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THE USE OF FUNGICIDE SEQUENCES TO MAXIMISE THE CONTROL OF EYESPOT IN CEREALS AND MINIMISE THE RISK OF SHARP EYESPOT

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THE USE OF FUNGICIDE SEQUENCES TO MAXIMISE THE CONTROL OF EYSPOT IN CEREALS AND MINIMISE THE RISK OF SHARP EYESPOT

by

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CONTENTS

1. ABSTRACT

This project report describes the results of a one year field trail carried out to investigate the control of common eyespot and sharp eyespot. Controlling one disease may allow another disease to colonise the clean stem base and one aim of this project was to investigate if controlling common eyespot would lead to an increase in sharp eyespot and if this could be suppressed.

Previous work on common eyespot has shown that the two most effective fungicides with activity against common eyespot have different optimum timings of application. Prochloraz works best when applied during the period of mid tillering to the start of stem extension. Cyprodinil works best when applied later at the second node stage of stem extension. Both fungicides cause an initial suppression of the eyespot population, but levels of eyespot then increase again. Successful treatment depends on getting a large enough initial reduction in the population coupled with a more sustained period of reduction before the eyespot population recovers. This project aimed to establish if using the two fungicides in sequence at their optimum timings would allow for a longer period of reduction and hence a more successful eyespot treatment.

The project found the most effective treatment for common eyespot control of those evaluated in the trial was cyprodinil applied at GS 32 as a single full dose treatment. Splitting this dose of cyprodinil between GS 30 and GS 32 was not as effective as the single full rate application. Prochloraz applied at full dose rate at GS 25 also reduced the levels of eyespot assessed at the end of the season. Splitting the prochloraz treatment between GS 25 and GS 31 did not improve eyespot control.

Splitting the eyespot treatment and applying half dose rate prochloraz at GS 25 and half dose rate cyprodinil at GS 32, so that each was applied at it's optimum timing, was not as successful at reducing visual eyespot as cyprodinil either as a single full dose application at GS 32 or as a split treatment as GS 30 and GS 32. PCR analysis, however, shows lower levels of eyespot DNA in the prochloraz followed by cyprodinil treatment than in these other treatments, which may support the theory that better eyespot control could be achieved by using both products at their optimum timing than could be achieved using either one straight. The yield from this split treatment of prochloraz and cyprodinil was also higher than cyprodinil applied on its own.

Analysis of the eyespot DNA present showed that the R strain was the dominant strain at the site and that the W strain of eyespot was only present at very low levels. In this trial eyespot was not seen until the crop was heading with no eyespot present at the critical time for making an eyespot spray choice, of stem extension. This shows how a threshold approach to treating this crop would not have been successful, and also demonstrates how the fungicides worked well as protectants in reducing final eyespot levels in the plots.

Sharp eyespot levels in the trial were very low, but there was a small increase in sharp eyespot levels following the most successful eyespot treatments and there was a negative correlation between sharp eyespot and common eyespot at the end of the season. A sequence of azoxystrobin sprays were applied and, of the timings evaluated, the spray applied at GS 32

was the most successful at reducing sharp eyespot as well in increasing yield and reducing lodging.

2. SUMMARY

A complex of diseases can infect the stem base of wheat and as common eyespot is the more damaging disease many studies have concentrated on controlling this pathogen. Other studies have observed, however, that where eyespot is controlled sharp eyespot tended to increase, successfully colonising the clean tissue from which common eyespot had been controlled. The aim of this study was to develop a fungicide program that would control common eyespot without increasing the risk of sharp eyespot. This was done by following the diseases both through visual assessments and by using DNA probes through out the season following sprays with the fungicides prochloraz, cyprodinil and azoxystrobin. The use of PCR in this way has proved a useful tool in tracking common eyespot epidemics through a season and this project aimed to apply the same techniques to track sharp eyespot and follow the progress of the two diseases together.

Prochloraz and cyprodinil are the two fungicides used to target common eyespot in wheat but they have little or no activity against sharp eyespot. Azoxystrobin on the other hand does have activity against sharp eyespot but no activity against common eyespot. By using the fungicides both in sequence and as mixes it was hoped that control of both diseases would be achieved. Azoxystrobin was therefore applied at a range of timings with the aim of establishing if it would reduce sharp eyespot levels and to determine the optimum timing for this.

Previous work on common eyespot has shown that prochloraz and cyprodinil have different optimum timings of application. Prochloraz has been shown to give the largest reduction in eyespot levels when applied during the period of mid tillering to the start of stem extension. Cyprodinil works best when applied later at the second node stage of stem extension. Both fungicides cause an initial suppression of the eyespot population, but levels of eyespot then recover again. Successful treatment depends on getting a large enough initial reduction in the population coupled with a longer period of reduction before the population recovers. This project aimed to establish if using the two fungicides in sequence at their optimum timings would allow for a longer period of reduction and hence a more successful eyespot treatment.

The most effective treatment for eyespot control of those evaluated in the trial was cyprodinil applied at GS 32 as a single full dose treatment. Splitting this dose of cyprodinil between GS 30 and GS 32 was not as effective as the single full rate application. Prochloraz applied at full dose rate at GS 25 also reduced the levels of eyespot assessed at the end of the season at GS 71. Splitting the prochloraz treatment between GS 25 and GS 31 did not improve eyespot control.

One aim of the work was to investigate if applying cyprodinil at it's optimum time of application for eyespot control of GS 32 as a split treatment with prochloraz, also applied at it's optimum time of application (GS 25 - 30). Visually this treatment was not as successful at reducing eyespot as cyprodinil, either as a single full dose application at GS 32 or as a split

treatment as GS 30 and GS 32. The PCR analysis however showed lower levels of eyespot DNA in the prochloraz followed by cyprodinil treatment than in these other treatments, which may support the theory that better eyespot control could be achieved by using both products at their optimum timing than could be achieved using either one straight. The yield from this split treatment of prochloraz and cyprodinil was also higher than that in the straight or split cyprodinil treatments. The result would support further work being done to confirm, or otherwise, the theory that splitting the treatments at their optimum timings would improve eyespot control.

Sharp eyespot levels in the trial were very low, but a reduction in sharp eyespot was seen following an application of azoxystrobin. Despite levels of sharp eyespot being so low there was a negative correlation between sharp eyespot and common eyespot levels at the end of the season. There was a small but not significant increase in sharp eyespot levels following the most successful treatments to control common eyespot and this increase was reduced by tank mixing azoxystrobin with the eyespot treatment. A sequence of azoxystrobin sprays were applied and, of the timings evaluated, the spray applied at GS 32 was the most successful at reducing sharp eyespot as well in increasing yield and reducing root lodging.

This finding is important as it emphasises the importance of correctly identifying stem base pathogens. Treatment for common eyespot if, in fact, sharp eyespot was the problem would make a sharp eyespot infection worse. Where common eyespot is the dominant pathogen then at present a sharp eyespot treatment (azoxystrobin) is probably not merited as the cyprodinil plus azoxystrobin mix would still require the addition of a triazole fungicide for foliar disease protection at GS 32. The resultant three way mix required to target foliar diseases, common and sharp eyespot would unlikely to be cost effective.

Analysis of the eyespot DNA present using the PCR technique showed that the R strain was the dominant strain at the site and that the W strain of eyespot was only present at very low levels. This is now felt to be typical of the situation in the UK where most sites surveyed have either only the R strain or, if a mixed population, the R strain dominating. Only a very few sites in the UK have any significant level of the W strain. The W strain is more easily controlled with fungicides and tends to show symptoms earlier in the season. The R strain typically infects later and increase rapidly, and this is thought to be the reason why thresholds for eyespot treatment no longer work. In this trial eyespot was not seen until the crop was heading with no eyespot present at the critical time for making an eyespot spray choice at stem extension. This trial demonstrated how a threshold approach to treating this crop would not have been successful, and also demonstrated how the fungicides worked well as protectants in reducing final eyespot levels in the plots.

3. INTRODUCTION

A complex of diseases infects the stem base in wheat of which common eyespot, caused by the organism *Pseudocercosporella herpotrichoides,* is the most common and the most damaging. Sharp eyespot, *Rhizoctonia solani*, also attacks the stem base as do several Fusarium species of fungi. All these diseases can weaken the stem base and reduce uptake, reducing yields and causing shrivelled grains and white heads. In severe cases they can also causes lodging, further reducing both yield and quality.

Common eyespot, *Pseudocercosporella herpotrichoides,* causes much larger yield losses than the other disease in the stem base complex. The severity of disease development as a result of infection by common eyespot is determined by agronomic as well as environmental factors, and is greatest under cool, moist conditions and where wheat and / or barley is grown in close rotation. Eyespot is conventionally controlled in winter wheat crops with a fungicide spray at early stem extension between growth stages Zadoks 30 to 32 (Anon, 1987, Burnett *et al.*, 1998), often applied as a split treatment.

In the asexual form the eyespot fungus survives in the soil in crop debris, where it can persist for more than a season so that a two year break from cereals is required for effective rotational control. Eyespot is worse where cereals are grown continuously or in short rotations. Conidia are spread to the host plant by rain splash where the mycelium penetrates the coleoptiles or leaf sheaths of the host plant. Infection is localised at the stem base; it seldom infects above the second node and does not colonise leaf or root tissue. The infection can proceed through several leaf layers to eventually penetrate the stem. After infecting the stem the characteristic eye or pupil shaped lesion can form. Surrounding tissue becomes discoloured. The development of the disease is favoured in the UK by mild, wet weather in winter and cool damp weather in spring. Eyespot is most severe in early-sown crops and can be reduced in high risk fields by late sowing and crop rotation (Cook *et al.*, 1993).

The sexual stage of the eyespot fungus is now suspected to more significant than it was in the past. Over recent seasons first wheat crops that would not be perceived to be at risk from the asexual, trash borne phase of eyespot have sometimes been severely affected by eyespot. The cause of these infections can be the airborne, sexual stage of the fungus. Airborne apothecia are produced on surrounding trash, stubble or crops and can then blow into and infect first wheat crops. Conditions which favour apothecia discharge are cool temperatures of 3-8°C and high rainfall. Standing stubble and set aside are common sources of the sexual stage, as is rye grass pasture land.

There are two species of eyespot which commonly occur in the UK, one the W strain is highly pathogenic on wheat, but less so on barley and on rye, the second the R strain is equally pathogenic on wheat, barley and rye (Scott *et al.*, 1975). The sexual stages of these two strains have recently been reclassified as two distinct species, *Tapesia yallundae* (W strain) and *Tapesia acuformis* (R strain). The R strain is the most common in the UK and is present at much greater levels that the W strain at most sites. The W strain is only present in significant proportions at a very few sites (Novartis Crop Protection Ltd. pers. comm.)

Recent work showed that there is a strong correlation between eyespot levels and yield (HGCA Project Report 150). Trials carried out by SAC before this, in the course of an HGCA funded project looking at the biology and control of eyespot (Project No. 0015/1/91), also found that there was a significant association between eyespot levels and yield. Although lodging was also associated with yield loss, the correlation was not as strong as that between eyespot and yield. There was also a significant correlation between eyespot and lodging (Burnett & Oxley, 1996, Burnett *et al.*, 1998).

Control of eyespot

Eyespot is conventionally controlled in winter wheat crops with a fungicide spray at early stem extension between growth stages Zadoks 30 to 32 (Anon, 1987, Burnett *et al.*, 1998), often applied as a split treatment. Previous work has identified prochloraz and cyprodinil as the two most effective fungicides for control of common eyespot and resultant yield benefit.

Work carried out at SAC (HGCA Project Report 150) showed that both fungicides were more effective at controlling the W strain than the R strain. The greater efficacy of the two fungicides against the W strain in that study concurs with reports in the literature. Prochloraz has been reported to control the W strain better than the R strain (Bateman *et al.*, 1986) and cyprodinil showed better control of the W strain in work carried out in France (Migeon *et al.*, 1995).

HGCA Project Report 150 reports that cyprodinil gave a more persistent reduction in R strain eyespot, in both seasons the project, ran than prochloraz and although control of the R strain with prochloraz was initially good the population often recovered. Recovery of the R strain population was slower after cyprodinil treatment in both seasons. The PCR technology used in the project demonstrated that fungicide treatments work by reducing the levels of both strains present. Control was temporary, and the populations recovered, so the key to effectively reducing the degree of visual symptoms and the damage to the plant at the end of the season, is timing the fungicide application to achieve the longest respite from the disease possible.

Treatment too early or too late allowed the populations to recover, and visual eyespot symptoms to develop to severe levels despite the treatment. Prochloraz applied too early led to a recovery of the W population that eventually exceeded the levels in the untreated controls. Control of the R strain had to be made early with prochloraz. Application too late did not significantly reduce the R strain eyespot levels after application. Cyprodinil could give large reductions in R and W strain eyespot, but the populations could recover fast, particularly the W strain, so again it was evident that cyprodinil used late could reduce the populations over the remainder of the season.

Prochloraz therefore has to be used early in the season, during tillering, for maximum effect on eyespot levels. Cyprodinil works best if applied after the start of stem extension. Spraying outside the optimum window could allow the eyespot populations to recover following treatment even if initial reductions in eyespot were achieved. Prochloraz applied too late did not reduce the eyespot population sufficiently to affect the levels at the end of the season. In contrast cyprodinil applied too early achieved an initial reduction that was not be maintained until the end of the season. The findings of the work would suggest the potential for using sequences of fungicides to achieve season long control of the eyespot pathogen.

Control of eyespot therefore remains a compromise between targeting the site of infection at early stem extension where this part of the plant is still exposed, but not going in so early that the eyespot populations can recover and eventually exceed the initial disease prognosis.

The use of thresholds

As treatment decisions have to be made early in the season if eyespot is to be targeted, disease risk assessment and prediction has been the aim of many research projects, with the objective of determining a threshold level of eyespot early enough in the season to identify crops where control of eyespot would be economic. Some schemes have relied on weather data, but this does not allow for the loss of lesions that either die out or are shed with the outer leaves and never penetrate the stem. The ADAS scheme for identifying crops at risk of eyespot was based on assessing the number of stems infected at the start of stem extension and recommending treatment if an incidence of more than 20% is found (Anon, 1987, Jones, 1994)).

Eyespot assessment in the spring, however, has long been recognised as an unreliable indicator of subsequent disease progress (Scott and Hollins, 1978). Hughes et al., 1999 demonstrated the fallibility of this threshold method and concluded that while it would identify correctly those crops that passed the threshold at stem extension as being those that would benefit from treatment is would miss all those that had not passed the threshold but would go on to develop serious infections. In view of the changes in fungicides, in wheat cultivars and in the pathogen population itself since the currently recommended threshold was devised it clearly needs to be updated.

This threshold was developed when the W strain of eyespot predominated whereas the R strain is now more common. The fungicides most commonly used on wheat over the last 20 years were members of the DMI group which act differentially on the two strains, and are far more effective in controlling the W strain. This may be one reason why the R strain now predominates throughout the UK. The R strain often infects later and then increases fast which may make it less suitable for meeting the threshold criteria. The wheat strain tends to cause more cell browning as it infects the stem and therefore may have been easier to assess as a visual threshold. HGCA Project Report 150 found that in one season there was a significant correlation between W strain levels at stem extension and the final levels at the end of the season, indicating how thresholds may have been more effective when the W strain was the dominant strain of eyespot in the UK.

Identifying crops at risk form eyespot requires further study. At present taking account of other risk factors such as sowing date and previous cropping would seem to be a more successful approach to identifying crops that would benefit from an eyespot spray, than would the use of thresholds.

Diagnostics

PCR technology now means that it is possible to detect and quantify the amount of fungal DNA present in the stem base. HGCA Project Report 150 investigated the use of PCR technology to assess eyespot levels. Until that time eyespot infections could only be quantified visually. Visual differentiation between other diseases of the stem base such as sharp eyespot and Fusarium was often difficult. In addition it was only possible to differentiate between the two eyespot strains using conventional mycological techniques which were often not definitive and could not quantify the levels of each pathotype present. Techniques developed at the John Innes Centre enable the type and quantity of each pathotype to be determined by extracting the pathogen DNA from the host tissue (Nicholson & Rezanoor, 1994) and it became possible to study the differential effect that different fungicides had on the eyespot pathotypes. It was also possible to plot the levels of each pathotype throughout the season and to study how they fluctuated following fungicide application.

The findings were that this technology was a useful tool when researching treatment efficacy as it was possible to chart the initial efficacy of the fungicides following application, and the duration of control. However, the levels of DNA measured were variable between plots even within treatments and the differences between treatments were only occasionally significant. Eyespot is patchily, rather than evenly, distributed in fields (N. McRoberts, pers. comm.) and the variation in the PCR results may be a factor of the sampling required to reduce variation between plots.

Another problem identified was that in very severely infected stems the levels of fungal DNA actually fall as the dead stem can no longer support the pathogen. This means that PCR results should always be taken together with visual assessments so that one can aid the interpretation of the other. ELISA (enzyme linked immunosorbent assay) technology also exists to measure eyespot levels in the plants. Commercial work at SAC has shown that this has the advantage of measuring total eyespot (whether dead or alive) and thereby overcomes this effect of low levels of fungal DNA being found in severe lesions, seen late in the season with PCR technology. The disadvantage is that it is less sensitive and does not differentiate between R and W strains.

Although diagnostics for eyespot have proved a useful research tool they have not, however, improved the accuracy of a threshold approach to treatment or been helpful in determining a new one.

Sharp eyespot

Sharp eyespot is caused by the soil borne fungus *Rhizoctonia solani*. The fact that it is ubiquitous in soils and also has a very wide host range means that there is no form of rotational control. All cereal crops can be affected, but as with other stem base diseases spring crops tend not to be severely affected. Winter wheat is the most susceptible of the cereals and there is no form of varietal resistance. The disease tends to be favoured by cool, dry conditions and therefore some fields are more prone to the disease than others.

Sharp eyespot causes symptoms very similar to those of common eyespot. The disease infects through outer leaf sheaths and causes eye-like lesions which have a much more defined edge and paler centre than those of common eyespot. Early in the season the lesions may have a more shredded appearance on the leaf sheaths than common eyespot. Mature lesions on the stem with sharp eyespot often contain a purplish mycelial growth which can be scraped off and later in the season flat sclerotia or resting bodies forms against the stem and between leaf sheaths. Lesions have a slightly oblique shape are often seen as multiple lesions extending far up the stem. As with the other stem base diseases, sharp eyespot reduces uptake through the stem and as a consequence can cause shrivelled grains, reduced yields and whiteheads as well as weakening the stem so that lodging is more likely. It is generally perceived to be less damaging than common eyespot in terms of yield losses.

Objectives

A complex of diseases can infect the stem base of wheat and as common eyespot is the more damaging disease many studies have concentrated on controlling this pathogen. Other studies have observed, however that where eyespot is controlled sharp eyespot tended to increase, successfully colonising the clean tissue from which common eyespot had been controlled. The aim of this study was to develop a fungicide program that would control common eyespot without increasing the risk of sharp eyespot. This was to be done by following the diseases both through visual assessments and by using DNA probes through out the season following sprays with the fungicides prochloraz, cyprodinil and azoxystrobin. The use of PCR in this way has proved a useful tool in tracking common eyespot epidemics through a season and this project aimed to apply the same techniques to track sharp eyespot and follow the progress of the two diseases together.

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