Institution: University of Edinburgh and SRUC, Scotland's Rural College

Unit of Assessment: 6

Title of case study: Discovery that *Ramularia collo cygni* causes leaf spotting in barley and development of a diagnostic to target fungicide use, saving the industry £5.4M per annum.

1. Summary of the impact (indicative maximum 100 words)

Impact: Economic: The first fungicide-based control schemes minimising UK barley yield losses (saving approx. 516K tonnes / £95.1M per annum). A risk assessment method, which minimised pesticide usage.

Significance: Barley is the second most popular cereal crop grown in the UK - in 2012, 5.52 million tonnes of barley were grown (market value £1.02 billion). The research led to savings to the UK farming industry of ~£5.4 million per annum

Beneficiaries: Farmers, malting and brewing industries, UK tax revenue.

Attribution: Drs. Oxley, Havis, Hughes, Fountaine, and Burnett (SRUC) identified the pathogen and produced a field test for early identification of infestation.

Reach: Barley growing, malting and brewing sectors, seed and agrochemical industries UK-wide and in Ireland.

2. Underpinning research (indicative maximum 500 words)

In 1998 barley crops in the north of the UK suffered from an unknown disease, which caused extensive premature leaf death and resulted in poor yields and low quality. Although a similar problem had affected crops in Germany and Ireland the previous year, there was little information on the cause, the economic importance, or on methods to manage the problem. As barley is the main cereal crop grown in Scotland, and the most valuable, it was vital this new disease threat was managed.

- We began researching the disease in 1998 (the team included Drs. Oxley (Senior Researcher, employed 1985-onwards), Havis (Researcher, employed 1996-onwards), Hughes (Researcher, employed 2003-2008), Fountaine (Plant Microbial Ecologist, employed 2007-onwards), and Burnett (Team Leader, employed 1992-onwards) identified the pathogen and produced a field test for early identification of infestation), and initial work was funded by the Home-Grown Cereals Authority (HGCA). This first project led to the discovery that *Ramularia collo-cygni* (Rcc) was the main biotic factor involved in leaf spotting in barley, causing a disease now called Ramularia Leaf Spotting (RLS) [3.1].
- A subsequent three-year HGCA-funded project focused on the effect of oxidative stress on the barley crop and the most effective chemical control for RLS. It developed the first fungicide programmes aimed at controlling RLS in barley crops.
- This initial research was followed by a three-year project, funded by the Scottish Government (SG; 2000-3), which aimed to design a test that would detect the fungus before symptoms appeared on the plant.
- The first diagnostic for the pathogen [3.2] was developed by the research team and this nested PCR test was able to detect the fungus at a molecular level. Field experiments showed that the test enabled the detection of the fungus in the leaves two to four weeks before the crop appeared to be affected. These findings demonstrated the importance of applying fungicides to protect crops that had no visible disease symptoms.
- Subsequent SG funded work (2005–2011) indicated that seed-borne infection is the most important source of Rcc infection in crops. A new PCR test developed and validated in our laboratories [3.3] indicated widespread contamination of seed stocks throughout the UK.
- The fungus was found to be situated deep within seed tissue and therefore not easily controlled by commercial seed treatments. Current research projects are examining options for eliminating or reducing pathogen inoculum in barley seed. Joint studies with the James Hutton Institute, which involved genetic transformation of the fungus with fluorescent tags [3.4], greatly aided study of the movement and localisation of the pathogen in the plant.

- A joint PhD with John Innes Centre led to establishment of a reliable method for inoculating plants with Rcc enabling experimental infection studies. The same study showed that the appearance of Rcc was not related to the introduction of lines resistant to the pathogen *Blumeria graminis* f.sp. *hordei* [3.5]. This allowed breeders to continue to use this genetic material in crossing studies aimed at identifying resistance to RLS.
- Since 2007, joint research with HGCA has been directed towards control of RLS through varietal resistance, seed health and forecasting.
- Our research showed that although most spore dispersal coincided with leaf wetness late in the growing season, epidemic severity was strongly correlated with sustained periods of leaf wetness earlier in the development of the crop. This important discovery was used to produce a risk forecast.
- Testing of archive barley samples from Rothamsted Research revealed that Rcc in barley crops in England increased greatly after 1998, while resistance to a major group of fungicides, the strobilurins, could be detected from 2001 onwards (3.5).
- The rapid appearance of resistance to this group of fungicides in Rcc has led to new work in the area. Thus, two PhD students, funded by agrochemical companies, are now examining the development of resistance to fungicides and the method of reproduction of the fungus.

3. References to the research (indicative maximum of six references)

3.1 Havis, N. D., Oxley, S. J. P., Piper, S. R. and Langrell, S. R. H. (2006). Rapid nested PCR-based detection of *Ramularia collo-cygni* direct from barley (*Hordeum vulgare*). FEMS Microbiology Letters. 256: 217-223. http://dx.doi.org/10.1111/j.1574-6968.2006.00121.x

3.2 Taylor, J. M. G., Paterson, L. and Havis, N. D. (2010). A quantitative real-time PCR assay for the detection of *Ramularia collo-cygni* from barley (*Hordeum vulgare*). Letters in Applied Microbiology. 50: 493-499. http://dx.doi.org/10.1111/j.1472-765X.2010.02826.x

3.3 Thirugnana Sambandam, A., Wright, K. M., Havis, N. D. and Newton, A. C. (2010). Agrobacterium-mediated transformation of regenerated protoplasts of the barley pathogen *Ramularia collo-cygni* with fluorescent marker tags. Plant Pathology. 60: 929-937. http://dx.doi.org/10.1111/j.1365-3059.2011.02440.x

3.4 Makepeace, J. C., Oxley, S. J. P., Havis, N. D., Hackett, R., Burke, J. I. and Brown, J. K. M. (2007). Associations between fungal and abiotic leaf spotting and the presence of mlo alleles in barley. Plant Pathology 56: 934-942. http://dx.doi.org/10.1111/j.1365-3059.2007.01680.x

3.5 Fountaine, J. M. and Fraaije, B. A. (2009). Development of QoI resistant alleles in populations of *Ramularia collo-cygni*. The second European Ramularia Workshop - A New Disease and challenge in barley production. Aspects of Applied Biology. 92: 123-126. (Copy available on request.)

4. Details of the impact (indicative maximum 750 words)

Impact on the Economy

Malting and brewing are very important to the UK economy with 1.65 million tonnes and 53,000 tonnes of barley used for these purposes respectively in 2012. Premiums for grain of a quality suitable for malting can be valued at an additional £5/tonne. Prior to 1998 RLS was not a significant disease problem in the UK but now it is regarded as the second most destructive foliar pathogen in the North and West of the UK. With a sudden, rapid increase in the incidence of the disease in the country's barley crops, especially in Scotland, there was an urgent need for information on the disease and how to control it. The first HGCA-funded research quickly provided growers with the first fungicide programmes aimed at controlling RLS in barley crops, saving the industry an estimated £5.4 million per annum from 2001 onwards.

Impact on Disease Control

The development of the first PCR diagnostic allowed the fungus to be detected in leaves two to four weeks before symptoms appeared. This enabled growers to target fungicide application, providing protection to crops that had not yet developed visible disease symptoms. In Scotland the

area protected from Ramularia by an effective fungicide rose from 4,000 ha to over 160,000 ha in just two years and more than trebled in the entire UK to just over 350,000 ha. Identification of seed as a major inoculum source enabled seed health schemes and treatments to be developed, aimed at reducing or eliminating Rcc in barley seed stocks.

Impact on the Agricultural Industry

The increasing importance of RLS in spring and winter barley led to its inclusion in the list of pathogens examined in HGCA-funded research into Appropriate Fungicide Doses (2001-onwards). As a result, RLS is now considered to be a major pathogen by the Chemical Regulation Directorate and agrochemical companies must now screen for activity in new chemicals used against the pathogen. Ongoing developments will have an impact on reducing the current £57 million annual yield loss in UK barley production.

Subsequent HGCA-funded work allowed determination of resistance ratings for Ramularia leaf spot in commercial UK barley varieties. Official resistance ratings for Ramularia in spring barley varieties were published for the first time in 2013. This is a significant step, as this information, along with all project summaries, is sent out to 26,000 barley growers in the UK (over 3,000 of them in Scotland) and is used when selecting the most appropriate varieties to grow. This reduces reliance on pesticides and has environmental as well as economic benefits.

A forecasting scheme, based on leaf wetness at a key period in early crop development was developed in 2010. The risk scheme allows farmers to adjust fungicide programmes if they are located in a low or high-risk region. The scheme has been publicised for 3 growing seasons via our Crop Protection Report (http://www.sruc.ac.uk/info/120118/crop_clinic/500/crop_protection_report) which received over 2,000 web page hits a month at the relevant publishing dates, and through 600 Crop Protection Report subscriptions.

Providing practical information to the industry (farming, malting and brewing, seed and agrochemical) has been an integral part of research undertaken in the past 14 years. Our research findings are disseminated in a number of ways; via press releases and articles, the SRUC website, SRUC Crop Protection Reports and Advisory Newsletters, Open Days (approx. 250 attendees at each) and winter disease roadshows (approx. 200 attendees at each) and farmer meetings (approx. 150 attendees at each). Feedback from Open Days indicates that 95 to 100% of respondents are happy with the findings presented and are confident it can make a positive impact on their production systems. New scientific information has been disseminated through peer reviewed papers and European and international scientific conferences and workshops. We have promoted the research at two European workshops organised specifically on RLS.

At the outset of this research, losses due to RLS in Scotland were calculated to be £13M on an annual basis. Our research led to targeted and effective fungicide programmes for growers offering major reductions in yield losses due to RLS. By 2012, 98% of Scottish barley crops received fungicides with the major products used highly active against RLS. The pathogen decreases both yield and grain quality.

5. Sources to corroborate the impact (indicative maximum of 10 references)

5.1) Professor James Brown, JIC. http://tinyurl.com/p4oy3rg

5.2) Dr Vicky Foster, HGCA (letter of support available on request). http://tinyurl.com/qfcjljk

5.3) Davies, D. H. K., Evans, E. A. and Oxley, S. J. P (2008). Changes in pests weeds and disease in Scotland over the past 20 years Proceedings Crop Protection in Northern Britain 2008. 12-22. http://tinyurl.com/pe5ynam

5.4) Pesticide usage survey-Arable crops SASA. This reference corroborates the increase in fungicide usage described in the text. http://tinyurl.com/noy4xpn

5.5) Oxley, S. J. P., Havis, N. D., Sutherland, K. G. and Nuttall, M. (2002). Development of a rationale to identify the causal of necrotic lesions in spring barley and to identify control mechanisms. HGCA Project Report No 282. http://tinyurl.com/nds4ag6

5.6) Oxley, S. J. P. and Hunter, A. E. (2005). Appropriate fungicide doses on winter barley: producing dose-response data for a decision guide. HGCA Project Report No. 366. http://tinyurl.com/n9gtew9

5.7) Oxley, S. J. P., Havis, N. D., Brown, J. K. M., Makepeace, J. C. and Fountaine, J. (2008). Impact and interactions of *Ramularia collo-cygni* and oxidative stress in barley (HGCA Project report 431 July 2008). http://tinyurl.com/pkptaym

5.8) Oxley, S. J. P. and Burnett, F. J. (2010). Barley Disease Control Technical note TN611 ISBN 1 85482 867 3. http://tinyurl.com/ocx8kx4

5.9) Oxley, S. J. P. and Havis, N. D. (2010). Managing *Ramularia collo-cygni* through varietal resistance, seed health and forecasting. (HGCA Final Report 463 March 2010). http://tinyurl.com/pot5zla