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Abstract and Summary

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Understanding fungicide mixtures to control Rhynchosporium in barley and minimise resistance shifts

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Abstract

The objective was to investigate the impact of fungicides used in mixtures and sequences of barley in terms of eradicant and protectant activity against rhynchosporium leaf scald (rhynchosporium) caused by *Rhynchosporium secalis*. Since resistance to several fungicide groups is becoming a major problem, the research also tested for potential resistance shifts to ensure that the most effective fungicide mixtures did not increase resistance. New molecular techniques were used to identify the presence of *R secalis* prior to symptom development to determine their practical use as a guide to disease risk.

Treatment with a single fungicide did not achieve the best disease control, yield or margin. Prothioconazole (Proline) was the key fungicide component in a fungicide mixture for both disease control and yield in winter and spring barley. Cyprodinil (Unix) was also key for yield in winter barley, but less important in spring barley. Pyraclostrobin (Vivid) was an important component of a mixture where rhynchosporium eradication was required. Chlorothalonil (Bravo) was a useful mixing partner, but in two-way mixtures, rhynchosporium eradication was reduced where the dose ratio was 1:1. This effect was not seen in a three-way mixture where the dose ratio of chlorothalonil to other fungicides was 0.5:1.

Using prothioconazole alone shifted the rhynchosporium population towards greater resistance compared to using the fungicide in mixture with a second active ingredient. Mixtures therefore will limit the increase in resistance occurring. Prothioconazole provided good control of rhynchosporium in these situations, but pyraclostrobin and cyprodinil also gave favourable disease control.

At grain prices of £175/tonne, two-way fungicide mixtures were the most cost effective approach for spring barley and three-way mixtures for winter barley. At lower grain prices of £75/tonne, two-way mixtures were the most cost effective for both.

Fungicide diagnostics were a useful tool to determine disease levels in high pressure crops by testing leaves and shoots before treatment. Visual assessments were effective, but a diagnostic test was more sensitive where disease symptoms had yet to appear. By testing rhynchosporium levels late in the season, it can be concluded that a yield response to fungicide occurs both in crops where visual symptoms are present and also where rhynchosporium DNA levels were high in the absence of symptoms. The lowest yield responses occurred where DNA levels and symptoms were low in the upper leaves. Plant breeders will need to redefine a resistant variety as one where visual symptoms are not present and where the fungus cannot be detected inside the plant. These results suggest varieties can respond to fungicide in the absence of visual disease symptoms but where the fungus is detectable at 10-40 pg DNA inside symptom free plants.

Summary

Rhynchosporium leaf scald (rhynchosporium) is a major wet weather disease in winter and spring barley caused by the fungus *Rhynchosporium secalis,* which can lead to extensive leaf death causing losses in yield. The aim for a successful grower is to achieve optimum yield, quality, and margin from a crop of barley. Crop disease will impact on this and varietal resistance alone is insufficient to deal with rhynchosporium.

Barley seed is a key source of *R secalis* alongside spores in barley trash, volunteers, rain splash spores and potentially airborne spores (Zhan *et al*. 2008). Rhynchosporium symptoms can develop in the autumn both on barley volunteers and in the crop, but widespread infection develops in January to February as a consequence of seed infection. It is common for disease symptoms to be present in a crop at the time of fungicide treatment, so effective disease eradication is required with fungicides. A typical timing for the first fungicide in winter barley is at stem extension (GS31-32). In high disease pressure situations this may be too late and earlier treatments are recommended at GS25-30 in the spring. Established disease in the winter may also warrant fungicide action with fungicides in exceptional situations (Oxley & Burnett 2008).

Spring barley sown in the winter is likely to follow disease patterns observed in winter barley, but spring barley sown in March to April will grow rapidly leading to a situation where no rhynchosporium symptoms are present at the time a fungicide treatment is applied at mid to late tillering (GS25-30). In this situation, fungicides are required to protect the crop from disease – a scenario which is more successful for most fungicides compared to attempts to eradicate established disease.

In the absence of robust varietal resistance for high disease pressure regions, fungicides play an important role in disease management and this research aims to understand how to use fungicides in mixtures to achieve effective eradication, protection, yield and margin.

One aspect of fungicide use which is of less immediate interest to a grower is fungicide resistance. For a grower, short term gains through using a particular fungicide programme may override the longer term risk of a build up in fungicide

resistance. This research looks at the impact fungicide mixtures have on resistance build up and looks at programmes which may achieve both the growers aims and minimise the risk of a build up in resistance.

Different growth and disease development patterns mean the management of rhynchosporium in winter barley requires a different approach to the spring crop. Relying on visual symptoms as a trigger to treat a crop with fungicide will mean disease is already well established and potentially causing damage to yield. New diagnostics can help identify *R secalis* DNA both in seed and in symptom-free plants. Part of this research therefore looked at the potential to use diagnostics as part of a decision process to treat a crop before disease symptoms were visible.

Effective fungicide mixtures to control rhynchosporium and achieve yield

Eradication of rhynchosporium is a greater challenge for fungicide mixtures than protection. No individual fungicide was sufficiently effective to be used alone either for disease control or optimum yields. The key components of a mixture under these circumstances were pyraclostrobin (Vivid) and prothioconazole (Proline). Fungicide mixtures to avoid for eradication include most two-way mixtures where chlorothalonil (Bravo) was a component (e.g. chlorothalonil + pyraclostrobin, chlorothalonil + cyprodinil, chlorothalonil + fenpropimorph). This negative effect on disease eradication was less of an issue in the two-way mixture with prothioconazole and in three-way mixtures where there was a higher dose of alternative fungicides.

Rhynchosporium protection was straightforward and all two and three-way mixtures achieved good protection. Some mixtures did however increase yield and margin more than others, so this should be taken account of in choosing mixtures (see summary below).

Increasing the components in a mixture led to an increase in yield. For winter barley at £175/tonne, three-way mixtures were the most cost effective. At grain prices of £75/tonne and for spring barley, two-way mixtures were the most cost effective.

Active ingredients use in mixtures

	Active ingredients g / ha		
Code	**Active ingredient 1**	**Active ingredient 2**	**Active ingredient 3**
PC	prothioconazole 100	fenpropimorph 375	-
PV	prothioconazole 100	pyraclostrobin 125	-
PU	prothioconazole 100	cyprodinil 300	-
PB	prothioconazole 100	chlorothalonil 500	-
CV	fenpropimorph 375	pyraclostrobin 125	-
CU	fenpropimorph 375	cyprodinil 300	-
CB	fenpropimorph 375	chlorothalonil 500	-
VU	pyraclostrobin 125	cyprodinil 300	-
VB	pyraclostrobin 125	chlorothalonil 500	-
UB	cyprodinil 300	chlorothalonil 500	-
PCV	prothioconazole 100	fenpropimorph 375	pyraclostrobin 125
PCU	prothioconazole 100	fenpropimorph 375	cyprodinil 300
PCB	prothioconazole 100	fenpropimorph 375	chlorothalonil 500
PVU	prothioconazole 100	pyraclostrobin 125	cyprodinil 300
PVB	prothioconazole 100	pyraclostrobin 125	chlorothalonil 500
PUB	prothioconazole 100	cyprodinil 300	chlorothalonil 500
VCU	pyraclostrobin 125	fenpropimorph 375	cyprodinil 300
VCB	pyraclostrobin 125	fenpropimorph 375	chlorothalonil 500
VUB	pyraclostrobin 125	cyprodinil 300	chlorothalonil 500
UCB	cyprodinil 300	fenpropimorph 375	chlorothalonil 500

Effective mixtures for rhynchosporium control and yield

Code	Rhynchosporium eradication	Rhynchosporium protection	Yield & value (winter barley)	Yield & value (spring barley)
PC	+ +	+ + +	+ + +	+ +
PV	+ + +	+ + +	+ + +	+ + +
PU	+ + +	+ + +	+ + +	+ + +
PB	+ + +	+ + +	+ + +	+ +
CV	+ +	+ + +	+	+ +
CU	+ + +	+ + +	+	+ +
CB	+	+ + +	+	+ +
VU	+ + +	+ + +	+	+ +
VB	+	+ + +	+ +	+ +
UB	+	+ + +	+ + +	+ +
PCV	+ + +	+ + +	+ + +	+ +
PCU	+ + +	+ + +	+ + +	+ + +
PCB	+ +	+ + +	+ + +	+ + +
PVU	+ + +	+ + +	+ + +	+ + +
PVB	+ + +	+ + +	+ + +	+ + +
PUB	+ + +	+ + +	+ + +	+ + +
VCU	+ + +	+ + +	+ +	+ + +
VCB	+ + +	+ + +	+ + +	+ +
VUB	+ + +	+ + +	+ +	+ + +
UCB	+ +	+ + +	+ +	+

Good	+ + +
Average	+ +
Poor	+

Fungicide resistance in *Rhynchosporium secalis*

There is a wide range of sensitivity to triazole fungicides (epoxiconazole and prothioconazole). This suggests there are populations of *Rhynchosporium secalis* which are resistant to both these key barley fungicides. There was a significant correlation between the sensitivities of isolates to epoxiconazole and to prothioconazole. This shows that using either of these fungicides will also increase resistance to the other.

The majority of *Rhynchosporium secalis* isolates were sensitive to the strobilurin fungicides pyraclostrobin and fluoxastrobin and they fell within a narrow band of sensitivity. Some isolates appeared less sensitive and tests will be done to see if this is a real effect. It is suggested that this is an artefact since there was no correlation between the sensitivities of these isolates to the two strobilurin fungicides. It can be concluded therefore that *Rhynchosporium secalis* remains highly sensitive to this group of fungicides.

Rhynchosporium secalis isolates were generally very sensitive to cyprodinil (Unix). Some isolates were outside this range however and were more resistant. Fewer isolates were tested against fenpropimorph (Corbel) than for other fungicides. Most were within a narrow band, but a few isolates were less sensitive.

Rhynchosporium secalis sensitivity ranged widely between sites, but no drift in sensitivity was seen between the years. The greatest effect between sites was observed with the triazole fungicides. *R. secalis* was more resistant to epoxiconazole in the north of Scotland on winter barley compared to the South Scotland or Northern Ireland.

Using prothioconazole alone caused the biggest shift in resistance during the season. This was not the case where prothioconazole was applied in a two-way mixture with chlorothalonil, cyprodinil, pyraclostrobin or fluoxastrobin (data not shown) or fenpropimorph. Sensitivity data from three-way mixtures are limited due to the effective control of disease, but it can be assumed three-way mixtures will behave similarly to the two-way mixtures.

In conclusion, the biggest concern in resistance is with triazole fungicides. There is evidence that using one will lead to an increase in resistance of another. Use of prothioconazole alone can increase resistance within a season, but use of prothioconazole in a two-way mixture will stop this effect. Prothioconazole must always be used in a mixture as an effective anti-resistance strategy.

Diagnostics as an aid to disease risk

Rhynchosporium DNA can be detected in the leaves, shoots and stems of barley before symptoms appear. DNA levels were higher in winter barley compared to spring barley where the subsequent level of symptoms was also higher. Weather plays an important part in disease infection and in the three seasons of trials, higher disease pressures occurred in a wet spring as opposed to a dry spring. DNA levels alone are therefore an insufficient trigger to determine a high risk crop. Diagnostics were as effective as visual assessment to determine the potential high risk of an outbreak. Diagnostics are however more sensitive than visual assessment at the early stages of an epidemic before symptoms appear. Since seed is known to be an important source of infection, testing leaves and shoots over the winter will be a useful guide to the crops with the greatest risk of disease developing. This information will be used in risk decision tools currently being developed in Scottish Government funded research.

Importance of asymptomatic infections

The detection of *Rhynchosporium secalis* DNA inside plants which show no symptoms leads to the question of the relative importance of symptom versus symptomless infection. To address this question, trials were categorised into high and low visual disease late in the season (based on spring rainfall) and high and low DNA levels at the end of the season. Where visual symptoms were high, yield responses to fungicide were also high. However, the same yield response was seen where symptoms were low, but *R secalis* DNA levels were high in the leaves. This observation requires further study, but if the effect is consistent, future advice on late fungicide use may be based upon the level of DNA in the upper leaves to determine risk of yield loss from disease.