Project Report No. 463

Managing *Ramularia collo-cygni* through varietal resistance, seed health and forecasting

by

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1. ABSTRACT

Ramularia leaf spot is a fungal disease of barley caused by *Ramularia collo-cygni*. The disease has become widespread in the north of Europe but it also occurs further south and has become endemic in France. An assessment of seed from harvested grain showed the causal fungus to be present throughout the UK. Levels on seed can vary by season and also by region with the highest levels detected in the north and west of the UK and the lowest levels in the east of England. Winter and spring barley varieties showed different levels of Ramularia leaf spot and on the basis of these observations a varietal resistance rating can be made. No varieties exhibited total resistance. The causal fungus developed inside plants of both resistant and susceptible varieties. This was detected using Polymerase Chain Reaction (PCR) diagnostics which can detect *R. collo cygni* DNA before symptoms are seen. This phase of the disease (asymptomatic) occurs both in resistant and susceptible varieties and it shows DNA levels of the fungus develop initially in the lower leaves and then in the upper leaves, but symptom expression is most extensive on the upper leaves.

Field trials using seed stocks with different levels of contamination with *R. collo-cygni* show that the level of seed infection can have a part to play in the final level of symptoms seen. This was greatest in the spring barley variety NFC-Tipple where a 50% reduction in visible symptoms was observed by using seed with less than 1pg *R. collo-cygni* DNA. It was not possible to determine the impact of sowing seed where *R. collo-cygni* was absent, since no seed stock was completely free from infection. It is recommended however that seed levels are kept as low as possible in regions where the disease is not endemic (1pg DNA England).

Site had the greatest impact on the disease epidemic with disease severity varying by 78% depending upon the site. The main differentiation between high and low disease pressure sites was leaf wetness in early June. A correlation between leaf wetness events in early June with subsequent symptom expression in July was determined on the basis of this research and this corroborates with results taken from previous trials since 2005. In early June, spring barley crops were at Growth Stages (GS) 25-32 and had yet to reach the optimum timing for fungicide control (at GS49). This new information can therefore be used as a risk forecast to warn growers about the potential risk of disease in time to take preventative action. It provides a big step forward in forecasting the risk of Ramularia leaf spot when used alongside other

factors including variety and seed contamination. It will enable action to be taken to protect crops most at risk with fungicide before symptoms appear.

Extending the spring barley risk forecast to other UK and European regions and extending the risk forecast for winter barley, will be the focus priority of future research. Since seed contamination had a lower impact on symptom expression compared to site, using seed contaminated with *R. collo cygni* is likely to have a low impact on the varietal resistance score, but it is recommended that seed stocks used for Recommended List trials have as low a level of seed contamination as is possible. Understanding the impact of seed treatments to eradicate disease from seed is the focus of new research.

2. SUMMARY

Ramularia leaf spot is a disease of barley which is caused by the fungus *Ramularia collo-cygni*. The aim of this proposal is to understand the relationship between *R. collo-cygni*, varietal resistance and seed infection under field conditions to provide information on current varietal resistance.

These aims are a challenge for a project comprising a single season, but this research provides new information which complements previous HGCA research (Oxley & Havis 2008) and also feeds into a new LINK project on the disease.

2.1 Variety resistance

Varieties vary in their susceptibility to Ramularia leaf spot. Tables I and II show the derived resistance ratings and comments on green leaf area retention based on the information from the 2008 season. No variety shows excellent resistance to Ramularia leaf spot and the winter barley varieties tend to show greater susceptibility than spring barley varieties on the current HGCA Recommended List. All data collected from this research and previous research since 1992 has been supplied to Cereal Evaluation Limited.

2.2 Contamination of seed

Seed samples taken at harvest from the UK were tested for presence of the causal fungus of Ramularia leaf spot (*Ramularia collo cygni*). The fungus was detected on harvested grain throughout the UK in both 2006 and in 2007 (see Figure I for 2007 distribution). An investigation of the amount present on seed showed *R. collo cygni* levels were greater in 2006 averaging 48.3 pg DNA compared to 2007 where seed levels average 9.9 pg DNA. It is proposed that weather conditions the previous July and August play a part in the level of seed contamination and this can be used as part of the risk forecast. Seed contamination levels were higher in samples from Scotland (32.7 pg DNA) and lowest in England (6.2 pg DNA).

Table I .Winter barley varieties 2008

Variety	Resistance rating (1-9)	Comment on Ramularia resistance	Comment on green leaf retention
Accrue	6	Intermediate	Good
Amarena	7	Good	Good
Boost	6	Intermediate	Good
Bronx	6	Intermediate	Good
Carat	6	Intermediate	Good
Cassata	5	Poor	Intermediate
Colibri	6	Intermediate	Good
CPBT B81	4	Poor	Poor
Daybreak	4	Poor	Poor
Flagon	5	Poor	Good
Karioka	6	Intermediate	Good
LP6-342	6	Intermediate	Good
MH 97 CQ2	5	Poor	Good
NSL 03-7442	5	Poor	Intermediate
Pearl	7	Good	Good
Pelican	5	Poor	Good
Retriever	5	Poor	Poor
Saffron	6	Intermediate	Good
Sequel	6	Intermediate	Intermediate
Suzuka	5	Poor	Intermediate
Volume	7	Good	Good
WB 031090	6	Intermediate	Intermediate
Wintmalt	6	Intermediate	Intermediate

Table II. Spring barley varieties 2008

Variety	Resistance rating (1-9)	Comment on varietal resistance	Comment on green leaf retention
Appaloosa	8	Good	Good
Azalea	7	Good	Intermediate
Belgravia	8	Good	Good
Berlioz	5	Intermediate	Intermediate
Cocktail	6	Intermediate	Good
Concerto	5	Intermediate	Good
Cropton	6	Intermediate	Good
Decanter	7	Good	Good
Doyen	6	Intermediate	Good
Fairytale	6	Intermediate	Good
Forensic	6	Intermediate	Intermediate
Jolika	5	Intermediate	Intermediate
Knightsbridge	6	Intermediate	Poor
LP1159.3.03	4	Poor	Intermediate
Maltby	8	Good	Good
NFC 405-103	5	Intermediate	Poor
NFC-Tipple	8	Good	Good
Optic	5	Intermediate	Intermediate
Oxbridge	6	Intermediate	Intermediate
Power	7	Good	Intermediate
Publican	7	Good	Good
Quench	6	Intermediate	Good
Rebecca	8	Good	Good
Riviera	6	Intermediate	Intermediate
Scout	7	Good	Good
Snakebite	6	Intermediate	Poor
Sweeney	6	Intermediate	Intermediate
Troon	4	Poor	Intermediate
Virgil	4	Poor	Intermediate
Waggon	8	Good	Good
Westminster	7	Good	Good
Wicket	7	Good	Intermediate

1-9 rating where the higher the number the more resistant the variety

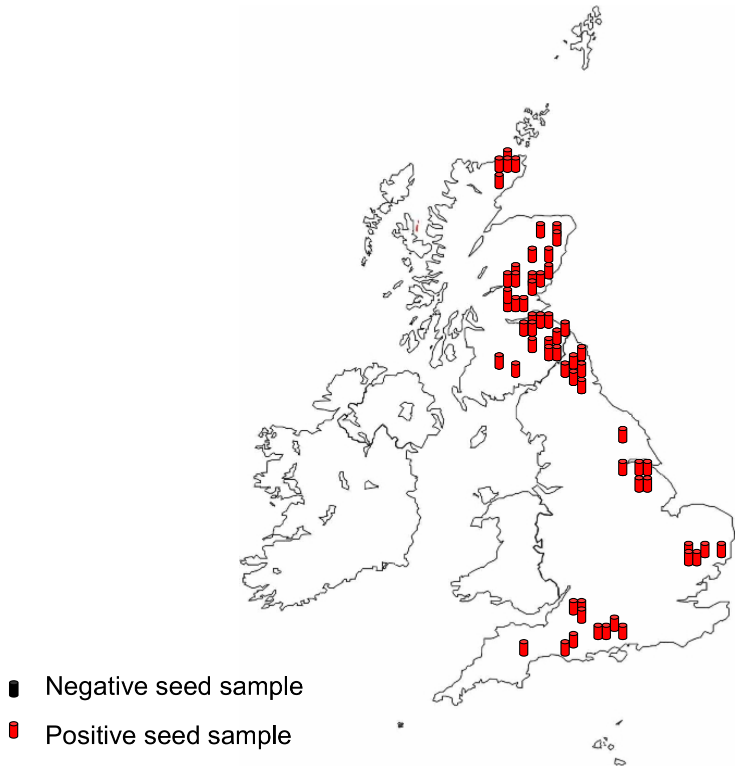

Figure I. Ramularia distribution on harvested seed in 2007

No seed stock used in the field trials was completely free from infection with *R. collo-cygni*. Minimum detection levels for this test were determined by the lowest standard on the calibration curve (0.128 pgrams). All seed samples tested from the 2007 harvest had *R. collo-cygni* levels above the min level. It is recommended that seed used for Recommended List variety trials has as low a level as is possible, since seed contamination can influence the overall level of symptoms observed on a variety. The impact of having a greater level of infection on a particular variety was, however, lower than the impact of site on symptom development. For regions where the disease is not endemic, seed health may be a more important factor to ensure the disease does not spread. The greatest impact of seed contamination was observed on the spring barley variety NFC-Tipple where a 50% reduction in visible symptoms was observed by using seed with less than 1pg *R. collo cygni* DNA. It was not possible to determine the impact of sowing seed where *R. collo cygni* was absent, since no seed stock was completely free from infection. It is recommended that seed levels or *R. collo cygni* DNA contamination are kept as low as possible in regions where the disease is not endemic (1pg DNA England, 5pg DNA north and west UK).

2.3 Impact of weather on symptom development and spore release

Previous research shows a correlation between leaf surface wetness in July with spore dispersal (Havis *et al.* 2009). This is thought to be important for seed contamination, taking the disease into the next season. The greatest impact on Ramularia symptom expression in this research proved to be the site. Having field trials with high and low levels of disease enabled us to determine the risk factors involved. Although this project comprised a single year's trialling, linking these results with others from previous research shows a correlation between leaf wetness in June with symptom expression in July in spring barley in Scotland. Analysis of leaf wetness events in the first two weeks of April also correlate to Ramularia leaf spot symptoms in late June in winter barley. These leaf wetness events are not associated with airborne spore dispersal events, therefore this knowledge can be used to help predict the risk of an outbreak of Ramularia leaf spot in time to apply protectant fungicides. Why these weather events impact on disease is not fully understood. One possibility could be related to the rate of movement of fungal mycelia within the plant or alternatively there could be small localised spore release from senescing leaves at the bottom of the crop canopy. However, the exact effect of the weather on the movement of the fungus requires further investigation.

These results have enabled us to improve the risk forecast for Ramularia leaf spot (Table III). This information will be used with other information as part of Scottish Government funded research and a new LINK project titled CORACLE to extend its use throughout the UK. It will also be used to modify the potential spread of the disease using current weather models and future climate change models.

Table III. Risk assessment for Ramularia leaf spot

Determining risk	Comment
Leaf wetness in 1-14 June at GS25 to GS32. Where leaf wetness events exceed 4000 minutes, spring barley crop is at high risk from Ramularia leaf spot. **Leaf wetness** in 1-14 April. Where leaf wetness events exceed 6000 minutes, winter barley crop is at high risk from Ramularia leaf spot.	Site has the greatest impact on disease risk and leaf wetness events are an important measurement to differentiate these sites. Calculating leaf wetness can be done centrally for weather stations, but new research can determine methods to get in-field data
Variety: Choose variety with greatest level of resistance for specific market.	Quality and yield are the main reasons to choose a variety, but longer term aims in plant breeding may make this an important option.
Seed contamination: Choose seed with less than 5pg DNA (Preferably less than 1 pg DNA).	Seed can be tested using molecular diagnostics and it is recommended that seed is tested for *Ramularia collo cygni*.
Seed Treatment:–existing seed treatments are unlikely to affect risk, but if new ones are developed, they should be used to ensure seed contamination is below 1pg DNA.	New research is required to investigate methods to control *Ramularia collo cygni* on seed.
Fungicides at GS25: If leaf wetness events are high in May/June and variety is susceptible, ensure GS25-30 fungicide comprises fungicide with effective activity.	Recommended option for high risk crops.
Fungicides at GS45: GS45 essential for high risk crops.	Essential option for high risk crops.
Leaf wetness at flowering: Increases the risk of seed infection. Home saving seed following extensive leaf wetness event in July (to be quantified) is not recommended in absence of effective seed treatment.	This will help determine the potential risk of seed contamination in the absence of a seed test.

3. TECHNICAL DETAIL

3.1 Introduction

Ramularia collo-cygni has become an established barley disease in the north of Europe. The disease has the capability to spread further south, based on knowledge of the climatic conditions that favour the pathogen. The aim of this proposal is to understand the relationship between *R. collo-cygni*, varietal resistance and seed infection under field conditions to provide information on current varietal resistance.

New research shows seed infection is a key method of spreading the disease within a season and potentially into new areas via seed movement. This research will determine the level of contamination in UK seed stocks in winter and spring barley. It will also look at the importance of seed infection on symptom development in the field.

Barley leaf spots were first reported in the north of Britain in 1996 and have now become a recognised economic problem in northern Europe and New Zealand. Barley leaf spots can cause extensive damage to crops in all UK regions including East Anglia, which is typically a low risk area. Forecasting high and low risk areas or seasons is still in it infancy, but leaf wetness and spore dispersal events have been recognised as important factors in previous research (Oxley & Havis, 2008). This research will determine how these factors can be used to forecast risk in time for growers to take action on crops at greatest risk.

Varieties can also differ in their susceptibility to leaf spots (Oxley *et al.,* 2002). The two key types of leaf spots are known as oxidative stress (abiotic spots) and Ramularia leaf spot or RLS (biotic spots). Other factors defined in the report include weather conditions conducive to leaf spot development, differences between fungicides in reducing or increasing the severity of leaf spots and optimum timing of fungicides to reduce the disease. The current research project has measured the yield loss from leaf spots. In 2002, yield loss from leaf spots ranged from 0.25 – 0.8 t/ha and an increase in screenings of 3%. In 2006, yield losses ranged from 0.1 t/ha up to 0.9 t/ha with a loss in screenings of 4%. The high yield loss values were from the variety Optic and the low yield loss from a resistant variety which does not meet market requirements. The figures show the potential savings which could occur if current malting varieties were replaced by Ramularia resistant varieties which meet market requirements. Research has observed a third type of leaf scorch caused by specific fungicide formulations. Fenpropimorph (e.g. Corbel) can cause rapid leaf death under certain weather conditions, while chlorothalonil (Bravo) helps maintain green leaf area.

The development of a diagnostic test specific to the biotic spot *R. collo-cygni* (Havis *et al.,* 2006), demonstrated the presence of *R collo-cygni* in the seed and monitored progress of the pathogen into developing leaves and up to symptom development at flowering. Symptom expression only occurs however when the crop is stressed. Stress from light, moisture or physiological events such as flowering as well as stress associated with fungicides triggers a change in Ramularia. *R. collo-cygni* produces a toxin in response to this stress which is activated by light. Symptoms then develop in areas of the plant exposed to light and the leaf rapidly dies back leading to loss in yield. The role of the *mlo* gene is not as straightforward as was originally thought. Where the crop is not stressed, the m*lo* gene provides the plant with resistance against *R. collo-cygni*. Under heat or light stress however, the *mlo* gene appears to accelerate symptom development making varieties carrying it more susceptible.

In order to give growers resistant varieties to Ramularia leaf spot and an accurate risk forecast, it is important to understand the role Ramularia leaf spot plays within a plant and what triggers the change from an endophyte, living in the plant without causing any apparent damage, to an aggressive pathogen. Information on seed health will also enable an accurate resistance rating to be developed for current and future HGCA Recommended List varieties with knowledge on how seed contamination impacts on varietal resistance. The development of seed treatments to kill Ramularia in seed will be explored through approaches to the major agrochemical companies.

The critical point in our current state of knowledge about Ramularia leaf spot is that we believe that control requires improved seed health and improved fungicide applications in the short term but will also need varieties with better resistance to Ramularia leaf spot in the medium to long term.

3.2 Materials and methods

3.2.1 Monitoring of R. collo-cygni spores in the environment

A Burkard seven day spore sampler was set up at the three trial sites. These machines sample air from the environment which is drawn through a small aperture and passes over coated Mellinex tape. After seven days the tape is removed and divided into segments which correspond to 24 hour periods. These were then halved lengthways and stored at -20 °C. DNA was extracted from the tape using the method described in Fountaine *et al.* (2007). Extracted DNA was tested for the presence and quantity of Ramularia DNA using a real time PCR test recently developed in our laboratory (Taylor *et al.,* 2009). An automatic weather recording station was situated next to the spore sampler to provide local meteorological data. Spore movement was compared to disease development in adjacent barley trials. Area under disease progress (AUDPC) values from untreated plots were calculated using the trapezoidal rule.

3.2.2 Seed survey

Seed and harvested grain from different UK regions and from different varieties was obtained from the official seed testing stations and agronomy groups and tested for the presence and amount of Ramularia contamination using the established PCR test. Up to 50 samples were tested. The results will show the current incidence of the disease in the UK including areas thought to be at low risk.

3.2.3 Field trials

Field trials were set up at three geographical sites in 2008. Details of the sites are listed below in Table 1.

Table 1 Site details for field trials and variety screens

	Perth	**Lanark**	**Bush**	**East Lothian**	**Borders**
Trials	Field trial	Field trial	Field trial	No	No
Variety screens	Yes	Yes	Yes	Yes	Yes
Sown date	17 Mar 08	17 April 08	17 Apr 08	7 Mar 08	18 Mar 09
Seed rate (seeds m^2)	360	360	360	360	360
Harvest	26 Aug08	23 sep 08	21 sep 08	24 Aug 08	28 Aug 08
Location	Gloagburn Farm, Tibbermore, Perth	Drumalbin, Lanark	Hayknowes field, Boghall farm, Edinburgh EH10	Stenton, East Lothian	Swinton Bridge End, Swinton, Berwickshire
Grid ref	NO 048 235	NS 904 383	NT 248 650	NT 622 738	NT 818 468
Soil texture (series)	Medium (Balrownie)	Sandy loam	Medium (Macmerry)	Loan (Pressmennan)	Clay loam
elevation	50m	230m	200 m	115m	55 m
Soil P	High	Low	High	Mod	Mod
Soil K	Mod	Low	High	Low	Mod
Soil Mg	Mod	Moderate	High	High	High
Soil S	Low	High	Low	Low	Low
pH	5.8	5.7	6.7	6.2	6.2
Previous crop 2007	Spring barley	Spring barley	Spring barley	Winter wheat	Spring beans
Previous crop 2006	Winter wheat	Grass	Spring barley	Winter wheat	Winter wheat
Previous crop 2005	Vining peas	Grass	Winter wheat	Spring barley	Winter oilseed rape
Previous crop 2004	Spring barley	Grass	Spring barley	Spring barley	Winter barley
Treatment1	31 May 08 (GS31)	7 June 08 (GS31)	11 June 08 (GS31)	20 May 08 (GS26-30)	22 May 08 (GS25)
Treatment2	11 Jun 08 (GS37)	13 June 08 (GS37)	16 Jun 08 (GS37)	5 Jun 08 (GS39-45)	5 June 09 (GS37)
Treatment3	16 Jun 08 (GS49)	21 Jun 08 (GS45-49)	23 Jun 08 (GS49)	-	16 Jun 08 (GS47)

The aim of the field trials was to determine the importance of site, varietal resistance, fungicide treatment, seed contamination with R. collo-cygni on the disease epidemic.

3.2.4 Importance of seed contamination: Impact of three varieties with two levels of seed contamination at three sites

Three varieties with different Ramularia resistances were chosen. The varieties Optic and Cocktail are defined as susceptible and the variety Decanter resistant to Ramularia leaf spot. Seed stocks used for the recommended list trials were sown alongside seed of the same variety which had been harvested from untreated plots in 2007. The interaction of variety and seed contamination with foliar fungicides was measured. Fungicides were chosen which were effective against the disease and applied at different timings (see Table 2). Disease development, green leaf area and yield were measured.

These experiments were sited on SAC sites where spore traps and weather stations are situated. The experiments will demonstrate the relative importance of varietal resistance, seed infection, external infection with air borne spores, fungicide activity and stress on symptom development and yield.

Fungicide treatments for the spring barley trials are described in Table 2. Previous research showed that the later fungicide treatments gave the best disease control. The untreated control (Treatment 1) was 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.

Table 2 Fungicide treatments in yield loss trials (Dose rates in l/ha)

	Fungicide treatment			
Treatment	**GS31-32**	**GS37**	**GS49**	**Comment**
1	Nil	Nil	Nil	Untreated
2	Proline 0.4 + Acanto 0.5 + Flexity 0.25	Nil	Nil	No late treatment
3	Proline 0.4 + Acanto 0.5 + Flexity 0.25	Nil	Proline 0.4+ Bravo 1.0 + Amistar 0.5	Well timed late treatment
4	Proline 0.4 + Acanto 0.5 + Flexity 0.25	Proline 0.4+ Bravo 1.0 + Amistar 0.5	Nil	Poorly timed late treatment

Amistar – active ingredient azoxystrobin 200 g/l
Acanto – active ingredient picoxystrobin 250 g/l
Proline – active ingredient prothioconazole 250 g/l
Bravo – active ingredient chlorothalonil 500 g/l
Flexity - - active ingredient metrafenone 300 g/l

3.2.5 Importance of variety and fungicide on R. collo-cygni and R. leaf spot

The spring barley varieties used in the trials were selected to cover a spread of disease susceptibility to Ramularia leaf spots. Poker, Decanter and Oxbridge were the most resistant. Prestige, Cocktail and Optic were the most susceptible. Trials were fully randomised in complete blocks with three replicates.

3.2.6 Varietal resistance of the Recommended List to R. leaf spot

Seed from a selection of Recommended List varieties for 2008 was obtained from Cereal Evaluation Limited (CEL) and tested for Ramularia DNA. Small disease observation plots were sown at 4 sites in Scotland using the two stocks of seed. Observations on Ramularia leaf spot and green leaf area were assessed. Seed contamination levels greater than 5 pg were defined as high, and levels below 5pg DNA were defined as low. No seed stock was completely free from the disease.

3.2.7 Monitoring R. collo-cygni movement within the plant

Three spring barley varieties with differing susceptibility to *R. collo-cygni* were sown in small hill plots at the Lanark trial site in Central Scotland in 2008. The varieties used were the same as those used for the yield loss experiments. Cocktail (susceptible), Decanter (resistant) and Optic (susceptible). Ramularia DNA levels were measured in seeds prior to sowing (Cocktail 29.1 picograms (pg), Decanter 20.5 pg and Optic 20.8 pg). Ramularia levels were measured in each leaf layer throughout the growing season. Visual symptoms were also recorded in the leaves. Seed was harvested at the end of the trial and Ramularia DNA quantified. Area under the curve for Ramularia DNA was calculated using the trapezoidal rule. A spore sampler and weather station at the site allowed Ramularia spore movement to be measured and periods of high leaf surface wetness recorded.

3.3 Results

3.3.1 Monitoring of *R. collo-cygni* spores in the environment

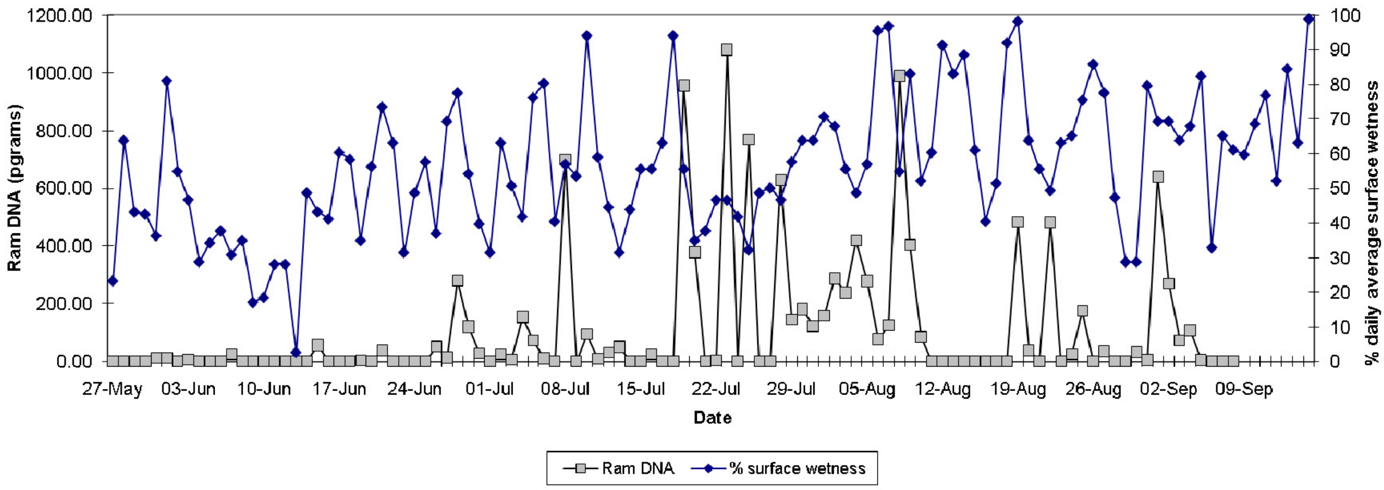

Figure 1 Changes in Ramularia DNA and daily average leaf surface wetness over a 5 month period at the Lanark site in 2008.

Ramularia spore production peaked in late July at Lanark in 2008 and continued up to early September, when the crop was still to be harvested (Figure 1). Late July was the same time that symptoms were widespread on the top two leaves of spring barley varieties. These spore peaks coincided with periods of leaf wetness when the % daily average leaf wetness was greater than 70% (Figure 1 diamond symbols).

Leaf surface wetness events in early June did not correspond with detectable spore release events (Figure 1). Leaf wetness events in early June were, however, important regarding the eventual symptom expression in July. Figure 2 shows the correlation between leaf wetness in June with eventual symptom development in July for the Lanark site in 2008.

This result has been added to results collected since 2005 and it can be concluded that leaf wetness periods in early June can be used as part of a risk forecast where an increase in leaf wetness from 1-14 June (growth stage 25-32) correlates to the eventual severity of disease in July. This provides sufficient warning to advise growers to apply a protectant fungicide at GS45-49 to crops at risk from the disease. Applying a fungicide in early June has some impact on final symptom development, but it is insufficient to provide effective control of the upper leaves.

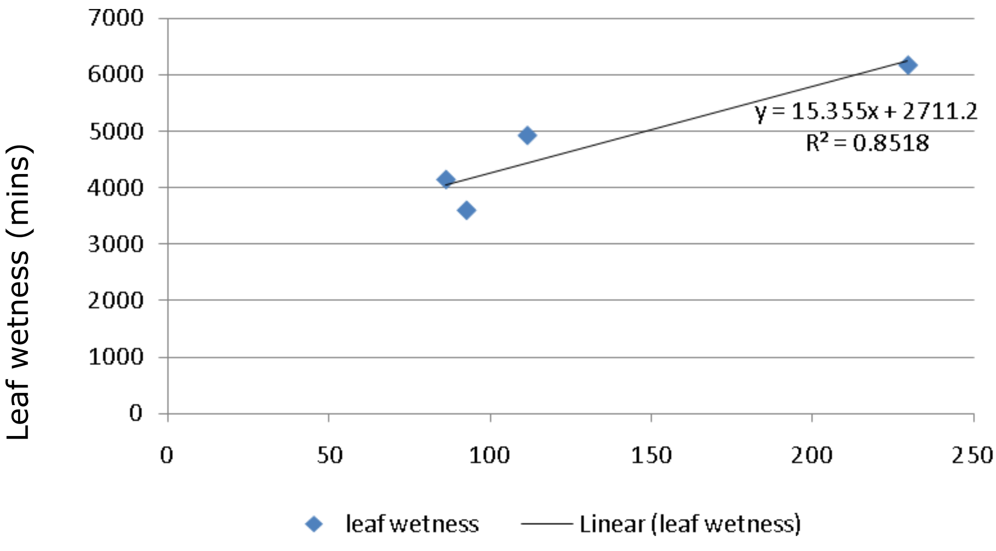

Figure 2 Correlation between total cumulative duration of maximum leaf wetness between 1 -14 June (in minutes) and Area under disease progress for Ramularia leaf spot in spring barley (Mean of all varieties assessed in each year).

A similar correlation for winter barley was seen in 2008 correlating leaf wetness in 1-14 April with Ramularia leaf spot development (Figure 3). More work is required to determine if this correlation is present in previous research.

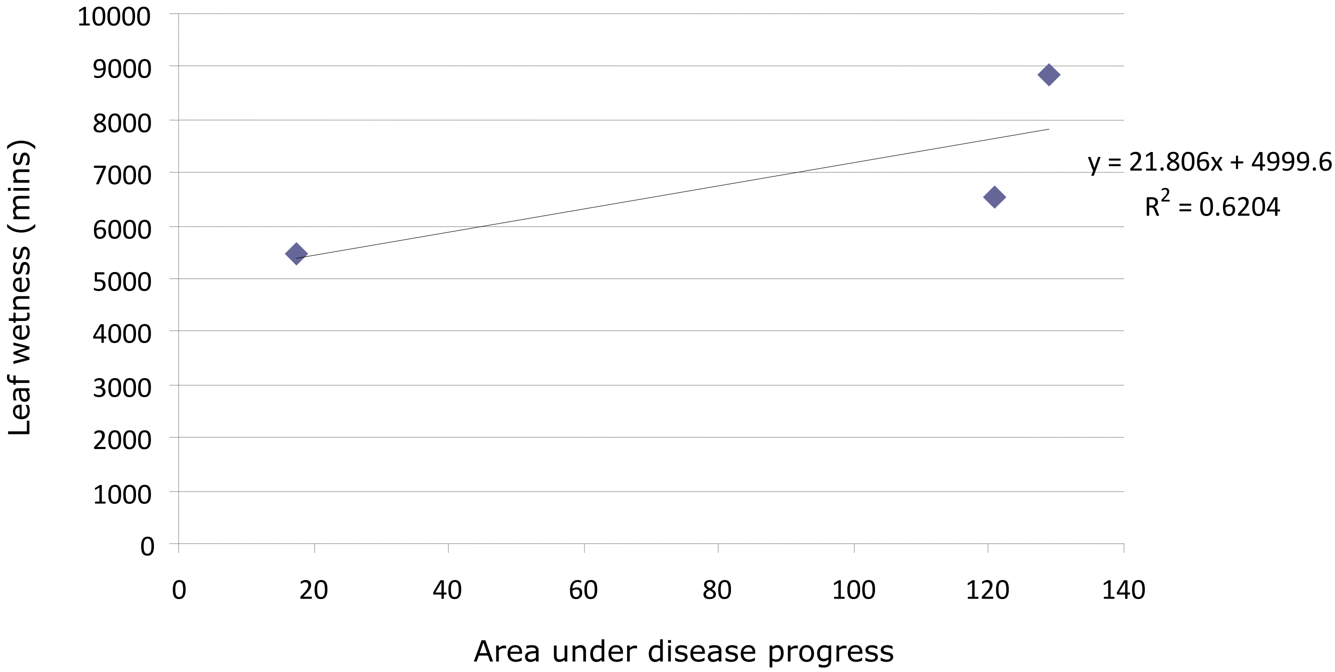

Figure 3 Correlation between total cumulative duration of maximum leaf surface wetness between 1 -14 April (in minutes) and Area under disease progress for Ramularia leaf spot in winter barley.

3.3.2 Asymptomatic development of *R. collo cygni* in barley leaf

The qualitative nested PCR test was able to detect the fungus in the barley canopy up to four weeks prior to disease symptoms appearing (Havis *et al.,* unpublished). In the same trial at Lanark in 2008 utilising the real time PCR method *R. collo-cygni* was detected in leaf tissue 60 days after sowing (1st July 2008). The build up of *Ramularia* DNA over time was quantified with the fungus reaching ngram/gram leaf levels in leaf layer Flag minus 3 and Flag minus 4 after 90 days. This substantial increase in fungal DNA was not correlated with high symptom severity on the lower leaves, probably due to the low light levels reaching the Flag-2 and Flag-3 leaf layers (data not shown). Final Ramularia DNA levels in the varieties at the end of the trial were as follows:

Cocktail - 15.2 pg; Decanter - 60 pg and Optic 15.3 pg (Figure 4). Although there were a number of significant spore release events in July the majority of Ramularia DNA was lower in the canopy indicating that upward movement of fungus is still a contributing factor to final levels in the ear. Future control measures against *R. collo-cygni* may need to be targeted towards protecting the seed while still in the ear and also in preventing the movement of the fungus into the developing plant from infected seed.

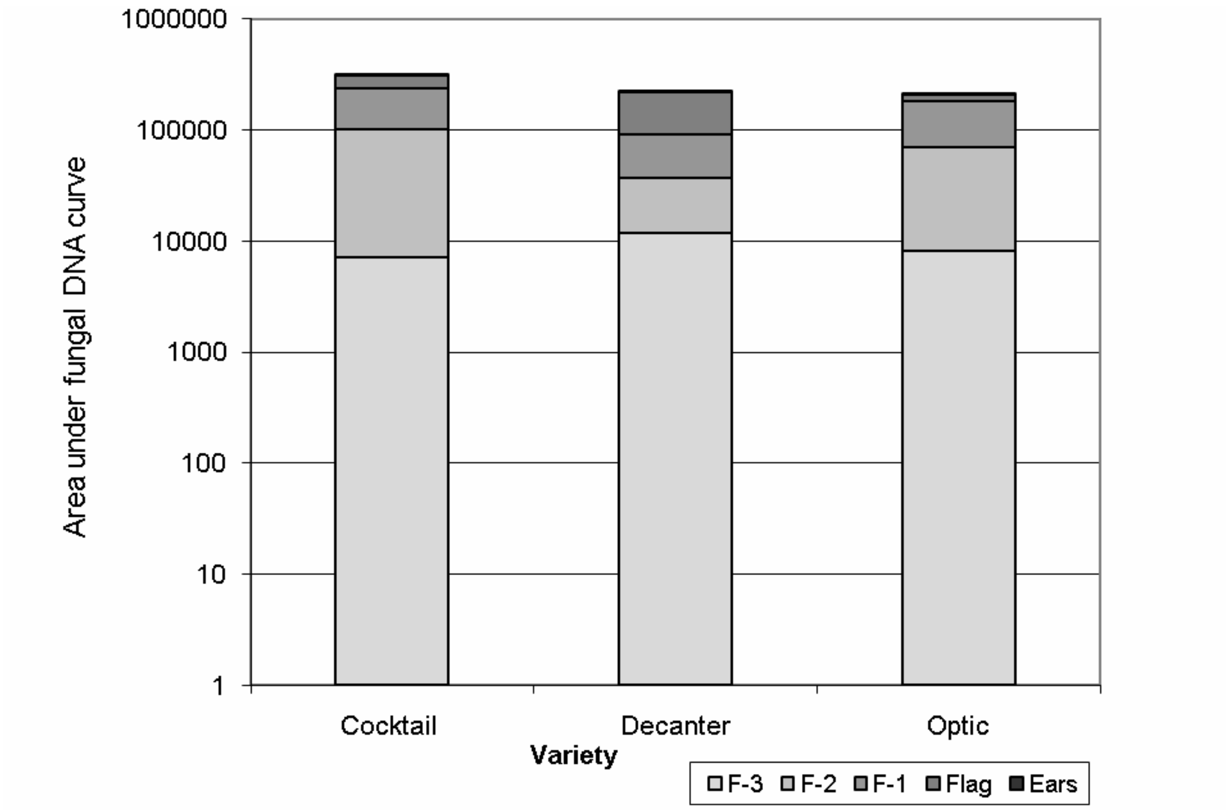

Figure 4 Area under curves for Ramularia DNA at Lanark site. The majority of Ramularia DNA recorded is in the lower leaves.

3.3.3 Survey of harvested grain 2006 and 2007

Table 3 shows that seed contamination levels varied from 0.08 pg DNA to 90 pg DNA of *R. collo-cygni*. There were no significant differences between the varieties.

Table 3 Levels of DNA of harvest seed (mean 2006 and 2007 harvest)

Variety	Ramularia (pg DNA)
Amarena	1.27
Braemar	0.08
Camion	58.90
Carat	0.09
Cellar	5.82
Chalice	21.70
Cocktail	0.22
Candy	25.29
Decanter	23.29
Doyen	24.85
Manitou	2.46
Maresi	4.62
Optic	8.69
Oxbridge	4.50
Pastoral	0.63
Pearl	2.99
Pict	2.36
Publican	3.40
Rebecca	16.19
Riviera	15.57
Saffron	22.86
Sequel	90.08
Static	18.90
Suffolk	8.18
Unknown	1.70
Unknown	1.26
Waggon	2.46
Westminster	6.12
SED	52.30
Significance	ns

There was a significant difference between the two seasons and seed contamination levels were higher in 2006 compared to 2007 (Table 4 & 5).

Regional differences in DNA levels on harvested grain were also observed. When data was categorised by Scotland (north) and England (south), there were significant differences with greater levels of contamination seen in Scotland.

Splitting the samples by RL region was less successful since so few samples came from the north west. There is, however, a trend towards lower DNA level in the east compared to the north.

Table 8 Levels of Ramularia DNA on harvested seed in 2006 and 2007

	Harvested year	
	2006	2007
pg DNA	48.31	9.92
Number	30	59
SED	10.76	
Significance	<.001	

Table 9 Levels of Ramularia DNA on harvested seed in 2006 and 2007 by regions

	Location			
	England	Scotland		
Level of Ramularia DNA (pg)	6.20	32.68		
Number of samples	33	56		
SED	10.92			
Significance	0.02			
	Region			
	North East	East	West	North west
Level of Ramularia DNA (pg)	27.47	9.26	20.34	10.47
Number of samples	57	12	15	5
SED	21.55			
LSD	42.85			
Significance	ns			

If seed is important for the initial spread of Ramularia leaf spot, levels of seed contamination vary significantly by season and also by region. Seed sourced from England, particularly in the east will have lower levels of contamination compared to seed sourced from wetter regions of England or from Scotland.

3.3.4 Ramularia collo-cygni contamination of harvested commercial seed

Results from the survey of barley seed taken at harvest in 2006 are sown in Figure56 and for 2007 in Figure 6.

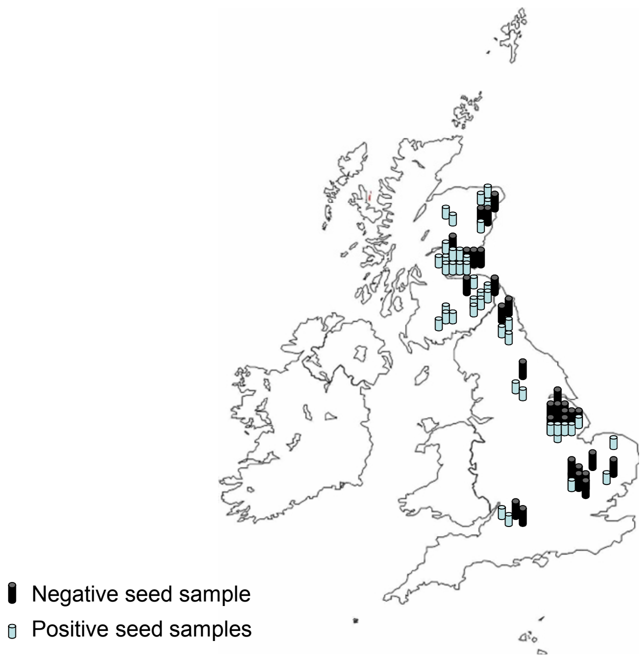

Figure 5 Ramularia distribution on harvested seed 2006. All green columns had Ramularia levels greater than 0.128 pgrams. Black columns has levels below minimum detection.

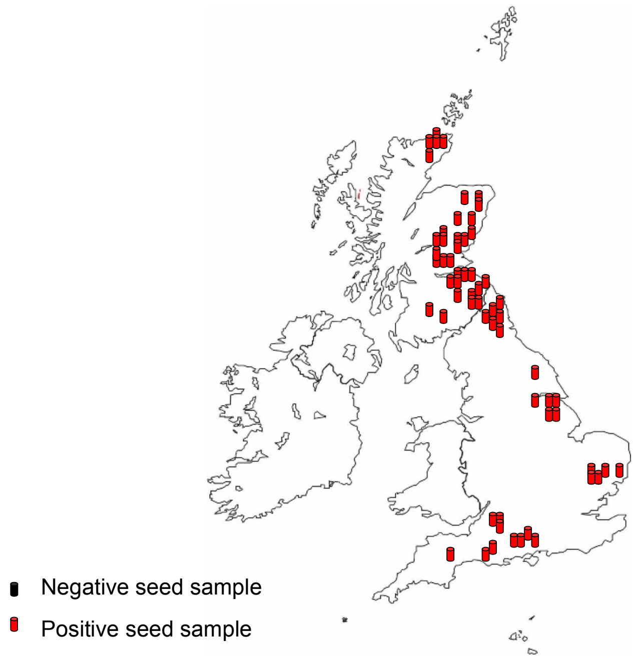

Figure 6 Ramularia distribution on harvested seed 2007. All red columns had Ramularia DNA levels above 0.128 pgrams. There were no samples below this threshold in 2007.

The field work occurred over a single season and the relative importance of site, variety, seed contamination and fungicide were determined. The results have been divided to look at these different aspects.

3.3.5 R. collo-cygni contamination of Recommended List seed

Seed stocks used in these experiments were tested for contamination with *Ramularia collo cygni*. Results are reported in Table 6. Levels of contamination less than 5pg DNA were categorised as low, whilst levels greater than 5 pg DNA were categorised as high. Most seed stocks used in the RL trials were categorised low, but some were as high as 31 pg Dna.

Table 6 Levels of Ramularia collo-cygni DNA in seed stocks (pg DNA)

2007 Spring Barley Seed (Harvested from untreated RL trials in 2007)			2008 Spring Barley Seed (Supplied by CEL)		
Variety name	R collo cygni concentration	Comment on contamination	Variety Name	R collo cygni concentration	Comment on contamination
Appaloosa	Nd	-	Appaloosa	0.3	Low
Belgravia	Nd	-	Belgravia	2.3	Low
Cellar	Nd	-	Berlioz	16.1	High
Cocktail	17.8	High	Cocktail	1.3	Low
Decanter	20.47	High	Concerto	31.7	High
Doyen	134.0	High	Cropton	8.4	High
Fairytale	40.0	High	Decanter	29.5	High
Jolika	1.1	Low	Doyen	1.6	Low
Knightsbridge	24.1	High	Forensic	10.9	High
Maltby	Nd	-	Jolika	1.1	Low
NFC Tipple	21.1	High	LP 11593.03	0.8	Low
Optic	29.1	High	NFC Tipple	0.8	Low
Oxbridge	47.9	High	Optic	4.2	Low
Power	2.0	Low	Oxbridge	2.6	Low
Publican	52.4	High	Publican	0.3	Low
Quench	Nd	-	Quench	0.8	Low
Rebecca	25.9	High	Rebecca	0.1	Low
Riviera	6.8	High	Scout	0.1	Low
SB02146	Nd	-	Sweeney	0.1	Low
Scout	39.3	High	Virgil	2.6	Low
Snakebite	2.3	Low	Waggon	0.8	Low
Sweeney	Nd	-	Westminster	18.8	High
Troon	3.7	Low			
Waggon	Nd	-			
Westminster	38.8	High			
Wicket	Nd	-			

ND – No Data

3.3.6 Importance of variety and seed stock on Ramularia leaf spot

Three spring barley varieties, Cocktail, Decanter and Optic were sown at three different sites in 2008.

Table 7 Seed DNA levels in two seed stocks (pg/100 ng DNA extracted from seed)

Variety (resistance to Ramularia leaf spot)	Cocktail (susceptible)	Decanter (resistant)	Optic (susceptible)
Home-Saved seed from 2007	17.8	20.5	29.1
Commercial seed from 2008	1.3	29.5	4.2

Two seed stocks were used for each variety. The Home saved seed from 2007 was selected from untreated field trials to ensure high seed contamination. The commercial 2008 seed was seed submitted for Recommended List trials. Ramularia DNA was detected in all the seed stocks. Contamination levels (i.e. high and low) were defined on the basis of DNA levels; less than 5pg are low and levels greater than 5pg high.

The 2008 commercial seed has lower levels of DNA compared to the 2007 seed in the case of Optic and Cocktail. The Decanter 2008 seed had higher levels. Since Decanter is a variety used in the north of the UK, it is likely this seed was produced in a region where Ramularia levels are high.

Table 8 Ramularia assessment based on area under the disease progress curve

Variety	Cocktail	Decanter	Optic
	131.2	103.7	145.5
Seed	Commercial seed	Home-saved seed	
	126.5	127.1	
	Commercial seed	Home-saved seed	
Cocktail	135.0	126.6	
Decanter	102.3	105.2	
Optic	141.4	149.7	
SED Variety	15.2		
SED Seed	10.2		
SED Variety x Seed	17.7		
Significance Variety	0.004		
Significance Seed	ns		
Significance Variety x Seed	ns		

The area under disease progress curve provides a useful indicator of the overall disease levels through the season (Table 8). Varietal differences were significant and in the order expected with Decanter showing lowest levels of disease and Optic the most. The differences between the seed stocks were not significant suggesting different levels of contamination had no affect on overall symptom expression throughout the time period of this experiment.

Table 9 Percentage Ramularia leaf spot symptoms on upper leaves in July

Variety	Cocktail	Decanter	Optic
	9.5	7.6	10.7
Seed	Commercial seed	Home-saved seed	
	9.1	9.4	
	Commercial seed	Home-saved seed	
Cocktail	9.7	9.4	
Decanter	7.4	7.7	
Optic	10.3	11.2	
SED Variety	0.92		
SED Seed	0.75		
SED Variety x Seed	1.31		
Significance Variety	0.003		
Significance Seed	ns		
Significance Variety x Seed	ns		

Table 10 Ramularia leaf spot symptoms on leaves in July (log10)

Variety	Cocktail	Decanter	Optic
	0.89	0.81	0.960
Seed	Commercial seed	Home-saved seed	
	0.88	0.90	
	Commercial seed	Home-saved seed	
Cocktail	0.89	0.89	
Decanter	0.79	0.83	
Optic	0.93	0.98	
SED Variety	0.05		
SED Seed	0.04		
SED Variety x Seed	0.06		
Significance Variety	0.005		
Significance Seed	ns		
Significance Variety x Seed	ns		

A similar pattern was seen when looking at a single late assessment (Table 9 & 10). Varietal resistance had a greater impact on disease than seed contamination.

Table 11 Crop yields at harvest (T/ha) 85% DM

Variety	Cocktail	Decanter	Optic
	5.02	5.48	5.13
Seed	Commercial seed	Home-saved seed	
	5.384	5.04	
	Commercial seed	Home-saved seed	
Cocktail	5.28	4.74	
Decanter	5.60	5.36	
Optic	5.26	5.01	
SED Variety	0.124		
SED Seed	0.101		
SED Variety x Seed	0.175		
Significance Variety	<.001		
Significance Seed	<.001		
Significance Variety x Seed	ns		

The yields were significantly different between the seed stocks and the varieties (Table 11). In all three varieties, the home-saved seed produced lower yields. Decanter, the most resistant variety to Ramularia leaf spot achieved the highest yield out of the three varieties.

Table 12 Quality assessment at harvest % Screenings 2.5 mm sieve

Variety	Cocktail	Decanter	Optic
	12.3	13.9	12.6
Seed	Commercial seed	Home-saved seed	
	12.8	13.1	
	Commercial seed	Home-saved seed	
Cocktail	12.1	12.5	
Decanter	13.7	14.1	
Optic	12.5	12.8	
SED Variety	1.55		
SED Seed	1.27		
SED Variety x Seed	2.19		
Significance Variety	Ns		
Significance Seed	Ns		
Significance Variety x Seed	Ns		

The quality of the grain was similar between the varieties and seed stocks (Table 12).

Table 13 Percentage green leaf area on upper leaves in July

Variety	Cocktail	Decanter	Optic
	62.9	70.9	63.0
Seed	Commercial seed	Home-saved seed	
	64.7	66.5	
	Commercial seed	Home-saved seed	
Cocktail	61.3	64.4	
Decanter	70.1	71.8	
Optic	62.8	63.2	
SED Variety	1.81		
SED Seed	1.48		
SED Variety x Seed	2.56		
Significance Variety	<.001		
Significance Seed	ns		
Significance Variety x Seed	Ns		

Significant differences were observed between the varieties in green leaf area retention. No differences were observed between the seed stocks.
On the basis of the experiments on these three varieties, varietal choice has a greater impact on disease development than level of contamination with *R. collo-cygni*.

3.3.7 Impact of fungicides and varietal resistance on Ramularia symptoms and yields using six varieties of spring barley

These trials comprised six varieties using one seed stock. Fungicide programmes detailed in the materials and methods are listed below to assist interpretation (Table 14).
Significant differences were observed between the varieties in green leaf area retention. No differences were observed between the seed stocks. On the basis of the experiments on these three varieties, varietal choice has a greater impact on disease development than level of contamination with *R. collo-cygni*.

Table 14 Fungicide treatments

Treatment	GS31-32	GS37	GS49	Comment
1	Nil	Nil	Nil	Untreated
2	Proline 0.4 + Acanto 0.5 + Flexity 0.25	Nil	Nil	No late treatment
3	Proline 0.4 + Acanto 0.5 + Flexity 0.25	Nil	Proline 0.4+ Bravo 1.0 + Amistar 0.5	Well timed late treatment
4	Proline 0.4 + Acanto 0.5 + Flexity 0.25	Proline 0.4+ Bravo 1.0 + Amistar 0.5	Nil	Poorly timed late treatment

Table 15 Ramularia assessment showing Area under disease progress curve

	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
Variety	135.8	102.3	141.4	94.0	88.9	105.6
	1	2	3	4		
Fungicide	146.1	125.0	73.4	100.6		
	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
1	177.7	131.2	162.9	134.7	141.0	129.0
2	145.1	114.8	51.7	120.2	97.5	120.9
3	91.9	73.6	109.1	52.9	43.0	69.7
4	126.6	89.8	141.7	68.1	74.2	102.8
SED Variety	20.66					
SED Fungicide	16.87					
SED Variety x Fungicide	41.33					
Significance Variety	0.05					
Significance Fungicide	<.001					
Significance Variety x Fungicide	ns					

The impact of fungicides on Ramularia leaf spot was greater than varietal resistance (Table 15). An early fungicide treatment has some influence on the disease epidemic, but the second treatment applied at GS45 continues to provide the best control. Disease levels on varieties with better resistance (Decanter, Poker and Oxbridge) were lower when compared to the more susceptible varieties (Cocktail, Optic, Prestige). Fungicides were also more effective on the resistant varieties.

Table 16 Ramularia leaf spot assessment on leaves in July (LOG10)

Variety	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
	0.90	0.79	0.93	0.56	0.49	0.70
Fungicide	1	2	3	4		
	0.91	0.82	0.57	0.62		
	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
1	1.01	0.98	1.01	0.83	0.78	0.84
2	0.98	0.90	0.96	0.60	0.66	0.80
3	0.75	0.57	0.87	0.24	0.33	0.66
4	0.84	0.73	0.90	0.55	0.18	0.52
SED Variety	0.105					
SED Fungicide	0.085					
SED Variety x Fungicide	0.209					
Significance Variety	<.001					
Significance Fungicide	<.001					
Significance Variety x Fungicide	ns					

Table 17 Percentage Ramularia leaf spot symptoms on leaves in July

Variety	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
	9.7	7.4	10.3	6.8	6.0	7.2
Fungicides	1	2	3	4		
	11.0	8.7	5.3	6.5		
	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
1	13.5	10.4	12.2	10.6	9.9	9.6
2	11.0	8.7	10.2	8.0	6.4	8.0
3	5.8	4.9	8.6	3.7	3.3	5.4
4	8.4	5.6	10.2	4.9	4.2	5.6
SED Variety	1.31					
SED Fungicide	1.07					
SED Variety x Fungicide	2.61					
Significance Variety	0.005					
Significance Fungicide	<.001					
Significance Variety x Fungicide	ns					

A similar pattern of disease control was seen in the single late assessment (Tables 16 and 17) compared to the disease progress results (Table 15). This demonstrates the importance varietal resistance can play in conjunction with fungicides to achieve the best level of control. Timing of fungicide is however important, with the greatest effect seen with the GS45 treatment.

Table 18 Yields at harvest (T/ha) 85% DM

Variety	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
	5.3	5.6	5.2	5.4	6.0	5.6
Fungicides	1	2	3	4		
	5.2	5.5	5.7	5.7		
	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
1	4.7	5.5	4.8	5.2	5.8	5.3
2	5.7	5.4	5.4	5.1	5.9	5.7
3	5.3	5.8	5.4	5.7	6.3	5.9
4	5.6	5.6	5.3	5.8	6.1	5.7
SED Variety	0.17					
SED Fungicide	0.14					
SED Variety x Fungicide	0.35					
Significance Variety	<.001					
Significance Fungicide	.002					
Significance Variety x Fungicide	ns					

Best yields were seen in the fungicide treatments which achieved the best control of Ramularia leaf spot (Table 18). Yield responses to disease control ranged from 0.6 t/ha down to 0.3 t/ha. The lowest response was seen in the most resistant variety Decanter.

Table 19 Quality of grain at harvest % Screenings 2.5 mm sieve

Variety	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
	12.1	13.7	12.5	8.1	8.5	7.8
Fungicide	1	2	3	4		
	10.1	10.7	9.8	11.1		
	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
1	11.8	12.2	12.1	7.9	9.7	7.3
2	13.5	12.3	13.0	10.0	8.7	6.5
3	11.7	15.1	10.4	7.01	8.0	6.3
4	11.5	15.1	14.5	7.5	7.4	10.4
SED Variety	1.57					
SED Fungicide	1.92					
SED Variety x Fungicide	3.85					
Significance Variety	ns					
Significance Fungicide	0.003					
Significance Variety x Fungicide	ns					

Screenings provide a good measure of quality, particularly from malting barley markets. Although screenings levels were similar in these trials, (Table 19), the best reduction was achieved with the fungicide applied at GS45. This was the same treatment which achieved best control of Ramularia leaf spot

Table 20 Percentage green leaf area on upper leaves in July

Variety	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
	61.3	70.1	62.8	61.3	67.9	56.3
Fungicide	1	2	3	4		
	53.9	62.4	70.1	66.8		
	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
1	46.9	62.5	52.7	55.8	58.0	47.2
2	62.3	69.6	64.6	59.1	65.9	53.0
3	72.6	77.4	68.6	66.7	75.1	60.1
4	63.5	70.8	65.3	63.7	72.4	65.2
SED Variety	2.35					
SED Fungicide	1.92					
SED Variety x Fungicide	4.71					
Significance Variety	<.001					
Significance Fungicide	<.001					
Significance Variety x Fungicide	ns					

Green leaf area levels were highest in the optimum fungicide timing for Ramularia leaf spot (Table 20 GS45, treatment 3).

These experiments show that fungicides had a greater impact on Ramularia leaf spot than the variety. There were however differences in the timing of the treatment. There were also interactions between variety and fungicide. Ramularia leaf spot was easier to control on a resistant variety and the overall yield response to disease control was greater on a susceptible variety.

3.3.8 Impact of site on Ramularia symptoms using five varieties

Table 21 Ramularia assessment Area Under Disease Progress Curve

Variety	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
	136	102	141	94	89	106
Site	Bush	Lanark	Perth			
	121	185	28			
	Bush	Lanark	Perth			
Cocktail	170	198	40			
Decanter	151	104	53			
Optic	174	202	49			
Oxbridge	82	193	7			
Poker	74	187	6			
Prestige	76	226	15			
SED Variety	12.7					
SED Site	9.0					
SED Variety x Site	21.9					
Significance Variety	<.001					
Significance Site	<.001					
Significance Variety x Site	<.001					

Levels of disease varied widely at the three sites (Table 21, 22 & 23). Disease levels were low at Perth, but high at Bush and Lanark. There were variety by site interactions however. At a low disease pressure site, Decanter exhibited relatively more disease when compared to Prestige. One reason for this may be the high level of seed contamination on Decanter. Under low disease pressure situations therefore, the importance of seed contamination will increase.

Table 22 Ramularia leaf spot on leaves in July (log10)

	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
Variety	0.89	0.79	0.93	0.56	0.49	0.70
	Bush	Lanark	Perth			
Site	0.83	1.03	0.32			
	Bush	Lanark	Perth			
Cocktail	0.91	1.15	0.63			
Decanter	0.90	0.74	0.75			
Optic	0.96	1.17	0.68			
Oxbridge	0.79	1.04	-0.16			
Poker	0.70	0.99	-0.24			
Prestige	0.75	1.09	0.27			
SED Variety	0.07					
SED Site	0.05					
SED Variety x Site	0.118					
Significance Variety	<.001					
Significance Site	<.001					
Significance Variety x Site	<.001					

Table 23 Percentage Ramularia leaf spot on leaves in July

Variety	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
	9.7	7.4	10.3	6.8	6.0	7.2
Site	Bush	Lanark	Perth			
	7.3	12.8	3.6			
	Bush	Lanark	Perth			
Cocktail	8.3	16.2	4.6			
Decanter	8.5	7.2	6.5			
Optic	9.3	15.6	6.0			
Oxbridge	6.3	12.9	1.0			
Poker	5.7	11.2	1.0			
Prestige	5.8	13.4	2.3			
SED Variety	0.97					
SED Site	0.68					
SED Variety x Site	1.68					
Significance Variety	<.001					
Significance Site	<.001					
Significance Variety x Site	<.001					

Table 24 Yields at harvest T/ha 85% DM

	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
Variety	5.3	5.6	5.2	5.5	6.0	5.6
	Bush	Lanark	Perth			
Site	5.8	5.3	5.5			
	Bush	Lanark	Perth			
Cocktail	5.6	5.0	5.3			
Decanter	4.5	5.5	5.8			
Optic	5.4	4.7	5.6			
Oxbridge	6.1	5.4	4.9			
Poker	6.6	5.6	6.0			
Prestige	5.7	5.6	5.6			
SED Variety	0.16					
SED Site	0.11					
SED Variety x Site	0.28					
Significance Variety	<.001					
Significance Site	<.001					
Significance Variety x Site	0.003					

Despite the Perth site exhibiting the lowest levels of Ramularia leaf spot, highest yields were achieved from a site where the disease levels were higher (Table 24). The susceptible variety Optic did however achieve a higher yield at Perth compared to the higher disease pressure sites.

Table 25 Grain quality as harvest as % Screenings (2.5 mm sieve)

	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
Variety	12.3	13.5	13.1	8.0	8.4	7.7
	Bush	Lanark	Perth			
Site	8.8	18.7	4.2			
	Bush	Lanark	Perth			
Cocktail	10.6	23.0	3.7			
Decanter	13.0	24.2	3.8			
Optic	10.3	25.7	3.8			
Oxbridge	5.7	13.3	5.3			
Poker	6.8	15.6	3.1			
Prestige	6.4	11.3	5.6			
SED Variety	1.03					
SED Site	0.73					
SED Variety x Site	1.79					
Significance Variety	<.001					
Significance Site	<.001					
Significance Variety x Site	<.001					

The quality of the grain was best at the low disease pressure site (Table 25).

Table 26 Percentage green leaf area in July

	Cocktail	Decanter	Optic	Oxbridge	Poker	Prestige
Variety	61.3	70.0	62.8	661.3	67.9	56.3
	Bush	Lanark	Perth			
Site	65.1	64.3	60.5			
	Bush	Lanark	Perth			
Cocktail	62.0	60.6	61.5			
Decanter	71.3	76.3	62.5			
Optic	65.8	56.3	66.4			
Oxbridge	62.2	64.2	57.6			
Poker	70.4	66.1	66.1			
Prestige	57.9	62.4	48.8			
SED Variety	2.63					
SED Site	1.86					
SED Variety x Site	4.56					
Significance Variety	<.001					
Significance Site	0.03					
Significance Variety x Site	0.02					

Variety had the greatest impact on late green leaf area (Table 26).

Results from these experiments show site had the greatest influence on Ramularia leaf spot, followed by fungicide treatments and timings and varietal resistance. The impact seed contamination was lower, but no seed stock was fully free from the disease.

3.3.9 Impact of Ramularia on Recommended List varieties

Assessments of Recommended List variety trials were undertaken in July 2008. Table 27 shows the results based on an average of five spring barley sites (Perth, Lanark, Bush, Fife, Borders). Winter barley trials were assessed at four sites in June 2008 (Fife, Bush, Lanark, Perth). Results are shown in Table 28.

Table 27 Spring barley varieties 2008 showing levels of Ramularia leaf spot and green leaf area retention in July and comments on varietal resistance

	% Ramula-ria	Ramularia (Log10)	Comment on varietal resistance	% Green leaf area	Green leaf area (log10)	Comment on green leaf retention
Appaloosa	11.5	1.02	Good	55.3	1.69	Good
Azalea	14.3	1.11	Good	44.3	1.60	Intermediate
Belgravia	11.4	1.01	Good	64.0	1.78	Good
Berlioz	17.3	1.19	Intermediate	43.5	1.58	Intermediate
Cocktail	16.7	1.18	Intermediate	51.3	1.65	Good
Concerto	17.8	1.22	Intermediate	50.5	1.65	Good
Cropton	15.2	1.15	Intermediate	55.2	1.70	Good
Decanter	13.1	1.08	Good	57.1	1.72	Good
Doyen	15.7	1.14	Intermediate	54.7	1.68	Good
Fairytale	15.2	1.14	Intermediate	53.3	1.68	Good
Forensic	15.7	1.15	Intermediate	43.6	1.46	Intermediate
Jolika	17.5	1.19	Intermediate	45.8	1.59	Intermediate
Knightsbridge	16.8	1.12	Intermediate	39.1	1.48	poor
LP1159.3.03	20.2	1.26	Poor	43.9	1.55	Intermediate
Maltby	10.4	0.95	Good	52.9	1.66	Good
NFC 405-103	18.4	1.23	Intermediate	40.5	1.54	poor
NFC-Tipple	11.4	0.96	Good	51.9	1.60	Good
Optic	18.0	1.17	Intermediate	48.3	1.61	Intermediate
Oxbridge	16.5	1.16	Intermediate	47.2	1.61	Intermediate
Power	12.6	1.04	Good	48.9	1.63	Intermediate
Publican	15.0	1.14	Good	54.8	1.70	Good
Quench	15.5	1.15	Intermediate	54.1	1.71	Good
Rebecca	11.3	0.95	Good	56.3	1.68	Good
Riviera	16.6	1.18	Intermediate	48.3	1.64	Intermediate
Scout	14.0	1.08	Good	56.4	1.72	Good
Snakebite	16.4	1.13	Intermediate	38.1	1.46	poor
Sweeney	16.0	1.14	Intermediate	46.6	1.58	Intermediate
Troon	20.4	1.23	Poor	43.3	1.56	Intermediate
Virgil	20.2	1.25	Poor	43.3	1.60	Intermediate
Waggon	11.5	1.01	Good	53.3	1.66	Good
Westminster	13.8	1.07	Good	54.2	1.68	Good
Wicket	12.6	1.06	Good	44.9	1.55	Intermediate
SED	2.95	0.0901		6.32	0.082	
LSD	5.82	0.1776		12.45	0.061	
Significance	<.001	<.001		<.001	<.001	

Differences between the varieties were significant and the varieties have been differentiated as having good resistance (0-15%), intermediate (16-20%) and poor (greater than 20%).

Table 28 Winter barley varieties 2008

	% Ramularia	Ramularia (Log10)	Comment on Ramularia resistance	% Green leaf area	Green leaf area (log10)	Comment on green leaf retention
Accrue	21.87	1.30	Intermediate	25.67	1.38	Good
Amarena	19.87	1.21	Good	37.07	1.46	Good
Boost	22.67	1.35	Intermediate	31.40	1.38	Good
Bronx	22.20	1.32	Intermediate	36.67	1.50	Good
Carat	24.07	1.35	Intermediate	26.00	1.35	Good
Cassata	27.60	1.41	Poor	24.00	1.32	Intermediate
Colibri	23.53	1.37	Intermediate	28.40	1.34	Good
CPBT B81	32.00	1.47	Poor	13.33	1.11	Poor
Daybreak	33.44	1.50	Poor	15.86	1.18	Poor
Flagon	27.07	1.42	Poor	34.00	1.52	Good
Karioka	23.35	1.36	Intermediate	28.78	1.41	Good
LP6-342	23.53	1.37	Intermediate	31.33	1.48	Good
MH 97 CQ2	27.92	1.44	Poor	29.88	1.53	Good
NSL 03-7442	27.92	1.44	Poor	24.88		Intermediate
Pearl	16.93	1.21	Good	30.00	1.39	Good
Pelican	25.00	1.39	Poor	34.00	1.48	Good
Retriever	25.00	1.39	Poor	15.67	1.17	Poor
Saffron	21.00	1.30	Intermediate	28.07	1.31	Good
Sequel	24.40	1.37	Intermediate	25.33	1.35	Intermediate
Suzuka	30.33	1.46	Poor	20.67	1.24	Intermediate
Volume	19.33	1.28	Good	42.67	1.62	Good
WB 031090	21.27	1.31	Intermediate	23.33	1.32	Intermediate
Wintmalt	22.87	1.35	Intermediate	20.07	1.20	Intermediate
SED	5.800	0.110		9.12	0.1463	
LSD	11.5	0.219		18.16	0.2931	
Significance	ns	ns		0.07	0.08	

Differences in Ramularia leaf spot are not significant but differences in green leaf area are significant. Varieties have been differentiated as having good (0-15%), intermediate (16-20%) and poor (greater than 20%) resistance.

3.3.10 Impact of seed contaminated with R. collo-cygni on Ramularia symptoms on 10 varieties grown at 5 sites

Table 29 shows the *R. collo-cygni* DNA levels on the seed of 10 spring barley varieties. Comments on high and low levels of contamination were based on the threshold of greater than 5pg DNA high and less than 5 pg DNA low.

Table 29 Assessment of DNA on seed stocks

	R. collo-cygni DNA on seed (pg)	R. collo-cygni dna on seed (pg)	R. collo-cygni on seed (pg)	Comment on difference
Variety (DNA contamination)	Average	2007 seed stock	2008 seed stock	
Cocktail	9.56	17.80	1.33	Greater in 2007
Decanter	24.98	20.46	29.50	Greater in2008
Doyen	67.82	134.00	1.63	Greater in 2007
Jolika	1.06	1.07	1.05	Similar
NFC-Tipple	10.96	21.10	0.82	Greater in 2007
Optic	16.67	29.10	4.23	Greater in 2007
Oxbridge	25.24	47.90	2.59	Greater in 2007
Publican	26.35	52.40	0.30	Greater in 2007
Rebecca	12.95	25.90	0.01	Greater in 2007
Scout	19.67	39.30	0.04	Greater in 2007
Westminster	28.80	38.80	18.80	Similar, but greater in 2007
Seed	22.19	38.89	5.48	

Table 30 Assessment of Ramularia leaf spot in July in two seed stocks labelled 2007 and 2008 for different spring barley varieties

	% Ramularia	% Ramularia	% Ramularia	Ramularia log10	Ramularia log10	Ramularia log10
	Average	2007	2008	Average	2007	2008
Cocktail	15.3	14.4	16.2	1.137	1.143	1.130
Decanter	12.9	11.0	14.8	1.075	1.028	1.121
Doyen	14.2	14.2	14.2	1.103	1.105	1.100
Jolika	15.7	15.8	15.6	1142	1.156	1.127
NFC-Tipple	12.1	15.9	9.2	1.007	1.086	0.927
Optic	17.1	19.2	15.0	1.133	1.185	1.080
Oxbridge	16.4	16.6	16.2	1.148	1.179	1.115
Publican	13.4	12.6	14.2	1.095	1.082	1.107
Rebecca	12.3	10.8	13.8	1.003	0.971	1.035
Scout	13.2	14.8	11.6	1.052	1.084	1.018
Westminster	12.4	11.6	13.2	1.041	1.015	1.066
Seed	14.09	14.18	14.0	1.085	1.095	1.076
SED Seed	4.499			1.130		
SED Variety	1.943			0.057		
SED Seed x Variety	5.206			0.163		
LSD Seed	10.375			0.329		
LSD Variety	3.867			0.114		
LSD Seed x Variety	11.153			0.351		
Significance Seed	Ns			Ns		
Significance Variety	Ns			0.08		
Significance Seed x Variety	ns			Ns		

Table 31 Assessment of abiotic leaf spots in July in two seed stocks labelled 2007 and 2008 for different spring barley varieties

	% Abiotic leaf spots	% Abiotic leaf spots	% Abiotic leaf spots
	Average	2007	2008
Cocktail	5.0	2.5	7.5
Decanter	0	0	0
Doyen	4.3	3.5	5.0
Jolika	8.8	7.5	10.0
NFC-Tipple	2.8	5.5	0
Optic	14.5	19.0	10.0
Oxbridge	7.5	5	10.0
Publican	2.5	0.1	5.0
Rebecca	2.5	0.1	5.0
Scout	5.0	7.5	2.5
Westminster	5.0	0	10.0
Seed	5.25	4.59	5.91
SED Seed	6.08		
SED Variety	3.86		
SED Seed x Variety	5.71		
LSD Seed	26.15		
LSD Variety	8.05		
LSD Seed x Variety	19.83		
Significance Seed	Ns		
Significance Variety	0.08		
Significance Seed x Variety	ns		

Table 32 Assessment of Green leaf area in July in two seed stocks labelled 2007 and 2008 for different spring barley varieties

	% Green leaf area	% Green leaf area	% Green leaf area	Green leaf area (log10)	Green leaf area (log10)	Green leaf area (log10)
	Average	2007	2008	Average	2007	2008
Cocktail	49.8	51.4	48.2	1.625	1.628	1.621
Decanter	53.7	59.4	48.0	1.695	1.751	1.639
Doyen	51.3	53.6	49.0	1.648	1.684	1.6111
Jolika	44.2	43.1	45.3	1.565	1.517	1.612
NFC-Tipple	45.5	42.2	48.8	1.533	1.489	1.577
Optic	44.2	41.2	47.2	1.568	1.567	1.568
Oxbridge	43.9	47.1	40.7	1.568	1.638	1.497
Publican	56.0	55.6	56.4	1.707	1.699	1.714
Rebecca	51.8	55.4	48.2	1.637	1.688	1.585
Scout	54.0	48.6	59.4	1.699	1.652	1.745
Westminster	54.3	59.0	49.6	1.679	1.748	1.610
Seed	49.9	50.6	49.2	1.629	1.639	1.619
SED Seed	15.1			0.1634		
SED Variety	4.3			0.0642		
SED Seed x Variety	16.1			0.1849		
LSD Seed	34.8			0.3768		
LSD Variety	8.5			0.1278		
LSD Seed x Variety	35.8			0.3995		
Significance Seed	ns			ns		
Significance Variety	0.02			0.06		
Significance Seed x Variety	ns			ns		

3.3.11 Ramularia seed stocks x varieties

Table 33 Assessment of Ramularia leaf spot in July in seed stocks categorised by the amount of R collo-cygni DNA

	% Ramularia	% Ramularia	% Ramularia	Ramularia log10	Ramularia log10	Ramularia log10
	Average	Seed DNA 5-134 pg	Seed DNA <5 pg	Average	Seed DNA <5 pg	Seed DNA 5-134 pg
Cocktail	15.2	15.2	15.3	1.138	1.162	1.111
Decanter		12.9			1.074	
Doyen	14.2	15.0	13.3	1.104	1.124	1.080
Jolika		15.6			1.193	
NFC-Tipple	12.4	15.8	8.3	1.016	1.106	0.907
Optic	17.3	20.0	14.1	1.134	1.204	1.061
Oxbridge	16.4	17.4	15.3	1.152	1.198	1.096
Publican	13.4	13.4	13.3	1.095	1.101	1.088
Rebecca	12.2	11.6	12.9	1.002	0.990	1.016
Scout	13.4	15.6	10.7	1.056	1.103	0.999
Westminster		12.4			1.041	
SED Variety	1.940				0.057	
SED Variety X DNA level	2.969				0.087	
LSD Variety	3.859				0.114	
LSD Variety x DNA level	5.907				0.174	
Significance Variety	0.06				0.05	
Sig Variety x DNA level	ns				ns	

Ramularia seed stocks x varieties

Table 34 Assessment of green leaf area in July in seed stocks categorised by the amount of R collo-cygni DNA

	% Green leaf area	% Green leaf area	% Green leaf area	Green leaf area (log10)	Green leaf area (log10)	Green leaf area (log10)
	Average	Seed DNA 5-134 pg	Seed DNA <5 pg	Average	Seed DNA <5 pg	Seed DNA 5-134 pg
Cocktail	49.6	48.3	51.3	1.623	1.603	1.647
Decanter		53.7			1.694	
Doyen	51.2	50.5	52.1	1.648	1.658	1.637
Jolika		41.2			1.520	
NFC-Tipple	44.9	39.1	51.9	1.526	1.463	1.603
Optic	43.6	38.1	50.3	1.565	1.541	1.594
Oxbridge	43.9	44	43.8	1.571	1.612	1.523
Publican	55.6	52.5	59.5	1.703	1.673	1.739
Rebecca	51.8	52.3	51.3	1.639	1.662	1.611
Scout	53.2	45.5	62.5	1.692	1.626	1.771
Westminster		54.3			1.679	
SED Variety	4.29			0.06524		
SED Variety X DNA level	6.57			0.0999		
LSD Variety	8.54			0.1298		
LSD Variety x DNA level	13.08			0.1987		
Significance Variety	0.02			0.07		
Sig Variety x DNA level	ns			ns		

3.4 Discussion

New information from this research can help to address the two principal aims which were to understand the relevance of seed contamination on varietal resistance and the measures required to clean up seed stocks and produce UK data for risk forecast based on variety, stress factors and accurate variety resistance scores for the HGCA Recommended list.

Different factors which can influence the severity of Ramularia leaf spot in a high disease pressure region are discussed and their relative importance quantified. The relative importance is likely to be different in a region where the disease is not endemic.

3.4.1 Site

Some sites in this project and in field trials in previous research resulted in high levels of disease. Other sites only exhibited low levels of disease. Attempting to determine differences at the sites is therefore an important aim to help determine future risk. Previous research showed that airborne spores occur at the end of the season when symptoms are visible in the field and these airborne spore dispersal events are linked to leaf wetness periods. Since these spore events occur in July when symptoms are already present in the crops it is not possible to use this information to forecast an epidemic. It only confirms that an epidemic has occurred and that there is a high risk that seed will become contaminated with *R. collo-cygni* spores.

Looking earlier in the season and comparing the weather patterns of sites which exhibited high and low levels of disease, this project shows that certain weather patterns in May and June can be used to forecast high risk crops before symptoms appear and in time for preventative fungicide applications to be applied. There is a higher risk of an epidemic occurring where there are a high number of cumulative leaf wetness minutes earlier in the season when the crop is tillering. This can be observed in this research, where the Perth site exhibited low levels of disease compared to the Lanark and Bush site. Other differences were seen between the sites (crop elevation, sowing date etc.) so the evidence from this single season is limited. Looking back at previous research, a similar pattern can be seen with a link between leaf wetness events early in the crop development and later disease epidemic. It is not fully understanding why early leaf wetness leads to a disease epidemic. It is possible that spores are released as a consequence of the leaf wetness and these spores infect the crop. Reasons why this may not be the case are: 1) early fungicides applied at tillering are not as effective as later applied fungicides in controlling the disease; 2) significant spore dispersal associated with these early leaf wetness events are not evident from spore trap data and 3) *R. collo-cygni* is already present within leaves in the form of mycelia as a consequence of seed-borne infection so there is already potential inoculum inside the plants regardless of external weather patterns. (It is hypothesised that early leaf wetness events are causing stress to the plants which will eventually lead *R. collo cygni* to exhibit extensive symptoms.). Although the reason for the link between early leaf wetness and symptom expression is not fully understood, it can be used to differentiate high and low disease risk sites as part of the disease forecast. Understanding the reason for its importance is the focus of new research.

3.4.2 Foliar fungicides

The importance of foliar fungicides to control disease has been well recognised. Timing of the treatment is also recognised and a later application at booting (GS45) before visible symptoms appear is the main time to achieve the best yield. Earlier foliar treatments at mid-tillering to first node stage (GS25-31) also has an impact on disease and yield, but this is of minor importance. In high disease pressure situations, particularly where a susceptible variety is sown with a high level of contamination with *R. collo- cygni* this earlier treatment is likely to have greater importance compared to lower risk situations.

3.4.3 Varietal resistance and seed contamination

Varietal resistance is the next major method to manage the disease and differences have been observed in symptom expression in both spring barley and winter barley varieties. The level of varietal resistance in the current Recommended List is not sufficient to manage the disease alone, but in common with other diseases, controlling Ramularia leaf spot on a more resistant variety is more straight forward compared to controlling the disease on a susceptible variety. Susceptible varieties are likely to require higher doses, more applications and more critical timing of application compared to a resistant variety.

One aim of this project was to look at the impact seed infection had on symptom expression. This was important since at some sites, varieties exhibit high levels of disease whilst at others they achieve low levels. Is this difference due to seed infection? This research shows that site has a greater role to play in symptom expression than seed infection. This means that as part of the varietal evaluation system, seed contamination may not play a major part in symptom expression. Looking at some of the more resistant varieties however (e.g. NFC-Tipple), a stock with 0.82 pg DNA exhibited 8.3 % symptoms compared to a stock with 21.1 pg of DNA which averaged 15.8% Ramularia leaf spot. It is recommended that for Recommended List testing, seed stocks are used with as low a level of contamination as can be sourced. The same advice should be given to regions where the disease is not endemic (i.e. east England).

R. collo cygni developed asymptomatically in resistant and susceptible varieties. Greater levels developed on lower leaves, but no symptoms occurred. This suggests *R. collo cygni* colonises plants via seed and that it can coexist within barley plants without producing symptoms. The stress that triggers necrosis and spotting are not fully understood, but leaf wetness events in early April or June are related to symptom expression. Future research questions are to determine if *R. collo cygni* can be eradicated or if this coexistence can be extended to beyond flowering delaying symptom expression and yield losses.

Since no stocks used in the research were totally free from the disease, it was not possible to compare a seed stock with no seed contamination with one with high levels of contamination. Since the initial source of the pathogen is thought to be the seed, excluding the pathogen from a region must be a major priority and therefore attempting to achieve seed with no viable R. collo cygni DNA is seen to be of major importance to reduce the spread of disease into new regions and of high priority. Even in the low risk region of England, the average level of seed contamination was 6.2 pg DNA compared to Scotland where levels averaged 32.7 pg DNA. Seasonal differences in seed contamination were also high. It is suspected that wet weather in July when symptoms are present, is an important weather factor determining the level of seed contamination, despite the fact it is too late to influence the epidemic in the current season. Our aim should be to reduce contamination through the use of seed treatments to ensure the disease does not become established throughout the UK.

On the basis of this research, a risk forecast can be produced (Table 35) based on the relative importance of factors (Table 32 & Figure 8).

Table 35 Risk forecast for Ramularia leaf spot in spring barley (endemic regions e.g. Scotland)

Determining risk	Comment
Leaf wetness: particularly in early June at the GS25 to GS32 stage. Where leaf wetness events exceed 4000 minutes, crop is at high risk from Ramularia leaf spot	Site has the greatest impact on disease risk and leaf wetness events. Calculating leaf wetness can be done centrally for weather stations, but new research can determine methods to get in-field data
Variety: choose variety with greatest level of resistance for specific market	Quality and yield are the main reasons to choose a variety, but longer term aims in plant breeding may make this an important option
Seed contamination: choose seed with less than 5pg DNA (Preferably less than 1 pg DNA)	This can be tested and it is recommended seed is tested for *Ramularia collo cygni*
Seed Treatment: existing seed treatments are unlikely to affect risk, but if new ones are developed, they should be used to ensure seed contamination is below 1pg DNA	New research is required to investigate methods to control *R. collo cygni* on seed.
Fungicides at GS25: if leaf wetness events are high in May/June and variety is susceptible, ensure GS25-30 fungicide comprises fungicide with effective activity.	Recommended option for high risk crops.
Fungicides at GS45: GS45 essential for high risk crops.	Essential option for high risk crops
Leaf wetness flowering: increased risk of seed infection. Home saving seed following extensive leaf wetness event in July (to be quantified) is not recommended in absence of effective seed treatment.	This will help determine the potential risk of seed contamination in the absence of a seed test.

Table 36 Relative importance of risk factors in region where Ramularia leaf spot is common

Variable	**Definition of differentiation of variable**	**% Reduction in Ramularia leaf spot symptoms**
Site	Lanark v Perth (Leaf wetness difference in early June)	73%
Fungicide applied at GS49	Applied v not applied	52%
Spring barley variety resistance	Best v least resistant (Virgil v Maltby)	48%
Winter Barley variety resistance	Best v least resistant variety (Daybreak v Pearl)	47%
Seed health	Above v below 5pg DNA seed contamination	28%
Fungicide applied at GS30	Applied v not applied	21%

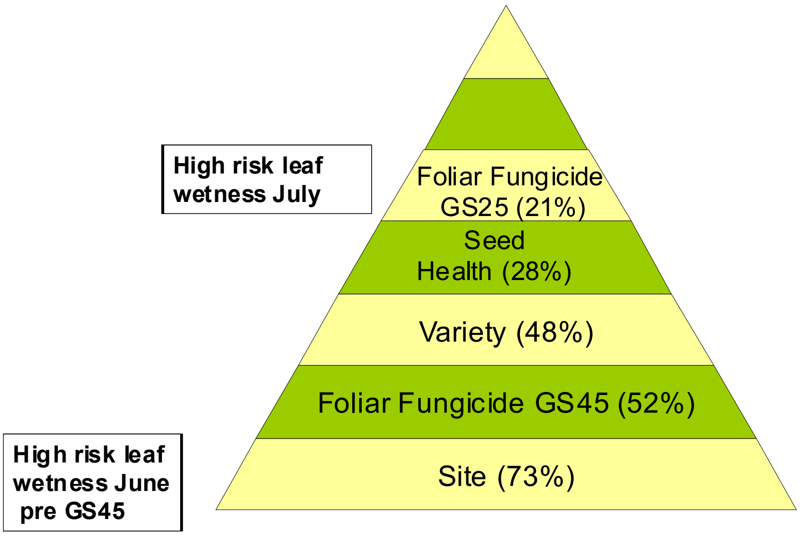

Figure 7 Main factors associated with Ramularia risk and management. The factors in wider part of triangle are of greater importance (Percentages in brackets show the best reduction of symptoms for each factor). Value for seed treatment is unknown.

The risk forecast will need to be modified to assist growers in regions where the disease is endemic and also regions where the disease has yet to be found, or where it only occurs sporadically. As with all biological systems, caution is required, since there are unknowns which may impact on the risk. The presence of a second fungal body on barley straw (known as astromella) is not taken account in the risk forecast and its importance is not fully understood. It is possible the leaf wetness periods early in the season are having an impact on the disease.

For UK regions where the disease is not yet established, the relative importance of the risk factors are likely to be different. In these situations, it becomes important to keep potential sources of inoculum out of the system. To address this, the risk forecast can be modified as follows.

Risk forecast (regions where disease has not been seen) East England
No routine fungicides are required for Ramularia leaf spot
Variety: Choose variety with greatest level of resistance for specific market
Seed Treatment –existing seed treatments are unlikely to affect risk, but if new ones are developed, they should be used to ensure seed contamination is below 1pg DNA
Fungicides at GS25: Unlikely to be required for Ramularia leaf spot in lower risk region.
Fungicides at GS45: Recommended where leaf wetness events were high in May/June and variety is susceptible. More information is required to determine the dates where leaf wetness is correlated to disease in these regions.

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5. APPENDIX

Protocol

Crops Division Study Number 001173
Study Title: **HGCA Managing Ramularia collo-cygni through seed health**
Name of Sponsors / Contacts: HGCA

Study Objectives:

Study Timetable: March – Oct 08

Location of test facilities:

Study number	Crop	Type	Site	
001173 (0801)	Spring barley	Variety/Seed Stock exp	Perth	72 plots
001173 (0802)	Spring barley	Variety x Treatment exp	Perth	36 plots
001173 (0803)	Spring Barley	Variety/Seed Stock exp	Bush	72 plots
001173 (0804)	Spring Barley	Variety x Treatment exp	Bush	36 plots
001173 (0805)	Spring barley	Variety/Seed Stock exp	Lanark	72 plots
001173 (0806)	Spring barley	Variety x Treatment exp	Lanark	36 plots

Study status: non regulatory.
QA SAC responsibility

The study will be conducted within SAC Crops Division Quality Assurance System

001173(0801) Spring barley Perth
3 variety x 3 replicates x 2 stocks x 4 treatments

Plot	Block	Variety	Stock	Fungicide
1	1	Optic	Commercial seed	3
2	1	Decanter	Home saved seed	1
3	1	Cocktail	Commercial seed	4
4	1	Decanter	Home saved seed	2
5	1	Cocktail	Home saved seed	2
6	1	Cocktail	Home saved seed	1
7	1	Decanter	Home saved seed	3
8	1	Optic	Home saved seed	4
9	1	Decanter	Commercial seed	1
10	1	Optic	Home saved seed	1
11	1	Decanter	Home saved seed	4
12	1	Cocktail	Home saved seed	4
13	1	Optic	Commercial seed	1
14	1	Optic	Home saved seed	3
15	1	Cocktail	Commercial seed	2
16	1	Optic	Home saved seed	2
17	1	Decanter	Commercial seed	3
18	1	Optic	Commercial seed	2
19	1	Decanter	Commercial seed	2
20	1	Optic	Commercial seed	4
21	1	Decanter	Commercial seed	4
22	1	Cocktail	Home saved seed	3
23	1	Cocktail	Commercial seed	1
24	1	Cocktail	Commercial seed	3
25	2	Optic	Home saved seed	3
26	2	Cocktail	Commercial seed	4
27	2	Cocktail	Commercial seed	2
28	2	Cocktail	Commercial seed	1
29	2	Optic	Commercial seed	3
30	2	Optic	Commercial seed	2
31	2	Cocktail	Home saved seed	3
32	2	Decanter	Commercial seed	4
33	2	Optic	Home saved seed	2
34	2	Optic	Home saved seed	4
35	2	Decanter	Commercial seed	3
36	2	Cocktail	Home saved seed	2
37	2	Decanter	Commercial seed	2
38	2	Cocktail	Home saved seed	4
39	2	Optic	Home saved seed	1
40	2	Cocktail	Commercial seed	3
41	2	Optic	Commercial seed	1
42	2	Decanter	Home saved seed	3
43	2	Decanter	Commercial seed	1
44	2	Cocktail	Home saved seed	1
45	2	Decanter	Home saved seed	2
46	2	Decanter	Home saved seed	1
47	2	Optic	Commercial seed	4
48	2	Decanter	Home saved seed	4

Plot	Block	Variety	Stock	Fungicide
49	3	Decanter	Commercial seed	2
50	3	Cocktail	Commercial seed	4
51	3	Optic	Commercial seed	1
52	3	Cocktail	Home saved seed	3
53	3	Optic	Home saved seed	3
54	3	Decanter	Commercial seed	3
55	3	Optic	Commercial seed	4
56	3	Optic	Commercial seed	3
57	3	Optic	Home saved seed	1
58	3	Optic	Home saved seed	2
59	3	Cocktail	Commercial seed	2
60	3	Cocktail	Commercial seed	1
61	3	Cocktail	Commercial seed	3
62	3	Decanter	Home saved seed	4
63	3	Cocktail	Home saved seed	1
64	3	Decanter	Commercial seed	4
65	3	Decanter	Home saved seed	3
66	3	Decanter	Home saved seed	2
67	3	Decanter	Commercial seed	1
68	3	Optic	Commercial seed	2
69	3	Optic	Home saved seed	4
70	3	Decanter	Home saved seed	1
71	3	Cocktail	Home saved seed	2
72	3	Cocktail	Home saved seed	4

001173 – 0802 Spring Barley Perth
3 variety x 3 replicates x 1 stocks x 4 treatments

Plot	Block	Variety	Stock	Fungicide
1	1	Oxbridge	Commercial seed	2
2	1	Prestige	Commercial seed	3
3	1	Poker	Commercial seed	4
4	1	Oxbridge	Commercial seed	4
5	1	Prestige	Commercial seed	2
6	1	Poker	Commercial seed	1
7	1	Poker	Commercial seed	3
8	1	Prestige	Commercial seed	4
9	1	Poker	Commercial seed	2
10	1	Prestige	Commercial seed	1
11	1	Oxbridge	Commercial seed	1
12	1	Oxbridge	Commercial seed	3
13	2	Prestige	Commercial seed	4
14	2	Prestige	Commercial seed	2
15	2	Prestige	Commercial seed	1
16	2	Oxbridge	Commercial seed	3
17	2	Poker	Commercial seed	4
18	2	Poker	Commercial seed	2
19	2	Oxbridge	Commercial seed	2
20	2	Poker	Commercial seed	3
21	2	Oxbridge	Commercial seed	4
22	2	Oxbridge	Commercial seed	1
23	2	Prestige	Commercial seed	3
24	2	Poker	Commercial seed	1
25	3	Poker	Commercial seed	3
26	3	Oxbridge	Commercial seed	1
27	3	Oxbridge	Commercial seed	4
28	3	Oxbridge	Commercial seed	3
29	3	Prestige	Commercial seed	1
30	3	Oxbridge	Commercial seed	2
31	3	Poker	Commercial seed	2
32	3	Prestige	Commercial seed	2
33	3	Prestige	Commercial seed	4
34	3	Poker	Commercial seed	4
35	3	Prestige	Commercial seed	3
36	3	Poker	Commercial seed	1

001173 – 0803 Spring Barley – Bush
3 variety x 3 replicates x 2 stocks x 4 treatments

Plot	Block	Variety	Stock	Fungicide
1	1	Cocktail	Home saved seed	2
2	1	Cocktail	Home saved seed	3
3	1	Optic	Commercial seed	4
4	1	Optic	Commercial seed	3
5	1	Cocktail	Commercial seed	4
6	1	Cocktail	Home saved seed	1
7	1	Decanter	Commercial seed	2
8	1	Decanter	Home saved seed	4
9	1	Cocktail	Commercial seed	3
10	1	Optic	Home saved seed	4
11	1	Decanter	Home saved seed	1
12	1	Decanter	Commercial seed	4
13	1	Decanter	Commercial seed	3
14	1	Optic	Commercial seed	2
15	1	Optic	Commercial seed	1
16	1	Cocktail	Home saved seed	4
17	1	Optic	Home saved seed	3
18	1	Decanter	Home saved seed	2
19	1	Decanter	Home saved seed	3
20	1	Cocktail	Commercial seed	2
21	1	Optic	Home saved seed	1
22	1	Decanter	Commercial seed	1
23	1	Optic	Home saved seed	2
24	1	Cocktail	Commercial seed	1

Plot	Block	Variety	Stock	Fungicide
25	2	Optic	Commercial seed	1
26	2	Optic	Home saved seed	3
27	2	Optic	Home saved seed	4
28	2	Cocktail	Home saved seed	4
29	2	Cocktail	Home saved seed	2
30	2	Cocktail	Commercial seed	2
31	2	Decanter	Commercial seed	2
32	2	Decanter	Home saved seed	2
33	2	Cocktail	Home saved seed	3
34	2	Optic	Home saved seed	1
35	2	Optic	Commercial seed	2
36	2	Decanter	Commercial seed	3
37	2	Cocktail	Home saved seed	1
38	2	Cocktail	Commercial seed	3
39	2	Decanter	Commercial seed	1
40	2	Decanter	Home saved seed	4
41	2	Decanter	Home saved seed	1
42	2	Optic	Commercial seed	4
43	2	Decanter	Home saved seed	3
44	2	Cocktail	Commercial seed	1
45	2	Decanter	Commercial seed	4
46	2	Optic	Home saved seed	2
47	2	Optic	Commercial seed	3
48	2	Cocktail	Commercial seed	4
49	3	Cocktail	Commercial seed	1
50	3	Optic	Commercial seed	1
51	3	Decanter	Commercial seed	1
52	3	Decanter	Commercial seed	2
53	3	Decanter	Home saved seed	4
54	3	Cocktail	Commercial seed	2
55	3	Optic	Home saved seed	2
56	3	Optic	Commercial seed	3
57	3	Optic	Commercial seed	4
58	3	Optic	Home saved seed	1
59	3	Decanter	Home saved seed	1
60	3	Cocktail	Home saved seed	4
61	3	Cocktail	Home saved seed	2
62	3	Decanter	Home saved seed	2
63	3	Cocktail	Home saved seed	1
64	3	Decanter	Commercial seed	4
65	3	Cocktail	Commercial seed	3
66	3	Cocktail	Home saved seed	3
67	3	Optic	Home saved seed	4
68	3	Optic	Home saved seed	3
69	3	Decanter	Home saved seed	3
70	3	Optic	Commercial seed	2
71	3	Cocktail	Commercial seed	4
72	3	Decanter	Commercial seed	3

001173 (0804) – Spring Barley Bush
3 variety x 3 replicates x 1 stock x 4 treatments

Plot	Block	Variety	Stock	Fungicide
1	1	Poker	Commercial seed	1
2	1	Poker	Commercial seed	3
3	1	Prestige	Commercial seed	1
4	1	Oxbridge	Commercial seed	1
5	1	Prestige	Commercial seed	3
6	1	Oxbridge	Commercial seed	2
7	1	Oxbridge	Commercial seed	4
8	1	Poker	Commercial seed	2
9	1	Prestige	Commercial seed	4
10	1	Prestige	Commercial seed	2
11	1	Oxbridge	Commercial seed	3
12	1	Poker	Commercial seed	4
13	2	Prestige	Commercial seed	3
14	2	Prestige	Commercial seed	4
15	2	Poker	Commercial seed	3
16	2	Oxbridge	Commercial seed	3
17	2	Oxbridge	Commercial seed	1
18	2	Prestige	Commercial seed	2
19	2	Poker	Commercial seed	2
20	2	Prestige	Commercial seed	1
21	2	Poker	Commercial seed	1
22	2	Poker	Commercial seed	4
23	2	Oxbridge	Commercial seed	2
24	2	Oxbridge	Commercial seed	4
25	3	Poker	Commercial seed	2
26	3	Oxbridge	Commercial seed	3
27	3	Poker	Commercial seed	3
28	3	Poker	Commercial seed	1
29	3	Prestige	Commercial seed	3
30	3	Prestige	Commercial seed	2
31	3	Oxbridge	Commercial seed	1
32	3	Prestige	Commercial seed	1
33	3	Prestige	Commercial seed	4
34	3	Oxbridge	Commercial seed	2
35	3	Oxbridge	Commercial seed	4
36	3	Poker	Commercial seed	4

01173 (0805) – Spring Barley – Lanark
3 variety x 3 replicates x 2 stocks x 4 treatments

Plot	Block	Variety	Stock	Fungicide
1	1	Optic	Home saved	4
2	1	Optic	Home saved	1
3	1	Decanter	Home saved	4
4	1	Optic	Commercial seed	2
5	1	Optic	Commercial seed	3
6	1	Cocktail	Home saved	4
7	1	Decanter	Commercial seed	4
8	1	Cocktail	Commercial seed	1
9	1	Optic	Commercial seed	4
10	1	Decanter	Commercial seed	3
11	1	Decanter	Home saved	3
12	1	Decanter	Home saved	2
13	1	Cocktail	Commercial seed	2
14	1	Optic	Home saved	3
15	1	Decanter	Commercial seed	2
16	1	Cocktail	Commercial seed	4
17	1	Decanter	Home saved	1
18	1	Optic	Home saved	2
19	1	Optic	Commercial seed	1
20	1	Cocktail	Home saved	2
21	1	Cocktail	Home saved	3
22	1	Cocktail	Commercial seed	3
23	1	Decanter	Commercial seed	1
24	1	Cocktail	Home saved	1
25	2	Cocktail	Commercial seed	2
26	2	Optic	Home saved	4
27	2	Optic	Commercial seed	2
28	2	Optic	Home saved	3
29	2	Optic	Home saved	1
30	2	Decanter	Commercial seed	4
31	2	Decanter	Home saved	3
32	2	Cocktail	Commercial seed	3
33	2	Cocktail	Home saved	3
34	2	Decanter	Home saved	4
35	2	Decanter	Commercial seed	3
36	2	Decanter	Home saved	2
37	2	Optic	Commercial seed	4
38	2	Optic	Commercial seed	3
39	2	Decanter	Commercial seed	1
40	2	Optic	Home saved	2
41	2	Cocktail	Commercial seed	1
42	2	Decanter	Home saved	1
43	2	Cocktail	Home saved	2
44	2	Cocktail	Home saved	4
45	2	Optic	Commercial seed	1
46	2	Cocktail	Home saved	1
47	2	Cocktail	Commercial seed	4
48	2	Decanter	Commercial seed	2

49	3	Decanter	Commercial seed	2
50	3	Optic	Home saved	1
51	3	Cocktail	Commercial seed	3
52	3	Optic	Home saved	3
53	3	Decanter	Commercial seed	3
54	3	Cocktail	Commercial seed	4
55	3	Decanter	Home saved	1
56	3	Optic	Home saved	4
57	3	Optic	Commercial seed	4
58	3	Optic	Commercial seed	1
59	3	Cocktail	Home saved	1
60	3	Cocktail	Commercial seed	2
61	3	Decanter	Commercial seed	1
62	3	Decanter	Commercial seed	4
63	3	Decanter	Home saved	2
64	3	Optic	Commercial seed	2
65	3	Optic	Commercial seed	3
66	3	Decanter	Home saved	3
67	3	Cocktail	Home saved	4
68	3	Optic	Home saved	2
69	3	Decanter	Home saved	4
70	3	Cocktail	Home saved	2
71	3	Cocktail	Home saved	3
72	3	Cocktail	Commercial seed	1

001173 – (0806) Spring barley – Lanark
3 variety x 3 replicates x 1 stock (commercial seed) x 4 treatments

Plot	Block	Variety	Stock	Fungicide
1	1	Poker	Commercial seed	1
2	1	Oxbridge	Commercial seed	1
3	1	Oxbridge	Commercial seed	3
4	1	Oxbridge	Commercial seed	2
5	1	Prestige	Commercial seed	3
6	1	Poker	Commercial seed	3
7	1	Poker	Commercial seed	2
8	1	Poker	Commercial seed	4
9	1	Prestige	Commercial seed	1
10	1	Prestige	Commercial seed	2
11	1	Prestige	Commercial seed	4
12	1	Oxbridge	Commercial seed	4
13	2	Oxbridge	Commercial seed	2
14	2	Prestige	Commercial seed	3
15	2	Poker	Commercial seed	3
16	2	Prestige	Commercial seed	1
17	2	Poker	Commercial seed	1
18	2	Oxbridge	Commercial seed	4
19	2	Poker	Commercial seed	2
20	2	Poker	Commercial seed	4
21	2	Oxbridge	Commercial seed	3
22	2	Oxbridge	Commercial seed	1
23	2	Prestige	Commercial seed	2
24	2	Prestige	Commercial seed	4
25	3	Oxbridge	Commercial seed	3
26	3	Oxbridge	Commercial seed	2
27	3	Oxbridge	Commercial seed	1
28	3	Poker	Commercial seed	3
29	3	Poker	Commercial seed	2
30	3	Poker	Commercial seed	4
31	3	Prestige	Commercial seed	1
32	3	Prestige	Commercial seed	2
33	3	Prestige	Commercial seed	3
34	3	Oxbridge	Commercial seed	4
35	3	Poker	Commercial seed	1
36	3	Prestige	Commercial seed	4

Treatments:

	GS31-32	GS37	GS49
1	Nil	Nil	Nil
2	Proline 0.4 + Acanto 0.5 + Flexity 0.25	Nil	Nil
3	Proline 0.4 + Acanto 0.5 + Flexity 0.25	Nil	Proline 0.4+ Bravo 1.0 + Amistar 0.5
4	Proline 0.4 + Acanto 0.5 + Flexity 0.25	Proline 0.4+ Bravo 1.0 + Amistar 0.5	Nil

Trial to be yielded
Comments:
Visual and PCR assessments for leaf spots/Ramularia will be done on the trials.

Spore traps trapping Ramularia and Rhynchosporium will be present on the sites
Weather data is being recorded on the sites
There are large scale plots on these sites looking at the spread of Rhynchosporium and potential seed borne infection of Rhynchosporium

Sample labelling

Label all samples with the study number, date of sampling, plot number and crop growth stage. Please put the full study number on since this identifies the site and year.

Study personnel: Study Director, Simon Oxley. Sponsor HGCA

END of PROTOCOL