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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).

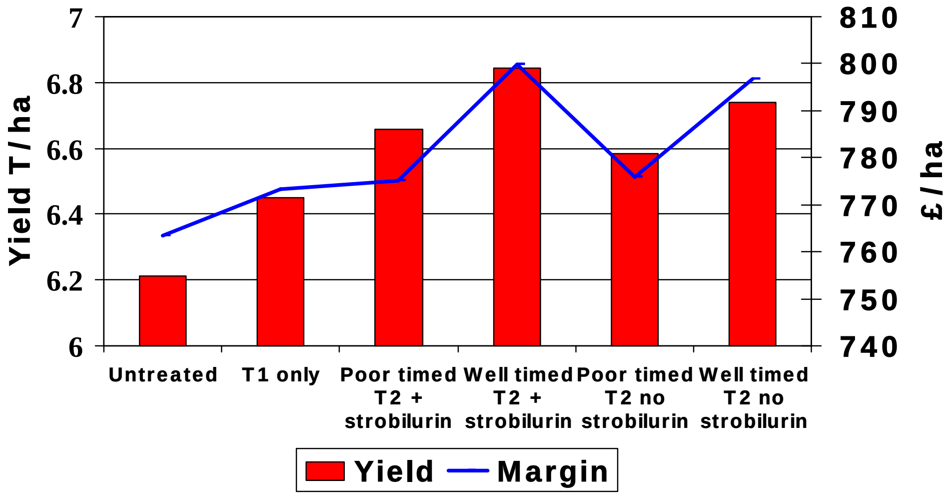

Figure 1 Crop yields and margin over fungicide in spring barley £125/t 05-07

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).

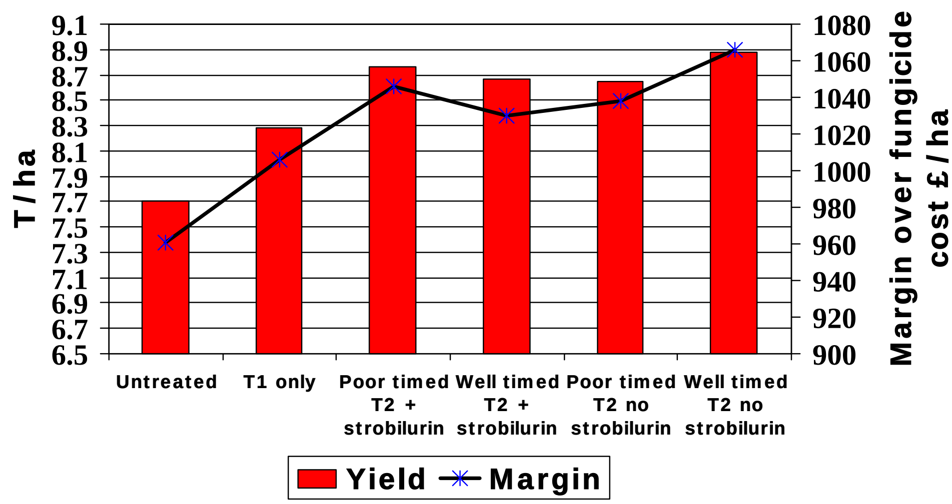

Figure 2 Crop yields and margin over fungicide cost in winter barley £125/t 2005-07

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.

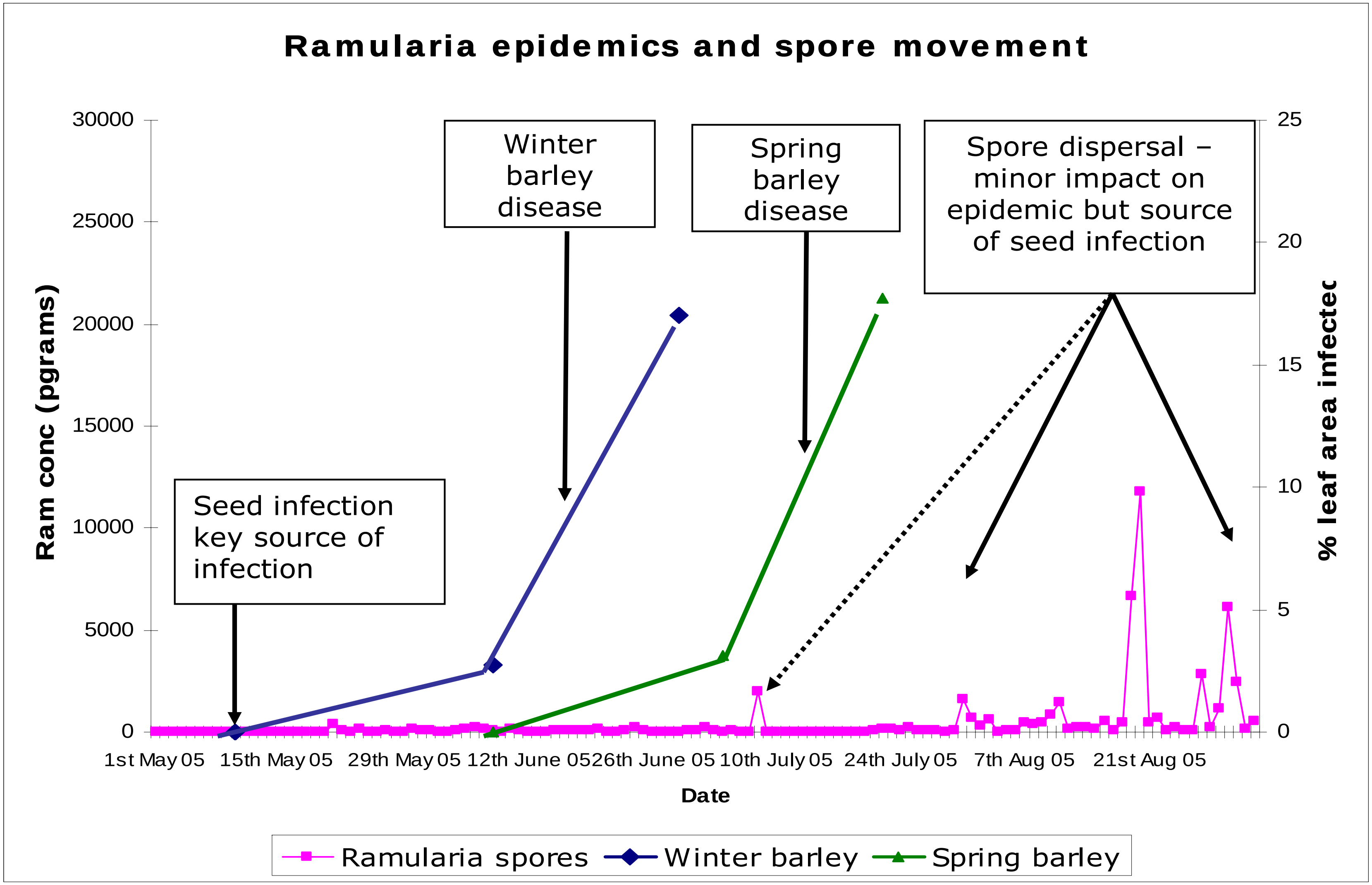

Figure 3 spore dispersal and disease development for 2005

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).

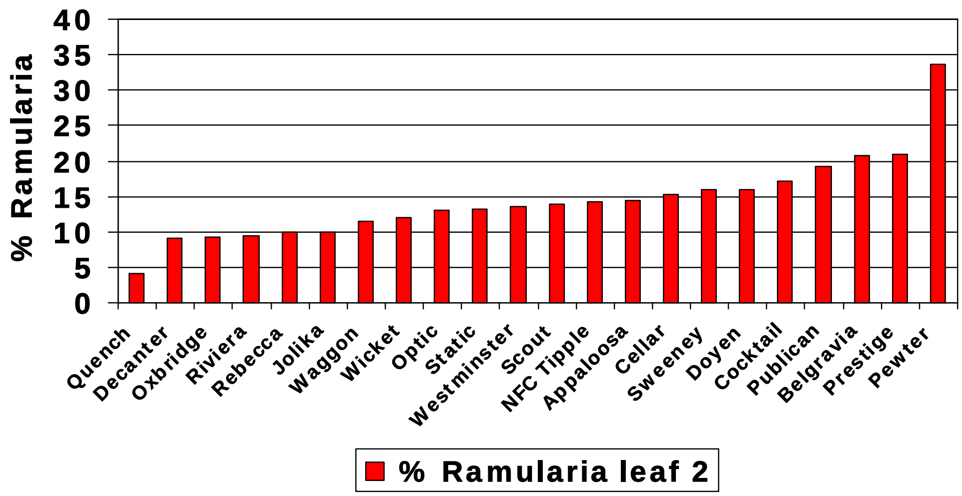

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Figure 4 % Ramularia on spring barley recommended List varieties 2008 and two susceptible varieties

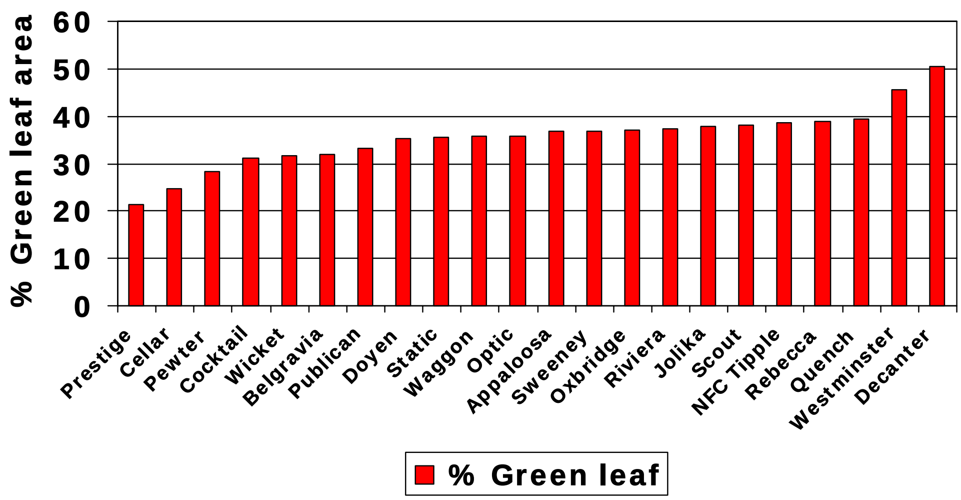

Figure 5 % Green leaf on spring barley recommended List varieties 2008 and two susceptible varieties

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.

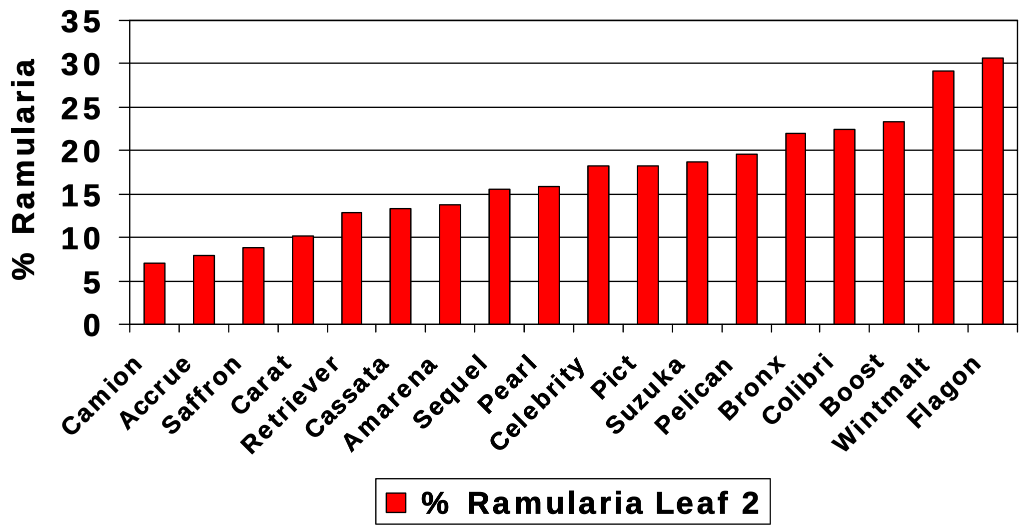

Figure 6 % Ramularia on winter barley recommended List varieties 2008

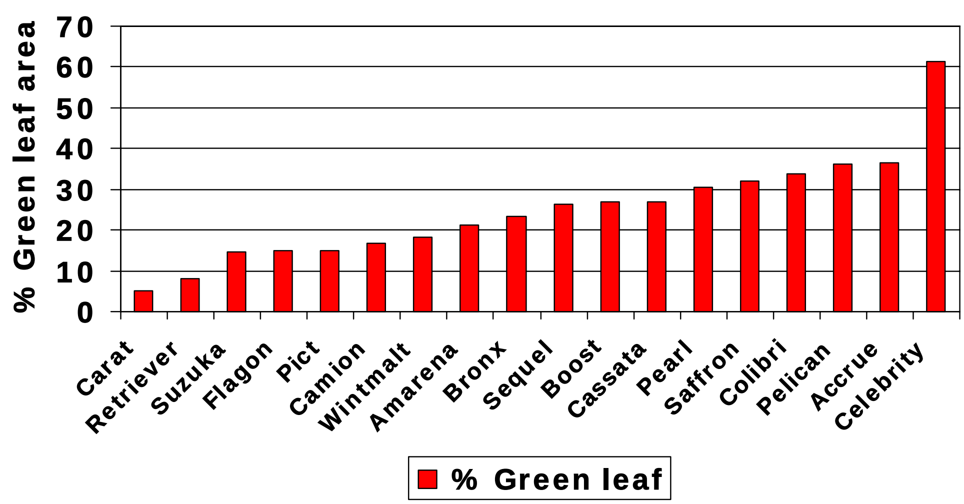

Figure 7 %Green leaf on winter barley recommended List varieties 2008

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

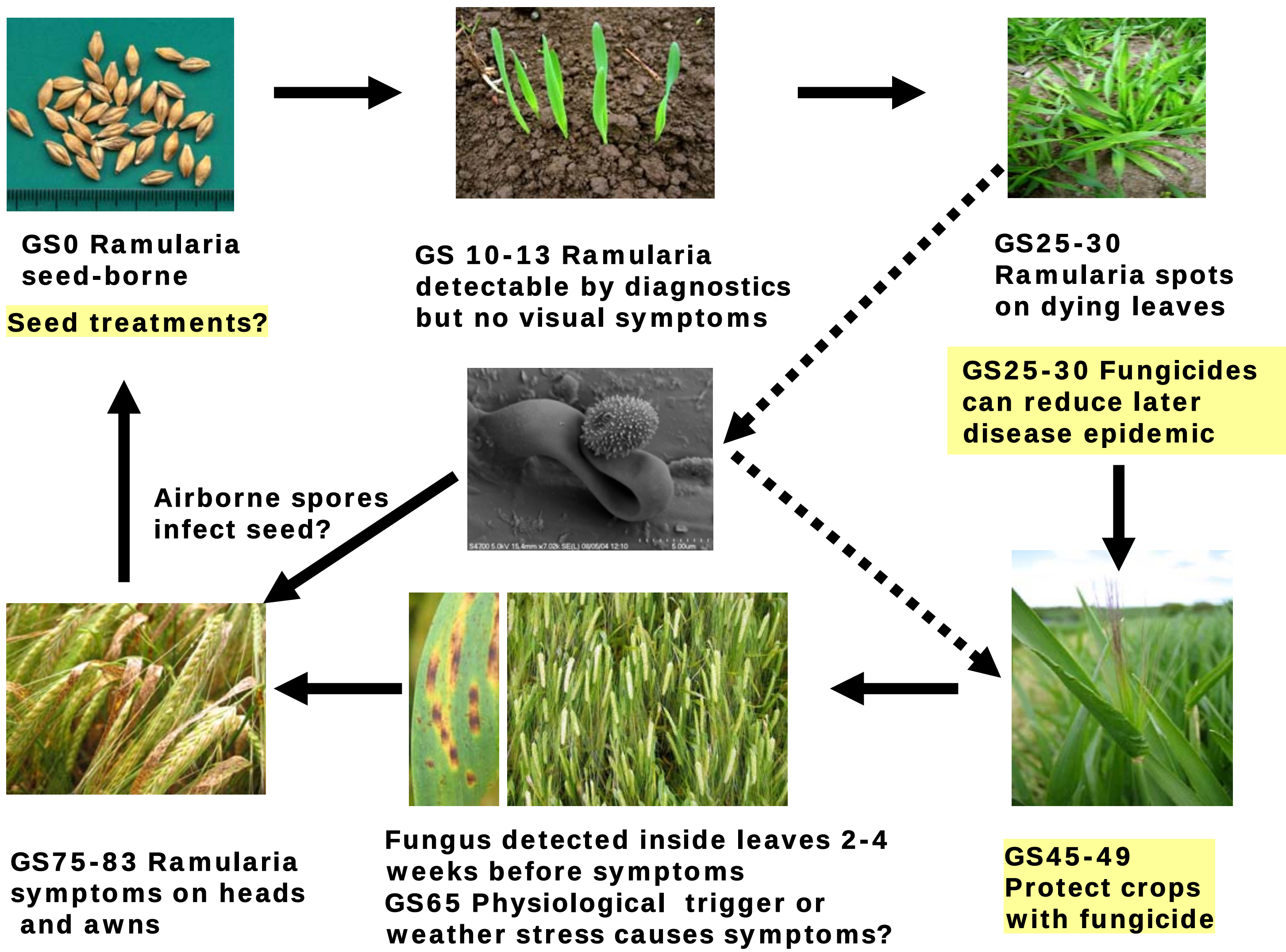

Figure 8 Ramularia lifecycle in barley