Project title:

What impact does the Barley disease Ramularia leaf spot have on malting quality?

Project Summary:

A key factor in the economic and environmental sustainability of the brewing sector is controlling barley diseases and the effects that they have on supply chains. Every year, the UK barley industry loses millions of pounds in revenue due to disease. Most of these losses are caused by fungi, and in the last 30 years we have seen the emergence of the fungus *Ramularia collo-cygni* (Rcc), the causal agent of the disease known as Ramularia leaf spot (RLS), the economic cost of this disease is currently estimated at £10m/year. Given the apparent ubiquity of *Rcc within* European barley it is essential to assess the impact of this pathogen on grain quality and processability in addition to the known consequences for agronomic yield to help mitigate risk to the malting industry and reliant sectors. Prior to this study Rccs' relationship with grain structure and malting had received little attention. Whilst it is well-established that some types of fungal infection in barley can detrimentally impact malt and beer quality (e.g., *Fusarium* mediated gushing), no definitive study has been conducted to investigate what impact RLS has on malting quality. This project therefore set out to assess what impact the presence of Rcc in barley grains has on key measures of malting quality and any potential risk this pathogen may pose to intermediary and final product quality.

Objectives and outcomes

Two key objectives were created at the outset of the project:

1. Does the presence of seed borne *Rcc* in barley grain affect malting quality?
2. How does the malting process influence the dynamics of *Rcc* infection?

Several different barley varieties were sourced from Scotland's Rural College (SRUC). These were grown in East Lothian during the 2021 season and prior to their use, the level of Ramularia infection was calculated using a well-established qPCR method Four of the varieties with different levels of infection (Table 1) were then micro-malted and subject to a full analysis. Raw barley and malted grains were characterized by measuring: moisture, skinning, β-glucan, germinative energy, germinative capacity, free amino nitrogen (FAN), sieving fractionation, and 100 grain weight using standard ASBC/SBC methods. Barley malt grain enzymatic activity was evaluated by determination of α-amylase activity, β-amylase activity, β-glucanase activity and limit dextrinase activity (Megazyme Int., Ireland). Wort was produced by the standard EBC congress mash (method 4.5.1)

Table 1: Level of Ramularia collo-cygni infection (pg of DNA) in selected varieties

	Franklin	**Power**	**Tankard**	**Braemar**
Level of infection (pg of DNA)	69.42	155.9	201.91	451.79

A summary of how an increasing level of fungal infection impacts malting parameters is shown in Table 2. It was found that with increasing Rcc infection, the germination energy and index of grains did not meet the standards underlined for quality malt production reducing by 25 and 35% between the lowest and highest levels of infection. 100 grain weight is significantly reduced with increasing infection, and therefore loss of material during grading was also found to increase with higher levels of Rcc infection which is consistent with work at SRUC who showed that Rcc infection can increase the rate of small grains by up to 4%. An increase in wort density was established with increasing Rcc infection while free amino nitrogen content of the wort produced was shown to decrease, with a decline of 27% seen in the grains with the highest level of Rcc.

Table 2: The impact of increasing Ramularia infection on key malting parameters and enzymatic activity.

Analysis	**Method**	**Result of increasing infection level**	**Δ%**
Germinative capacity	EBC 3.5.1	↓ ***	-25
Germinative energy (GE)	EBC 3.6.2	↓ ***	-35
Moisture	EBC 3.2	—	0
100 grain weight	EBC 3.4	↓ **	-12
Sieving test for barley	EBC 3.11.1	↓ **	-50
FAN	EBC 4.10	↓ *	-27
Density	EBC 9.43.2	↑ *	2
β-glucan	MEGAZYME© Azo-Barley Glucan	↓ ***	-89
Starch	MEGAZYME© Total starch (AA/AMG)	↓ *	-50
Amylose	MEGAZYME© K-AMYL method	↑ ***	220
α-amylase	MEGAZYME© Ceralpha	↑*	30
β-amylase	MEGAZYME© Betamyl-3	↑ *	79
Limit dextrinase	MEGAZYME© PullG6	↓ *	-46
Skinning	SMMG method	↑ **	50

Δ% represents change from lowest to highest infection. Significant differences $p \leq 0.05$ (*), $p \leq 0.01$(**), $p \leq 0.001$ (***).

Significant differences were also found in the enzyme activity within the grains with considerable changes to both the amylolytic(amylase) and cytolytic (β-glucan) behavior of the malt. High initial levels of Rcc led to an increase in both α and β-amylase levels in the final malt product. It must be noted that these elevated levels may be coming from fungal infection as an exact allocation of the respective enzymes is not possible because the cleavage products of these are often the same whether of cereal or microbial origin. Further work using methods that aim at the gene expression of the corresponding enzymes could be used to classify the origin of the enzymes (cereal or fungal).

For other fungal species it has previously been demonstrated that the steeping process, high water availability, and aeration associated with malting can induce the germination of fungal spores, leading to an increase in the number of barley grains infected. Therefore, we set out to track the path of Rcc infection during malting and examine any changes to grain ultrastructure. An increase in pink pigmentation of grains during malting was shown to increase with Rcc infection (Figure1), it is important to note that pink grains while indicative of fungal infection are not limited to Ramularia infection however noticeable changes were observed in grain structure. During microscopy, there was little pink pigmentation in lower-mid levels of infection as can be seen in Figure 1a, b, c and d. Grains of Franklin and Power exhibited typical starch morphology at the surface level. In contrast, Braemar grains showed deep pink pigmentation, particularly in the area surrounding the pericarp. To distinguish whether starch morphology is perturbed with Rcc infection, we carried out environmental scanning electron microscopy (Figure 2)

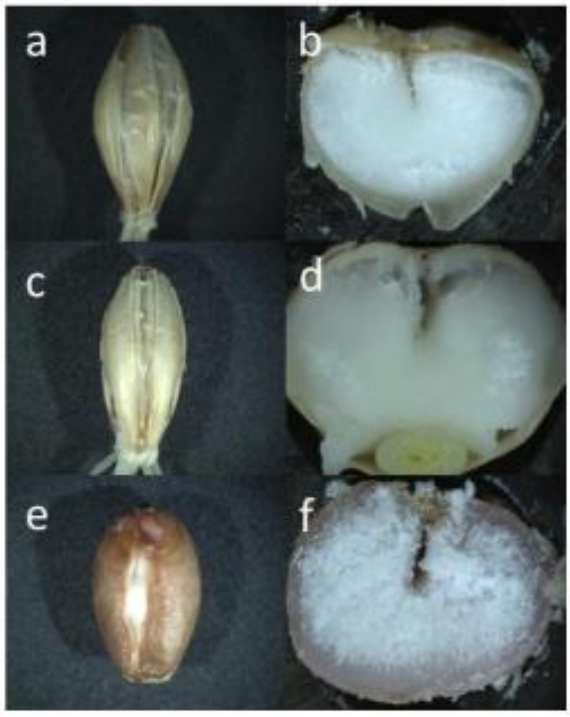

Figure 1: Visualization of grains through digital microscopy after 3 days of germination*:* A, C, E: Frontal plane of Franklin, Power and Braemar. B, D, F: Cross section of Franklin, Power and Braemar

THE SEM results (Figure 2) showed uniform and compact storage of starch granules within low-infected barley grains. The starch granules in grains with higher infection were more detached from the cell boundaries. Damage to the starch could also be seen at the equatorial plane of larger A granules (Figure 3), suggesting potential fungal amylolytic degradation. Micrographs also showed lower levels of B granules in the grains with a higher infection level, - this was confirmed by an analysis of the A (large) to B (small) starch granule ratio. A reduction of 1:15.38 to 1:3.40 being recorded.

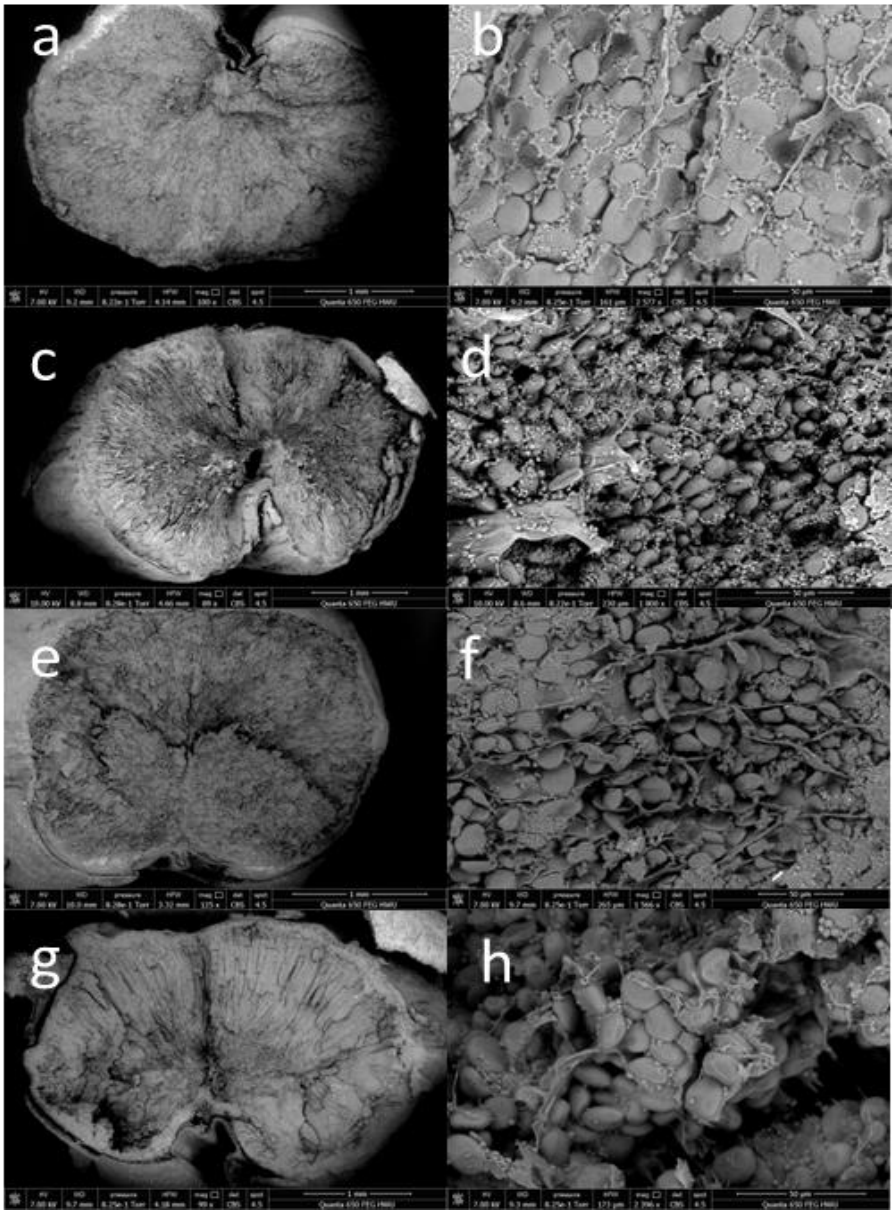

Figure2. SEM micrograph of a. transverse section of unmalted and malted barley a. unmalted Franklin, b. magnified unmalted Franklin, c. transverse section of malted Franklin, d. magnified Franklin malt, e. transverse section of unmalted Braemar, f. magnified cross-section of unmalted Braemar, g cross-section of malted Braemar, h. magnified Braemar Malt

This could be on account of B granule usage during the infection of the grain, as smaller granules would require less energy to degrade. Even so, Rcc would require enzymes to break down the starch granules for usage. The theoretical enzymatic activity of Rcc needs to be investigated further. Studies in Fusarium spp. have indicated the capacity of *F. culmorum* and *F.graminearum* to produce degradative cell wall enzymes including cellulases, xylanases and pectinases during the colonization of barley. A similar study could be adopted to investigate Rcc enzyme biosynthesis and its potential role in barley colonization and grain degradation. SEM also showed the presence and localization of fungal hyphae deep within the grain. Previous studies have shown the presence of Rcc-related hyphal growth in barley leaves through compound microscopy, but this study is the first to show the potential for Ramularia to penetrate the grain so deeply.

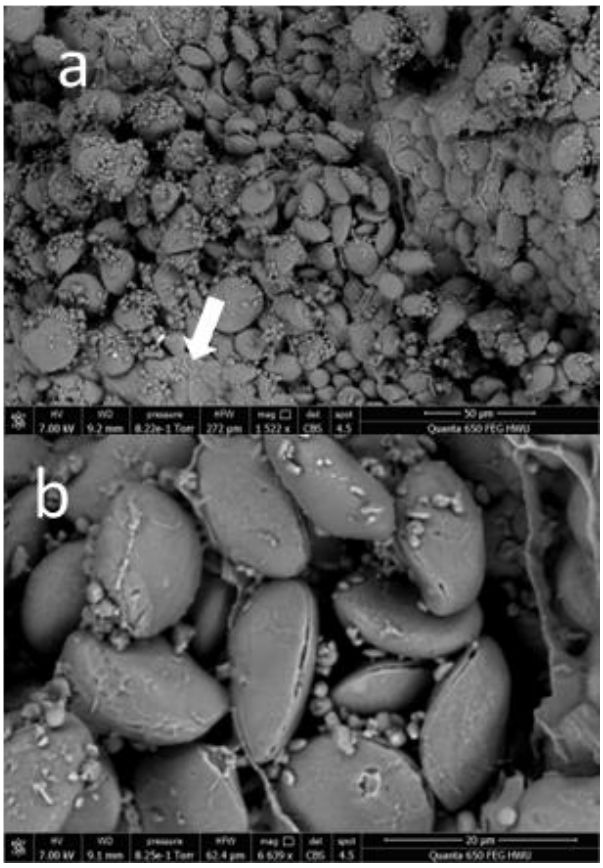

Figure 3: SEM micrograph of malted barley displaying a. hyphal growth between granules and b. Damage of A granules across the equatorial plane.

Impact and conclusions

This study shows for the first time that field sourced barley grains that are infected with high levels of *Ramularia collo-cygni* can fail to meet the standards required for use in malting. The work reported here illustrates the overall changes occurring in mature barley grains because of infection and how the level of infection spreads during malting. We have shown how the fungus proliferates during malting and can colonizes healthy kernels, and the consequences of this contamination to the final malt and wort quality. The contribution of enzymes,

produced exogenously or induced in grains by fungal stress, is not fully understood. Additionally, the impact that infection has on the aroma-volatile profile of beer and spirits as well as potential impact on downstream process warrant further study. The impact on markers of final product quality such as foam stability, gushing and flavor stability are also unknown and need to be assessed considering these results.