


Figure 19 % Green leaf area in winter barley 2005-07

Highest green leaf area was recorded with the well timed fungicide with strobilurin.

Leaf wax - Impact of fungicides on ramularia

Yields

Proline achieved the best yield of the treatments (Table 24 and Figure 20), and increased the yield in all varieties except Decanter (a Ramularia resistant variety). Bravo achieved the lowest yield. This occurred in 7 of the 9 varieties used. Both Corbel and Amistar achieved similar yields to the No T2 treatment. No yield reduction was observed with Corbel and no yield benefit from the Amistar.

Table 24 Crop yields T/ha

Trial	No T2	Bravo 1.0	Proline 0.4	Corbel 0.5	Amistar 0.5	Tracker 0.75
2005 Bush	5.831	5.724	6.128	5.946	5.863	6.048
2005 Lockerbie	5.594	5.488	5.892	5.709	5.627	5.812
2006 Bush	7.821	7.714	8.119	7.936	7.854	8.039
2006 Lockerbie	6.164	6.057	6.462	6.279	6.196	6.382
Average	6.3525	6.24575	6.65025	6.4675	6.385	6.57025
Mean sed for comparison of treatments within trial				0.1254		
LSD				0.2508		

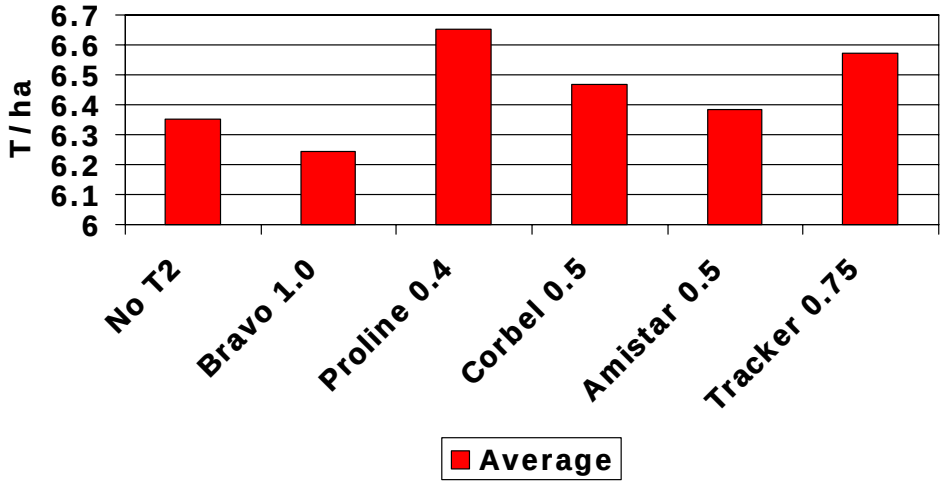


Figure 20 Crop Yields T/ha

Margin over fungicide cost

Although the treatments would not be commercially used, they show best margin with Proline (the fungicide which achieved the best control of leaf spots) and the lowest were from Bravo. This is unusual, since this treatment achieved good control of ramularia symptoms (Table 25 and Figure 21).

Table 25 Margin over fungicide cost £125/t grain cost

Trial	No T2	Bravo 1.0	Proline 0.4	Corbel 0.5	Amistar 0.5	Tracker 0.75
2005 Bush	684.9	670.3	715.1	694	681	701.6
2005 Lockerbie	645.2	630.6	675.4	654.3	641.4	661.9
2006 Bush	950.8	936.2	981	959.9	946.9	967.5
2006 Lockerbie	740.5	725.9	770.7	749.6	736.6	757.1
Average	755.35	740.75	785.55	764.45	751.475	772.025

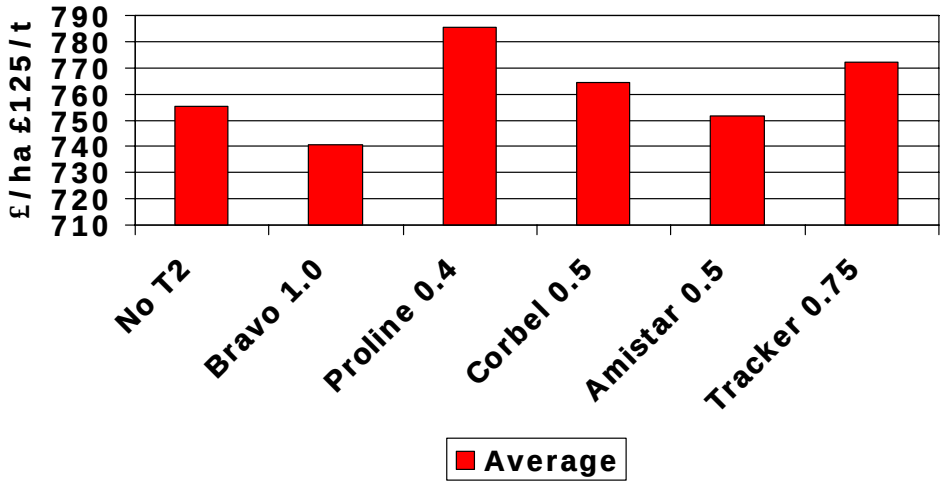


Figure 21 Margin over fungicide cost at £125/t grain price

Ramularia development

Tables 26, 27 and Figure 22 show ramularia development in the trials.

Table 26 % Log Late Ramularia Leaf 2

Trial	No T2	Bravo 1.0	Proline 0.4	Corbel 0.5	Amistar 0.5	Tracker 0.75
2005 Bush	3.279	2.108	2.162	3.296	3.323	2.818
2005 Lockerbie	3.11	1.793	1.528	3.13	3.107	2.199
2006 Bush	3.335	1.517	1.499	3.365	3.348	2.313
2006 Lockerbie	2.322	1.636	1.325	2.411	2.291	1.805
Average	3.0115	1.7635	1.6285	3.0505	3.01725	2.28375
Mean sed for comparison of treatments within trial				0.1615		
LSD				0.323		

Table 27 % Late Ramularia Leaf 2

Trial	No T2	Bravo 1.0	Proline 0.4	Corbel 0.5	Amistar 0.5	Tracker 0.75
2005 Bush	25.55	7.23	7.69	26.00	26.74	15.74
2005 Lockerbie	21.42	5.01	3.61	21.87	21.35	8.02
2006 Bush	27.08	3.56	3.48	27.93	27.45	9.10
2006 Lockerbie	9.20	4.13	2.76	10.15	8.88	5.08
Average	19.32	4.83	4.10	20.13	19.44	8.81

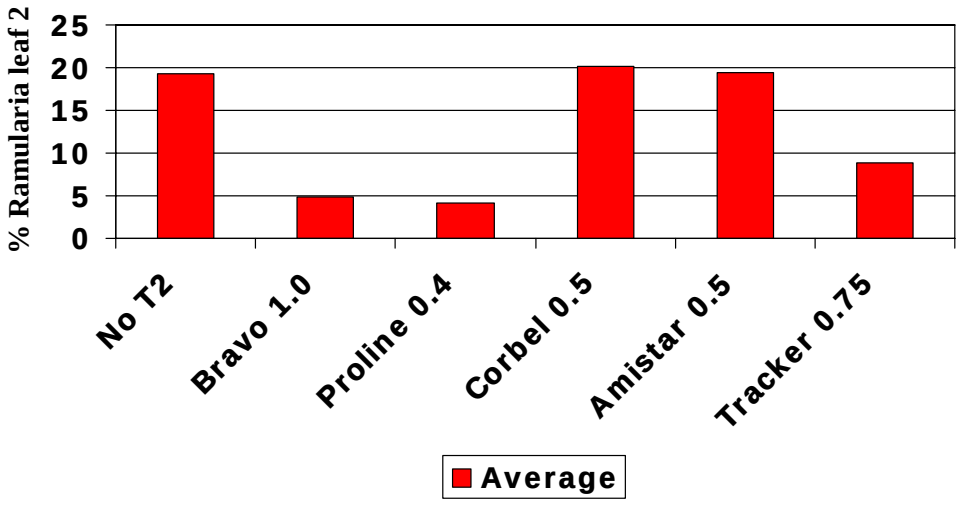


Figure 22 % Late ramularia on fungicide treatments

Table 28 Log Area Under Disease Progress Curve (AUDPC)

Trial	No T2	Bravo 1.0	Proline 0.4	Corbel 0.5	Amistar 0.5	Tracker 0.75
2005 Bush	5.705	5.074	4.721	5.798	5.819	5.23
2005 Lockerbie	4.797	4.195	3.895	4.809	4.816	4.239
2006 Bush	4.96	4.344	3.974	5.041	5.081	4.394
2006 Lockerbie	4.028	3.457	3.083	4.129	4.093	3.514
Average	4.872	4.267	3.918	4.944	4.952	4.344
Mean sed for comparison of treatments within trial				0.09289		
LSD				0.18578		

Table 29 Area Under Disease Progress Curve (AUDPC)

Trial	No T2	Bravo 1.0	Proline 0.4	Corbel 0.5	Amistar 0.5	Tracker 0.75
2005 Bush	299.37	158.81	111.28	328.64	335.64	185.79
2005 Lockerbie	120.15	65.35	48.16	121.61	122.47	68.34
2006 Bush	141.59	76.01	52.20	153.62	159.93	79.96
2006 Lockerbie	55.15	30.72	20.82	61.12	58.92	32.58
Average	129.65	70.34	49.31	139.37	140.49	76.03

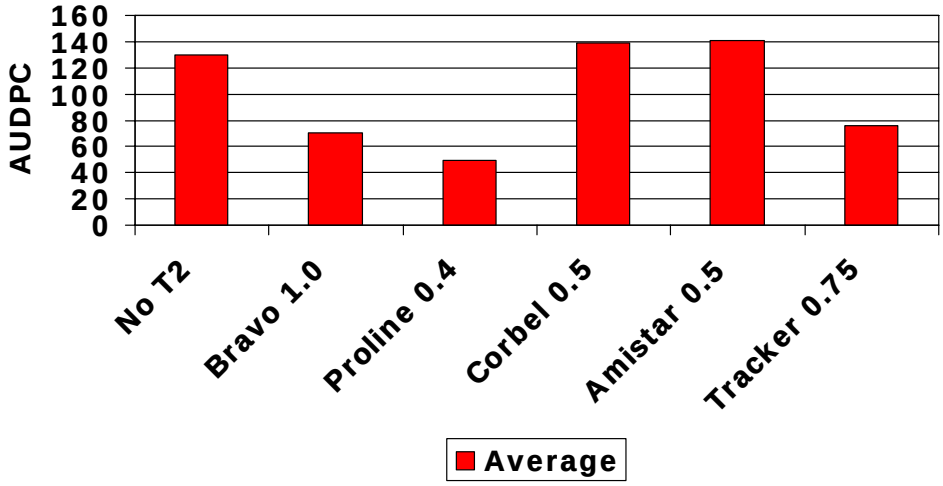


Figure 23 Area Under Disease Progress Curve (AUDPC)

The three fungicides which achieved most control were Proline, Bravo and Tracker. Corbel and Amistar gave no control.

Differences between varieties were also significant with lowest levels from Decanter, Oxbridge, Poker and Westminster and most disease from Troon, Cellar and Prestige.

The area under disease progress gives a better picture of control over time. Proline achieved best control followed by Bravo and Tracker. Corbel and Amistar were the same as the untreated.

Abiotic leaf spots were highest in the variety Prestige and in the fungicide treatment Corbel. Proline achieved the best reduction.

Screenings

The lowest screenings were seen in the Proline treatment and the highest in the untreated. Both Corbel and Amistar had screening levels similar to the untreated control (Table 30, 31 and Figure 24).

Table 30 Log screenings (<2.5 mm sieve) for spring barley varieties

Trial	No T2	Bravo 1.0	Proline 0.4	Corbel 0.5	Amistar 0.5	Tracker 0.75
2005 Bush	2.462	2.378	2.366	2.549	2.495	2.362
2005 Lockerbie	2.848	2.717	2.492	2.767	2.737	2.695
2006 Bush	1.174	0.997	0.925	1.057	1.105	1.068
2006 Lockerbie	1.843	1.794	1.79	1.986	2.02	1.861
Average	2.082	1.971	1.893	2.089	2.089	1.99
Mean sed for comparison of treatments within trial				0.1125		
LSD				0.225		

Table 31 % screenings (<2.5 mm sieve) for spring barley varieties

Trial	No T2	Bravo 1.0	Proline 0.4	Corbel 0.5	Amistar 0.5	Tracker 0.75
2005 Bush	11.73	10.78	10.65	12.79	12.12	10.61
2005 Lockerbie	17.25	15.13	12.09	15.91	15.44	14.81
2006 Bush	3.23	2.71	2.52	2.88	3.02	2.91
2006 Lockerbie	6.32	6.01	5.99	7.29	7.54	6.43
Average	8.02	7.18	6.64	8.08	8.08	7.36

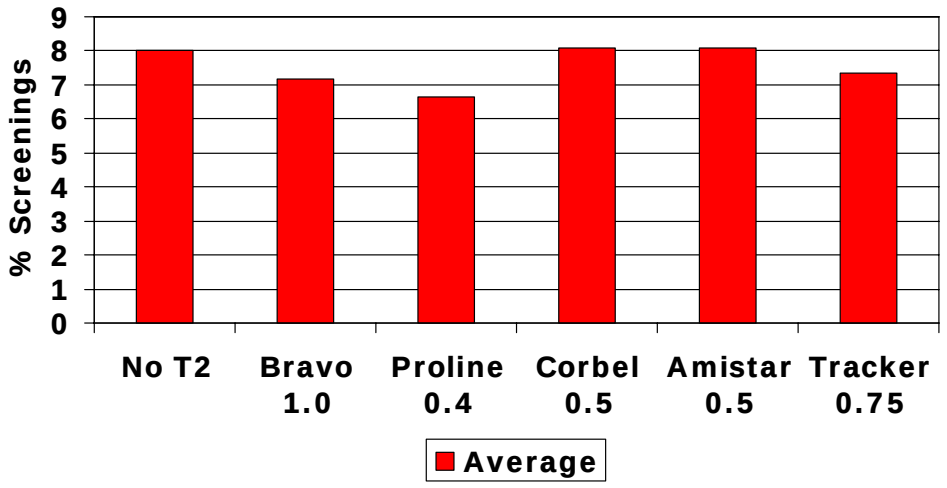


Figure 24 % screenings (2.5 mm sieve)

Green leaf

Green leaf area retention showed a similar pattern with highest green leaf area retention from the treatments which gave best control. Amistar and Corbel gave low green leaf area scores which were the same as the untreated. This suggests the greening effect is no longer seen with this strobilurin fungicide (Table 32,33 and Figure 25).

Table 32 Angular Green leaf area in spring barley varieties 2005-07

Trial	No T2	Bravo 1.0	Proline 0.4	Corbel 0.5	Amistar 0.5	Tracker 0.75
2005 Bush	32	48.83	53.29	29.69	31.4	47.45
2005 Lockerbie	48.37	69.3	75.27	53.08	52.19	66.05
2006 Bush	45.75	62.13	68.46	46.02	46.05	60.94
2006 Lockerbie	45.88	55.42	59	43.48	45.28	53.71
Average	43.00	58.92	64.00	43.06	43.73	57.03
Mean sed for comparison of treatments within trial				2.651		
LSD				5.302		

Table 33 %Green leaf area in spring barley varieties 2005-07

Trial	No T2	Bravo 1.0	Proline 0.4	Corbel 0.5	Amistar 0.5	Tracker 0.75
2005 Bush	28.08	56.66	64.27	24.53	27.15	54.27
2005 Lockerbie	55.87	87.51	93.53	63.92	62.42	83.52
2006 Bush	51.31	78.15	86.52	51.78	51.83	76.41
2006 Lockerbie	51.54	67.79	73.47	47.35	50.49	64.97
Average	46.51	73.35	80.79	46.63	47.78	70.40

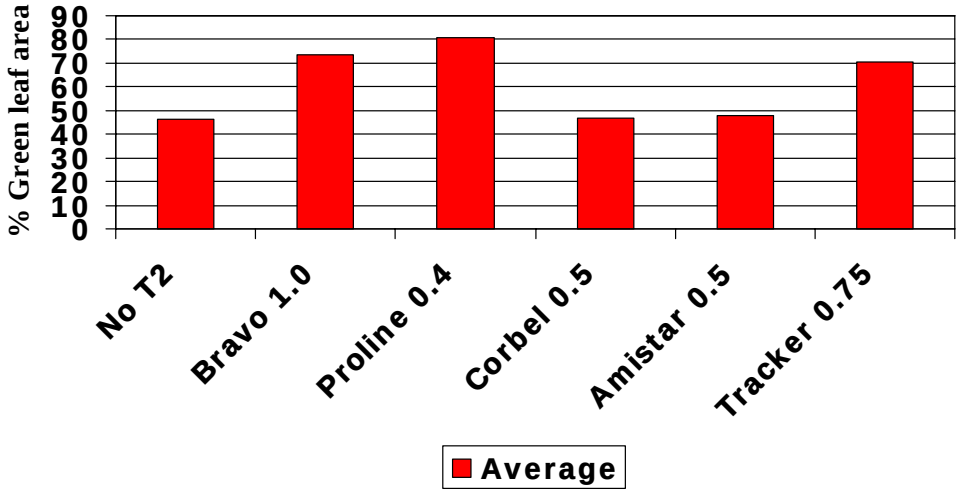


Figure 25 % green leaf area leaf 2

Discussion

Determining the loss in yield from a single disease is a challenge, since the fungicides applied control a wide range of diseases which will impact on yield. Using a "well timed" compared to a "poor timed" approach is one method used to apply the same amount of fungicide to a crop and manipulate ramularia levels on the upper leaves. This was a successful approach to manipulate the disease epidemic, but the "poor timed" treatment also influenced the ramularia epidemic.

Previous research demonstrated the importance of the later fungicide timings for the management of ramularia. A new development from this research is the importance of the earlier fungicide treatment on ramularia management. Earlier research suggested GS25-30 was of limited importance to manage ramularia and the focus on ramularia management kept until GS45-49. This research shows that the earlier treatment can influence the development of ramularia and new fungicide advice will take account of this contribution in high risk situations. This effect is seen most in the area under disease progress curve data. It is less noticeable on the disease levels on the final leaf 2 late in the season. One reason for the change maybe in the better activity of prothioconazole on ramularia compared to cyprodinil used in previous research.

The later fungicide timing at GS45-49 continues to be a key timing to target ramularia, but timing of this later fungicide is important to ensure the best margin. If this later treatment is applied too early at GS37, a grower may fail to realise an increase in the margin over the single early fungicide after taking account of fungicide costs.

The contribution of a strobilurin fungicide in a mixture has diminished in spring barley. An explanation for poor activity of strobilurins is discussed in the following section. Resistance developed in 2002, so was widespread in all trials in this project. A 0.1 t/ha response was the average response from 0.4 l/ha Amistar. This is not a significant increase in yield and at grain prices of £125/t this treatment will cost £10/ha for a return of £12.50/ha. There may be other diseases to consider which make the addition of the strobilurin fungicide important. If brown rust, for example, is a potential risk, the strobilurin component would be an important contribution to manage this disease. Although the contribution from Amistar on net blotch has diminished, other strobilurin fungicides may contribute to net blotch control at this timing.

The average yield difference between the "well timed" and "poorly timed" T2 treatment was 0.186 t/ha. At high disease sites, the difference was greater at 0.366 T/ha. Yield losses from Ramularia will be higher than this and a comparison of the well timed treatment with no late treatment achieved an average yield difference of 0.393 t/ha. At high disease sites this increased to 0.575 t/ha. The yield loss from ramularia may ranges between 0.186 – 0.575 t/ha. On the basis of a fungicide programme costing £30/ha, controlling ramularia is cost effective at the average yield loss Figure at grain prices greater than £80/t.

These values provide a guide to the potential yield loss in an average situation. Yield losses on a susceptible variety can be higher and the largest was seen with Cocktail at 1.256 t/ha.

Focussing on the individual fungicide components, Proline achieved the best control of disease, highest yield and lowest screenings. Bravo achieved good control of ramularia, and green leaf area retention, but yield and quality were not as high as expected. Proline achieves a yield above a level expected from disease control, or Bravo has the opposite effect. Both Corbel and Amistar achieved poor control of ramularia and green leaf area retention. Both fungicides also achieved a similar yield to the untreated. This suggests that beneficial physiological effects from a strobilurin fungicide in absence of disease control was not observed and any detrimental effects from the morpholine were also not seen in these trials. Care should however be taken if a morpholine is applied late to a crop, since this treatment can increase levels of abiotic leaf spots.

Conclusions

Yield losses due to ramularia leaf spots in spring barley based on six varieties from seven field trials over three seasons falls with the region of 0.186 – 0.575 t/ha (average 0.38 t/ha). On the basis of a fungicide programme costing £30/ha, controlling ramularia is cost effective even at the lower yield loss Figure at grain prices greater than £80/t.

Yield losses on a susceptible spring variety at specific sites were however higher and the largest was seen with Cocktail at 1.256 t/ha where no late fungicide was applied. This loss in yield equates to a monetary loss of £157/hectare at £125/tonne grain costs.

On the basis of a cropping area of 225,000 hectares of spring barley in Scotland (2006), ramularia leaf spot is costing the Scottish malting barley industry an average of 85,500 tonnes in lost barley grain valued at £10.68 million at a price of £125/tonne.

Although this loss can be minimised through the use of an effective fungicide treatment costing £30/ha, breeding for resistance could minimise the loss to the lower end of the spectrum.

Fungicide resistance to strobilurin fungicides

Introduction

QoI fungicides (inhibitors of mitochondrial respiration at QoI site of the cytochrome *bc*1 enzyme complex), including strobilurin A, found in mushrooms) are used in many countries world-wide, because they have a broad spectrum of control against a large number of pathogens on various crops (Ypema *et al*., 1997). These compounds inhibit the mitochondrial respiration by binding to the ubiquinol oxidation (Qo) site formed by domains of cytochrome *b* and the iron-sulphur within the cytochrome *bc*1 complex. Because ATP production is compromised, energy-demanding stages of fungal development, such as spore production, are greatly affected. The Qo inhibitors have become a key component of disease control in cereal crops. However, in 1998, the first field isolates resistant to this group of fungicides occurred in wheat powdery mildew (*Blumeria graminis* f. sp *tritici*) in northern Germany. All these fungal isolates contained a mutation in the cytochrome *b* to change a glycine to alanine at amino acid position 143 (G143A). This mutation was also found in a single resistant isolate of *B. graminis* f. sp. *hordei* (barley powdery mildew) again in Northern Germany in 1999. By 2001, resistance in cereal powdery mildews was widespread in NW-Europe. In 2002, the G143A mutation was detected in resistant field isolates of *Septoria tritici* (teleomorph Mycosphaerella graminicola) in the UK (Fraaije *et al*., 2003). However, up to 11 different amino acid exchanges have been reported to confer resistance to QoI in other organisms, but only mutations at codon 129 and 143 have been reported for plant pathogens (Gisi *et al*., 2002). The mutation at codon 129 was shown to reduce the efficacy of QoI fungicides, but the practical occurrence of this mutation does not appear to have a significant impact in field disease control. Other mechanisms have also been reported, such as alterations in the target site, induction of alternative respiration and unknown mechanisms in *Venturia inaequalis* (Steinfeld *et al*., 2001). However, practical disease failures have only ever been reported with the presence of the mutation at codon 143.

Ramularia collo-cygni first appeared in barley crops in Scotland in 1998 and was found to be easily controlled by the fungicide azoxystrobin (Amistar), as reported in the Oxley *et al* (2002) (Figure 26.). This type of fungicide was therefore recommended for Ramularia disease control.

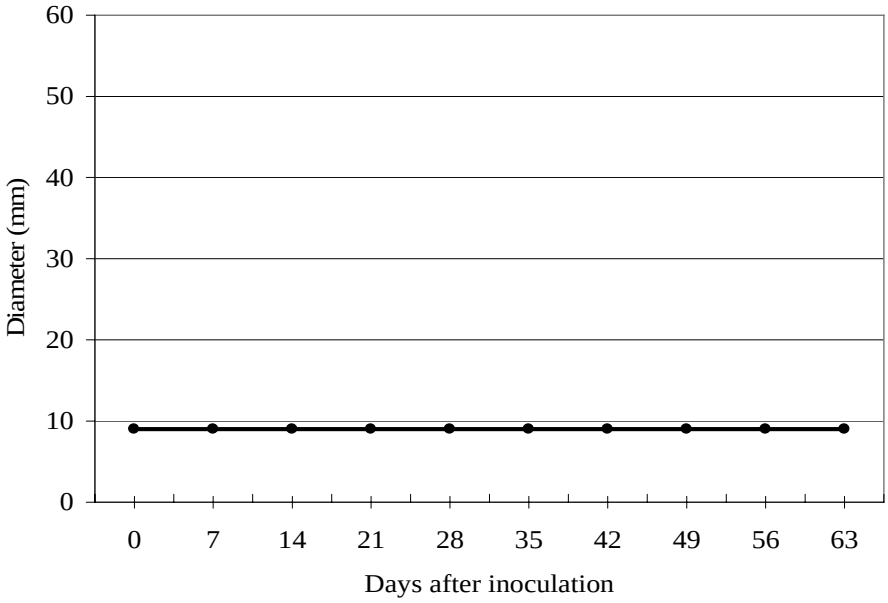


Figure. 26. The diameter of Ramularia fungal growth on QoI fungicide amended media.

However, by the summer of 2003 the control of Ramularia using QoI fungicides in Scotland showed a sharp decline in efficacy. Figure 27, shows a dose response curve for the QoI fungicide azoxystrobin (Amistar) and shows that control was not achieved in experimental field plots.

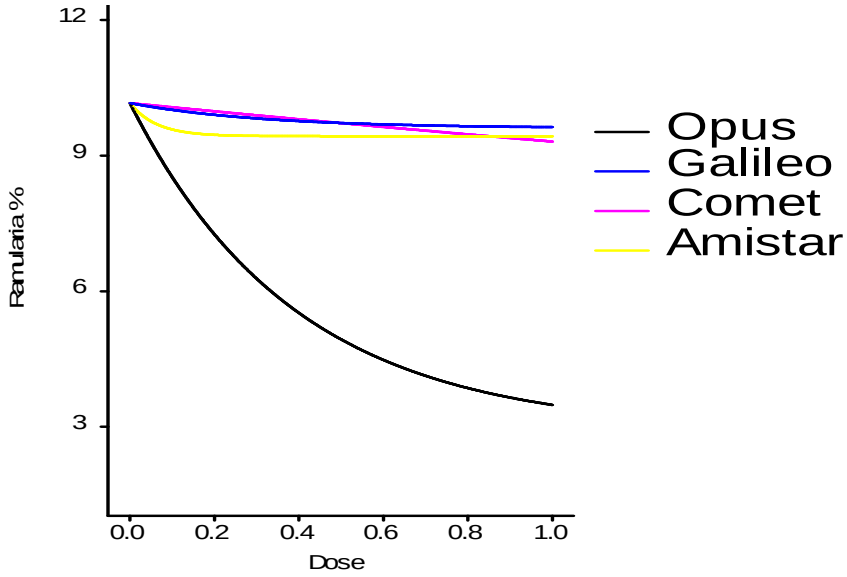


Figure 27. A dose response curve for Ramularia disease control, showing that control could not be achieved in the field during 2003-7 when using a QoI fungicide alone.

It was therefore assumed that resistance had developed, although not confirmed by either bioassay or sequencing. This work used both sequencing and PCR-RFLP diagnostics to demonstrate the development of specific mutations conferring QoI fungicide resistance and on an agar micro-plate bioassay to calculate the degree of resistance in individual isolates.

Materials and Methods

Fungal material

A selection of fungal material from different locations was used in this study and is shown in Table 34.

Table 34. Isolates of *Ramularia collo-cygni* used in both sequencing and primer design.

Isolate name	Collection location	Year of isolation
4/1	Reichenberg, Austria	1998
32	Freising, Bayern, Germany	1998
58	Aberdeen, Scotland	1998
56	Stadl-Paura, Austria	1998
S semi FL	Denmark	2007
B semi FL	Denmark	2005
P semi FL	Denmark	2007
P1	Denmark	2007
P2	Denmark	2007
B1	Denmark	2005
B2	Denmark	2005
S1	Denmark	2007
S2	Denmark	2007
7B23	Bush estate, Scotland	2007
7B22	Bush estate, Scotland	2007
7B37	Bush estate, Scotland	2007
7B41	Bush estate, Scotland	2007

Growth of fungal isolates

V8 agar was prepared by placing 200 ml of V8 vegetable juice and 20 g agar (Difco bacto agar) into 800 ml of distilled water. The pH was adjusted to pH 6.0 using sodium hydroxide and sterilised at 15 psi for 15 minutes. Once hand hot the agar was poured into sterile 9 cm plastic Petri-dishes and allowed to set. Isolates of Ramularia

were then sub-cultured on to the fresh media and grown in the dark at 18 ^{o}C for two weeks.

Isolation of total DNA

Total DNA was extracted from 0.1 g of freeze dried fungal material that was crushed to a fine powder using a mini pestle in a 2 ml eppendorf tube. using the method of Fraaije *et al*. (1999) except that the DNA extraction buffer was amended with 5 mM 1,10-phenanthroline monohydrate and 2 % (wt/vol) polyvinylpyrrolidone K30 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) to clean the DNA (Zhang and Stewart, 2000). The DNA was then quantified using an eppendorf spectrophotometer with the resulting solutions being diluted to 2.5 ng/µl.

Amplification and sequencing of the cytochrome *b* gene

Initially, a 675 bp fragment of the cytochrome *b* (cytb) gene of ramularia was amplified with the primer set CBF1 (5`-tattatgagagatgtaaataatgg-3`) /CBR3 (5`-cctaataatttattaggtatagatctta-3`). This was performed using a standard PCR carried out in a Applied Biosystems thermocycler with 1.25 units of Promega GoTaq in 40 µl of Green GoTaq reaction buffer, 1.25 µM each dNTP, 0.5 µM Primers, and 1 ng of template DNA in a final volume of 50 µl. The amplification conditions were 94 ^{o}C for 2 min 30 sec; followed by 40 cycles of 94 ^{o}C for 30 sec, 55 ^{o}C for 1 min, and 72^{o}C for 1 min 30 sec; and a final extension at 72 ^{o}C for 8 min 30 sec. The PCR products were separated on ethidium bromide-stained 1.3% (wt/vol) agarose gels run in Tris-borate-EDTA (TBE) buffer (89mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8.0) and exposed to UV light to visualize DNA fragments. The PCR products were purified with the High Pure PCR Product Purification Kit (Boehringer, Mannheim, Germany) and ligated into pGEM-T easy (Promega Corp., Madison, WI). Plasmids were transformed into Escherichia coli JM109 cells (Promega Corp.) as described by Sambrook *et al*., (1989). Plasmid DNA was extracted using the RPM Kit (Bio101 Inc, Caramulariabad, CA). Nucleotide sequences were determined by the dideoxy chain termination method, at Durham University Sequence Centre (Durham, UK).

Design of PCR-RFLP

The 11 isolates of ramularia were sequenced using the primer set CBF1/CBR3. Using the aligned sequence data, a new set of ramularia primers were designed (RamCytF1 (5`-ctcctagaacactggtgtgaa-3` and RamCytR1 (5`-gcgttgttaactgagaatcca-3`); these amplified shorter regions of the cytochrome b gene to cover both codons 129 and

143. The PCR was carried out in a GeneAmp PCR system 9700 (Applied Biosystems) with 1.25 units of Promega GoTaq, 10 µl of Green GoTaq reaction buffer, 1.25 µM each dNTP, 0.5 µM Primers, and 1 ng of template DNA in a final volume of 25 µl. The amplification conditions were 94 ^{o}C for 2 min 30 sec; followed by 40 cycles of 94 ^{o}C for 30 sec, 60 ^{o}C for 1 min, and 72 ^{o}C for 1 min 30 sec; and a final extension at 72 ^{o}C for 8 min 30 sec. The PCR products were separated on ethidium bromide-stained 1.3 % (wt/vol) agarose gels run in Tris-borate-EDTA (TBE) buffer (89mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8.0) and exposed to UV light to visualize DNA fragments. After checking for amplification the products were treated with the enzyme *Alu*I (Roche diagnostics) according the manufactures recommendation. The enzyme treated PCR products then were separated on ethidium bromide-stained 1.3 % (wt/vol) agarose gels run in Tris-borate-EDTA (TBE) buffer (89mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8.0) and exposed to UV light to visualize DNA fragments.

Fungicide sensitivity testing

Assays were performed in 24 well plates using Potato Dextrose media amended with technical grade azoxystrobin fungicide (Syngenta) at concentrations ranging from 0.0064 to 100 ppm (each individual treatment was replicated). Agar plugs of 4 mm where cut from two week old cultures of Ramularia pre-cultured on V8 agar plates and placed in the centre of the individual fungicide amended media. The plates were then incubated for two weeks at 15 ^{o}C in the dark.

As well as carrying out an experiment with azoxystrobin alone, another assay was also set up with azoxystrobin and an alternative oxidase inhibitor salicylhydroxamic acid (SHAM). This additional experiment was carried out to rule out any growth that may occur due to the role of the alternative oxidase pathway (AOX). No significant role for the AOX pathway was observed.

Results

Fungicide sensitivity bioassay

In total 8 individual isolates of ramularia were tested in a fungicide amended agar plate assay, these results showed that isolates collected in Scotland before 2002 were sensitive to QoI fungicides, three of these isolates can be seen in Figure 28 showing the range of growth in the resistant isolate, as compared to the sensitive. These results demonstrate that the resistant isolates can grow in the present of at least 4 ppm of technical grade azoxystrobin.

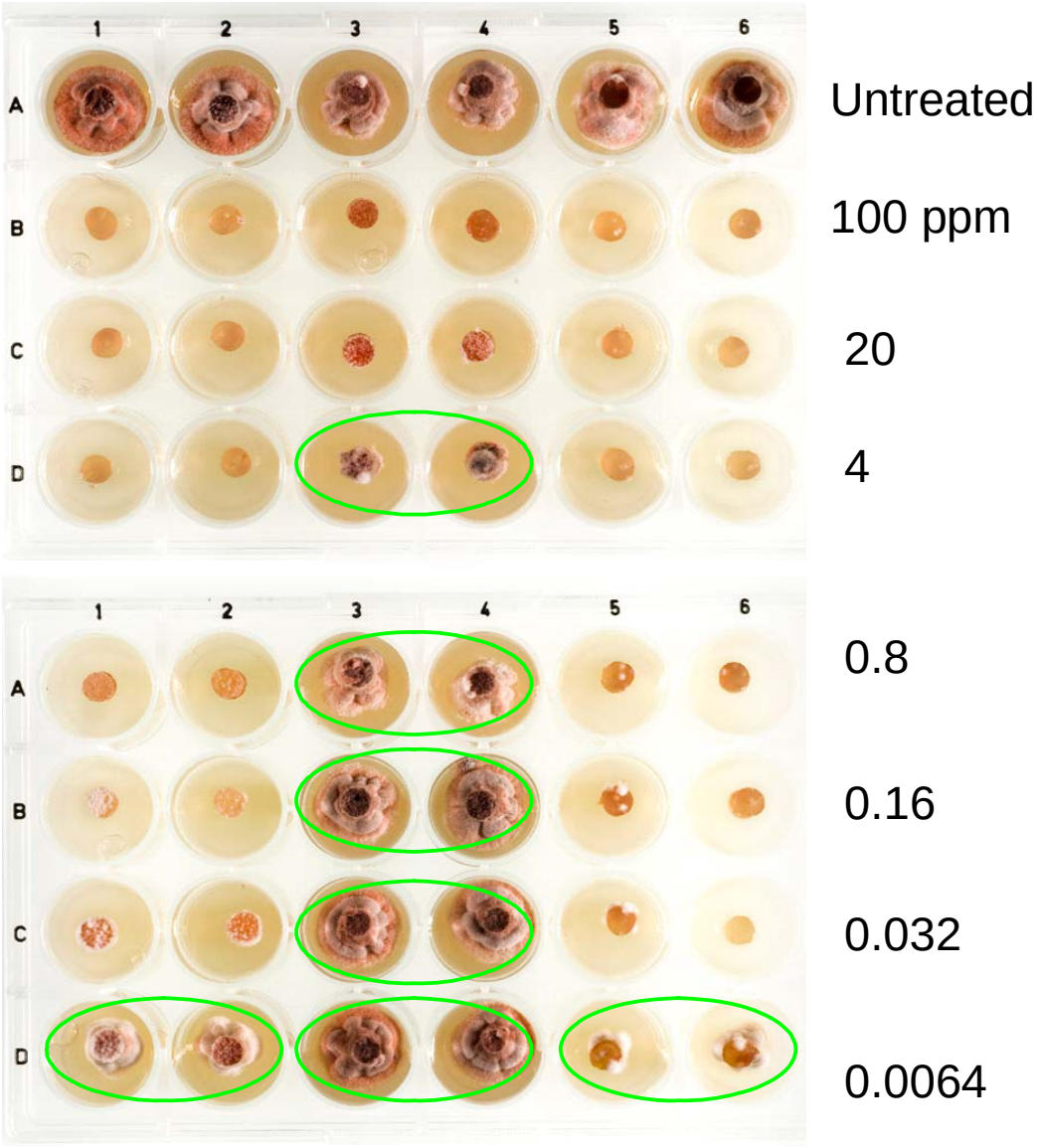


Figure 28 The comparison between two QoI sensitive isolates of Ramularia and one (centre) found to be highly resistant to all QoI fungicides using fungicide amended media.

PCR amplification and sequence analysis of cytochrome *b* gene

A partial sequence of the cytochrome *b* gene was obtained using the primer set CBF1/CBR3. This PCR product gave a partial sequence of 675 bp which was then used to align to a similar fungal DNA sequences found in the NCBI database using a BLAST search tool. This BLAST comparison allowed for the design of cytochrome *b* Ramularia specific primers. The partial sequencing of the cytochrome *b* gene enabled a selection of isolates from different locations to be screened for mutations at both codons 143

and 129. The sequence data obtained during these experiments showed that both resistant and sensitive isolates from the SAC Bush estate. However, the majority of isolates from Scotland showed the G143A mutation from isolates collect from the field in 2007. No mutations occurring at codon 129 were observed. Table 35, shows the partial sequence of a selection of isolates of ramularia isolates which demonstrate that samples collected in Scotland before 2002 were sensitive to QoI fungicides, but after this date a mutation at codon 143 developed.

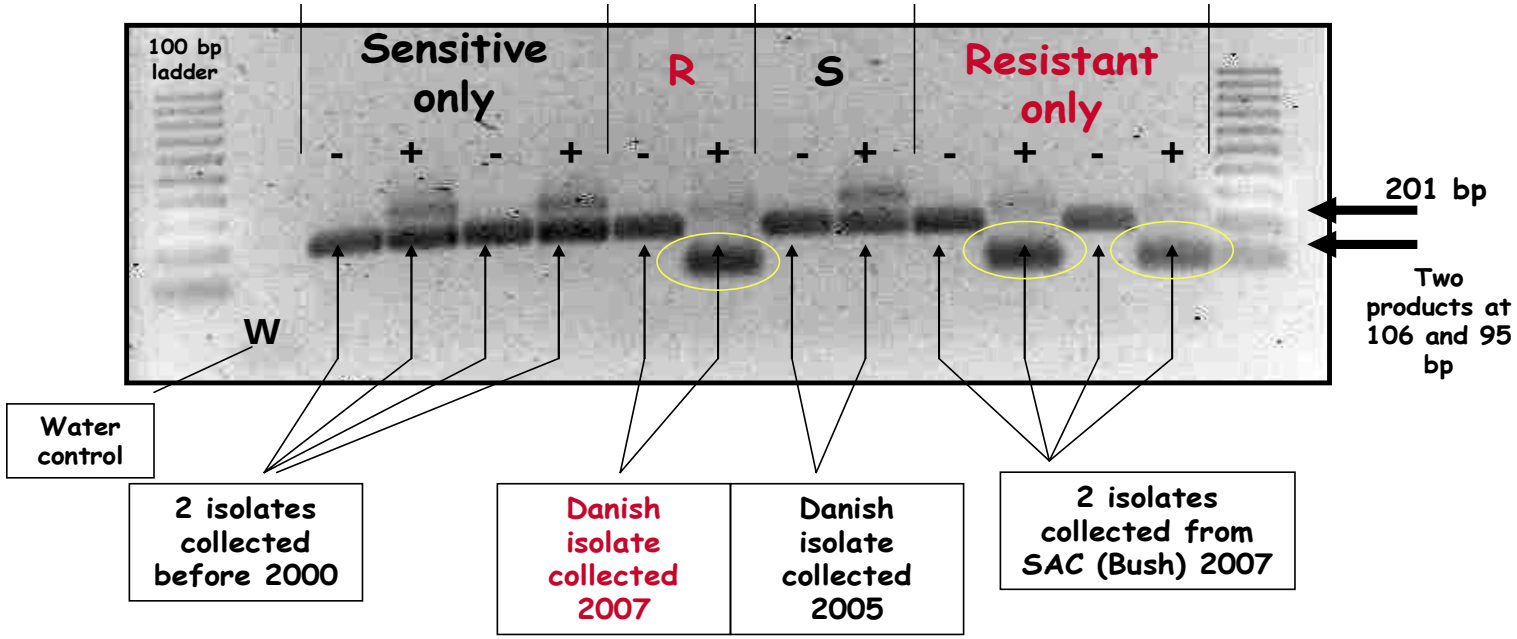
Table 35. Partial alignment of the cytochrome *b* gene from a range of resistant and sensitive isolates of Ramularia

Isolate name	Partial DNA sequence	Resistant or Sensitive	Year of isolation
4/1	5′-caaatgtccttatga**ggt**gctacacttat-3′	Sensitive	1998
32	5′-caaatgtccttatga**ggt**gctacacttat-3′	Sensitive	1998
58	5′-caaatgtccttatga**ggt**gctacacttat-3′	Sensitive	1998
56	5′-caaatgtccttatga**ggt**gctacacttat-3′	Sensitive	1998
S semi FL	5′-caaatgtccttatga**gct**gctacacttat-3′	Resistant	2007
B semi FL	5′-caaatgtccttatga**ggt**gctacacttat-3′	Sensitive	2005 Denmark
P semi FL	5′-caaatgtccttatga**gct**gctacacttat-3′	Resistant	2007
7B23	5′-caaatgtccttatga**gct**gctacacttat-3′	Resistant	2007
7B22	5′-caaatgtccttatga**gct**gctacacttat-3′	Resistant	2007
7B37	5′-caaatgtccttatga**gct**gctacacttat-3′	Resistant	2007
7B41	5′-caaatgtccttatga**gct**gctacacttat-3′	Resistant	2007

PCR-restriction fragment length polymorphism

Using the newly design primers specific products from ramularia were amplified and treated with the enzyme *Alu*I. The presence of the mutation at codon 143 changing the DNA sequence from AGGT to AGCT caused the 201 bp PCR product to be cut at the mutation into two products of 106 bp and 95 bp, the un-mutated samples were not cut by the enzyme *Alu*I. This result can be seen in Figure 29 showing a range of sensitive and resistant isolates. The strains collected from Denmark were isolated from the same location in both 2005 and 2007. Interestingly, this result shows that

the population has changed from the wild type (sensitive) allele to that of the mutated (resistant) type within two years. All recent isolates from Scotland show that only the resistant allele is present, therefore a high level of resistance exists in Ramularia population in Scotland. Currently the resistance levels in other areas of the UK are untested.



(-) PCR products untreated with restriction enzyme

(+) PCR products were treated with the restriction enzyme (Alu1). This enzyme specifically cuts in the presence of the sequence AGCT (resistant) at codon 143, but will not digest the PCR product in the presence of the wild type sequence AGGT

Figure 29. Simple PCR RFLP to detect Qoi Resistance. The samples that contain the mutated DNA were digested by the enzyme to produce two PCR products of around 100 bp each and are highlighted in yellow.

Discussion

These results have clearly shown that resistance to QoI fungicides has now developed in populations of Ramularia in both Scotland and Denmark. The mutation conferring complete resistance to QoI fungicides is the well documented and occurs at codon 143 in the cytochrome *b* gene. The occurrence of this mutation in the UK appears to have occurred around 2001-2002. This corresponds to the decline in field performance during this period. However, due to the limited nature of this study we do not know the extent of the distribution of these resistant alleles in the wider population. However, it is expected that levels have increased year on year since the initial development as seen in other cereal pathogens such as *M. graminicola* in wheat.
As a consequence, future work will be performed using a range of different molecular techniques (real-time PCR and Pyrosequencing) to measure the ratio of both the resistance and sensitive alleles conferring QoI resistance in populations of Ramularia infected crops. This information will enable us to understand the dynamics of resistance development and the possible role of spore and seed infection in the Ramularia life cycle. It is hoped that by gaining a greater understanding of the basic epidemiology and the ability for the fungus to develop fungicide resistance it may help in the development of effective control and anti-resistance strategies.

Varietal resistance to *Ramularia collo-cygni*

Introduction

The HGCA Recommended List contains information on the varietal resistance to common disease of barley including rhynchosporium, brown rust, powdery mildew and net blotch. Information on varietal resistance to ramularia is currently not available on the recommended list. The purpose of this section of the research was to provide new information to assist growers and breeders determine the range of varietal resistance to ramularia in existing and candidate varieties of spring barley.

Materials and Methods

Ramularia, abiotic leaf spots and green leaf area were assessed on spring barley Recommended List (RL) trials in 2002 - 2007. Average disease and green leaf scores were taken from the top two leaves in untreated plots in July, when crops were at milky ripe growth stages (GS73-77).

Green leaf, Ramularia and abiotic leaf spots averages were determined using analytical techniques used to analyse Recommended List trials since not all varieties were present in all the trials in all seasons.

Results

Winter barley

Table 36 shows the average levels of Ramularia, on the upper leaves of winter barley varieties between 2002, 2003, 2005 and 2006.. Figure 30 shows the information for varieties on the 2008 Recommended List.

Differences in ramularia levels between the varieties were not significant. This suggests no winter sown variety shows effective varietal resistance to the disease.

Table 36 Winter barley recommended List Leaf spotting averages

	Number of trials		Transformed data (log)		% Ramularia	
Variety	Flag leaf	Leaf 2	Flag leaf	Leaf 2	Flag leaf	Leaf 2
Accrue	4	4	1.5	2.2	3.3	7.9
Acctrice	2	2	2.7	3.1	14.1	21.9
Amarena	6	6	2.3	2.7	8.7	13.8
Angela	2	3	2.7	2.9	14.1	16.4
Antelope	1	1	3.2	2.9	22.9	17.1
Antonia	1	2	2.1	2.3	6.9	9.2
Blythe	2	2	2.5	2.4	11.0	10.3
Boost	6	6	2.5	3.2	11.4	23.4
Bronx	4	4	2.5	3.1	11.5	22.0
Camion	7	7	2.0	2.1	6.6	7.0
Cannock	5	5	1.6	2.3	4.0	8.6
Carat	8	9	1.7	2.4	4.5	10.1
Cassata	4	4	2.4	2.7	10.4	13.3
Celebrity	2	2	2.9	3.0	16.4	18.2
Clara	2	3	2.2	3.2	8.2	23.0
Colibri	6	6	2.7	3.2	14.3	22.5
Colossus	7	7	2.4	2.9	10.0	17.3
Connoiser	1	1	1.7	2.7	4.5	14.1
CPBT	1	2	3.0	3.2	19.6	23.3
Eden	1	1	2.2	2.2	8.3	8.0
Cypress	2	2	1.6	2.9	4.2	16.4
Diamond	1	2	2.1	2.7	7.0	14.0
Dolphin	2	2	1.0	2.4	1.8	10.1
Fahrenheit	2	2	2.7	2.8	13.8	14.7
Fanfare	1	2	2.3	2.9	9.5	16.6
Flagon	6	6	2.6	3.5	12.2	30.6
Haka	2	3	1.6	2.4	4.0	10.5
Heligan	1	2	2.6	3.3	12.9	27.2
Jewel	2	3	2.2	2.9	8.3	16.8
Kestrel	2	3	2.8	3.1	15.4	20.6
Kingston	1	1	2.3	2.2	9.1	8.3
Leonie	1	2	2.6	3.3	12.1	25.6
Manitou	2	2	2.6	3.1	12.4	20.6
Marado	2	2	2.1	2.4	7.2	10.3
Marcorel	2	2	2.0	2.9	6.5	16.8
Muscat	1	2	2.8	2.8	15.4	14.8
Nocturne	1	1	3.2	2.9	22.9	17.1
Opal	1	2	2.6	2.7	12.6	14.5
Parasol	1	2	1.5	2.9	3.6	17.5
Pastoral	3	4	1.6	2.5	4.0	11.0
Pearl	8	9	2.2	2.8	8.2	15.9
Pedigree	2	3	2.6	3.3	12.9	25.3
Pelican	4	4	3.0	3.0	19.7	19.6

Table 36 continued

	Number of trials		Transformed data (log)		% Ramularia	
Variety!	Flag leaf	Leaf 2	Flag leaf	Leaf 2	Flag leaf	Leaf 2
Pict	8	9	2.5	3.0	11.5	18.3
Regina	4	5	2.5	2.6	11.7	12.8
Retriever	4	4	2.3	2.6	9.2	12.8
Rounder	2	2	1.3	2.1	2.7	6.8
Saffron	6	6	2.1	2.3	7.0	8.8
Scylla	4	5	2.2	2.4	8.5	10.2
Sequel	8	9	2.8	2.8	15.2	15.6
Siberia	4	5	1.6	2.4	3.9	10.5
Spectrum	6	6	2.3	2.8	9.4	14.8
Sumo	2	3	2.7	2.7	14.0	13.9
Surtees	2	2	1.5	2.2	3.5	8.5
Suzuka	4	4	2.4	3.0	9.9	18.7
Swallow	2	3	2.6	2.8	11.9	14.8
Talica	1	1	2.3	2.2	9.1	8.3
Vanessa	1	2	2.3	3.0	8.5	19.2
Vertige	1	2	2.3	2.8	8.6	14.8
Wigwam	1	1	2.5	2.5	10.9	11.2
Wintmalt	2	2	3.2	3.4	23.1	29.2
Zzoom	2	2	2.6	2.4	12.1	9.8
SED						
Average:			0.7	0.6		
Maximum:			1.0	1.0		
Minimum:			0.3	0.3		
variety p-value			0.273	0.629		

Figure 30 shows ramularia levels in the 2008 winter barley Recommended List varieties. Disease levels range from 5% to 25 % on leaf 2. Note highest levels were seen on the varieties Flagon and Wintmalt and lowest levels on Camion, Accrue and Saffron.

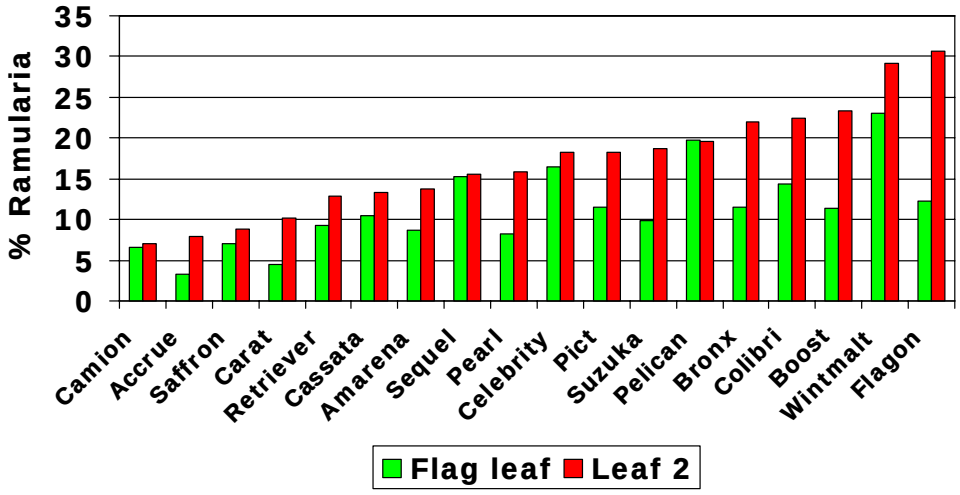


Figure 30 % Ramularia in winter barley recommended List Varieties 2005-07

Green leaf scores are equally important, since varieties which lose green leaf area rapidly (Figure 31). Care must be taken since varieties which suffer from early disease (i.e. rhynchosporium) or are early maturing, will show lower green leaf scores. Carat and Retriever recorded the lowest scores. Other field observations suggest Retriever can lose green leaf area rapidly late in the season, and this variety should be protected from late ramularia to prevent this.

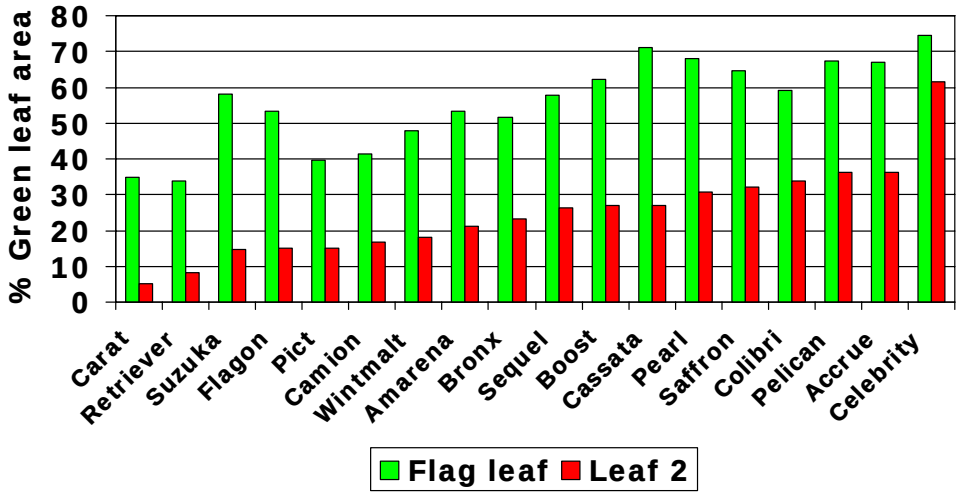


Figure 31 % Green leaf area in winter barley recommended List varieties 2005-2007

Spring barley

Table 37 shows the average levels of ramularia, on the upper leaves for a range of spring barley varieties in Recommended List trials between 2002- 2007. Figure 32 shows varieties on the 2008 Recommended List and two susceptible reference varieties, Pewter (susceptible to ramularia) and Prestige (susceptible to abiotic leaf spots).

Differences between the varieties in ramularia, abiotic leaf spots and green leaf are significant. Some varieties have only been in trial for one year, so care has to be taken in interpretation, but most of the varieties were in trial for three years.

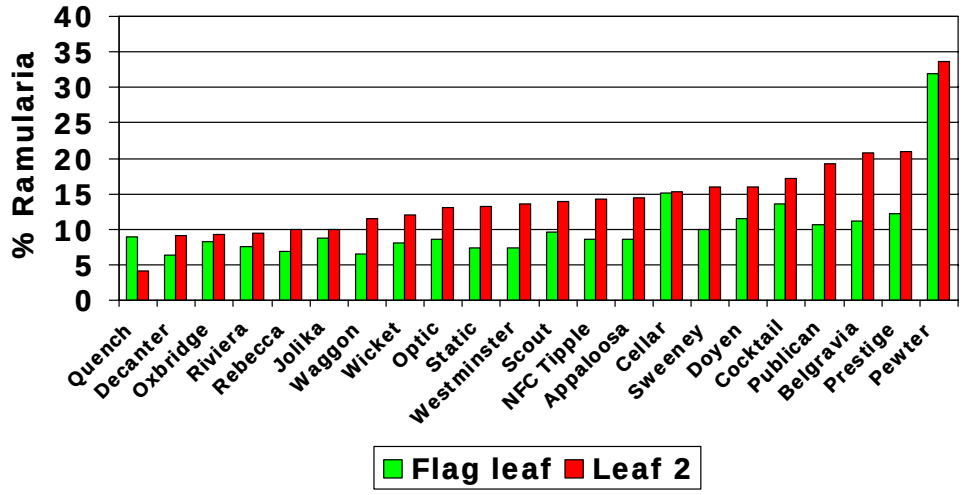


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Figure 32 Ramularia scores in spring barley Recommended list varieties 2008

Decanter, Quench Oxbridge, Riviera Rebecca and Jolika showed the lowest levels of ramularia leaf spots. Doyen, Cocktail, Publican and Belgravia showed the highest levels, but there is improvement compared to the reference susceptible variety Pewter. Disease levels can vary from season to season and in the case of Quench and Publican, data will be limited to 2007 data, so care is required to categorise a variety based on limited data. Cocktail has consistently shown high levels of leaf spots and Decanter has consistently shown lower disease levels. Optic is intermediate in this analysis, but in the most current data, disease levels are higher compared to earlier data, suggesting varietal resistance can change over the lifetime of a variety.

Table 37 Spring Barley Recommended List % Ramularia,

Variety	Number of trials		Log ramularia		% Ramularia	
	Flag	Leaf 2	Flag	Leaf 2	Flag	Leaf 2
Appaloosa	4	4	2.3	2.7	8.6	14.4
Aquila	3	3	2.1	2.2	7.0	8.4
Athena	2	2	1.9	2.4	5.5	10.4
Azalea	3	3	2.3	1.4	9.1	3.1
Barke	2	3	1.7	1.7	4.6	4.4
Beatrix	3	3	2.3	2.5	8.6	10.7
Belgravia	4	4	2.5	3.1	11.1	20.7
Beryllium	2	2	2.8	3.0	15.7	18.6
Braemar	0	1		3.7		37.5
Carafe	6	10	2.6	2.6	12.6	12.9
Cellar	9	14	2.8	2.8	15.1	15.3
Centurion	3	3	1.7	2.5	4.7	11.3
Chalice	8	13	2.6	2.7	12.6	14.3
Cocktail	11	16	2.7	2.9	13.6	17.1
Colston	2	3	2.4	2.9	9.9	17.7
County	2	3	2.6	2.6	12.1	12.3
Cribbage	3	3	1.8	2.1	5.1	7.6
Decanter	12	16	2.0	2.3	6.3	9.1
Doyen	8	12	2.5	2.8	11.5	16.0
Drum	3	3	2.6	2.9	12.4	17.0
Fairytale	3	3	2.4	2.6	9.7	12.7
Feltwell	2	2	2.6	2.8	12.4	15.3
Global	2	3	2.4	2.3	9.7	9.4
Henley	0	4		2.6		12.5
Hydra	3	3	2.9	3.3	17.4	25.0
Jersey	0	1		2.2		7.9
Jolika	3	3	2.3	2.4	8.8	10.0
Kirsty	5	10	2.2	2.6	7.7	12.0
Knightsbridge	4	4	2.3	2.7	9.2	13.7
Macaw	0	4		2.9		16.9
Maltby	4	4	2.3	2.8	8.8	15.5
Minstrel	0	4		3.1		21.6
NFC Tipple	7	11	2.3	2.7	8.5	14.2
Novello	2	3	3.1	3.2	20.2	23.0
Optic	12	17	2.3	2.6	8.6	13.1
Oxbridge	7	11	2.2	2.3	8.2	9.3
Pewter	2	3	3.5	3.5	32.3	33.6
Poker	3	3	2.3	2.5	8.6	10.7
Power	6	10	2.5	2.5	11.0	10.8
Prestige	5	10	2.6	3.1	12.2	20.9
Publican	4	4	2.5	3.0	10.7	19.3
Putney	3	3	2.3	2.4	9.4	9.6
Quench	4	4	2.3	1.6	9.0	4.2

Table 37 continued

Variety	Number of trials		Log ramularia		% Ramularia	
	Flag	Leaf 2	Flag	Leaf 2	Flag	Leaf 2
Rebecca	8	12	2.1	2.4	6.8	9.9
Riviera	10	15	2.1	2.3	7.5	9.4
Scout	4	4	2.4	2.7	9.6	13.9
Sebastian	2	3	2.7	2.6	13.8	11.8
Snakebite	3	3	2.1	1.8	7.6	4.9
Spire	7	12	2.2	2.5	8.4	11.5
Static	8	13	2.1	2.7	7.4	13.3
Sweeney	3	3	2.4	2.8	10.0	15.9
Tavern	1	1	2.7	1.9	14.4	5.8
Thetford	3	3	2.7	2.8	13.6	15.8
Toby	2	2	3.0	3.0	19.9	19.1
Tocada	3	7	2.6	2.5	12.4	11.0
Topic	2	3	2.3	3.2	9.2	23.5
Toucan	0	4		3.3		25.3
Troon	6	10	2.3	2.6	9.0	13.0
Vortex	2	3	2.4	2.2	10.1	8.4
Waggon	7	11	2.0	2.5	6.6	11.5
Westminster	6	10	2.1	2.7	7.4	13.5
Wicket	6	10	2.2	2.6	8.1	12.1
SED						
Average:			0.4	0.4		
Maximum:			0.6	0.8		
Minimum:			0.2	0.2		
variety p-value			0.013	<0.001		

Table 38 shows results for abiotic leaf spots. The 2008 recommended list varieties and the susceptible reference varieties are shown in Figure 33.

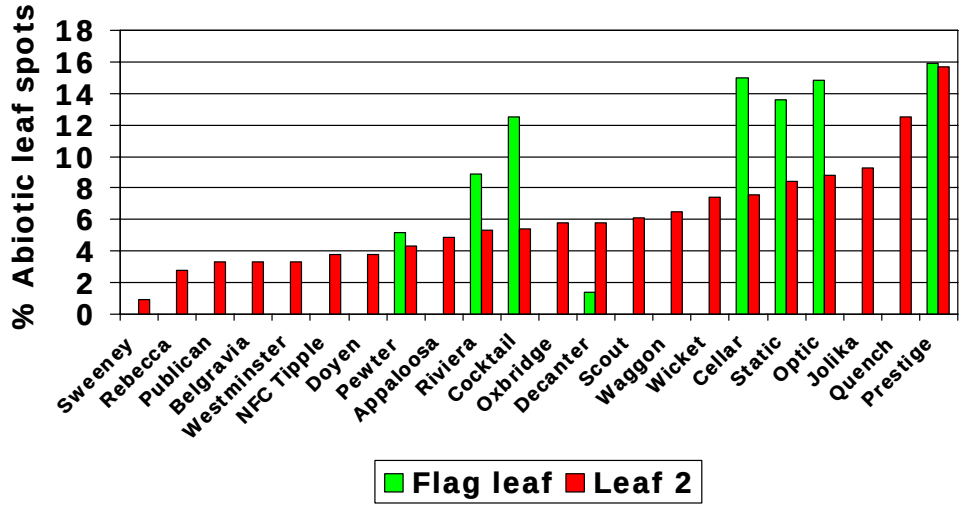

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Figure 33 % Abiotic leaf spots in spring barley recommended list varieties and Pewter and Prestige

Abiotic leaf spot levels are currently low in most RL varieties compared to the susceptible variety Prestige.

Table 38 Spring Barley Recommended List %, abiotic leaf spots

Variety	Number of trials		Log abiotic spots		% Abiotic spots	
	Flag	Leaf 2	Flag	Leaf 2	Flag	Leaf 2
Appaloosa	0	4		1.8		4.9
Aquila	0	1		1.4		3.0
Athena	0	0				
Azalea	0	3		1.6		4.0
Barke	2	2	1.9	2.0	5.7	6.6
Beatrix	0	1		2.2		8.0
Belgravia	0	4		1.5		3.3
Beryllium	0	0				
Braemar	1	1	2.1	1.7	7.3	4.5
Carafe	0	4		1.6		3.9
Cellar	2	7	2.8	2.2	15.0	7.6
Centurion	0	1		1.4		3.0
Chalice	2	6	2.0	1.9	6.2	5.8
Cocktail	2	9	2.6	1.8	12.5	5.4
Colston	2	2	2.5	2.5	11.0	11.3
County	2	2	2.3	2.4	8.7	10.0
Cribbage	0	1		1.6		4.0
Decanter	2	10	0.9	1.9	1.4	5.8
Doyen	0	7		1.6		3.8
Drum	0	0				
Fairytale	0	3		2.2		8.5
Feltwell	0	0				
Global	2	2	1.4	1.9	3.2	5.9
Henley	0	3		1.6		4.1
Hydra	0	1		1.1		2.0
Jersey	1	1	2.1	1.3	7.3	2.6
Jolika	0	3		2.3		9.3
Kirsty	2	5	1.9	1.9	5.5	5.8
Knightsbridge	0	4		2.4		10.5
Macaw	0	3		1.8		5.0
Maltby	0	4		1.5		3.6
Minstrel	0	3		1.5		3.5
NFC Tipple	0	8		1.6		3.8
Novello	2	2	2.1	1.9	6.9	5.7
Optic	2	10	2.8	2.3	14.8	8.8
Oxbridge	0	8		1.9		5.8
Pewter	2	2	1.8	1.7	5.2	4.3
Poker	0	1		1.6		4.0
Power	0	7		1.5		3.4
Prestige	2	5	2.8	2.8	15.9	15.7
Publican	0	4		1.5		3.3
Putney	0	1		2.1		7.0
Quench	0	4		2.6		12.5

Table 38 continued.

Variety	Number of trials		Log abiotic spots		% Abiotic spots	
	Flag	Leaf 2	Flag	Leaf 2	Flag	Leaf 2
Rebecca	0	7		1.3		2.8
Riviera	2	9	2.3	1.8	8.9	5.3
Scout	0	4		2.0		6.1
Sebastian	2	2	2.7	2.1	14.0	7.6
Snakebite	0	3		2.7		13.8
Spire	2	6	1.3	1.4	2.8	2.9
Static	2	6	2.7	2.2	13.6	8.4
Sweeney	0	3		0.6		0.9
Tavern	1	1	1.7	2.3	4.4	9.1
Thetford	0	0				
Toby	0	0				
Tocada	0	4		1.3		2.8
Topic	2	2	2.6	2.1	12.6	7.1
Toucan	0	3		3.3		25.1
Troon	0	7		2.1		7.1
Vortex	3	3	1.9	1.6	6.0	4.2
Waggon	0	8		2.0		6.5
Westminster	0	7		1.5		3.3
Wicket	0	7		2.1		7.4
SED						
Average:			0.7	0.6		
Maximum:			1.0	1.0		
Minimum:			0.6	0.3		
variety p-value			0.373	0.007		

Green leaf scores are shown in table 39 and Figure 34

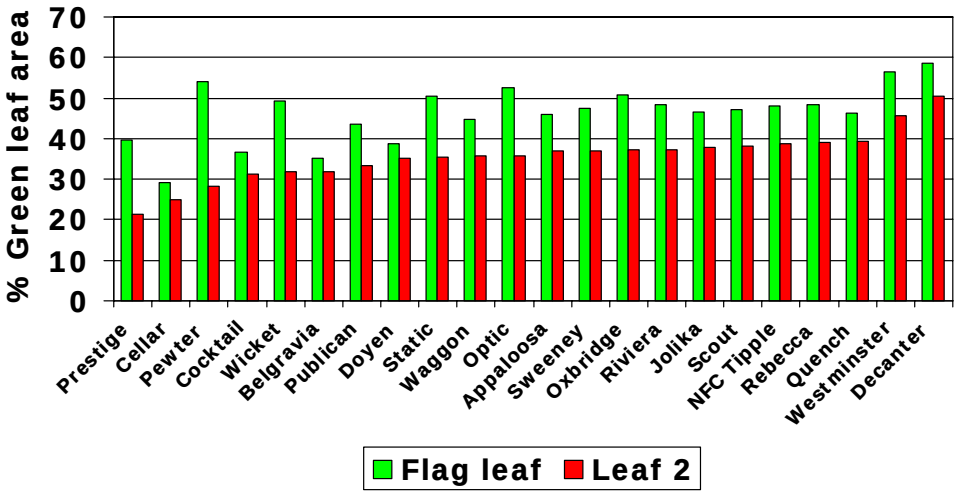


Figure 34 % Green leaf area in spring barley Recommended List Varieties 2005 and 2007

Green leaf scores are possibly a better way to reflect a varietal response to leaf spots, since accurate identification of ramularia can be challenging. Care has to be taken since an early variety will lose green leaf earlier than a late variety. Decanter Westminster and Quench show good green leaf retention, Cellar, Cocktail and the two reference varieties Prestige and Pewter show lowest green leaf scores. Note Quench had low ramularia, high abiotic leaf spots and high green leaf scores. The abiotic leaf spots have therefore had little impact on accelerating loss in green leaf for this variety.

Table 39 Spring Barley Recommended List % Green leaf area,

Variety	Number of trials		Angular transformation green leaf		% Green leaf area	
	Flag leaf	Leaf 2	Flag leaf	Leaf 2	Flag leaf	Leaf 2
Appaloosa	4	4	42.7	37.4	46.0	36.9
Aquila	3	3	45.1	39.7	50.3	40.7
Athena	2	2	52.0	52.8	62.1	63.4
Azalea	3	3	43.2	36.0	46.9	34.5
Barke	3	3	44.3	27.6	48.8	21.5
Beatrix	3	3	25.0	26.2	17.9	19.6
Belgravia	4	4	36.3	34.4	35.1	31.9
Beryllium	2	2	33.5	34.3	30.4	31.7
Braemar	1	1	42.0	26.7	44.7	20.1
Carafe	6	10	41.9	35.6	44.7	33.9
Cellar	10	14	32.6	29.8	29.0	24.8
Centurion	3	3	33.2	38.3	30.0	38.4
Chalice	9	13	37.9	31.8	37.7	27.8
Cocktail	12	16	37.3	33.9	36.8	31.1
Colston	3	3	40.9	27.6	42.9	21.5
County	3	3	34.1	27.1	31.5	20.7
Cribbage	3	3	51.1	50.7	60.6	59.8
Decanter	13	16	49.9	45.2	58.5	50.4
Doyen	8	12	38.5	36.4	38.7	35.2
Drum	3	3	43.5	35.4	47.4	33.5
Fairytale	3	3	42.5	39.1	45.6	39.8
Feltwell	2	2	46.1	37.1	52.0	36.5
Global	3	3	41.2	28.9	43.4	23.3
Henley	0	4		37.0		36.2
Hydra	3	3	41.0	29.1	43.1	23.6
Jersey	1	1	42.0	20.6	44.7	12.4
Jolika	3	3	43.0	37.9	46.6	37.8
Kirsty	6	10	35.4	36.7	33.6	35.7
Knightsbridge	4	4	44.5	41.1	49.1	43.3
Macaw	0	4		27.8		21.8
Maltby	4	4	43.5	37.9	47.4	37.7
Minstrel	0	4		31.9		27.9
NFC Tipple	7	11	44.0	38.5	48.2	38.7
Novello	3	3	51.2	44.1	60.8	48.4
Optic	13	17	46.4	36.7	52.5	35.8
Oxbridge	7	11	45.4	37.6	50.8	37.2
Pewter	3	3	47.3	32.1	54.0	28.2
Poker	3	3	53.0	43.5	63.7	47.4
Power	6	10	39.4	39.4	40.3	40.2
Prestige	6	10	39.1	27.5	39.8	21.3
Publican	4	4	41.3	35.2	43.5	33.2
Putney	3	3	33.1	25.0	29.9	17.8
Quench	4	4	42.8	39.0	46.2	39.5

Table 39 continued

Variety	Number of trials		Angular transformation green leaf		% Green leaf area	
	Flag leaf	Leaf 2	Flag leaf	Leaf 2	Flag leaf	Leaf 2
Rebecca	8	12	44.0	38.7	48.3	39.0
Riviera	11	15	44.1	37.7	48.5	37.3
Scout	4	4	43.4	38.2	47.2	38.2
Sebastian	3	3	29.8	23.6	24.7	16.1
Snakebite	3	3	54.7	46.9	66.6	53.4
Spire	8	12	48.3	36.7	55.7	35.8
Static	9	13	45.3	36.6	50.5	35.6
Sweeney	3	3	43.6	37.4	47.6	36.9
Tavern	2	2	41.0	25.1	43.0	17.9
Thetford	3	3	39.8	28.0	41.0	22.1
Toby	2	2	30.6	28.4	25.9	22.6
Tocada	3	7	42.9	32.9	46.4	29.5
Topic	3	3	37.5	27.0	37.0	20.6
Toucan	0	4		20.5		12.2
Troon	6	10	36.9	36.2	36.1	35.0
Vortex	3	3	42.3	40.4	45.3	42.0
Waggon	7	11	42.0	36.7	44.8	35.7
Westminster	6	10	48.7	42.5	56.4	45.7
Wicket	6	10	44.7	34.3	49.4	31.8
SED						
Average:			6.7	5.7		
Maximum:			12.3	10.9		
Minimum:			3.4	2.7		
variety p-value			<0.001	<0.001		

Discussion

No variety shows complete resistance to ramularia, but the variety Decanter has consistently shown lower levels of symptoms. The variety Cocktail at the other end of the spectrum has shown high levels of symptoms in recommended List trials. Compared to other varieties (Pewter), the current list shows how improvements have been made in varietal resistance recent years.

Green leaf area scores can be misleading, since later maturing varieties would be expected to have higher levels of green leaf area than early maturing varieties area on any given date. The most susceptible varieties Cocktail and Prestige were amongst the varieties with lower green leaf scores, whilst Decanter and Westminster which had lower levels of ramularia had higher green leaf area scores. This suggests the most susceptible varieties do senesce early as a result of high levels of ramularia.

The role of seed infection may change the varietal susceptibility; hence new research will focus on seed stocks with high or low levels of contamination. It may be concluded however that more susceptible varieties are likely to get higher levels of seed infection. A detailed study of these results could include the presence of the mlo5 resistance gene to determine if varieties with the gene show more symptoms in the field. The role of asymptomatic infection cannot be studied by visual observations alone. Future plant breeding programmes should make use of PCR tools to determine both symptom expression and also asymptomatic disease in developing resistant varieties.

Weather influences on Ramularia spore dispersal

Introduction

Weather influences on the spread and infection of *R. collo-cygni* are poorly understood. It has been suggested that the symptoms on barley leaves induced by *R. collo-cygni* are dependent on light intensity (E. Sachs, unpublished results, quoted in Heiser *et al.,* 2003). Shading of plants in the field has been shown to significantly reduce the number of *R. collo-cygni* lesions (Oxley *et al*., 2002). Fungal sporulation only occurs in necrotic tissue, with conidiophores emerging through stomata. *R. collo-cygni* grows preferably, although not exclusively, on abaxial (bottom) leaf surfaces and Huss (2004) estimates that a heavily infected leaf can produce up to 50, 000 conidia. However little work has been done on spore dispersal. Field monitoring has relied on the use of simple agar coated spore traps (Huss pers com). However the development of specific PCR based assays for *R. collo-cygni* has enabled more precise work to be carried out (Havis *et al.* 2006a). The capture, detection and quantification of plant pathogenic spores from environment samplers has been demonstrated in previous studies (Calderon *et al.* 2002)

Materials and Methods

Four automatic meteorological stations were situated at SAC trial sites within Scotland in 2004 and measurements of radiation, sunlight, rainfall, air temperature, surface wetness, wind direction and speed taken on an hourly basis. The four sites chosen were Aberdeen, Perth, Bush Estate (Midlothian) and Lockerbie. 14 day volumetric spore samplers (Burkard, UK) were situated at the Aberdeen, Perth and Lockerbie sites and a 7 day recording tape sampler was set up at the Bush Estate. DNA was collected form the 14 days samplers in 1.5 ml eppendorf tubes and extracted using the following protocol. The collected material was suspended in 1 ml of 0.1% Nonidet P-40 by vortexing for 2 min. A 0.5 ml aliquot was taken from the tube and placed into a 2cm screw top tube (Alpha labs). Approx 0.2 g of Ballotini beads were added to each tube and 440 μl of extraction buffer [Extraction buffer – Solution is 2xTEN buffer pH 8.0 with 5 mM Phenanthroline, Mercaptoethanol (1 μl/ml) and PVP (0.02g/ml)]. Samples were placed on Fastprep machine for two 40 second bursts (placed on ice for 2 minutes between runs) 800 μl of phenol/chloroform/IAA (25:24:1) was added to each tube and samples were centrifuged for 10 minutes at 13,000 rpm. The aqueous supernatant was transferred to another tube (approx 600 μl) and the DNA precipitated

by the addition of 40 μl of 0.5M Ammonium, 600 μl of isopropanol and 4 μl of Glycoblue. The samples were then centrifuged at 13,000 rpm for 20 minutes. The supernatant was discarded and the blue pellets washed with 600 μl of 70% ethanol. Following another centrifuge run at 13,000 rpm for 10 minutes the supernatant was discarded. Tubes were left to air dry before the pellet was resuspended in 100 μl of sterile distilled water. The tubes were placed in a 50 °C water bath for 10 minutes and pipetted gently to ensure complete resuspension. Tape samples from the Bush Estate were cut into shorter pieces corresponding to a 24 hour period. The segments were halved lengthways and one half stored in case further analysis was required. DNA from the tapes was extracted using the following protocol; Samples were cut into small 1 cm lengths using flamed scissors and placed into a 2cm screw top tube (Alpha labs). Approx 0.4 g of Ballotini beads were added to each tube and 440 μl of extraction buffer and DNA extracted as for the eppendorf sam Primer Premier software (version 5.0 ; Premier Biosoft International, Palo Alto, CA) was used to design forward primer RamF6 (5'-CGTCATTTCACCACTCAAG-3') and reverse primer RamR6 (5'-CCTCTGCGAATAGTTGCC-3').

Molecular beacon probe Ram6 designed by Sigma-Genosys (Sigma, UK) (5'-GCGATTCCGGCTGAGCGGTTCGTCATCGCG-3') was labelled at the 5' end with the reporter dye FAM (6-carboxy-fluorescein) while the 3' end was modified with the quencher dye BHQ1.

Real-time PCR assays were performed in a total volume of 25 μl. The reaction mixture contained the following components (final concentration) : 1x iQ^{TM} supermix (Bio-Rad, Hercules, CA), 400 nm forward and reverse primer, 150 nm Ramularia probe, 5μl DNA template (20ng μl^{-1}) and finally, PCR-grade water to make up the final volume. Three simultaneous, replicated amplifications were carried out for each sample. For negative (no template) control reactions, nuclease free water was substituted for the DNA.

The *qPCR* was carried out in an iCycler iQ (Bio-Rad) according to the following program: An initial hot start of 10 min at 95ºC was followed by 50 cycles of 95ºC for 20 s, 55ºC for 20 s and 72ºC for 20 s. A final extension step of 95ºC for 1 min was added. Fluorescence emission was measured in the annealing phase. Threshold cycles were calculated automatically by the Bio-Rad iCycler software (Version 3.1).

Results

DNA extracts from the 14 day volumetric spore samplers were poor in terms of quality, primarily due to contamination with insect DNA. Tests for *ramularia* indicated

only 4-5 periods where spores were detected. No pattern in spore dispersal was evident from these samplers. In contrast the 7 day tape sampler produced good quality DNA extracts and a pattern in *ramularia* spore dispersal appeared.

The major peaks in spore dispersal appeared to be in the summer months (Figure 35). Closer examination of DNA results and meteorological data from the adjoining station indicated that there was no relationship between spore levels and rainfall, radiation levels, sunlight and wind velocity. However, plotting *ramularia* DNA and surface wetness indicated that spore release events occurred in the 48 hour period following a period of near maximum surface wetness (Figures 36, 37 and 38).

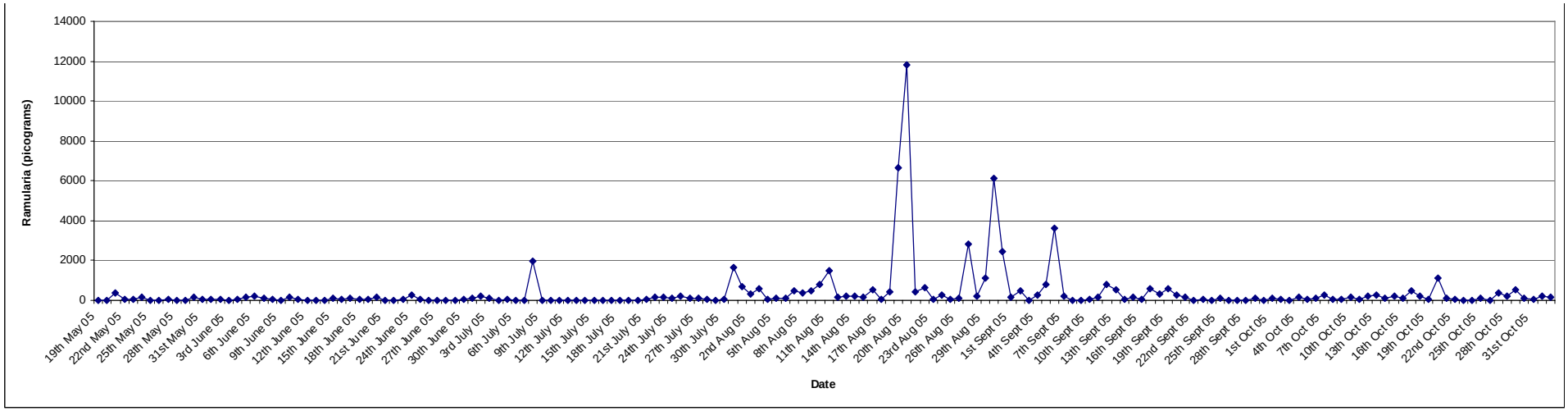


Figure 35. *R. collo-cygni* DNA concentration from daily reading at Bush spore trap(Nov 2004- Nov 2005)

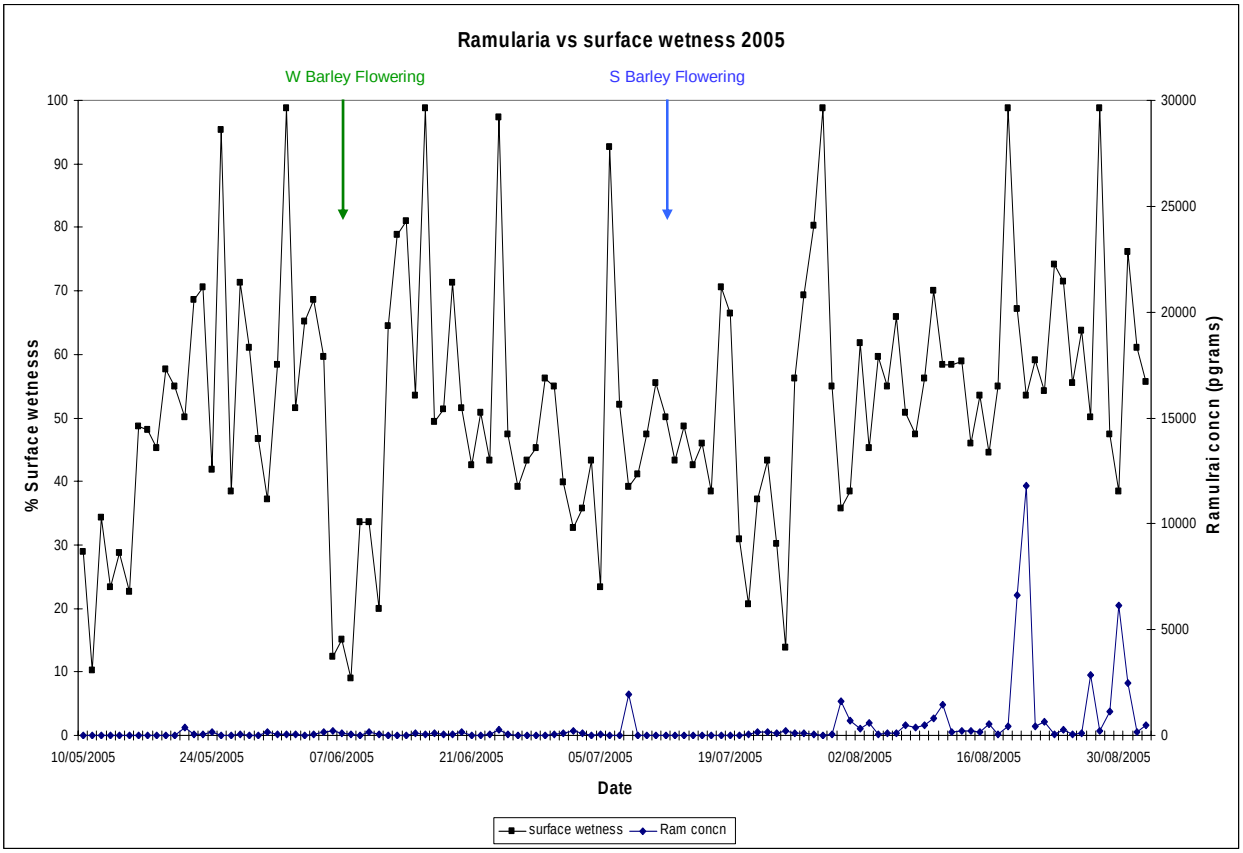


Figure 36 Ramularia spore dispersal (blue line) vs surface wetness (black line) 2005

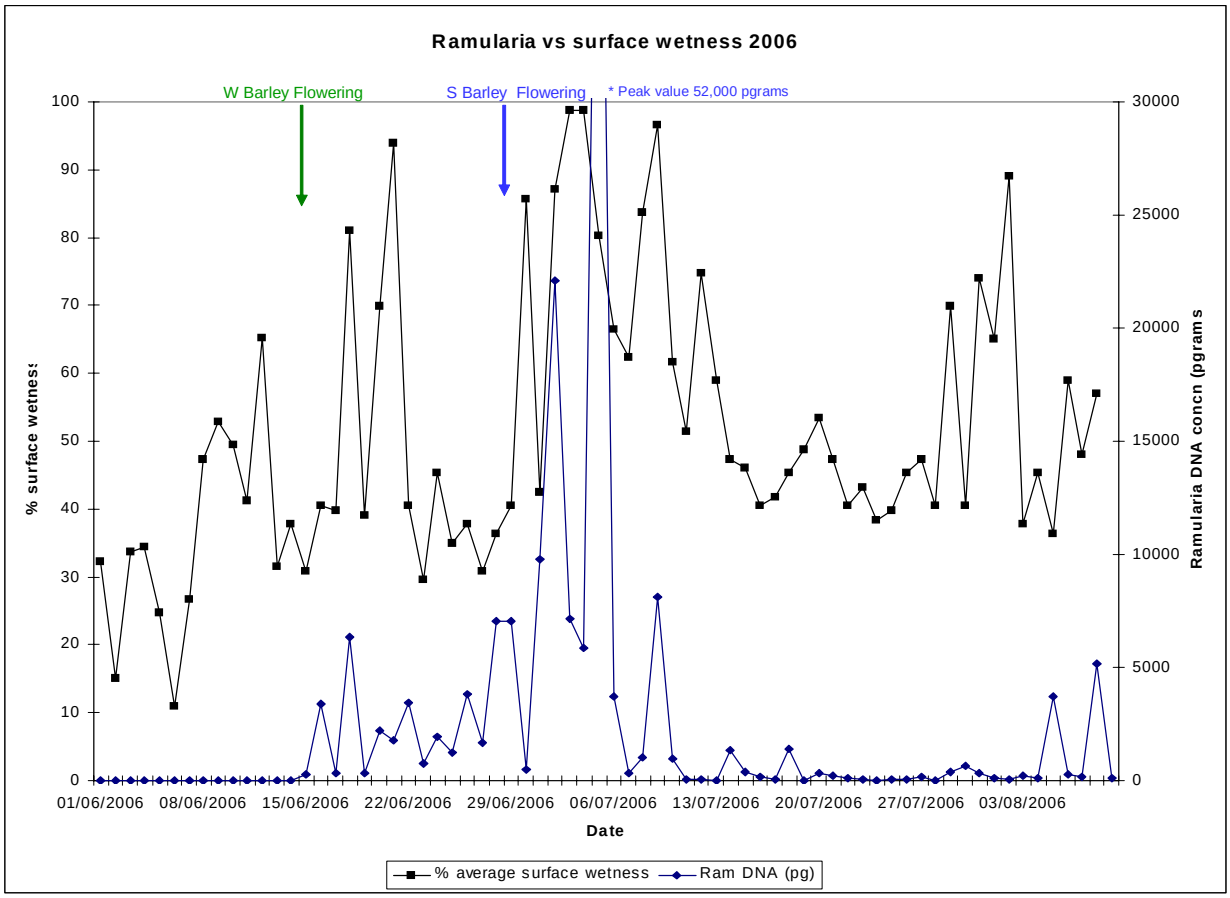


Figure 37 Ramularia spore dispersal (blue line) vs surface wetness (black line) 2006

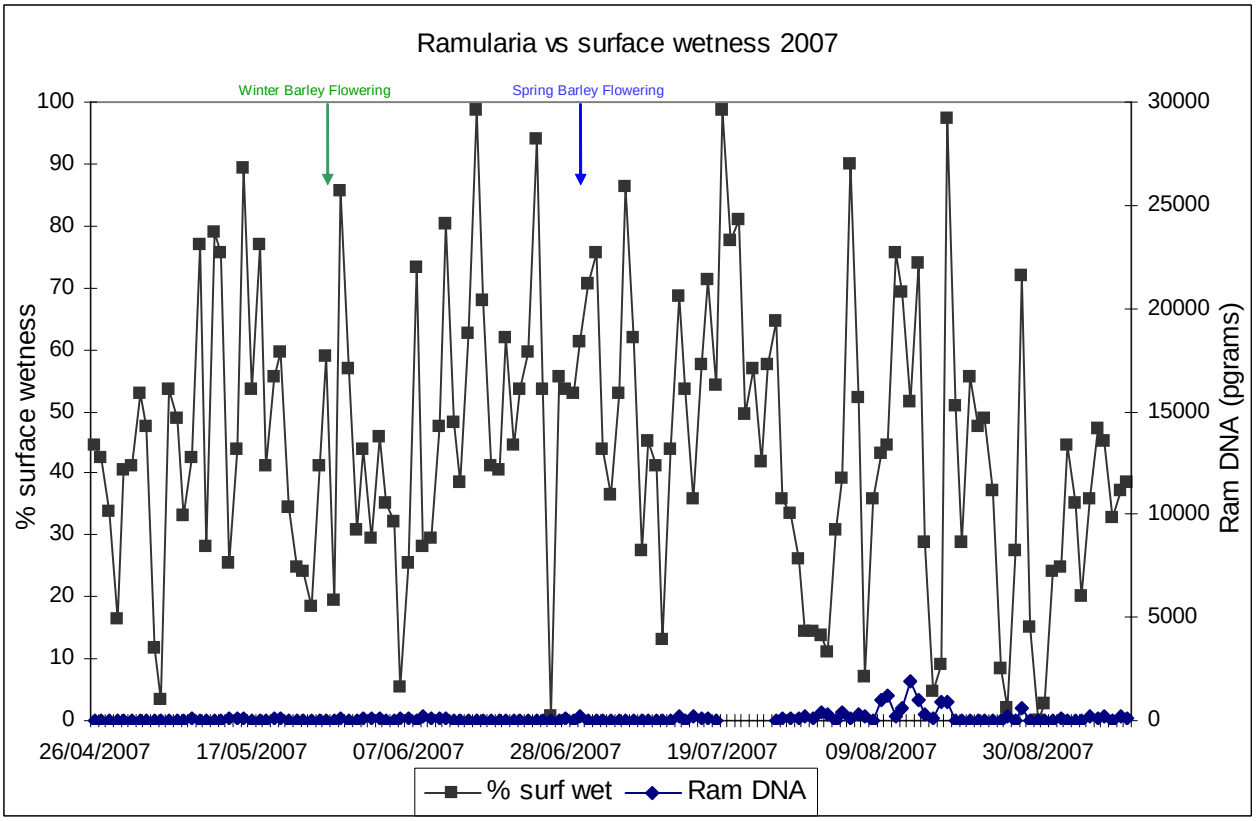


Figure 38 Ramularia spore dispersal (blue line) vs surface wetness (black line) 2007

DNA from the tape sampler was also plotted against the disease progress curves for winter and spring barley at the Bush trial site

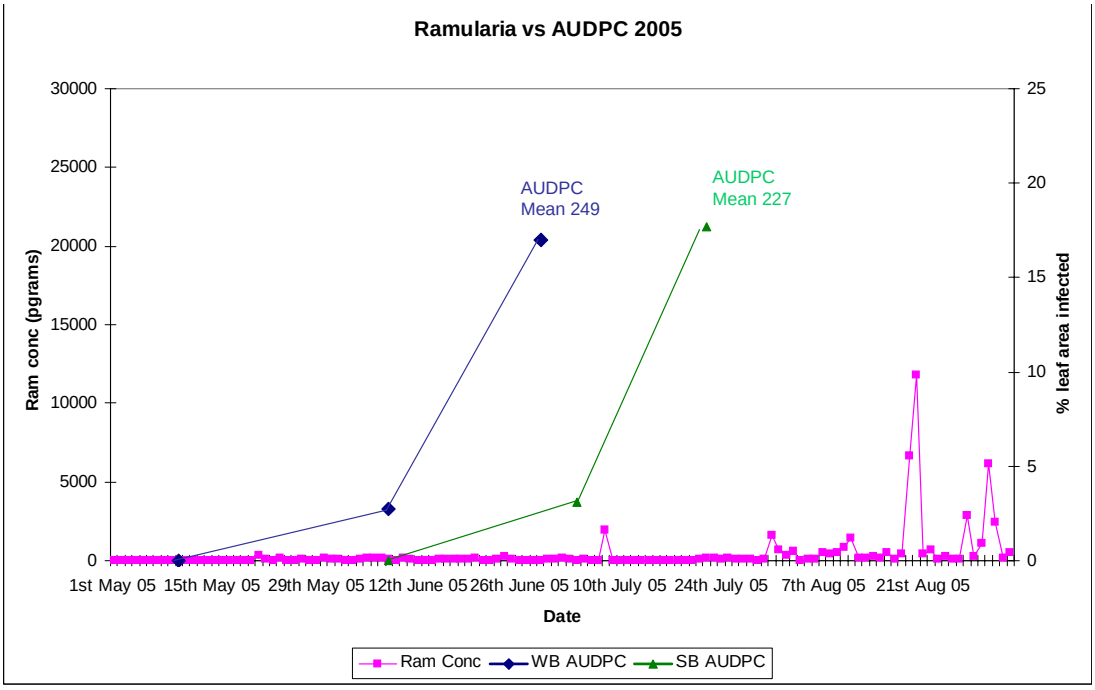


Figure 39 Ramularia DNA vs Area Under disease progress Curve (AUDPC) 2005

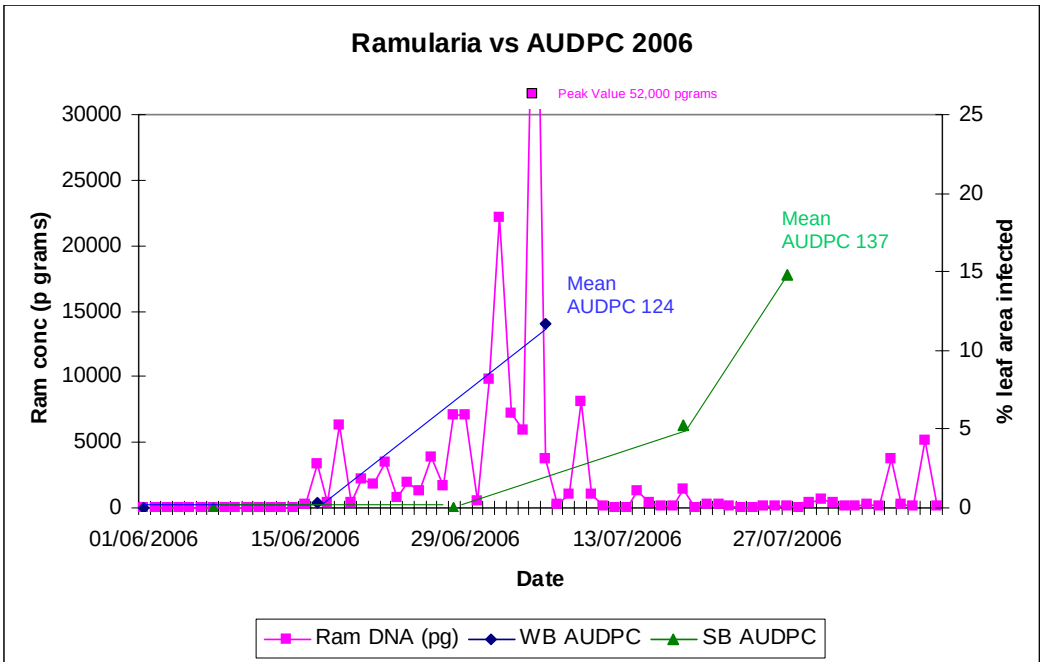


Figure 40 Ramularia DNA vs Area Under disease progress Curve (AUDPC) 2006

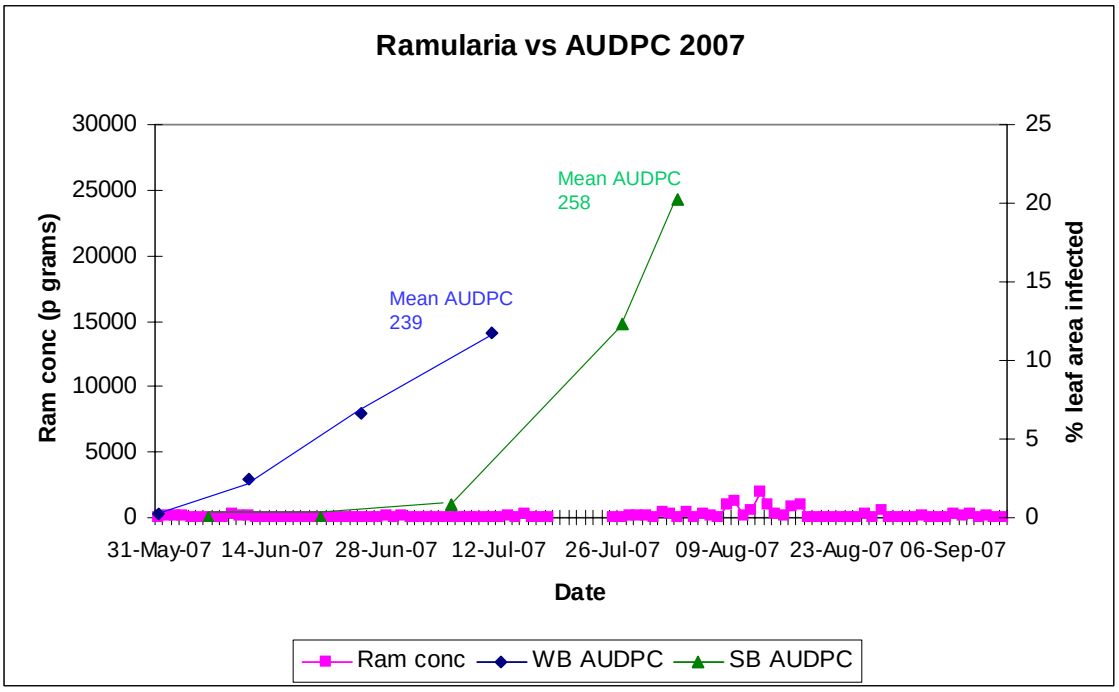


Figure 41 Ramularia DNA vs Area Under disease progress Curve (AUDPC) 2007

The results from the spore tape sampler indicated that the major spore release event in the growing season occurs late in the season, after flowering of either winter barley or spring barley. In 2005 the peak occurred in mid to late august well after spring barley flowering. Spores were released after a period of near 100% surface wetness. 2006 was a very different summer in Scotland with high temperatures leading to a rapid development and ultimately senescence of the crops. A massive peak in spore production occurred after a 48 hour period of 100% surface wetness in mid July. This peak was shortly after spring barley flowering. The summer of 2007 was noted for the very high levels of rainfall. The maximum peaks on *ramularia* spore production occurred in mid August, although recorded DNA levels were much lower than in previous years (Figures 35-38).

Discussion

Spore release events for *R. collo-cygni* were primarily in mid to late summer and were very different to the results obtained for the barley pathogen *Rhynchosporium secalis* and the closely related cereal pathogen *Mycosphaerella graminicola.*

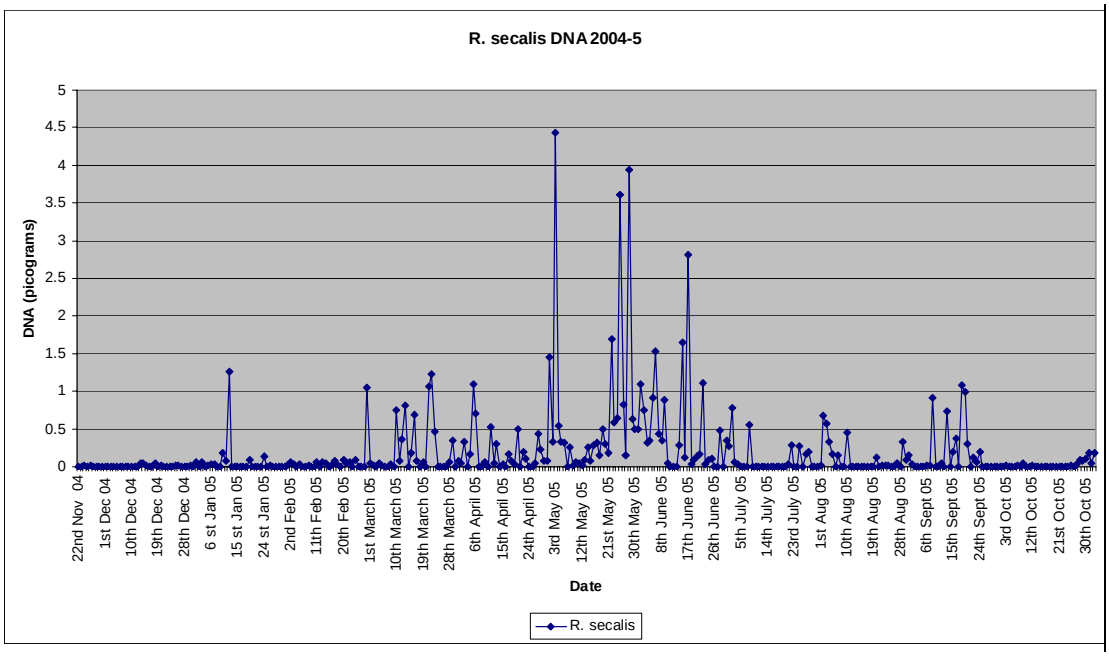


Figure 42 *R. secalis* DNA 2004-5

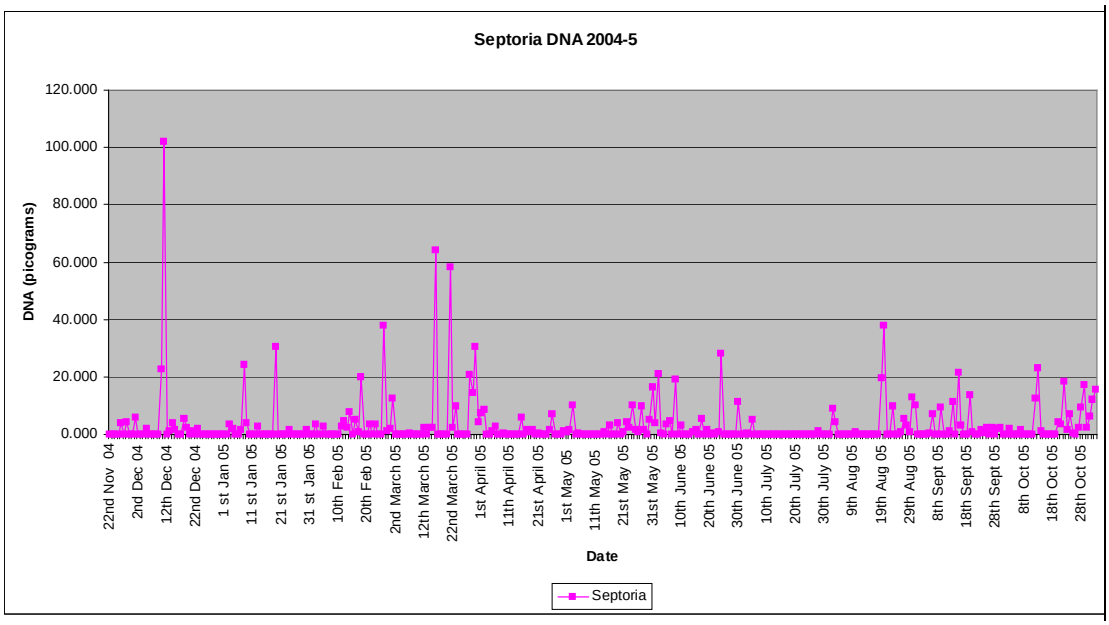


Figure 43 *M. graminicola* (*S. tritici*) DNA 2004-5

These pathogens exhibited a number of spore release events, however as they are both splash borne diseases there is greater potential for spore movement. Results for *ramularia* DNA indicate that there no movement via this method.

Early proposed life cycles for *R. collo-cygni* focused on the overwintering of the pathogen on winter crops and alternative hosts which produced inoculum to infect spring crops (Huss, 2006).

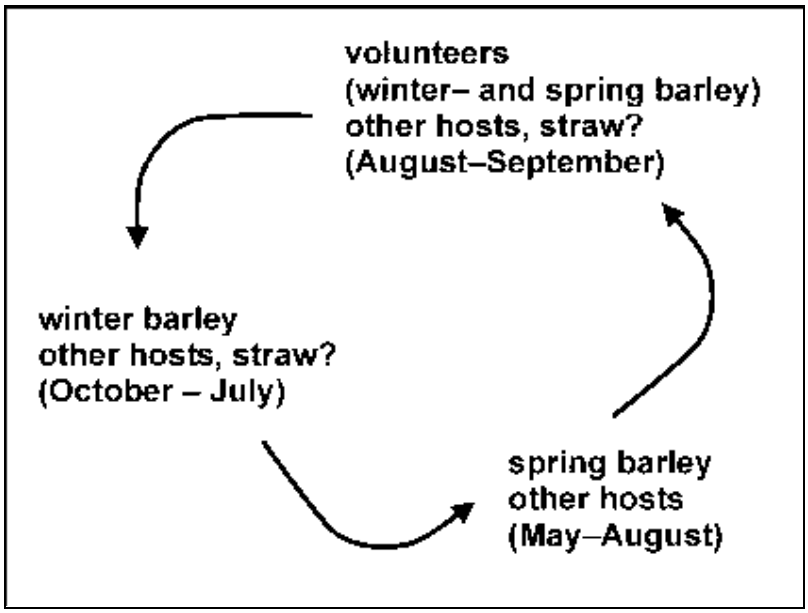


Figure 44 Ramularia Life Cycle (Frei 2007)

Frei *et al.* (2007) reported that in Switzerland winter barley crops acted as an inoculum source for spring crops. However, the results from the tape sampler indicate that in 2005 and 2007 symptoms developed in both winter and spring crops before any major spread of airborne spores. In the case of 2007 spore numbers were much reduced compared to previous years but AUDPC levels remained comparable to previous years and were greater than 2006. This would appear to indicate that *R collo-cygni* symptoms in crops could be due to infection from another source. The differences in AUDPC may also reflect the shortness of the 2006 season. Havis *et al.* (2006) demonstrated the existence of a seed borne stage for the pathogen and that symptom less movement from seed to developing leaves does occur. It is possible that the major spore release events detected during the growing season could be a major source of infection of ears and subsequent seed for the next season. A more detailed life cycle for *Ramularia collo-cygni* was proposed by Walters *et al.* (2008). This was modified in light of this research (Figure 45).

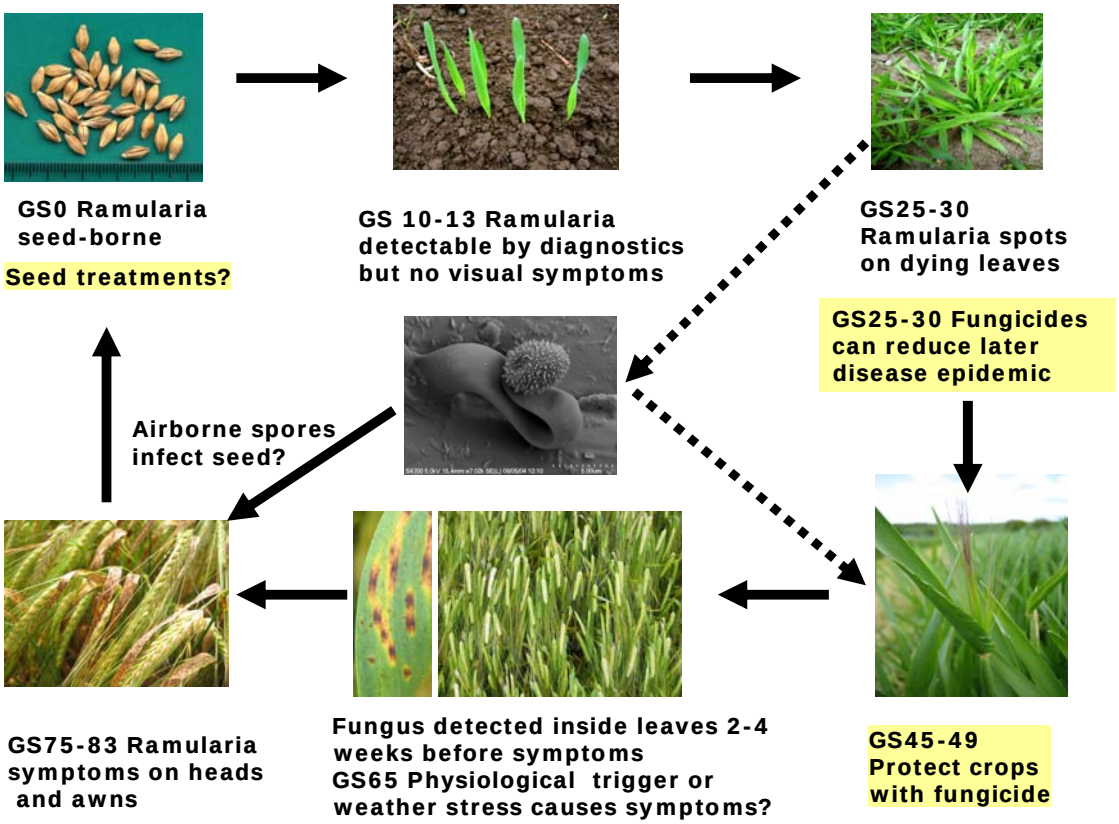


Figure 45 Proposed Ramularia lifecycle

The results from the spore sampler work would appear to indicate that disease symptoms may be due more to seed borne transmission than external infection via spores. Recent work from Germany has proposed that symptom expression in winter barley is independent of spore numbers or environmental conditions (Schützendübel *et al.*, 2008). This would imply that latent infections may be the major contributor to disease expression in Germany. Indeed study of disease development and *ramularia* DNA detection (Figures 39-41) indicated symptom development begins in winter barley prior to spore release events.

Continued sampling is ongoing as part of the Scottish Government funded Workpackage study into barley pathology, however, more work is now required into potential seed treatments to control or reduce ramularia infections. In addition late season fungicide applications to protect seed crops may have to be considered.

b) Investigate the role of mlo mildew resistance on barley leaf spots

Introduction

The effect of *mlo* mildew resistance on ramularia leaf spot

Research on the interaction of *mlo* mildew resistance with ramularia fell into two parts. Results relating to the first topic, the effect of *mlo* on symptoms of ramularia in field conditions, were published (Makepeace *et al.* 2007 Appendix 1). The second part has been accepted for publication (Makepeace *et al.* 2008 Appendix 2) and concerns the development of a laboratory method for testing varietal resistance to ramularia. The two papers describing the work are attached to this report. The following sections contain a summary of the key points in those publications. All references are cited in the papers. A summary is also provided of subsequent work on the effect of *mlo* on ramularia symptoms in laboratory conditions.

Effect of mlo mildew resistance on Ramularia in the field

Introduction

The *mlo* mildew resistance genes have been shown to increase the susceptibility of barley to spotting diseases common in the tropics, caused by *Magnaporthe grisea* and *Cochliobolus sativus*. The hypothesis tested here was that the extensive use of *mlo* in spring barley varieties in northern Europe has contributed to the substantial increase in spotting diseases over the last 20 years.

Materials and methods

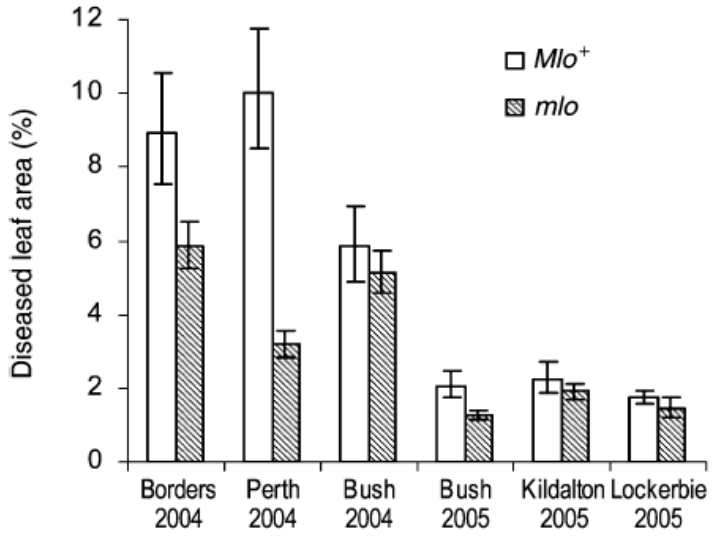
Near-isogenic lines of the barley varieties Pallas and Ingrid have been developed containing different alleles of the *Mlo* gene. Both varieties have the wild-type Mlo^{+} allele for susceptibility to mildew. Near-isogenics of Ingrid with the *mlo1*, *mlo3*, *mlo5* and *mlo9* resistance alleles are available, as well as a line of Pallas containing *mlo5*. These lines were trialled at four sites in Scotland and two in Ireland over three years and scored for symptoms of diseases and abiotic stress. Mildew was controlled by sprays of the mildew-specific fungicide quinoxyfen, to eliminate confounding of the effects of *Mlo* genes and of mildew infection on spotting diseases.

Results & Discussion

Although disease levels were generally quite low, levels of ramularia were high enough in six trials for comparisons between lines to be meaningful, five in Scotland and one in Ireland. In addition, levels of rhynchosporium were sufficiently high in three trials, all in Ireland. In contrast to the published reports of work on *M. grisea* and *C. sativus*, lines with *mlo* mildew resistance genes had consistently lower levels of ramularia symptoms than near-isogenic lines with the wild-type Mlo^+ mildew susceptibility gene (Figure 46). In addition, levels of leaf blotch were lower in *mlo* lines at two of the three sites analysed and similar at the third (Figure 2). The overall mean level of both diseases, averaged across sites, was lower in *mlo* lines. By contrast, *mlo* lines had consistently more abiotic spotting, consistent with numerous previous reports.

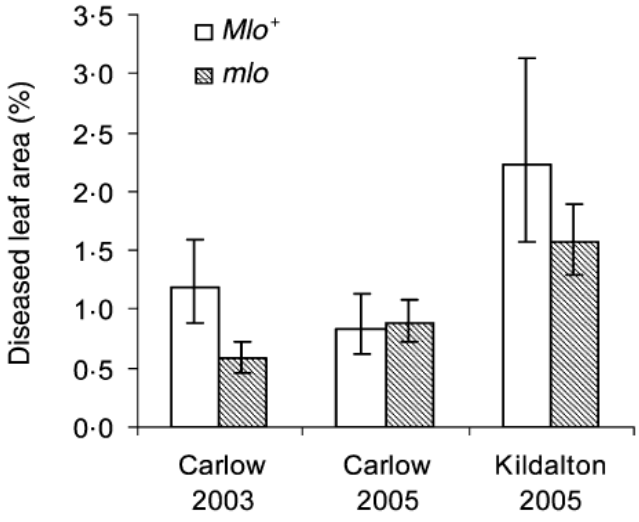
The implication of the results of this study is that, in itself, the *mlo* gene has not been solely responsible for the significant rise in spotting diseases, including ramularia, over the last 20 years. It is possible that the effect of *mlo* on spotting diseases in general and ramularia in particular depends on environmental conditions. Specifically, the environmental conditions used in research on *M. grisea* and *C. sativus* may have caused *mlo* to contribute to susceptibility to spotting diseases whereas the conditions in the field trials reported here may have contributed to resistance. This hypothesis was explored further in the third item of work reported here.

The results reported here contrast with other recent research which shows that cultivars with *mlo* tend to be more susceptible to ramularia. While the presence of *mlo* in varieties was associated with susceptibility to ramularia, the design of the latter experiments did not allow a genetic analysis and it is not appropriate to interpret those data as a firm indication that *mlo* causes susceptibility to ramularia. It is possible that interactions between different diseases may influence results of field trials. It was observed that lines attacked heavily by mildew do not show ramularia symptoms. In the experiments described here, quinoxyfen was used to control mildew levels, so defences against mildew would not operate and thus would not influence the plant's response to *R. collo-cygni*.



Bars +/- standard error

Figure 46 Ramularia collo-cygni symptoms on flag and F-1 leaves of Mlo$^+$ and mlo barley lines at the six sites.



Bars +/- standard error

Figure 47 Rhynchosporium secalis percentage disease leaf area on flag leaf and F-1 leaves of Mlo$^+$ and mlo barley backcross lines.

Environmental changes may alter plant fungal relationships. Depletion of the ozone layer has led to an increase in the levels of UVB radiation reaching the Earth's surface. *R. collo-cygni* produces toxins which induce the formation of reactive oxygen species (ROS) within the host through light-dependent photodynamic reactions, producing spotting symptoms. Increased UVB in these cases may increase the effectiveness of the toxins produced by *R. collo-cygni*, resulting in greater damage by the pathogen.

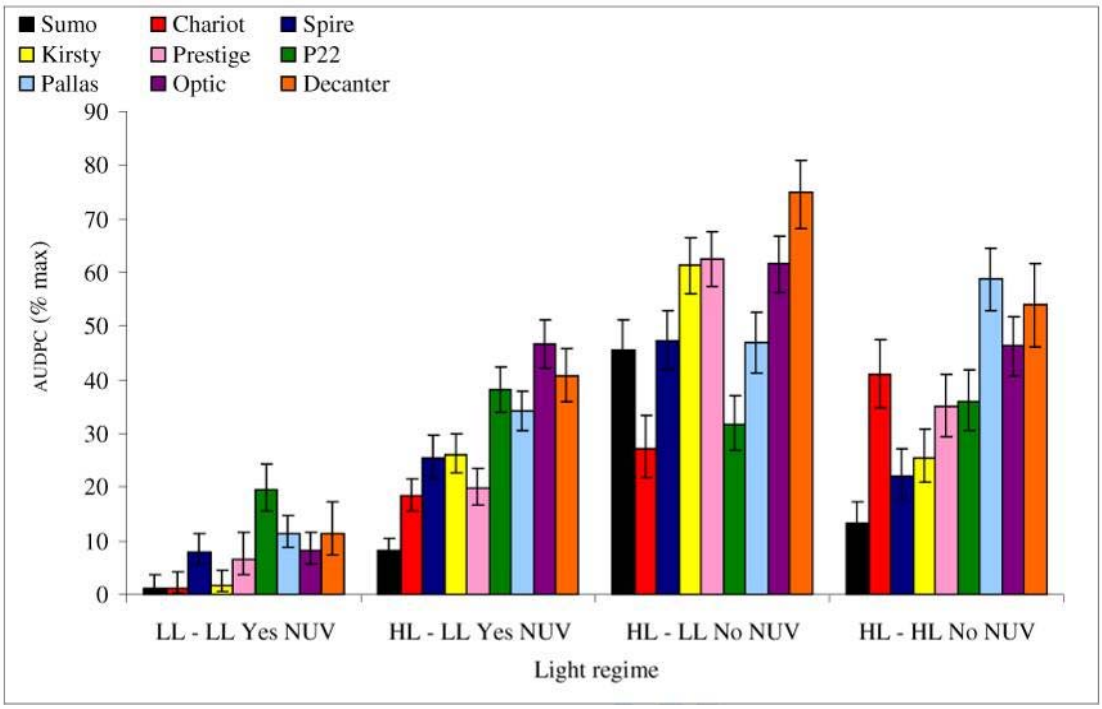
A laboratory method for testing varieties' resistance to ramularia

Introduction

Methods of handling *R. collo-cygni* in laboratory experiments, and therefore the science of both the fungus and the disease, are in their infancy. A particular limitation recognised early in this HGCA-funded project was the lack of a method of inoculating plants to produce ramularia symptoms, which could be used in testing barley varieties' resistance. In devising such a test, we were able to confirm two of Koch's postulates, thus contributing to confirmation that the ramularia symptoms observed on barley plants are indeed caused by *R. collo-cygni*.

Materials and methods

The method of inoculation was similar to one used for the fungus *Mycosphaerella graminicola*, the pathogen of septoria tritici blotch of wheat. This species is closely related to *R. collo-cygni* and it was thought that methods used in work on septoria would be applicable to ramularia. Barley seedlings were sprayed with a suspension of *R. collo-cygni* mycelium fragments, incubated at 15ºC, first in darkness for 48 hours then in a cycle of 16 hours light and 8 hours dark. Growth of plants in high light intensity (900 µmol m^{-2} s^{-1}) before inoculation increased disease symptoms but reduced disease when applied after inoculation (Figure 48).



Bars indicate standard errors. AUDPC: area under the disease progress curve, shown as percentages of the maximum possible AUDPC.

Figure 48 of Responses of barley lines grown in different light regimes to *Ramularia collo-cygni*

Results & Discussion

In contrast to *M. graminicola*, exposure of plants to near-ultraviolet light after inoculation reduced symptom development. It appears that for the full development of ramularia on barley seedlings, plants should be grown in a stressful environment before inoculation. The reason why the application of abiotic stress before inoculation greatly enhances the development of ramularia symptoms is not known, nor is the contrast between the effects of high light intensity applied before and after inoculation understood in terms of the plant's physiology.

Symptoms of ramularia similar to those found in the field were reproduced on seedlings, fulfilling the third of Koch's postulates. The fungus was then re-isolated from these lesions, which fulfils Koch's fourth postulate (Figure 49).

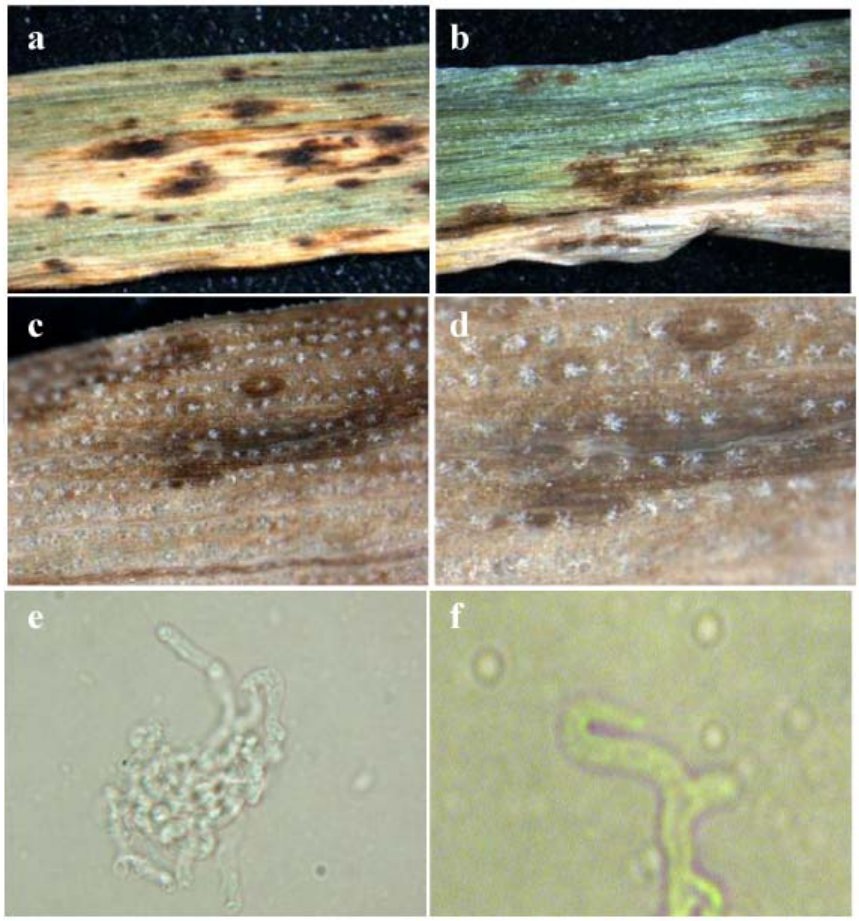


Figure 49. Symptoms and microscopical examination of *R collo-cygni*

(a) Symptoms of ramularia leaf spot on plants sampled from a field trial (magnification x1.25);

(b) ramularia leaf spot symptoms on first seedling leaves inoculated in the laboratory (x2);

(c) lines of sporulating fruiting bodies on the underside of a first seedling leaf inoculated in the laboratory (x5);

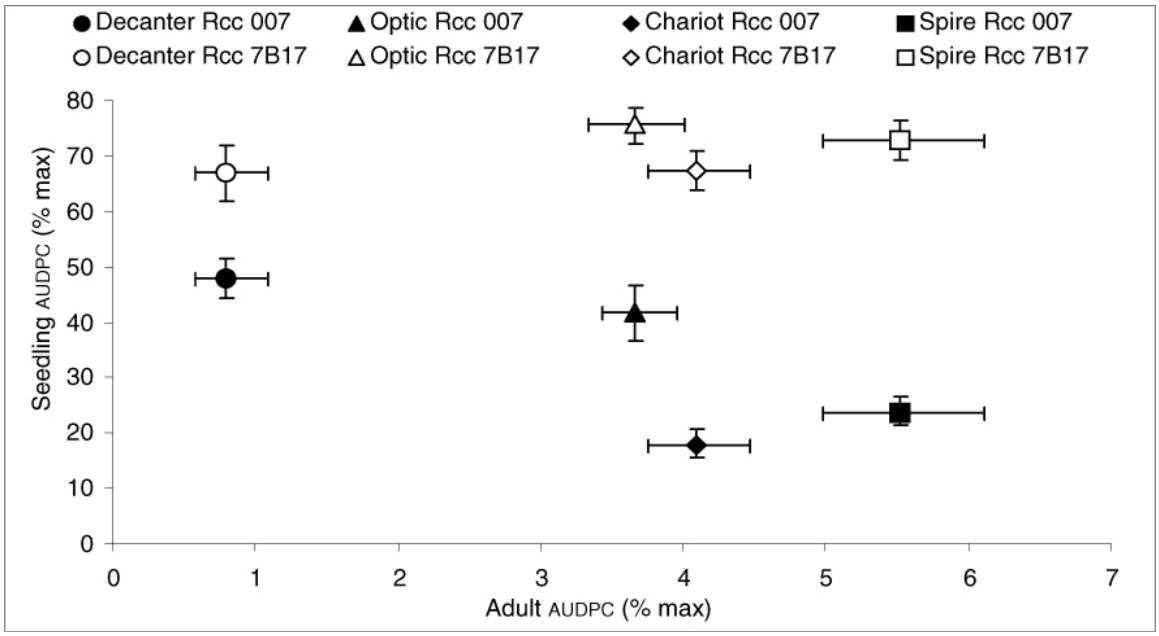
(d) lines of sporulating fruiting bodies on the underside of a first seedling leaf inoculated in the laboratory (x10);

(e) sporulating fruiting body picked from the surface of a inoculated leaf inoculated in the laboratory (x20);

(f) *Ramularia collo-cygni* conidiophore from the surface of a laboratory-inoculated leaf, with the typical swan's neck shape (x40)

These results contribute to resolving a controversy over the extent to which spotting symptoms on barley are caused by a pathogen rather than abiotic stress, indicating that the symptoms known as ramularia leaf spot are indeed caused by *R. collo-cygni*.

Nine barley lines were assessed for resistance to ramularia as seedlings and a sub-set were tested in field trials with natural infection by *R. collo-cygni*. There was variety-by isolate interaction in the infection of seedlings by two *R. collo-cygni* isolates and ramularia levels on adult plants in field plots were correlated with ramularia scores on seedlings formed by one isolate but not the other (Figure 50).



AUDPC: area under the disease progress curve, shown as percentages of the maximum possible AUDPC. Bars indicate standard errors.

Figure 50 Responses of barley lines grown in different light regimes to *Ramularia collo-cygni* isolates 00/7 and 2B17.

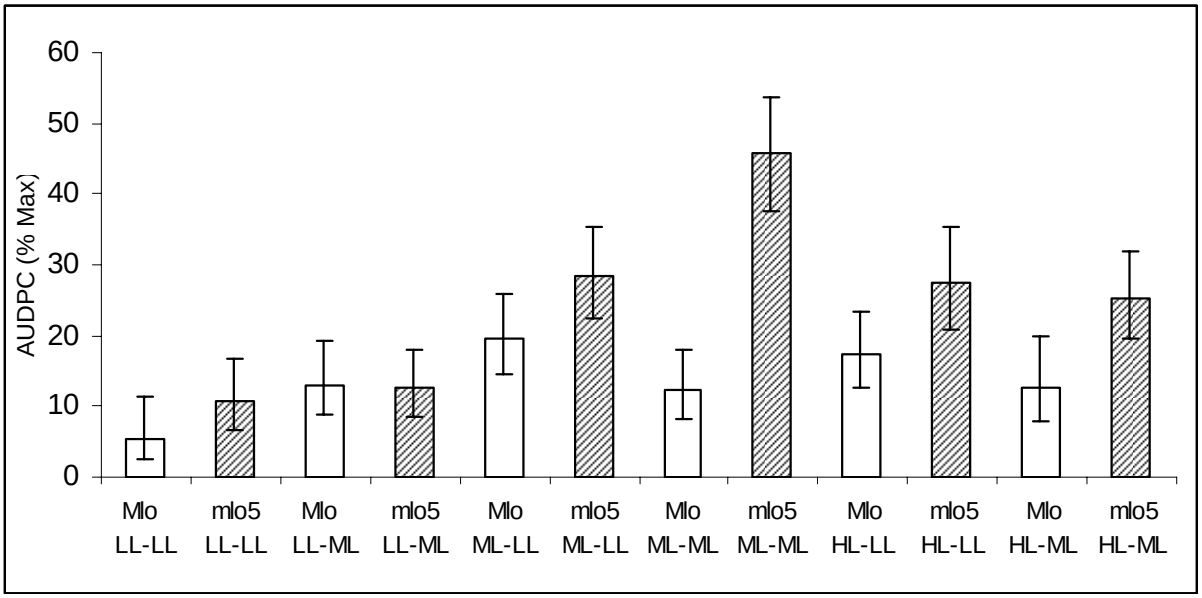
It is not known whether this is the result of variation between different genotypes of *R. collo-cygni*, in the length of time for which the isolates had been kept in culture (00/7 was isolated in 2000 but 2B17 in 2007) or other, unknown factors.

Effects of *mlo* on ramularia in laboratory conditions

The laboratory method described was then used to investigate further the effect of *mlo* mildew resistance genes on the development of ramularia symptoms. Methods were as described above but a low light (LL) level at 100, medium light (ML) at 300 or

high light (HL) at 900 µmol m^{-2} s^{-1} was applied. A cross-over design was used, in which plants were grown in one of the three environments before inoculation and placed in either the same environment or one of the other environments after inoculation. Near-isogenic lines of Ingrid and Pallas with the *Mlo*$^{+}$ mildew-susceptibility or *mlo5* mildew-resistance alleles were tested.

The results were unexpected, contrasting in a striking manner with those from field trials. Here, the *mlo5* mildew resistance allele was associated with increased susceptibility to ramularia (Figure 51). This effect was consistent across light treatments but was most notable with medium or high light before inoculation.



. (LL Low light, ML Medium light, HL High light).

Figure 51 Area under disease progress for Mlo^{+} and mlo5 isogenic lines under different light conditions before and after inoculation with *R collo-cygni*

The role of *mlo* in the susceptibility of barley to ramularia therefore remains an open question, but it clearly involves a three-way interaction between *mlo* genes in the plant, the pathogen and the environment.

Further work

Some key questions for future research on the resistance of barley to ramularia are as follows.

- Do stresses other than light induce ramularia symptoms? The physiology of stress caused by excessive light is well-understood (although its effects on ramularia are not) but it is difficult and expensive to apply to a large number of plants. A simpler, cheaper type of stress would be beneficial to plant breeders and others who need to assess plants' susceptibility to ramularia.

- Can a simple, efficient test for varieties' susceptibility to ramularia be devised? Ideally, such tests would complement, if not replace, cumbersome, expensive field trials for ramularia-resistance.

- Which varieties used in UK barley breeding have useful resistance to ramularia? Most importantly from the point of view of breeding for improved resistance, do different varieties have different genes for ramularia resistance?

- How does *mlo* mildew resistance interact with the environment to determine whether barley plants are more or less susceptible to ramularia?

- How do environmental conditions in the field elicit symptoms of ramularia? It would be especially useful to know if environmental variables affect the rating of varieties' susceptibility to ramularia?

- Is there pathogenic variation in the population of *R. collo-cygni*, such that some isolates are more aggressive to particular varieties? Such a pattern is seen in the related disease Septoria tritici blotch of wheat, controlled by gene-for-gene relationships and possibly more complex interactions.

General discussion

Ramularia leaf spot caused by the fungus *Ramularia collo-cygni* is a relatively new disease of economic importance in the north of Europe. When the first outbreaks caused economic loss in the north of the UK, the only advice available to growers was to protect crops with fungicides, but with little knowledge available to assist them on product choice, best timing, the varieties or regions most at risk.

Reliance on fungicides without knowledge of the disease quickly led to a serious problem when the main fungicide group became resistant to strobilurins. It also demonstrated the urgency for plant breeders to take the disease as a serious breeding aim for barley.

This research builds on previous HGCA funded research and it has increased our knowledge on the economic importance of the disease, the current status of varietal resistance in varieties submitted to Recommended List trials since 2002, new information on the source of the epidemic, new information on forecasting, fungicide resistance and environmental factors which induce symptom expression. This research can form the foundation of future research into plant breeding solutions, methods of forecasting high risk crops, and future fungicide strategies.

Diagnostic tests developed as part of the research will enable breeders to understand the importance of the pathogen within the plant prior to symptom expression. This is a new area which requires further exploration to determine the importance of the pathogen inside the plant prior to symptom expression. If this is important, the breeding solutions may differ compared to developing varieties which purely suppress symptom development. The same diagnostics can be used as tools to determine seed stocks most at risk from ramularia, measure the potential spread of the disease into other regions and measure the impact fungicide seed treatments have on seed health. Another novel approach to seed health is to focus on the control of disease on the heads in seed crops. This may lead to different approaches in the future to manage seed crops, since fungicide treatments maybe required beyond the stage currently allowed by product labels.

The laboratory bioassay has demonstrated the necessity for stress prior to symptom development as well as stress post infection. This can be developed further to assist

forecasting the disease in the field and also be used to test new varieties for their susceptibility to ramularia.

As with most research, new questions have to be addressed on the basis of this work, but the results presented here have increased our understanding of the pathogen leading to better disease management both in the short and longer term.

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