Relationships between disease control, green leaf duration, grain quality and the production of alcohol from winter wheat

Andrew M Watson,[a]* Martin C Hare,[a] Peter S Kettlewell,[a] James M Brosnan[b] and Reginald C Agu[b]

Abstract

BACKGROUND: Since demand for distilling wheat is expected to increase rapidly as a result of the development of the bioethanol industry, efficient production will become of increasing importance. Achieving this will require an understanding of the agronomic factors that influence both grain yield and alcohol yield. Therefore five field experiments using the winter distilling wheat variety Glasgow were conducted over three seasons (2006–2007, 2007–2008 and 2008–2009) to study the relationships between foliar disease and alcohol yield.

RESULTS: There was a significant relationship between alcohol yield and the severity of the disease septoria leaf blotch (*Septoria tritici*), which was present in the experiments from natural infection. Retention of green flag leaf area as affected by disease control following fungicide application was also shown to be important for achieving high alcohol yields. Measurements of grain quality showed that high thousand-grain weight and low grain protein concentration were significantly related to increased alcohol yield.

CONCLUSION: The experiments showed the importance of disease management to protect alcohol yields in the distilling wheat crop. Fungicides that provide greater disease control and improved green leaf retention are likely to be beneficial to alcohol yield.
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Keywords: grain quality; alcohol; bioethanol; wheat; disease; green leaf duration; fungicide

INTRODUCTION

Traditionally, distilling wheat in the UK has been grown for the Scotch whisky industry. This market requires an annual production of 7×10^5 t of wheat to produce approximately 3×10^8 L of alcohol (LA) each year.[1] There is growing interest in the production of distilling wheat for the emerging fuel alcohol market. Fuel alcohol, commonly known as bioethanol, is produced using similar techniques to those used by the potable alcohol industry. Growing interest in biofuels has led to concerns over the production of large areas of non-food crops threatening the availability and affordability of food.[2] There have also been concerns over the amount of CO_2 emitted during production and processing of the fuel.[3] If biofuels are to be produced, it is important to address these concerns by maximising efficiency both in the field and during processing. Achieving a high level of alcohol production per hectare will help minimise the cultivated area of non-food crops, easing pressures on both food production and the environment. Maximising the efficiency of crop production can also help limit the amount of CO_2 emitted during production. Achieving a high grain alcohol yield (LA t^{-1}) will also help maximise processing efficiency and reduce energy requirements. To achieve these objectives, it is necessary to have a thorough understanding of the agronomic factors that influence alcohol production. Previous investigations have studied relationships between disease control, green flag leaf area duration and grain quality,[4,5] but none has looked at the influence of these factors on alcohol yield. This study is therefore designed to expand on what is known to determine how these factors influence the production of alcohol.

MATERIALS AND METHODS
Experimental design
Five field experiments were conducted in Shropshire, UK at Harper Adams University College. During the study, experiment 1 was established for the 2006–2007 growing season, experiments 2 and 3 were conducted in the 2007–2008 growing season and experiments 4 and 5 were conducted in the 2008–2009 growing season. Each experiment used the soft winter wheat variety Glasgow recommended for distilling. All experiments were conducted using a randomised block design with seven replicates.

* Correspondence to: Andrew M Watson, Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire TF10 8NB, UK. E-mail: awatson@harper-adams.ac.uk

a Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire TF10 8NB, UK

b Scotch Whisky Research Institute (SWRI), Riccarton, Edinburgh EH14 4AP, UK

Table 1. Fungicide treatments applied at GS 59

Treatment	Fungicide treatment at GS 59
1	Untreated[a]
2	Prothioconazole + tebuconazole 62.5 : 62.5 g ha^{-1}
3	Prothioconazole + tebuconazole 62.5 : 62.5 g ha^{-1} + azoxystrobin 62.5 g ha^{-1b}
4	Prothioconazole + tebuconazole 62.5 : 62.5 g ha^{-1} + azoxystrobin 125 g ha^{-1}
5	Prothioconazole + tebuconazole 62.5 : 62.5 g ha^{-1} + azoxystrobin 187.5 g ha^{-1}
6	Prothioconazole + tebuconazole 62.5 : 62.5 g ha^{-1} + azoxystrobin 250 g ha^{-1}

[a] Received no fungicide at GS 59 (i.e. only received fungicides at GS 30, 32 and 39).
[b] Azoxystrobin 62.5 g ha^{-1} treatment was not present in experiment 1.

Each plot measured 12 m × 1.8 m with a 1.5 m buffer zone between replicates and a 0.2 m buffer zone between plots within each replicate.

All plots in all five experiments received a fungicide application of 300 g L^{-1} metrafenone (Attenzo, BASF, Cheadle Hulme, UK) at 0.2 L ha^{-1} applied with 375 g L^{-1} chlorothalonil + 62.5 g L^{-1} propiconazole + 50 g L^{-1} cyproconazole (Cherokee, Syngenta Crop Protection UK Ltd, Fulbourn, UK) at 0.75 L ha^{-1} at growth stage (GS) 30 (pseudostem erect).[6] A further 125 g L^{-1} epoxiconazole (Opus, BASF) at 0.6 L ha^{-1} + 500 g L^{-1} chlorothalonil (Bravo 500, Syngenta Crop Protection UK Ltd) at 1 L ha^{-1} was applied at both GS 32 (second node detectable) and GS 39 (flag leaf blade all visible). Experimental treatments were 125 g L^{-1} prothioconazole + 125 g L^{-1} tebuconazole (Prosaro, Bayer CropScience, Milton, UK) at 0.5 L ha^{-1} with varying rates of 250 g L^{-1} azoxystrobin (Amistar, Syngenta Crop Protection UK Ltd) applied at GS 59 (ear fully emerged) (Table 1). The base fungicide programme used for this study was designed to provide a high level of disease control to enable any potential physiological effects of the strobilurin fungicide azoxystrobin on green leaf duration, grain quality and alcohol to be detected.

Crop establishment and management
All five experiments were established as the first wheat crop after oilseed rape into a plough-based seedbed consisting of a free-draining sandy loam soil. The experiments were all drilled in early October using a seed rate of 300 seeds m^{-2}. In the autumn of each year a broad-spectrum herbicide along with an insecticide was applied to control weeds and aphids in each of the experiments. Soil nitrogen was measured prior to fertiliser applications, enabling nitrogen inputs to be adjusted accordingly. This ensured that crops grown in each experiment would have approximately 180 kg available nitrogen ha^{-1}. In experiment 1 (2007), nitrogen was applied on 8 March (GS 25) at 40 kg ha^{-1}, on 14 April (GS 32) at 60 kg ha^{-1} and on 27 April (GS 37) at 60 kg ha^{-1}. In experiments 2 and 3 (2008), applications were made on 6 March (GS 25) at 42.5 kg ha^{-1}, on 15 April (GS 32) at 42.5 kg ha^{-1} and on 3 May (GS 37) at 40 kg ha^{-1}. In experiments 4 and 5 (2009), nitrogen was applied on 13 March (GS 29) at 40.5 kg ha^{-1}, on 9 April (GS 31) at 45 kg ha^{-1} and on 15 May (GS 39) at 41.5 kg ha^{-1}. A growth regulator of chlormequat (1.29 kg ha^{-1}) was applied at GS 31. Fungicide treatments at GS 30, 32 and 39 were applied to all plots using a 12 m tractor-mounted sprayer. Experimental

treatments at GS 59 were applied using a hand-held field trial sprayer propelled by CO_2. Spraying was performed using a spray pressure of 2 bar to provide medium spray quality, with Lurmark F110 02 nozzles. Treatments were applied using a water volume of 200 L ha^{-1} and a forward speed of 1 m s^{-1}.

Weather data
Weather data were collected from a meteorological station within 1 km of the field experiments. Data for monthly rainfall and average minimum and maximum temperatures were compared with data collected over a period of 30 years (1961–1990) from the same meteorological station.

Preharvest assessments
Septoria leaf blotch (*Septoria tritici*) was the only foliar disease that caused significant symptoms in these experiments throughout the growing season. This disease was assessed on the flag leaf 60 days after leaf emergence on ten leaves per plot using visual assessment keys,[7] which provided a pictorial comparison of % disease symptoms at given levels (1, 2 and 5%). Despite being a first wheat, parts of experiment 2 became infected with take-all (*Gaeumannomyces graminis*). This was visually assessed using the method of Tilston *et al*.,[8] whereby the number of white heads within 1 m^2 were counted at three points in each plot using a systematic sampling method. These data were then used as a covariate when analysing the results for this experiment.

Towards the end of the growing season the decline in green flag leaf area was monitored by performing visual assessments of % green leaf area every 3 days throughout the period of senescence on ten randomly selected shoots per plot.

Harvest and postharvest assessments
Yield and grain weight
Experiments were harvested using a plot combine, a 1 kg sample of grain being taken from each plot; all further measurements conducted derived from this sample. Thousand-grain weight was measured using an automated seed counter with a known weight of grain. From this the average weight of a grain was determined and then multiplied by 1000.

Grain protein concentration
Grain nitrogen concentration was measured by near-infrared (NIR) using an Infratec 1241 grain analyser (Foss, Warrington, UK). Grain protein concentration was measured by multiplying the nitrogen concentration by 5.7.[9]

Predicted alcohol yield
The Scotch Whisky Research Institute (SWRI) has developed a calibration for the NIR grain analyser to predict alcohol yields, using grain samples from across the UK collected over 7 years. This technique is now commercially used within Scottish grain distilleries and was employed in this study to predict the alcohol yields on the plot grain samples. In each of the three years, ten samples were selected for their high and low alcohol yields. These selected samples were subjected to a wheat cook method[10] to confirm the accuracy of the predicted alcohol yield data. This method closely simulates the distillation process conducted in grain distilleries.

Table 2. Monthly rainfall, average minimum temperature and average maximum temperature for May, June, July and August 2007, 2008 and 2009 in comparison with 30 year mean (1961–1990) of recordings taken at Harper Adams University College, Shropshire, UK

Year	May	June	July	August
Rainfall (mm)				
2007	107.2	236.8	125.8	20.4
2008	47.5	35.2	94.4	83.6
2009	50.2	92.2	110.6	37.8
1961–1990	57.2	54.2	49.1	60.4
Average minimum temperature (°C)				
2007	7.2	11.4	11.4	11.1
2008	9.1	9.0	12.0	12.8
2009	7.2	9.7	11.5	11.8
1961–1990	6.0	8.8	10.6	10.5
Average maximum temperature (°C)				
2007	16.8	19.7	19.5	20.8
2008	18.2	19.2	21.4	20.6
2009	17.5	20.4	21.0	21.7
1961–1990	15.6	18.7	20.5	20.2

Table 3. Correlation matrix showing relationships between septoria leaf blotch on flag leaf 45 days after leaf emergence (SLB, %), green flag leaf area duration (GFLAD, days to Gompertz M), grain yield at 85% dry matter (GY, t ha^{-1}) and grain quality parameters thousand-grain weight (TGW, g), grain protein concentration (GPC, %) and predicted alcohol yield (PAY, LA t^{-1})

	SLB	GFLAD	GY	TGW	GPC
GFLAD	−0.4660***				
GY	−0.3047***	0.8117***			
TGW	−0.1307NS	0.3313***	0.3108***		
GPC	0.1807*	0.2085**	0.2660***	0.1318NS	
PAY	−0.4778***	0.3953***	0.4188***	0.2382**	−0.3349***

Data presented are from all five experiments. Significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant ($P > 0.05$).

Statistical analysis

Data analysis was performed using the statistical program Genstat Version 12.0. Green flag leaf area duration was assessed by using regression analysis with standard curves and fitting the Gompertz model.[11] This enabled data to be combined from assessments made throughout the period of green leaf decline to give the time (days) to 37% green flag leaf area (Gompertz M) for each plot. A large proportion of the plots had low levels of disease, which skewed the data. Log_e transformation was performed. The results from experiment 2 were analysed using take-all as a covariate.

All data from the five experiments were combined in a spreadsheet, and one-way analysis of variance (ANOVA) in randomised blocks was performed using experiment as a factor. The residual data from the ANOVA table were then used for further analysis. The residuals were used to remove the systematic effect of experiment, enabling overall regressions to be performed on the data. The general mean was then added to the residuals for presentation of the results. Correlation and linear regression were used to study the relationships between the various parameters.

RESULTS

The weather data collected showed that there was a high level of rainfall in May, June and July 2007 (experiment 1), July 2008 and June and July 2009 (Table 2). Temperature measurements showed no major variations from the 30 year mean. Therefore water does not appear to be a limiting factor to grain development within these experiments.

The experiments were designed to study how the alcohol yield of the grain was influenced by factors occurring in the field, such as disease infection, green leaf retention and the relationships these had with grain quality. The overall relationships from the five experiments are presented in Table 3. Increasing levels of septoria leaf blotch disease symptoms resulted in a significant ($P < 0.001$) reduction in green flag leaf area duration, which showed a significant ($P < 0.001$) relationship with increasing grain weight. Increases in thousand-grain weight were related

($P = 0.003$) to an increase in the predicted alcohol yield observed in the experiments (Table 3 and Fig. 1).

The data can be linked together through the physiological mechanisms involved. This shows how disease infection in the field results in a decline in green leaf area, which in turn is important for grain quality, which ultimately influences predicted alcohol yield (Fig. 1).

Where levels of septoria leaf blotch symptoms were less, green flag leaf area duration was significantly ($P < 0.001$) greater. Increased green flag leaf area duration showed a relationship with increased grain filling, resulting in a higher thousand-grain weight, which in turn resulted in an increased predicted alcohol yield.

Septoria leaf blotch and green flag leaf area duration can have a large impact on grain quality and were therefore directly linked to the predicted alcohol yield of the grain (Figs 2 and 3).

Septoria leaf blotch and green flag leaf area duration

Septoria leaf blotch was the only foliar disease present at high enough levels for assessments to be conducted. The average severity of symptoms in plots that received no fungicide at GS 59 across the five experiments was 17.0% on the flag leaf in mid-July (60 days after flag leaf emergence). Greater symptoms of the disease inevitably resulted in a decline in green leaf area ($P < 0.001$).

Septoria leaf blotch and grain quality

The disease appeared to decrease thousand-grain weight, but the relationship was not significant ($P = 0.157$). This is likely to be the result of the relatively low disease levels present in these experiments. A significant ($P = 0.050$) relationship between septoria leaf blotch and grain protein concentration was detected, however. This showed that, where there was a 1% increase in the level of septoria leaf blotch on the flag leaf, grain protein concentrations increased by 0.04%. In addition, it was shown that a 1% increase in the disease reduced alcohol yields by 1.12 LA t^{-1} ($P < 0.001$).

Green flag leaf area duration, grain quality and yield

Increased green flag leaf area duration had a significant ($P < 0.001$) relationship with thousand-grain weight, which showed an increase of 0.18 g for each day taken to reach Gompertz M. A significant ($P < 0.001$) increase in grain yield of 0.20 t ha^{-1} was
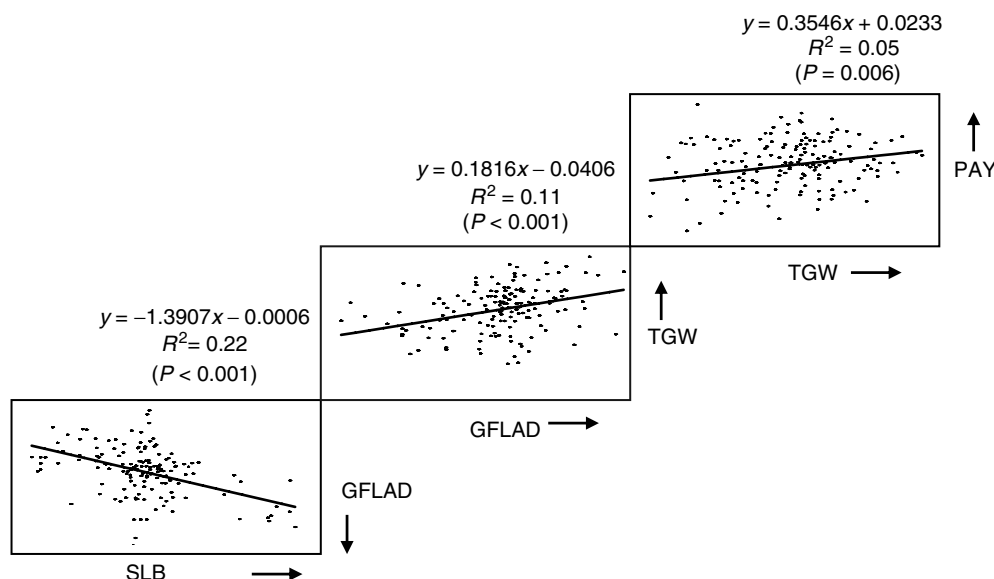
Figure 1. Effect of septoria leaf blotch (SLB) on green flag leaf area duration (GFLAD) and how this influences thousand-grain weight (TGW) to ultimately influence predicted alcohol yield (PAY) of grain.
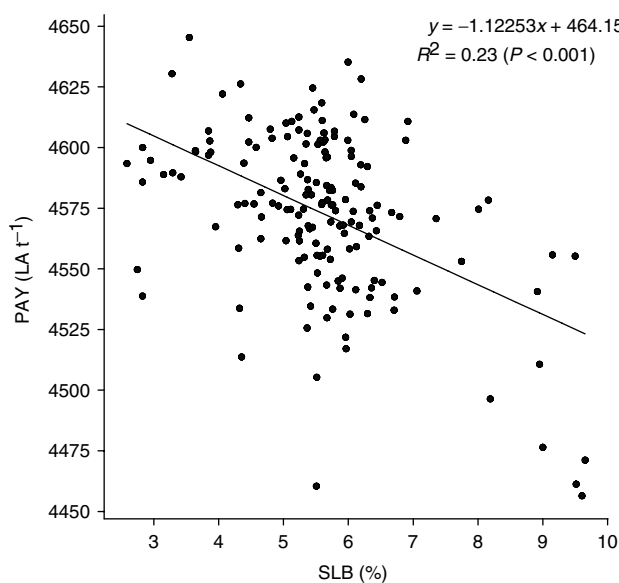

Figure 2. Overall relationship between septoria leaf blotch (SLB) on flag leaf (60 days after leaf emergence) and predicted alcohol yield (PAY) from all five experiments (data presented are residuals added to general mean).
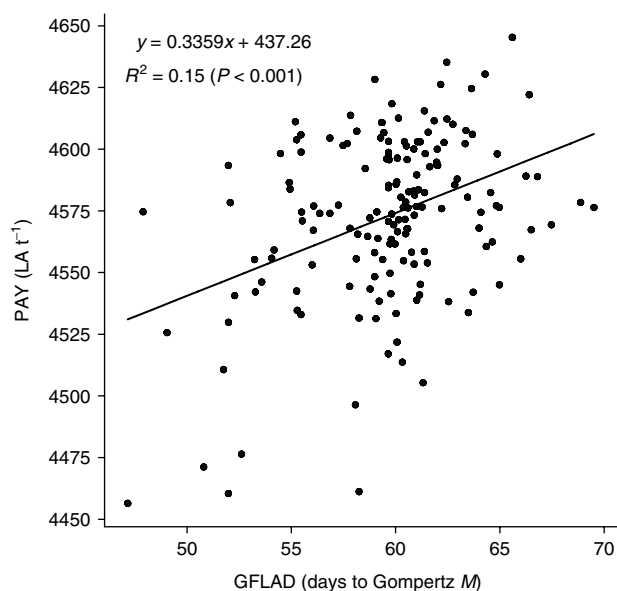

Figure 3. Overall relationship between green flag leaf area duration (GFLAD) and predicted alcohol yield (PAY) from all five experiments (data presented are residuals added to general mean).

also observed. Grain protein concentration increased ($P = 0.006$) by 0.02% for each day taken to reach Gompertz M. Predicted alcohol yield increased by 0.34 LA t^{-1} for each day taken to reach Gompertz M ($P < 0.001$).

Grain quality and predicted alcohol yield

Thousand-grain weight

Increasing thousand-grain weight appeared to result in a higher grain protein concentration in these experiments, but the relationship was not significant ($P = 0.096$). A significant ($P = 0.003$) relationship was seen with predicted alcohol yield, which increased by 0.36 LA t^{-1} for each 1 g increase in thousand-grain weight.

Protein and predicted alcohol yield

An inverse relationship ($P < 0.001$) between grain protein concentration and predicted alcohol yield was shown in these experiments. For every 1% increase in the concentration of protein within the grain, the predicted alcohol yield fell by 3.23 LA t^{-1}.

Predicted alcohol yield and actual alcohol yield

Distillations were conducted to determine the accuracy of the predicted alcohol yields. The results showed that there was a significant ($P < 0.001$) relationship between the two methods. For every 1 LA t^{-1} increase in predicted alcohol yield the actual alcohol yield increased by 0.71 LA t^{-1}.

DISCUSSION

The results show that factors affecting the crop later in the season, such as disease and green flag leaf area duration, can have substantial effects on alcohol yield. Maintenance of green flag leaf area is of key importance in achieving high crop yields (t ha^{-1}) as well as helping to maximise the alcohol yield of the grain (LA t^{-1}). Delaying senescence helps maximise the crop's ability to photosynthesise. In these experiments, green flag leaf area duration was manipulated by fungicide applications. Prolonged green flag leaf area duration is largely the result of disease control when nutrition and water are not limiting factors. In addition, fungicides have been shown to prolong green flag leaf area duration through physiological effects such as promoting the growth hormone cytokinin and delaying the inhibitor ethylene.[12] Other studies have shown increases in green flag leaf area duration through the control of phylloplane microflora such as *Cladosporium herbarum*.[13,14]

Prolonged green flag leaf area duration increases the potential grain-filling period and hence increases grain weight. In this study an average thousand-grain weight increase of 0.18 g occurred for each additional day the flag leaf retained above 37% green leaf area. However, studies by Dimmock and Gooding[15] showed that the ability of the crop to prolong grain filling with increased green flag leaf area duration was dependent on the variety grown. They found that early-maturing varieties benefited less from extending green flag leaf area duration, as these varieties had a limited grain-filling period compared with later-maturing varieties. The variety Glasgow has an average maturity date which is identical (0 days $\pm$) to the standard solstice.[16] Therefore it is likely that the application of fungicides that promote green flag leaf area duration would be more beneficial in increasing alcohol yield in late-maturing varieties such as Invicta (+3 days). However, it should be noted that green flag leaf area duration and grain filling will also be strongly influenced by the growing conditions of the crop, such as temperature and the availability of water and nitrogen.[17–19] Therefore this must be taken into account when making decisions on whether to apply additional fungicides to distilling wheat crops.

Where increases in thousand-grain weight occur, it could be assumed that grain protein concentration would be diluted as a result of greater accumulation of carbohydrates later in the season. However, despite increases in thousand-grain weight in this study, no dilution of grain protein was observed, suggesting a late season accumulation of nitrogen within the grain. Therefore grain protein increased at a comparable rate to starch. As a result, there was a relatively high level of protein at a given starch concentration. Therefore the relationship between protein and alcohol yield was less (−3.23 LA t^{-1} per 1% increase in grain protein) than in previous studies (−7.50 LA t^{-1}).[1] Weather data collected over the experiments showed a high level of rainfall in the later period of the growing season in each of the years. According to Monaghan *et al.*,[20] this can result in an increased proportion of nitrogen accumulated post-anthesis. In addition, Gooding *et al.*[21] and Ford *et al.*[22] observed that late season fungicide applications had the potential to delay senescence in the root system, thus providing crops with the potential to translocate a greater amount of nitrogen to the grain. This may therefore offer a potential explanation for the results in the current study.

Studies of the growing crop also showed that the control of septoria leaf blotch had a significant relationship with alcohol yield. For every 1% increase in septoria leaf blotch severity on the flag leaf (60 days after flag leaf emergence) there was a reduction in alcohol yield of 1.12 LA t^{-1}. Septoria leaf blotch is a facultative pathogen. Facultative pathogens are highly destructive, secreting enzymes that destroy the plant cell wall to enable them to feed on the glucose within the cell.[23] This reduces the plant's grain-filling potential, thus reducing the thousand-grain weight. According to Gooding *et al.*,[24] facultative pathogens are more detrimental to carbohydrate accumulation than to nitrogen. This was seen in the present study with an increase in grain protein concentration of 0.04% for every 1% increase in septoria leaf blotch severity. Previous studies[25–27] have also observed increases in grain protein concentration as a result of septoria leaf blotch. The ability of this disease to reduce carbohydrate accumulation, thereby increasing grain protein concentration, can be directly related to the reductions in predicted alcohol yield observed in the present study. These findings suggest that other facultative pathogens such as eyespot (*Oculimacula* spp.) and take-all (*G. graminis* var. *tritici*) may also have a similar relationship, resulting in reduced alcohol yields. The occurrence of obligate pathogens such as rust (*Puccinia* spp.) and mildew (*Blumeria graminis*), however, is likely to have a lesser effect. These pathogens are less destructive, developing a haustorial feeding complex to continuously absorb nutrients from the living plant cell,[28] which results in less of an effect upon starch translocation. Studies by Dimmock and Gooding[29] suggest that mildew retains nitrogen in the leaves, which is likely to reduce the grain protein concentration further. This is supported in studies by Johnson *et al.*[30] and Puppala *et al.*,[26] whose results appear to show a reduction in grain protein concentration with increased levels of mildew. Therefore infection with these diseases is likely to have little effect on grain alcohol yield per tonne but will reduce the overall crop yield and therefore alcohol yield per hectare.

In conclusion, this study has shown that fungicide applications have the potential to substantially increase the alcohol yield of wheat grain as a result of disease control and extended green flag leaf area duration when the crop is not sink-limited. In addition to increasing the alcohol yield per tonne of grain, the concomitant effect of increased crop yield will result in a substantial increase in alcohol yield per hectare. In the present study, disease levels remained relatively low in the later stages of crop development. This was the result of the robust fungicide programme applied; in situations where disease levels are greater, the influence of applying a late season fungicide is likely to be of increased importance. It should be noted that at the time of writing this paper there are no known price incentives in place for growers to produce crops with high alcohol yields in the UK. However, the cost of fungicide inputs in this study (£12.50/ha + £10.80/ha for application) was more than covered by the additional yield benefit observed (+0.85 t ha^{-1} at £100/t = £85/ha).[31]

ACKNOWLEDGEMENTS

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