

PROJECT REPORT No. 239

**EFFECTS OF STROBILURIN
FUNGICIDES ON THE MILLING
QUALITY OF BREADMAKING
WINTER WHEAT VARIETIES**

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**EFFECTS OF STROBILURIN FUNGICIDES ON THE MILLING
QUALITY OF BREADMAKING WINTER WHEAT VARIETIES**

by

N SAUNDERS & S SALMON

Campden and Chorleywood Food Research Association,
Chipping Campden, Gloucestershire, LG55 6LD

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FINAL REPORT : Strobilurins and milling quality

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ABSTRACT

Strobilurin fungicides are widely used within agriculture. They exert effects on both disease control of crop plants such as wheat and barley and increase yields. For this reason they now account for the majority of cereal fungicide treatments used in the UK, replacing the traditional triazole treatments which previously dominated fungicide usage. A number of strobilurin fungicide products are now available. Their precise mode of action is not known, but effects are largely preventative, as opposed to the curative effects offered by the triazoles. In practice, strobilurins are mixed with triazoles before application to crop plants, so that the relative merits of each are fully utilised.

Grain quality issues are a major concern for both the milling and processing industries, particularly in terms of protein content and *alpha*-amylase levels. There is little information available at present relating to the effects of strobilurin/triazole mixtures upon the subsequent quality of wheat samples. This study assessed the effects of a range of strobilurin /triazole applications on the subsequent quality of three wheat varieties.

This study was carried out in a season where there was severe pressure on wheat quality particularly in relation to *alpha*-amylase activity. Prolonged wet weather over the harvest period increased ear disease levels and provided crop protection challenges.

Aims

- ◆ Assess milling quality to determine relative yields from each fungicide treatment for each of the three wheat varieties.
- ◆ Examine whether any fungicide application leads to a “cleaner crop” by determining levels of Ochratoxin A, moulds, yeasts and viable counts on stored grain samples.
- ◆ Evaluate the effect on flour quality criteria e.g. protein and *alpha*-amylase content plus dough rheology.
- ◆ Assess breadmaking performance by the Chorleywood Bread Process, using loaf volume and crumb score as key quality indicators.

Conclusions and implications

- ◆ There were clear differences between varieties for the majority of quality tests carried out.
- ◆ The majority of wheat Hagberg Falling Number values were below 250s for Hereward and Rialto. This would cause rejection at mill intake and reduce subsequent processing options.
- ◆ Fungicide treatments had relatively few significant effects on quality parameters that were confined to susceptible varieties. For example, individual fungicide regimes tended to reduce Falling Number values in Rialto and Hereward, but had no effect on the more sprout resistant Malacca.
- ◆ A significant decrease in protein content was observed for Rialto under individual experimental fungicide regimes.
- ◆ Extraction rates for Rialto differed significantly between fungicide treatments, but there was no difference between treatments for Hereward or Malacca.
- ◆ There were no clear differences in breadmaking quality between fungicide treatments for any variety tested.
- ◆ There was no overall significant effect on the microbiological condition of grain samples resulting from any fungicide treatment. In general the variety Rialto, grown under the conditions of this trial, appeared to have slightly higher microbial counts than either Malacca or Hereward.
- ◆ Ochratoxin A was not detected in any of the samples after a period of 20 weeks storage under standard conditions.

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1. INTRODUCTION

The widespread use of fungicides to combat disease within agriculture has recently become dominated by the use of strobilurins. Since their commercial advent in 1997, strobilurins have become a dominant force within agriculture, accounting for approximately 47% of all cereal fungicides used in the UK. Manufactured from fungal derivatives, strobilurins have advantages over the traditional chemistry offered by triazoles, in that disease resistance is coupled with increasing yields. Strobilurins work primarily in 2 ways: offering superior disease control and extending green leaf retention, both of which encourage yield increases. The plant surface is protected preventing the admission of fungal infection. Such disease control increases the availability of energy to the plant for growth and yield, which would be otherwise diverted into fighting disease.

All strobilurin-related compounds have the same site of activity on the fungus, but they vary in their ability to stay on the leaf surface or enter the plant. There are three strobilurins commercially available at the moment: azoxystrobin (Amistar, Zeneca), kresoxim-methyl (Landmark, BASF) and trifloxystrobin (Twist, Novartis) all of which are taken into the plant. Exact effects within the plant are uncertain but the delaying of senescence, which is known to occur, has a direct effect on increasing yield.

Grain quality issues from the use of strobilurin fungicides, have as yet, not been fully investigated. There is evidence to suggest that specific weight and grain size can be increased with strobilurins (Konradt *et al.*, 1996). Conversely, any fungicide that increases grain yield is likely to reduce protein content. Strobilurins are no exception and dilution of grain protein and Nitrogen content has been reported in quality wheat for milling and malting barley respectively. In wheat, reductions in grain protein content of up to 1% and reductions in Hagberg Falling Number have been reported by Konradt and co-workers in 1996 for azoxystrobin use. Both effects can be expected to have an impact on end-use quality, particularly breadmaking. Furthermore, varietal responses to strobilurin application are known to occur, both in terms of yield and disease resistance (Bayles, 1999). It is therefore feasible that responses to quality will be affected in a similar way. Serious concerns existed within the milling industry that the effects of this new fungicide chemistry on end-use quality were unknown. This study set out to determine whether strobilurin/triazole applications affect wheat quality parameters that are critical to processing, using the 3 major breadmaking varieties currently in commercial use.

2. PROJECT OBJECTIVES

- ◆ Assess milling quality to determine relative yields from each fungicide treatment for each of the three wheat varieties.
- ◆ Examine whether any fungicide application leads to a “cleaner crop” by determining levels of Ochratoxin A, moulds, yeasts and viable counts on stored grain samples.
- ◆ Evaluate the effect on flour quality criteria e.g. protein and *alpha*-amylase content plus dough rheology.
- ◆ Assess breadmaking performance by the Chorleywood Bread Process, using loaf volume and crumb score as key quality indicators.

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3. MATERIALS AND METHODS

3.1 Wheat samples

Wheat was supplied by the National Institute of Agricultural Botany (NIAB) from a Fungicide Interactions Trial (FIT) grown at Gwent, Monmouthshire. Similar trials grown at Bridgets and Morley were rejected due to poor Falling Number values throughout the crop. Three varieties were selected namely Hereward, Rialto and Malacca from this trial to represent the major nabim Group 1 and 2 breadmaking varieties.

3.2 Fungicide Treatments

Ten fungicide mixtures were applied at growth stages 31/32 (T_1), 39 (T_2) and 59 (T_3). Nine of the ten treatments included applications at all three growth stages. Two fungicide regimes were triazole based only, the remainder included strobilurins in the mixture. No control, untreated sample was included in this study as this was considered to be commercially unrealistic.

There were 2 plots per treatment (with the exception of Rialto treatment 6, where only 1 plot was supplied) thus a total of 59 samples were examined.

Table 1. Fungicide treatments in study

Treatment no. and type	GS 31/32	GS39	GS59
2. Phthalimide/triazole	Bravo (1l/ha) Unix (1kg/ha) Opus Team (0.75 l/ha)	Opus Team (1.5 l/ha)	Folicur (0.75l/ha)
3. Strobilurin/triazole	Mantra (0.75 l/ha) Unix (1kg/ha)	Mantra (1l/ha)	Folicur (0.75l/ha)
4. Strobilurin/triazole	Mantra (1 l/ha)	Mantra (1l/ha)	Folicur (0.75l/ha)
5. Strobilurin/triazole	Mantra (0.75 l/ha)	Mantra(0.75l/ha)	Opus (0.5l/ha)
6. Strobilurin/triazole	Amistar (0.6 l/ha) Opus (0.5 l/ha)	Amistar (1l/ha) Opus (0.5l/ha)	Folicur (0.5l/ha)
7. Strobilurin/triazole	Opus (0.5 l/ha) Bravo 500 (1.5 l/ha)	Amistar (1l/ha) Opus (0.5l/ha)	Amistar (0.5l/ha)
8. Triazole/experimental strobilurin	Unix (0.67kg/ha) Menara (0.25 l/ha)	A9604A (1.2l/ha) Opus (0.5l/ha)	A9604A (0.6l/ha) Plover (0.2l/ha)
9. Triazole/experimental strobilurin	Unix (0.67kg/ha) Menara (0.25l/ha)	A9604A (1.2l/ha) Opus (0.5l/ha)	Bavistin DF(250g/ha) Plover (0.3l/ha)
10.Experimental strobilurin /triazole	A9805B (0.6 l/ha)	A9604A (1.2l/ha) Opus (0.5l/ha)	Bavistin DF(250g/ha) Plover (0.3l/ha)
11. Quinoline/triazole/ phthalimide	Fortress (0.15 l/ha) at G.S30/31	Opus (0.5l/ha) Bravo (1l/ha)	-

3.3 Grain quality

Grain samples were tested prior to milling in order to determine whether strobilurin/triazole treatments affected basic mill intake criteria and to ensure that samples were of the required quality for subsequent milling and breadmaking assessment. Samples were tested for moisture content, Hagberg Falling Number, specific weight and protein content (NIR and Dumas). There were three varieties (Hereward, Malacca and Rialto) and 10 treatments.

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3.4 Milling performance

Wheat samples were cleaned on a Carter-Day dockage tester. Following measurement of moisture content, samples were conditioned to 16% moisture by the addition of a pre-determined amount of water and allowed to condition for 16 hours prior to milling. Samples were milled to produce white flour with high starch damage levels using a laboratory Bühler mill, set to produce flour as close as possible to a commercial product. The bran and offal fractions were cleaned using a Bühler bran finisher to remove adhering endosperm particles. The following measures of milling performance were carried out:

- ◆ Recovery and extraction rates as a measure of "ease of milling".
- ◆ Flour colour grade as a guide to bran contamination levels and to measure any differences in the base colour of the "white" flour produced during milling
- ◆ Starch damage levels as an indication of differences in endosperm texture.

3.5 Flour quality evaluation

Flour quality was assessed by the following standard methods:

- ◆ Protein content by Dumas (Nx5.7, dry matter basis)
- ◆ Hagberg Falling Number to measure *alpha*-amylase levels
- ◆ Brabender Farinograph (600 line) to determine flour water absorption and rheological properties under gentle mixing conditions
- ◆ Brabender Extensograph to measure dough rheology under stretching conditions
- ◆ Gel protein weight and rheology to provide fundamental information on the quality of samples for breadmaking.

3.6 Breadmaking quality assessment

Test baking was carried out using a standard Chorleywood Bread Process (CBP). Ingredients were mixed using a Morton z-blade mixer to a fixed work input of 11 watt hours kg⁻¹ to produce 800g, four piece unliced white bread. All loaves were assessed for loaf volume and crumb structure score.

Each flour sample was baked in triplicate and each treatment was examined in duplicate i.e. each loaf measurement was based on six observations.

3.7 Contaminant testing

Entire samples were stored for 20 weeks under controlled conditions (temperature =10-15°C, relative humidity = 60-70%) after harvest prior to sampling for contaminant levels. Representative sub-samples from individual fungicide treatment plots were blended together for each variety to produce a sample for contaminant testing (500g from each plot, 30 samples in total).

Samples were tested for the presence of the mycotoxin Ochratoxin A plus for the following microbial contaminants: moulds and yeasts plus total viable counts. The latter allowed determination of the basic microbiological condition of each grain sample. High Performance Liquid Chromatography (HPLC) was used for measurement of Ochratoxin A, and standard agar plate incubation for total viable counts and yeasts and moulds.

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4. KEY FINDINGS

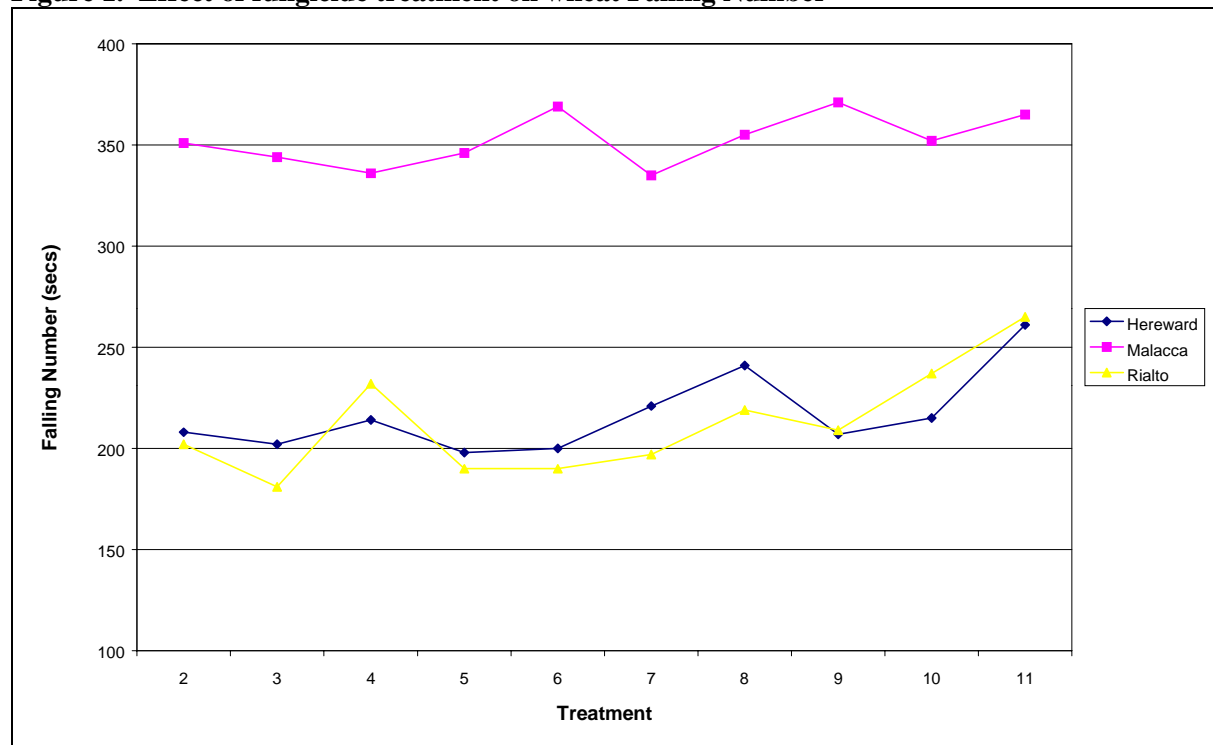
4.1 Grain quality

Wheat Hagberg Falling Number values were found to be in the range 181-265s for Rialto, 198-261s for Hereward and 335-371s for Malacca (Figure 1). Observed varietal differences are as expected, but some treatments resulted in Rialto and Hereward samples that would be rejected at mill intake where values of 250s are used as cut-off values for breadmaking wheat. In particular, trends suggested that treatments 3,5 and 6 (all 3-spray combinations involving strobilurin/triazole mixes) produced the lowest Falling Number values for Hereward and Rialto. These trends were not confirmed for Malacca, where Falling Number results were all within experimental error i.e. fungicide treatments had no effect.

Fungicide treatment produced little variation in specific weight in both Hereward and Rialto. Both varieties had acceptable specific weights (above 76 kg/hl) regardless of fungicide treatment (Figure 2). In contrast, Malacca produced a decrease in specific weight under fungicide regimes 10 and 11. The variety also had a lower average specific weight (75.5kg/hl) and thus some treatments produced samples that would be rejected at mill intake. The lowest specific weight of 73.5kg/hl was recorded for a non-strobilurin treatment No. 11.

As expected, there was a clear varietal effect on protein content. The variety Hereward produced significantly higher grain protein levels than Rialto that in turn was higher than Malacca (Figure 3). There was no overall consistent effect of treatment on protein content. Treatment 10 produced the lowest grain protein content in Rialto only and treatment 11 produced the highest for Malacca and Rialto. Such differences may be attributable to significant increases or decreases in grain yield.

Figure 1. Effect of fungicide treatment on wheat Falling Number



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Figure 2. Effect of fungicide treatment on specific weight

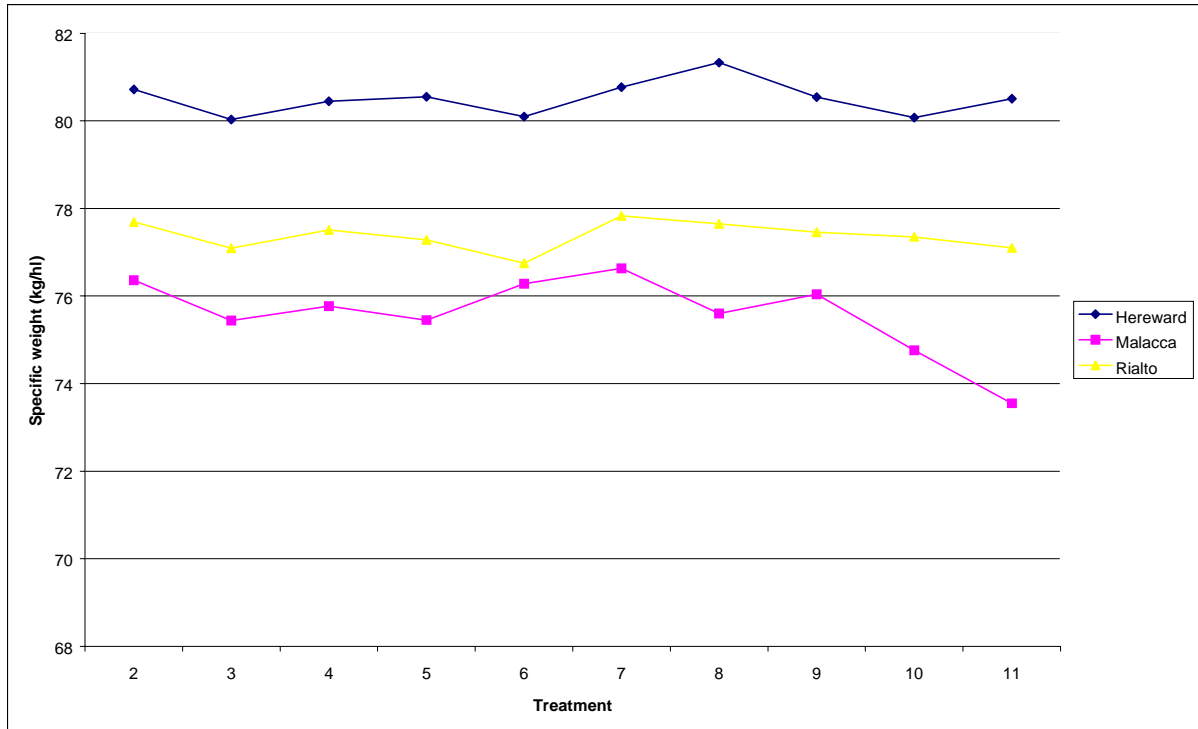
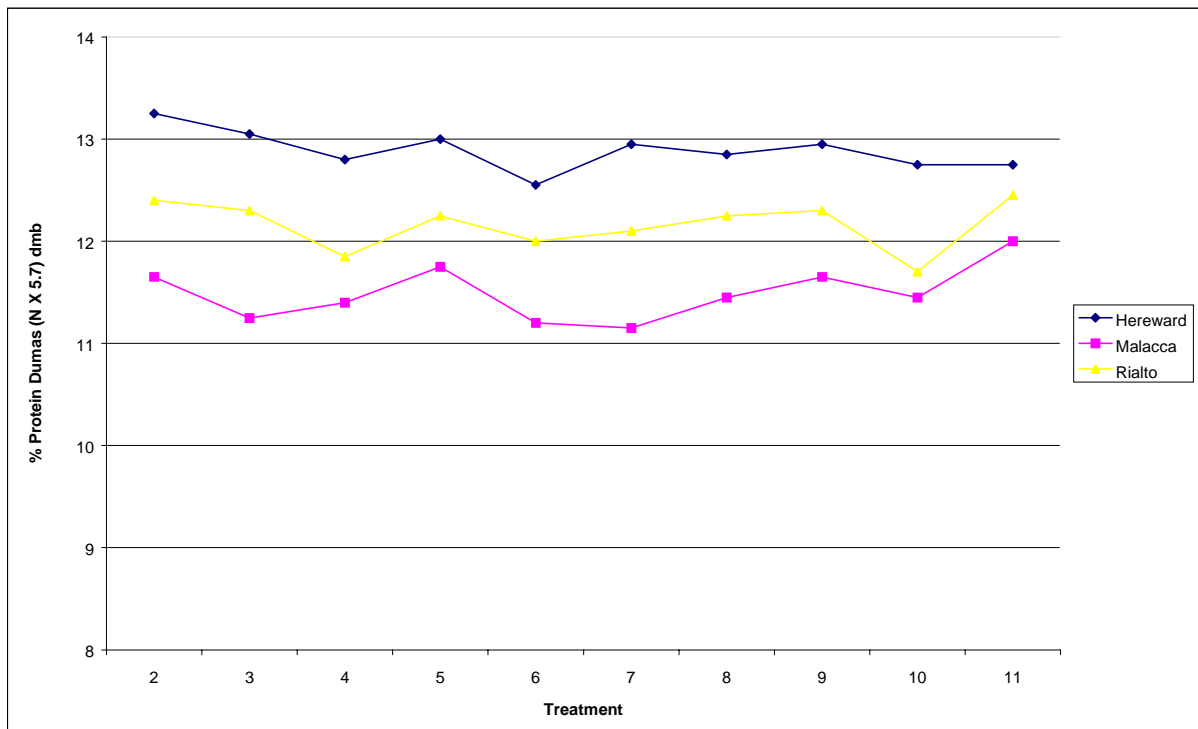


Figure 3. Effect of fungicide treatment on wheat protein content



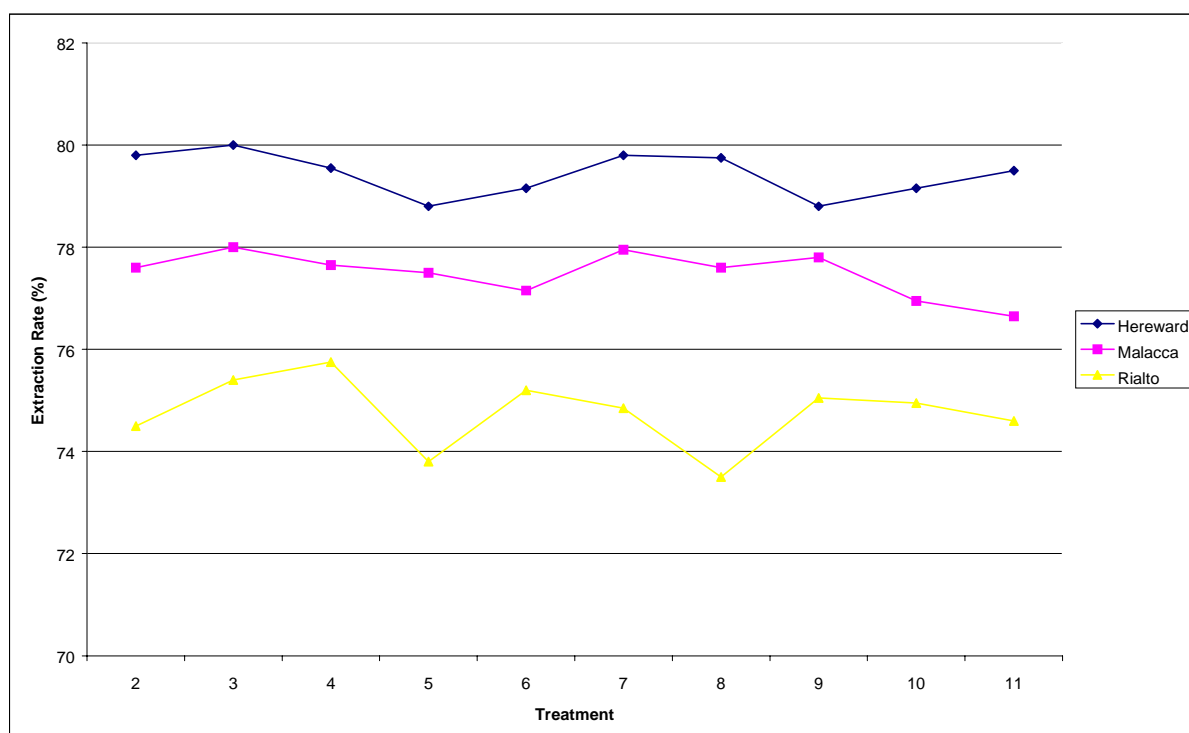
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4.2 Milling performance

Milling performance was assessed by calculation of total percentage extraction rate of white flour (based on products) and percentage straight-run flour (i.e. white flour produced before addition of finisher flours).

The only overall significant difference ($p < 0.01$) found was between treatment 5 (mean flour extraction rate approximately 2% below over the 3 varieties) compared to treatments 9 and 4 which, on average, produced higher extraction rates. However, it is clear from Figure 4 that the performance of Rialto is having a major effect on this result. Straight-run flour values mirror the pattern observed above with treatments 5 and 8 producing low extraction rates. Fungicide treatment had no significant effect on starch damage levels and thus extraction rate differences would not seem to be attributable to differences in endosperm texture. Similarly there were no significant effects on flour colour.

Figure 4. Effect of fungicide treatment on flour extraction rate (products basis)



4.3 Flour quality evaluation

Flour Hagberg Falling Number results followed the basic pattern shown for wheat with fungicide applications 2-4 producing significantly lower values than treatments 8-11 ($p < 0.05$) for all varieties. For the susceptible varieties Falling Number values, between treatments 2 and 11, ranged from 248 to 315 s for Rialto and 242 to 321s for Hereward. Treatments 2 and 6 produced Falling Number values below 250s for both Hereward and Rialto. For the variety Rialto only, treatment 11 produced a significantly higher flour Falling Number than all other treatments except treatment 10.

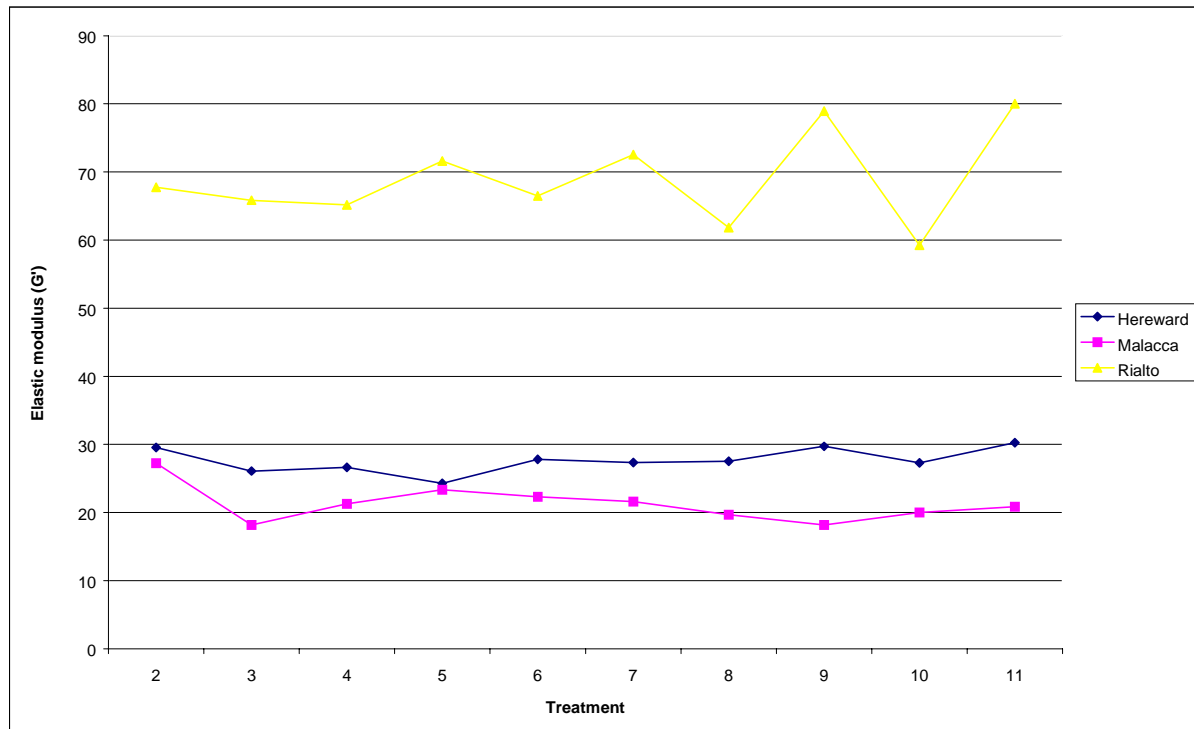
Flour protein data followed similar trends to grain protein for all three varieties (Figure 3). Values were approximately 1% lower than the equivalent wheat due to losses in milling and differences in the moisture basis of expression.

Protein quality was assessed by a number of different techniques namely Brabender Farinograph and Extensograph plus gel protein rheology. Major significant differences were observed between the three varieties tested, but no overall significant effect of fungicide treatment was observed by any of

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the three tests of protein strength. Within the variety Malacca (the weakest variety studied in this trial) treatment 11 produced a stronger dough which did not soften as much during continued gentle mixing in the Farinograph. A tendency for reduced 100E/R ratios were observed between treatments 2 and 11, but this failed to reach significance. The varieties Hereward and Malacca showed little variation in elastic modulus (G') values (figure 5). Treatments 8 and 10 produced below average and treatments 9 and 11 above average G' values for Rialto. G' values ranged from 59 to 80Pa for Rialto: values above 40 are often considered to be over strong for optimum performance in UK breadmaking processes. In treatment 10 this apparent reduction in protein quality was linked to low protein content. This may be expected to impact on overall breadmaking quality.

Figure 5. Effect of fungicide treatment on gel protein elastic modulus



4.4 Breadmaking quality assessment

Although there were clear varietal differences in terms of loaf volume, there were no significant differences due to fungicide treatments (Figure 6). On average, loaf volumes differed by approximately 240ml between the best and the worst examples of a variety, but there was no consistent pattern to the data. Generally, replicate variability was minimal within varieties. In this particular study, the highest average loaf volume was achieved for Rialto, followed by Hereward and Malacca. The positions of Rialto and Malacca are somewhat unexpected. However, other quality data shows the Malacca within this trial to be of below average protein content and quality. In contrast, Rialto is at the stronger end of the quality scale for this variety. Other measures of breadmaking quality suggested no significant difference between fungicide treatments within a variety.

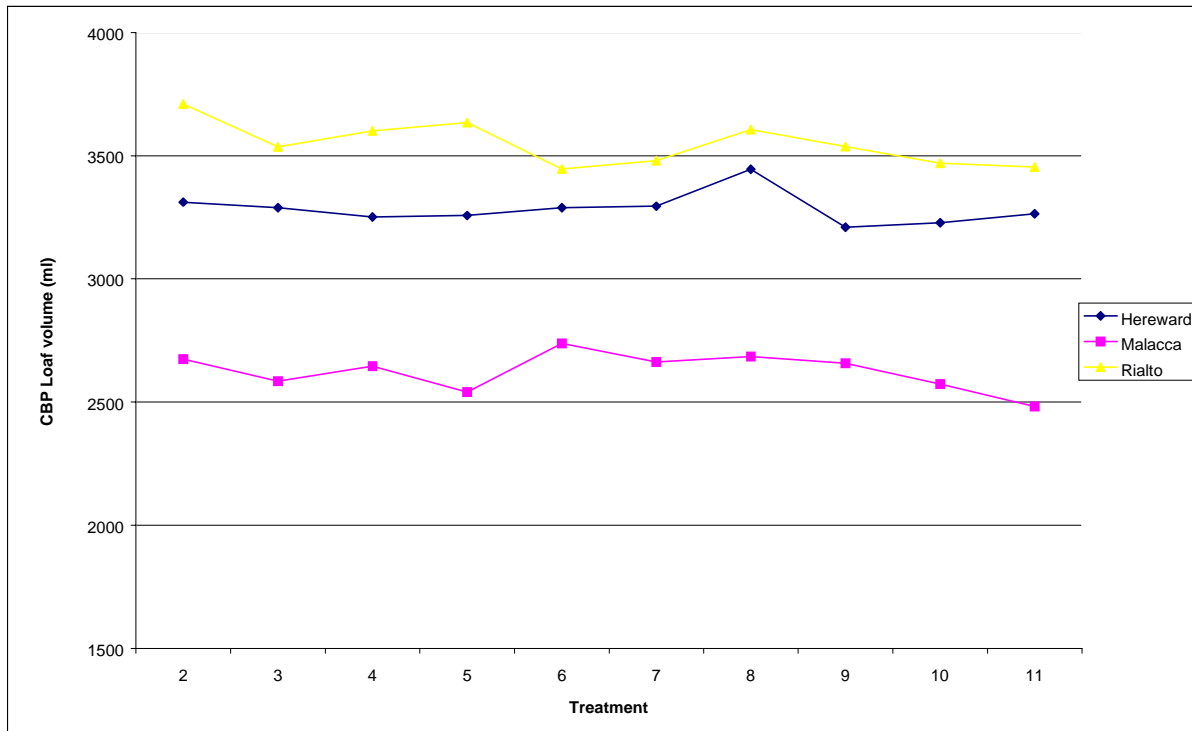
4.5 Contaminant testing

Ochratoxin A was not detected in any of the stored, bulked samples tested (i.e. $<1\mu\text{g}$ per kg or 1ppb, the limit of detection). Similarly, yeast levels were extremely low in all samples <5 per gram (the limit of detection).

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Overall, Rialto exhibited higher average mould levels and total viable counts compared to Hereward and Malacca. In particular, treatments 4 and 10 produced low levels of both moulds and total viable counts. However, there were no significant differences between treatments over all three varieties.

Figure 6. Effect of fungicide treatment on CBP loaf volume



5. CONCLUSIONS AND IMPLICATIONS

This study was carried out in a season where pressures on quality were particularly high due to prolonged wet weather over the harvest period. Such conditions may be expected to accentuate fungicide treatment differences, particularly in relation to *alpha*-amylase activity. Wet weather during ripening also increased ear disease levels with *Fusarium* and *Septoria* ear blights a particular problem. This may be expected to increase the microbial loading on harvested and stored grain.

- ◆ There were clear varietal differences in the majority of quality parameters measured.
- ◆ Fungicide treatments had relatively few significant effects on quality parameters and where present these were confined to susceptible varieties. For example, individual fungicide regimes tended to reduce Falling Number values in Rialto and Hereward, but had no effect on the more sprout resistant Malacca. Also a significant decrease in protein content was observed for Rialto under an experimental strobilurin/triazole treatment (treatment 10).
- ◆ Fungicide treatment significantly affected flour extraction rate for Rialto, but there was no difference between treatments for either Hereward or Malacca.
- ◆ The majority of wheat Hagberg Falling Number values were below 250s for Hereward and Rialto. This would cause rejection at mill intake and reduce subsequent processing options.
- ◆ Within a variety, there were no significant differences in breadmaking quality between fungicide treatments.
- ◆ There was no overall significant effect on the microbiological condition of grain samples resulting from any fungicide treatment. When samples were stored for a period of 20 weeks, no Ochratoxin A was detected in any samples.

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To summarise, individual treatments produced marginal effects on basic quality parameters e.g. Falling Number and protein content in specific varieties, but no significant effects were observed in end-use (breadmaking) quality. No consistent or significant effect could be attributed to application of a particular active ingredient or fungicide mixture. In a difficult harvesting season above average total viable counts were observed in this trial. Although fungicide treatments may be expected to improve disease control and crop cleanliness, no treatment had an overall significant effect on the level of microbial contamination.

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INTRODUCTION

The arrival of strobilurin fungicides in agriculture introduced new chemistry to combat cereal disease. Until 1997, triazoles were predominantly and successfully used within agriculture for their curative effects on a range of fungal infections. Strobilurin use is now widespread: the two main products on the market when this work was first proposed were azoxystrobin from Zeneca (sold as Amistar, a single active ingredient broad-spectrum product) and kresoxim-methyl from BASF (sold as Landmark in mixture with epoxiconazole, the active ingredient of Opus).

Strobilurins are fungal derivatives that interfere with electron transport within mitochondria, hence preventing ATP (Adenosine Tri-Phosphate) synthesis in disease-causing fungi of cereals. The effects following application to cereals are two-fold: disease control and yield enhancement. This combination has led to widespread use of strobilurins by growers and in the 1999 harvest they were thought to account for 47% of fungicides used in agricultural practices for cereals. One reported secondary effect of strobilurin application has been intense crop greening and delayed crop senescence with consequent concerns regarding harvest delays particularly when intemperate weather conditions make harvesting difficult in the UK.

High yields have been reported for azoxystrobin applications to wheat (Konradt *et al.*, 1996). Farm trial results from Zeneca have shown significant yield improvements for feed, biscuit and bread wheats following strobilurin use compared to other fungicide application programmes. Increases in hectolitre weight, with minimal effects on protein content have also been shown (HGCA, 1998a). The eradicator and protectant action of kresoxim-methyl on a range of crop diseases such as Septoria, rusts, net-blotch and mildew have been illustrated by the manufacturer. Independent agronomic studies involving azoxystrobin have shown 4-5% increases in yield for barley, enhanced rooting and increased nitrogen uptake with control of brown rust on rye, and maintenance of green leaf area (HGCA, 1998a). Studies carried out at the National Institute of Agricultural Botany (NIAB) have shown that strobilurin fungicides raise potential yields by at least 1t/ha for all wheat varieties (Bayles, 1999), but the level of this yield increase depended on the variety. It was suggested that for winter wheat, the benefit of strobilurin use was least for low yielding varieties that already possessed good resistance to fungal disease.

Kresoxim-methyl can induce alterations in the physiological state of the plant, such as enhancing concentrations of chlorophyll and increasing biomass production (HGCA, 1998b). Both azoxystrobin and kresoxim-methyl are thought to have the effect of reducing ethylene synthesis within plants (Grossman & Retzlaff, 1997). Ethylene is formed in response to plant stress and also during other processes such as germination, ripening and senescence (Abeles *et al.*, 1992). It has been suggested that kresoxim-methyl affects induction of ACC synthase (1-aminocyclopropane-1-carboxylic acid) activity, an enzyme crucial for ethylene biosynthesis, and this in turn delays senescence by preventing ethylene formation. The more recent advances in strobilurin chemistry have yielded trifloxystrobin (F279 or Twist), manufactured by Novartis, a fungicide with the ability to travel through the crop canopy, the so-called “mesostemic effect”. This new development offers improved control of such diseases as Septoria, larger yields and affords some curative activity (Abel, 2000). The precise modes of action of individual strobilurin fungicides at a physiological level within the plant have not yet been entirely investigated. Increases in yield from strobilurin use tend to occur in parallel with delays in plant senescence. At a physiological level, this could exert deleterious effects on grain quality, such as accentuation of *alpha*-amylase and dilution of protein content. Information on the effects of strobilurins on wheat quality has generally been restricted to specific weight, grain size, Falling Number with a limited amount of information of protein content and quality. The effect on quality parameters, following application of this new chemistry to wheat plants, therefore needed investigation.

The 1999 harvest provided particular disease and quality pressures due to a period of wet weather that increased ear disease levels and pre-harvest sprouting in many wheat crops. The varieties within the trial and site selected all produced Falling Number values in excess of 180s i.e. were not visibly sprouted but varied significantly in terms of *alpha*-amylase activity. Under these conditions the consequences of delayed ripening due to fungicide applications may be expected to accentuate quality

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differences. Therefore, this material can be considered to provide a very rigorous test of the impact of such treatments on milling and breadmaking quality of wheat. Reports have suggested that certain fungicide treatments produce a cleaner, safer product. The impact of weather conditions on ear disease levels may be expected to increase the microbial loading on harvested and stored grain.

PROJECT OBJECTIVES

This study set out to examine the effects of strobilurin/triazole applications to wheat on a range of quality parameters, utilising three wheat varieties. Primary aims included:

- ◆ Assessment of milling performance to determine the effect of fungicide treatments on relative yields.
- ◆ Evaluation of flour quality by chemical and rheological methods.
- ◆ Detailed examination of Chorleywood Bread Process (CBP) breadmaking performance using loaf volume plus crumb score as key quality indicators.
- ◆ Investigation of reported claims that fungicide application leads to reduced microbial contamination or levels of Ochratoxin A in treated crops.

MATERIALS AND METHODS

Wheat samples

Wheat samples of three varieties (Hereward, Malacca and Rialto) were supplied by the National Institute of Botany (NIAB) from the Fungicide Interaction Trial (FIT) series grown at Gwent, Monmouthshire. The site was chosen following basic quality testing (Falling Number and grain protein content) carried out by NIAB to establish suitability for breadmaking. Poor weather during the harvest period rendered some sites unsuitable due to pre-harvest sprouting problems. Varieties were selected from a total of 10 grown at the site as they represented current **nabim** Group 1 and Group 2 breadmaking wheat varieties (**nabim**, 1999).

Fungicide Treatments

The FIT trials were set up by NIAB to examine varietal responses to fungicide programmes involving new (strobilurin) and conventional (triazole) products. In addition to basic control of mildew and yellow rust across the whole trial (if required), various strobilurin/triazole treatments were applied at the following growth stages: start of stem extension (GS31/32), flag leaf emergence (GS39) and ear emergence (GS59). See Table 1 for details of fungicides and timings examined.

There were two replicate plots per treatment per variety, with the exception of Rialto treatment 6, for which only 1 plot was supplied. This provided a total of 59 separate samples. The winter wheat trial represented the first cereal crop after peas at the Gwent site. Fertilizer, herbicide and plant growth regulator (PGR) applications, are detailed in Table.2

Disease incidence and control

Septoria tritici was assessed as very severe at the Gwent site. *Septoria nodorum* (glume blotch) was severe and eyespot moderate. All other disease symptoms were absent.

Good control of *Septoria tritici* was achieved by all fungicide treatments, although treatment 11 was found to be least effective.

Septoria nodorum was reduced by all fungicide treatments but control was least effective with the 2-spray programme (treatment 11) where the ear-spray was omitted.

Treatments 2 and 3 reduced the severity of eyespot in all trials. Treatments 4, 5 (strobilurin rate applications) and 8-10 (experimental strobilurin treatments) were less effective, but still reduced eyespot compared with untreated plots. Treatments 6 and 7 (strobilurin timing applications) gave little reduction in eyespot levels, whilst treatment 11 did not reduce eyespot compared with untreated plots.

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Table 1. Fungicide treatments in study

Treatment no. and type.	GS 31/32	GS39	GS59
2. Phthalimide/triazole	Bravo (11/ha) Unix (1kg/ha) Opus Team (0.75 l/ha)	Opus Team (1.5 l/ha)	Folicur (0.75l/ha)
3. Strobilurin/triazole	Mantra (0.75 l/ha) Unix (1kg/ha)	Mantra (1l/ha)	Folicur (0.75l/ha)
4. Strobilurin/triazole	Mantra (1 l/ha)	Mantra (1l/ha)	Folicur (0.75l/ha)
5. Strobilurin/triazole	Mantra (0.75 l/ha)	Mantra(0.75l/ha)	Opus (0.5l/ha)
6. Strobilurin/triazole	Amistar (0.6 l/ha) Opus (0.5 l/ha)	Amistar (1l/ha) Opus (0.5l/ha)	Folicur (0.5l/ha)
7. Strobilurin/triazole	Opus (0.5 l/ha) Bravo 500 (1.5 l/ha)	Amistar (1l/ha) Opus (0.5l/ha)	Amistar (0.5l/ha)
8. Triazole/experimental strobilurin	Unix (0.67kg/ha) Menara (0.25 l/ha)	A9604A (1.2l/ha) Opus (0.5l/ha)	A9604A (0.6l/ha) Plover (0.2l/ha)
9. Triazole/experimental strobilurin	Unix (0.67kg/ha) Menara (0.25l/ha)	A9604A (1.2l/ha) Opus (0.5l/ha)	Bavistin DF(250g/ha) Plover (0.3l/ha)
10.Experimental strobilurin /triazole	A9805B (0.6 l/ha)	A9604A (1.2l/ha) Opus (0.5l/ha)	Bavistin DF(250g/ha) Plover (0.3l/ha)
11. Quinoline/triazole/ phthalimide	Fortress (0.15 l/ha) at G.S30/31	Opus (0.5l/ha) Bravo (1l/ha)	-

Table 2. Fertilizer, herbicide and PGR applications at the Gwent site

Growth stage	Product	Rate
23	N Top dressing 1	42.6 kg/ha
33	N Top dressing 2	106.5 kg/ha
37	N Top dressing 3	52.5 kg/ha
30	P2O5	59.28 kg/ha
30	K20	133.38 kg/ha
37	Starane (herbicide)	
30	New 5C Cycocel (PGR)	
33	Terpal (PGR)	

Grain yield

Grain yield was recorded for all varieties in t/ha at 85% dry matter. The values for the Gwent site are detailed in Table 3.

Table 3. Grain yield (t/ha at 85% dry matter) for the 3 varieties used in this study from the Gwent site.

Treatment	Hereward	Malacca	Rialto	Mean of 3 varieties
2	8.50	9.29	9.18	8.99
3	9.28	9.35	9.31	9.31
4	8.43	9.27	9.26	8.98
5	8.27	9.04	9.10	8.80
6	8.61	9.30	8.84	8.91
7	8.17	9.96	8.52	8.88
8	8.11	8.85	8.65	8.53
9	8.65	9.22	9.06	8.97
10	8.06	8.72	8.67	8.48
11	7.68	7.86	7.89	7.81
Mean	8.38	9.09	8.85	8.77

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Grain quality

All tests were carried out according to UKAS accredited methods unless otherwise specified. Methods indicated as FTWG have been agreed and collaboratively tested by representatives of the UK milling and baking industries (Salmon, 1997). Grain samples were tested prior to milling in order to determine whether strobilurin/triazole treatments affected basic mill intake criteria and to ensure that samples were of the required quality for subsequent milling and breadmaking assessment. Upon receipt, all 59 samples were tested for moisture content using a Sinar rapid moisture meter to ensure moisture levels were below 15% and samples were suitable for storage.

Subsequently, samples were tested for the following basic quality characteristics:

◆ Specific weight

The wheat sample was poured, in a controlled manner, from a hopper into a one-litre Kern chondrometer and weighed. The result obtained gives the amount of wheat (in kilograms) contained within a hectolitre in kg/hl.

◆ Falling Number

Falling Number was measured according to FTWG 0006. The weight of grain sample was adjusted according to the moisture content of the ground grain.

◆ Protein content

Grain protein content was measured by the Dumas combustion method (FTWG 0019). Results were corrected for moisture and reported as Dumas (N x 5.7) on a dry matter basis.

Milling performance

The samples were stored in controlled conditions at 15°C for 20 weeks before Bühler milling and subsequent testing.

◆ Cleaning and conditioning

Samples (10kg) were cleaned on a Carter-Day dockage tester to remove dust, husk, unthreshed grain, small and broken grains and weed seeds. Sample moisture content was measured using a Sinar rapid moisture meter. All grain samples ranged between 13.6-15.0 %. None of the grain required drying nor was it so dry as to require two-stage conditioning prior to milling.

Wheat was placed in an electrically driven concrete mixer and the requisite amount of water added to bring the moisture of the grain to 16±0.5%. After mixing for at least 15 minutes the wheat was stored in sealed plastic tubs and allowed to condition for 16 hours. Conditioning toughens the bran and mellows the endosperm, such that when milled, the bran is removed in large pieces, reducing contamination of the flour with bran specks.

◆ Bühler Milling

The wheat was milled to high starch damage using a Bühler laboratory mill type MLU-202 in conditions of controlled temperature and humidity. The bran and offal were cleaned using a Bühler laboratory bran finisher type MLU-302 to remove any remaining flour. All milling products were weighed, the flour blended and the relevant recovery and extraction rates calculated.

◆ Flour colour grade

Flour colour grade was measured according to FTWG 0007/4 and reported in flour colour grade units on a scale from -5 to +18. This test measures the amount of light at 530nm which is reflected from a flour/water paste contained in a glass cell and provides a measure of the level of bran contamination in a white flour sample. High values indicate greater bran contamination that may be expected to have a detrimental effect on breadmaking performance. Commercial bread flours will typically have flour colour grade values of 2 to 3 GCF units.

◆ Starch damage

Starch damage was determined according to FTWG 0005, based on the Farrand method and quoted in Farrand units (FU). The level of starch damage produced during the milling process

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is an important quality parameter due to its effect on flour water absorption. Commercial bread flours will typically have starch damage levels of above 35 FU.

Flour quality evaluation

Flour quality was assessed by a series of chemical and rheological methods as follows:

◆ *Falling Number*

As for wheat, Falling Number was measured according to FTWG method 0006. A graph relating Falling Number values to cereal *alpha*-amylase activity was used to estimate the cereal *alpha*-amylase of each flour and hence to calculate the amount of fungal *alpha*-amylase supplement required in subsequent baking tests.

◆ **Flour Protein**

Flour protein and moisture contents were measured by Near Infrared Reflectance (NIR) according to FTWG method 0014. Flour protein content (Dumas, N x 5.7) was then corrected to a 14% moisture basis.

◆ **Brabender Farinograph**

FTWG 0004 was used to determine water Absorption (% on the 600 line) and dough rheological properties: development time (min.), stability (min) and degree of softening (Brabender Units or BU). The Farinograph records torque on the mixer blades when flour and water are mixed at low speed. The added water hydrates the flour to form a dough and under the conditions of the test, the dough must be developed to a fixed consistency, i.e. 600 Brabender Units. The time between the start of mixing and the first point of weakness was taken as the development time and recorded in minutes. In order to assess protein strength, mixing was continued for a period of 12 minutes after the first signs of dough weakening. By measuring the position of the centre of the curve at dough development and the final mixing point, it was possible to obtain a measure of dough weakening (degree of softening) as determined by the ability of the flour to withstand continued mixing. The time in minutes during which the top of the curve remains above 600 line, was measured as the dough stability. The shape of the trace is primarily related to the quality of the gluten protein and, the Farinograph characteristics provide information on dough strength. Any evidence of dough weakening under the gentle mixing conditions in the Farinograph is likely to occur more rapidly under standard CBP mixing conditions and may be expected to influence final breadmaking performance.

Commercial CBP bread flours typically produce Farinograph characteristics in the following ranges:

Water absorption (600line) : 58.0 - 62.0 %

Development times: 2-4 minutes

Stability: 3-6 minutes

Degree of softening: 50-100BU

◆ **Brabender Extensograph**

The Extensograph, used according to FTWG 0003, measures the stretching properties of a flour-salt-water dough of standard consistency and provides an indication of breadmaking performance. A flour-salt-water dough was prepared under standard conditions in the Brabender Farinograph and moulded into a cylinder shape. After a fixed period of time (45 minutes) the dough was stretched and a curve drawn recording the Extensibility of the dough and its Resistance to stretching. Resistance(R) and Extensibility(E) measurements are frequently combined as a ratio such as the 100E/R value. The size and shape of the curves, as measured by E and R, are used as a guide to the baking characteristics of the flour.

◆ **Gel protein quantity and quality**

The weight of gel protein represents the amount of functional protein present in the flour and consists, principally, of glutenin. Thus, breadmaking wheats have higher levels than feed or biscuit-making varieties. 15g flour was defatted with 40ml petroleum ether (b.p. 40-60°C) for 1 hour, filtered and dried. 5g of defatted flour was mixed with 75ml of 1.8% sodium dodecyl sulphate for 10min. at 10°C and then centrifuged at 40000rpm for 35min. The gel protein layer was removed and weighed. A typical range for breadmaking would be 9-12g / 5g of flour (wet-weight basis).

The elastic modulus (G') of gel protein was measured using a Rheometric rheometer with a cone and plate configuration. The G' value can be used to distinguish between varieties in terms of quality for UK breadmaking. Optimum performance in the standard Chorleywood Bread Process (CBP) used in this study is generally produced when G' is between 15 and 40 Pascal. Low values indicate samples

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that may be too weak for the high-speed mixing process used and high values suggest excessive strength which results in under-mixing and under-performance in breadmaking.

Breadmaking quality assessment

◆ Chorleywood Bread Process (CBP)

A standard Chorleywood Bread Process was carried out using the ingredients shown in Table 2, a z-blade Morton Mixer and a work input of 11 watt hours kg⁻¹.

Table 4. Standard ingredients for the Chorleywood Bread Process

Ingredients	% of flour weight
Flour	100.0
Yeast (compressed)	2.5%
Salt	2%
Fat (Ambrex, slip point 45°C)	1%
Fungal Amylase	80 FU (Farrand units)
Ascorbic Acid	0.01% (100ppm AA)
Water	According to Farinograph 600 line water absorption

At the end of the mixing process, an assessment of the condition of the dough was made. A scale of 1 to 8 was used where 1= tight, 3=normal and 8 = soft and sticky.

All loaves were baked using standard tins used for the production of 800g four-piece loaves (unlidded). All loaves were stored in a closed cabinet at approximately 20°C overnight, prior to assessment of loaf volume and crumb structure.

◆ Loaf volume

Loaf volume was determined by seed displacement. Before use, the instrument was calibrated using a dummy loaf of nominal volume representing an 800g loaf. The weight of seed displaced by the test loaves was measured and converted to loaf volume. Each loaf volume reported was the mean of 6 loaves (2 loaves per bake x 3 bakes)

◆ Crumb Score

Crumb score was determined by a trained assessor. A scale of 1 to 10 was used, which is based upon the visual appearance of the cut surface of the bread. A summary of this scale is shown below:

Score Description

- < 5 **Crumb scores reflect totally unacceptable bread structure, i.e. crumb structure is very uneven and coarse, and cells are thick walled. Bread in this category normally exhibits poor oven lift and lacks volume, i.e. is rather dense.**
- 5,6 **Scores are given when the baked product is just bread-like. Crumb structure is poor with large areas of coarse and thick walled cells, visible cores or streaks.**
- 7 **Assigned to a sample that produces a loaf of acceptable appearance, but exhibits some faults in crumb structure terms, i.e. some areas of coarse, thick walled cells or streaks are evident.**
- 8 **This score is assigned to a loaf that exhibits good quality characteristics. The crumb is relatively uniform and the majority of cells are thin walled. No major faults such as cores or streaks are evident.**
- 9 **Given to a loaf with very good crumb structure i.e. uniform with thin cell walls and no evidence of major faults.**
- 10 **Only assigned to the perfect CBP loaf, i.e. where texture consists only of small, uniformly distributed in the crumb cross-section and cells are thin walled.**

Contaminant testing

Since Ochratoxin A is a storage mycotoxin and not generated in the field, samples were stored for 20 weeks under controlled conditions (temperature = 10-15°C and relative humidity = 60-70%) designed to represent good storage conditions (HGCA, 1999) after harvest and prior to sampling for contaminant levels. Representative sub-samples from individual fungicide treatment plots were bulked together for each variety to produce an average sample for contaminant testing i.e. 500 grams

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from each of the 2 treatment plots were blended thoroughly in plastic container to yield a 1kg average sample. This produced a total of 30 samples: 3 varieties (Hereward, Malacca and Rialto) x 10 treatments.

◆ Ochratoxin A

10g of each wheat sample was blended with 40ml of 50% v/v phosphate buffer solution in methanol and centrifuged at 3500rpm for 10 minutes at room temperature. 15ml of the supernatant was added to 35ml of phosphate buffered saline solution (PBS) and centrifuged for 5 minutes at 3500rpm. The resulting supernatant was removed to a clean vessel.

10-20ml of PBS was added to an immunoaffinity column at approximately 5mlmin⁻¹ followed by the prepared sample extract (added at the same rate) and finally the column was washed using 20ml of distilled water. 2ml of desorption solution was then added to the column reservoir in order to elute Ochratoxin A from the column. High-performance liquid chromatography (HPLC) was performed on the eluate using an Ultracarb 5 ODS (5µm Octadecylsilane, 250 x 3.2mm) column. The HPLC conditions were as shown in Table 5.

Table 5. HPLC conditions used in Ochratoxin A measurement.

Parameter	Setting
Fluorescence detector	Excitation 330nm Emission 477nm
Flow Rate	0.8mlmin ⁻¹
Injection volume	100µl
Mobile Phase	10ml Glacial Acetic Acid 700ml Methanol 300ml Water
Oven temperature	35°C

Results were quantified using peak areas and the concentration of Ochratoxin A calculated after correction for blanks. The limit of determination was 1µg/kg (i.e. 1part per billion).

◆ Total viable counts

20g of a prepared ground wheat sample was weighed into a Stomacher bag and 180g of maximum recovery diluent (MRD) added. Serial dilutions were then prepared using 9ml of MRD. Using the pour plate technique, 1ml volumes of appropriate dilutions were inoculated onto media in petri dishes. Approximately 15-20ml of molten plate count agar (PCA), tempered to 46°C, was then added, mixed and allowed to set. The plates were then inverted and incubated at 30°C for 48hours. All colonies on each plate were counted and the number of colonies per gram calculated to determine the total viable (TVC) or aerobic plate count.

◆ Yeasts and moulds

Yeasts and moulds were determined according to the method given for the TVC count except that 15ml of Rose Bengal Chloramphenicol Agar (RBCA) was used in place of PCA, and petri dishes were incubated at 25°C for 48 hours instead of 30°C. The number of yeasts and moulds on each plate were counted and used to calculate the number of yeasts and moulds per gram of product.

RESULTS

All data was tested for significance by two-way ANOVA (Analysis of Variance) in order to establish the effects of individual fungicide treatments on quality characteristics. The statistical model has taken all 3 varieties into account to determine significance of fungicide applications on the measured parameters. Statistical significance, where found, is indicated in the summarised results (Table 4). As expected, varietal differences on quality attributes are more consistent and significant than those attributable to differences in fungicide treatment. However, significant effects over all three varieties were observed for specific grain, flour extraction and quality parameters. Such effects on individual quality parameters are discussed in full in later sections of this report.

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Table 6. Analysis of variance for wheat quality parameters
(Significance levels are indicated as follows: *** = p<0.05, ** = p<0.01, * = p = <0.001 and ns = not significant)

TEST	VARIETY	TREATMENT	INTERACTION
Grain quality:			
Falling Number	***	***	*
Specific weight	***	**	**
Dumas protein	***	ns	ns
Milling performance:			
Total extraction (on product)	***	**	**
Straight-run extraction (on product)	***	**	**
Flour colour	***	ns	*
Starch damage	*	ns	ns
Flour quality evaluation:			
Falling Number	***	***	ns
Dumas protein	***	**	ns
Brabender Farinograph : Water absorption	***	ns	ns
Development time	***	ns	ns
Stability	***	**	ns
Degree of softening	***	**	ns
Brabender Extensograph : Resistance	***	ns	ns
Extensibility	***	*	ns
100E/R	***	ns	ns
Gel protein weight, g/5g flour	***	ns	ns
Gel protein elastic modulus, G'	***	ns	ns
Breadmaking quality assessment:			
Loaf volume	***	ns	ns
Crumb score	ns	ns	ns
Contaminant testing:			
Ochratoxin A	nd	nd	nd
Total Viable Counts	***	ns	-
Yeasts	nd	nd	nd
Moulds	**	ns	-

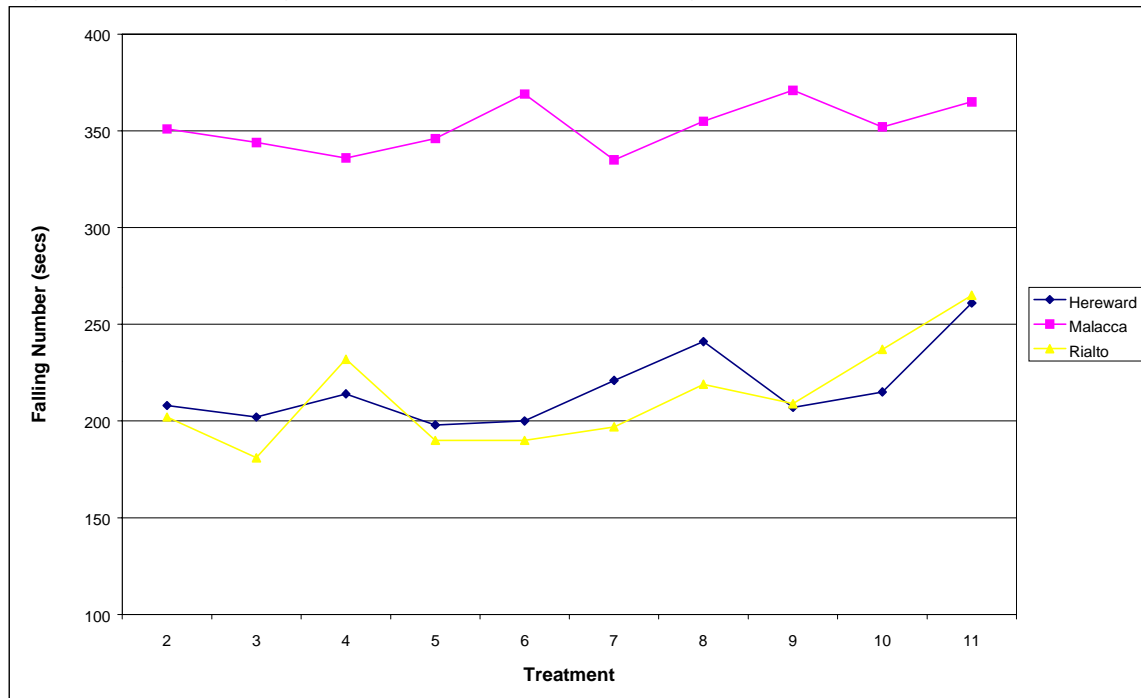
nd = not detected or below the limit of detection

Grain Quality

Wheat Hagberg Falling Number values (Figure 1) showed significant varietal effects. As expected, Malacca, a naturally high Falling Number variety (NIAB, 2000) produced markedly higher Falling Number values compared to Hereward and Rialto under the difficult harvesting conditions of 1999. Values for Malacca were in the region of 335-371s for all of the fungicide treatments. Thus, any differences observed were within the errors of the test and fungicide treatment was found to have no significant effect on Falling Number in this variety. Hereward and Rialto exhibited Falling Number values within the range 198-261s and 181-265s respectively i.e. both varieties produced samples that would be rejected at mill intake where a cut-off value of 250s is normally used. These varieties are more susceptible to increases in *alpha*-amylase activity when poor harvesting conditions prevail. For the more vulnerable Hereward and Rialto, treatment 3 (a conventional strobilurin treatment), treatment 5 (a three quarter rate strobilurin treatment) and treatment 6 (an early strobilurin treatment) produced the lowest Falling Number values. Treatment 11 (a 2-spray mixed triazole) produced the highest Falling Number.

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Figure 1. Effect of fungicide treatment on wheat Falling Number



Specific weight, Figure 2, showed marked varietal differences. Average specific weights were ~81kg/hl for Hereward, 77.5kg/hl for Rialto and 75.5kg/hl for Malacca i.e. both Hereward and Rialto produced specific weights above normal mill intake criteria of 76kg/hl. Against this background, fungicide treatment produced little variation in specific weight in either Hereward or Rialto. In contrast, fungicide treatments produced significant differences in specific weight in Malacca. In particular, treatments 10 and 11 produced unacceptably low specific weights with the specific weight of Malacca, grown under fungicide regime No. 11 (a non-strobilurin treatment), significantly lower than other treatments. However, there was no evidence that either treatment was less effective in disease control, which would cause shrivelling of grain and hence result in reduced specific weight.

Results of protein measurements by the Dumas combustion method are presented in Figure 3 (NIR data showed the same trends). As expected, there were clear varietal effects on protein content with Hereward>Rialto>Malacca. There was no overall significant effect of fungicide treatment on protein content. The largest differences between fungicides were observed for Rialto between treatment Nos. 2 or 11 and treatment No. 10 where differences of approximately 1 % were observed. Treatment 11 (a 2-spray non-strobilurin treatment) produced significantly lower grain yield with consequent effect on protein concentration. Treatment 2 (a 3-spray triazole) yielded 0.5t/ha higher than treatment 10 (experimental strobilurin treatment) at the Gwent site only. This combination of high yield and high protein content under treatment 2 cannot be confirmed at any other site.

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Figure 2. Effect of fungicide treatment on grain specific weight

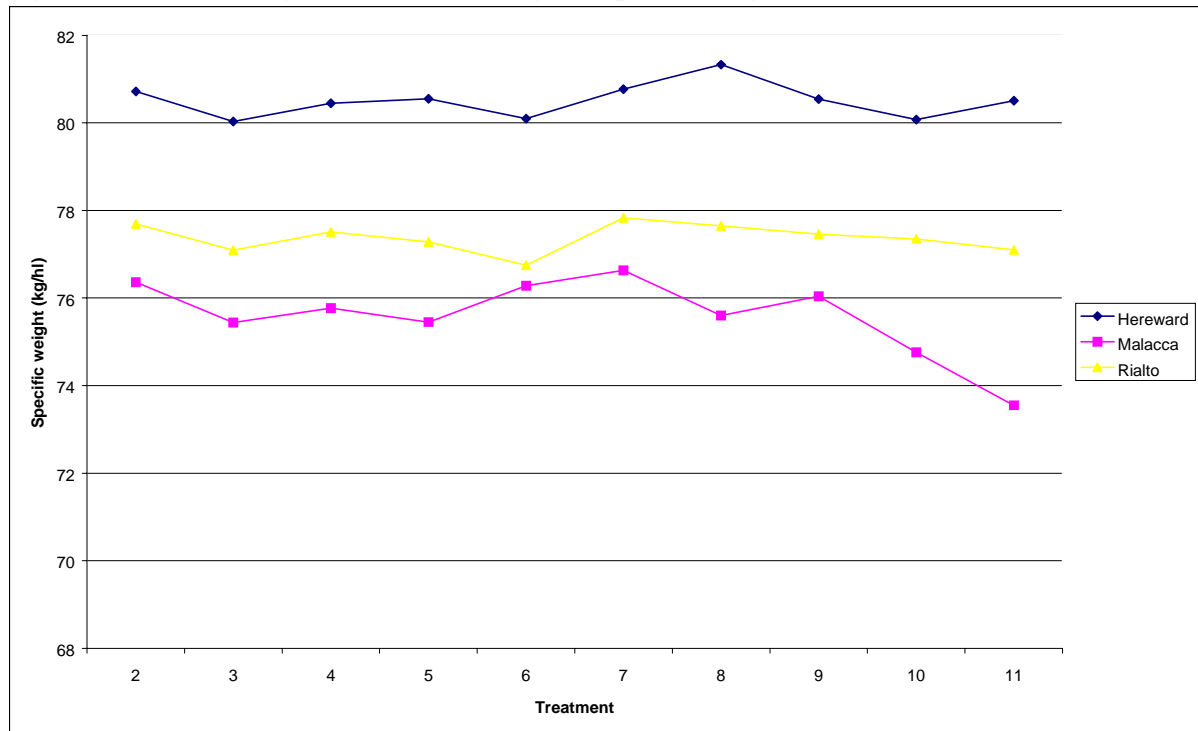
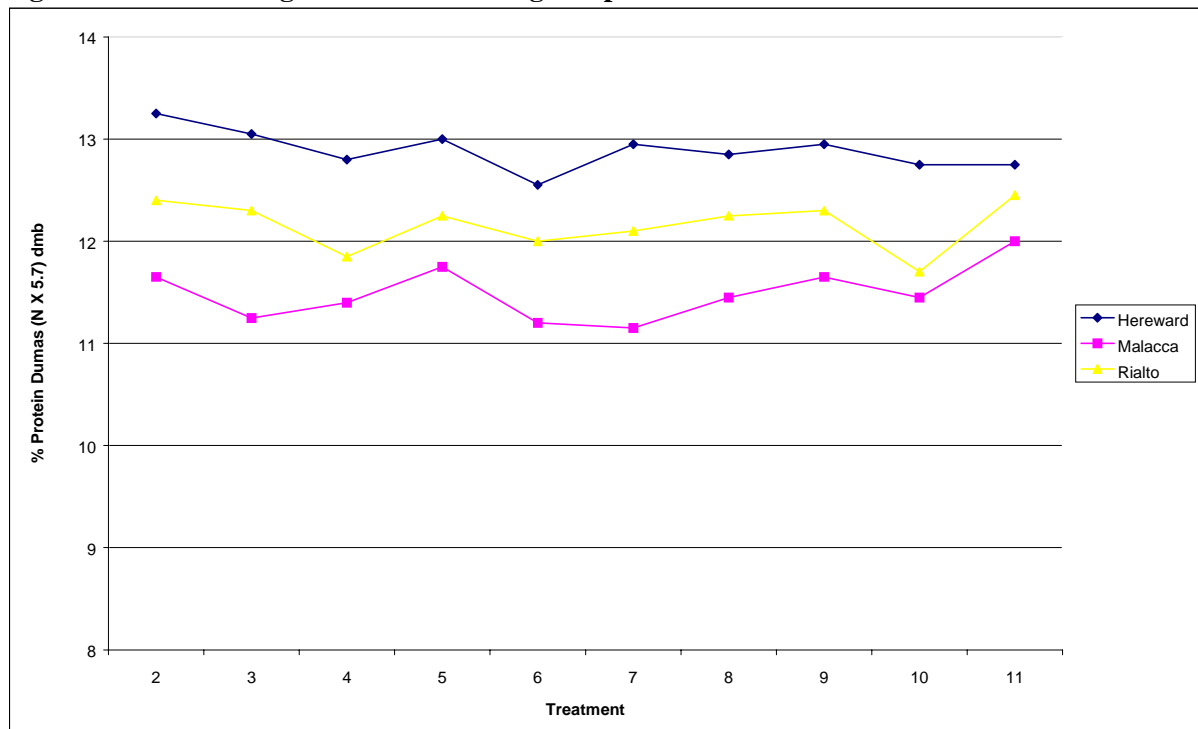


Figure 3. Effect of fungicide treatment on grain protein content



Milling performance

Milling performance was assessed by calculation of the total percentage extraction rate of white flour (based on products) and the percentage straight-run white flour (i.e. white flour from the break and reduction passages of the mill before addition of bran finisher flours). Results are presented for straight-run extraction rate (Figure 4) and total extraction rate (Figure 5), both on a total products basis.

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Figure 4. Effect of fungicide treatment on straight-run extraction rate (products, basis)

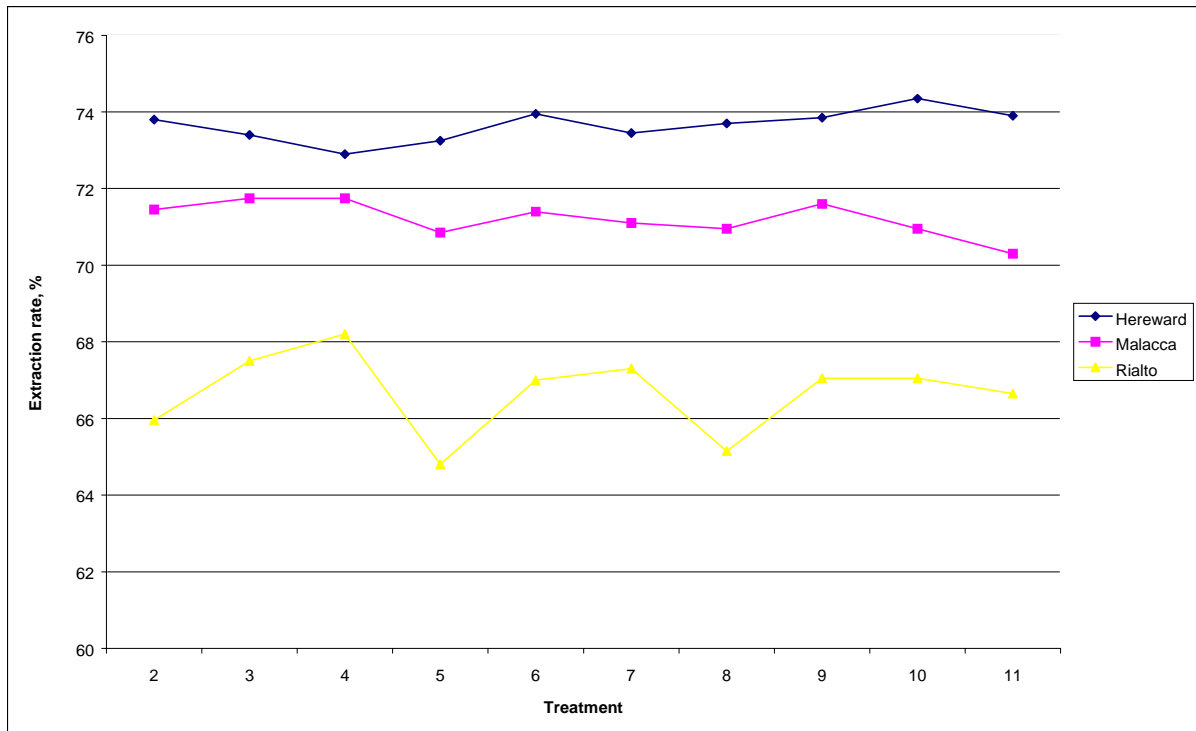
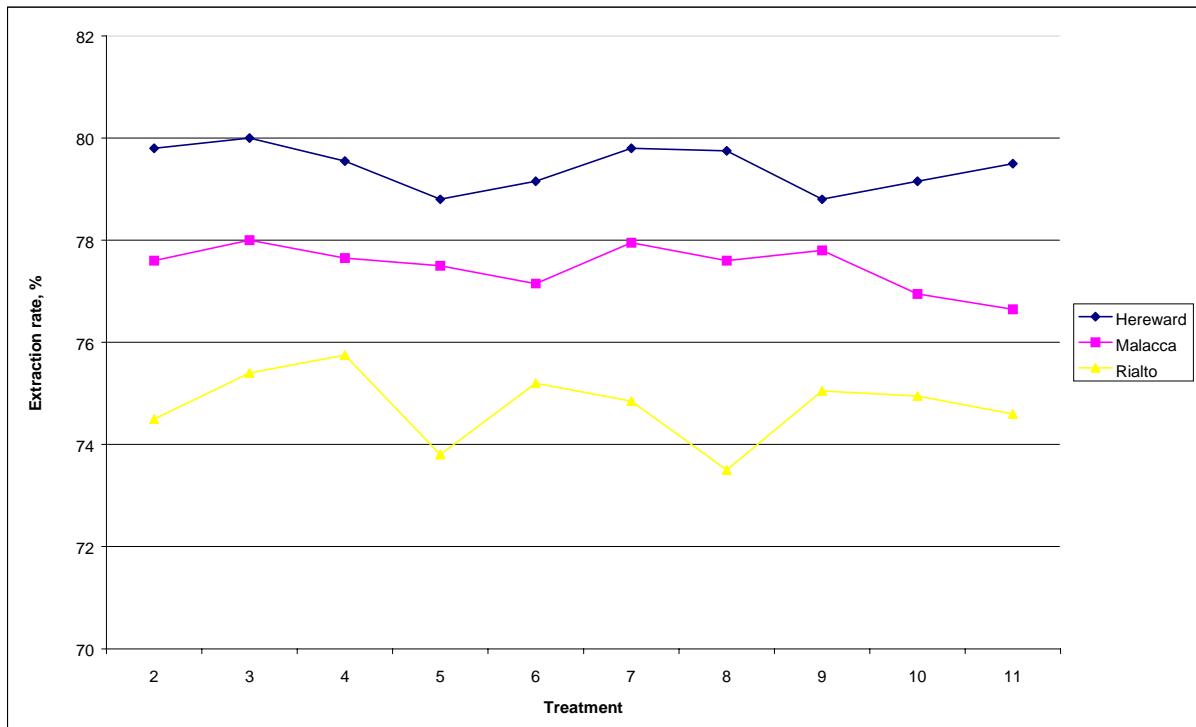


Figure 5. Effect of fungicide treatment on total extraction rate (products basis)



Significant varietal effects were evident in both measures of white flour extraction. Within this trial Hereward produced the highest straight-run and total extraction values, followed by Malacca with Rialto producing unacceptable yields of white flour under this fixed milling system. When total flour extraction results from this trial are compared with the averages obtained for these varieties in the 1999 Recommended List trials, the same ranking order is observed and close agreement obtained for both Hereward and Malacca. For Rialto, total flour extraction is nearly 3% below average and a

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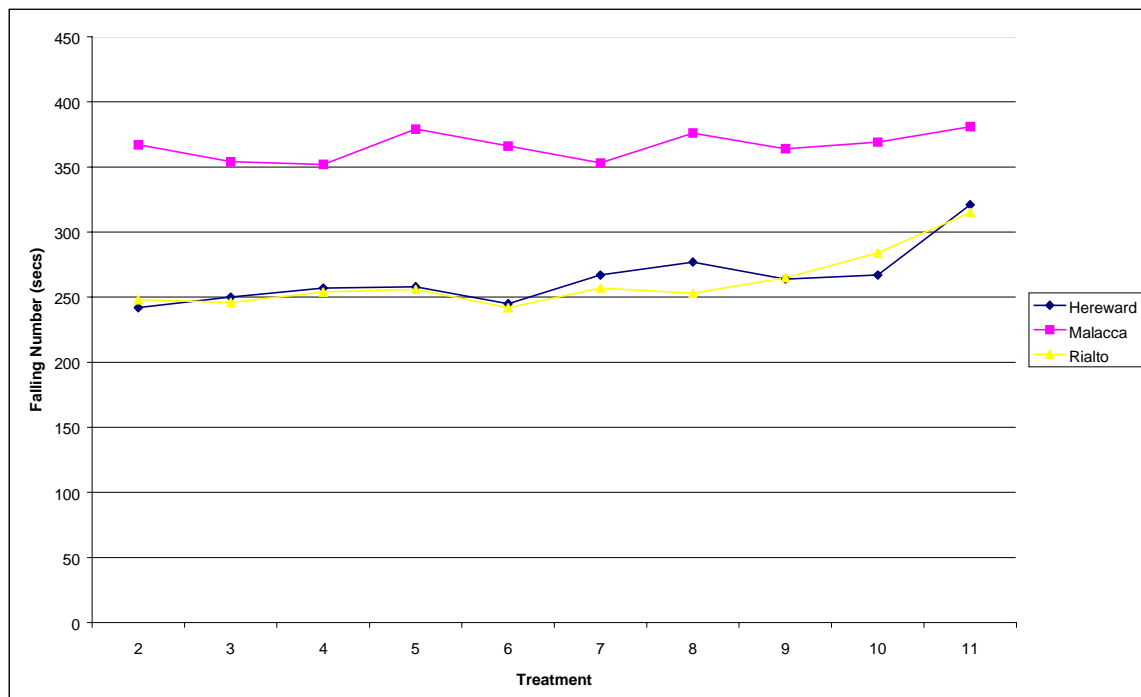
significantly higher amount is recovered during bran finishing (i.e straight-run yields are nearly 8% less than total extraction rates for Rialto compared with 5-6% for the other varieties under test). Trends indicated that extraction rates were lower under fungicide regimes 4 (a full rate strobilurin treatment) and 8 (an experimental strobilurin treatment). However, there was no common active ingredient to explain this effect. In particular, extraction rate was significantly lower under treatment No.8 compared with treatments 4 and 9 for Rialto only. Since results of intake testing did not indicate a reduction in grain size under this treatment it is difficult to explain this very low flour yield figure.

Flour Quality Evaluation

Results are presented for both chemical and rheological measurements carried out on white flour samples. Where results are not presented graphically, they are summarised in Table 4 which also included levels of significance observed.

Flour Falling Number data confirmed consistently high values for Malacca compared to Hereward and Rialto (Figure 6) and insignificant differences between treatments in this variety. All values for Malacca were above 350s and therefore well above a normal bread flour specification of around 250s. Flour Falling Number results for Hereward and Rialto were very similar and basically mirrored results observed for wheat. Interestingly, Flour Falling Number values for these two varieties showed a shift upwards compared with the corresponding wheat values (compare Figures 1 and 6). This suggests that for the more sprout susceptible varieties, milling fractions that are discarded in the production of white flour (i.e. bran and offal in the standard milling process used in this exercise) contain elevated levels of *alpha*-amylase resulting in reduced enzyme activity in the final milled product. Treatment No.11 (a 2-spray triazole) resulted in the highest Falling Number value, above 300s, for both Hereward and Rialto. This was significantly higher than Treatments 2 (conventional triazole), 3 (conventional strobilurin) or 6 (an early strobilurin treatment).

Figure 6. Effect of fungicide treatment on flour Falling Number

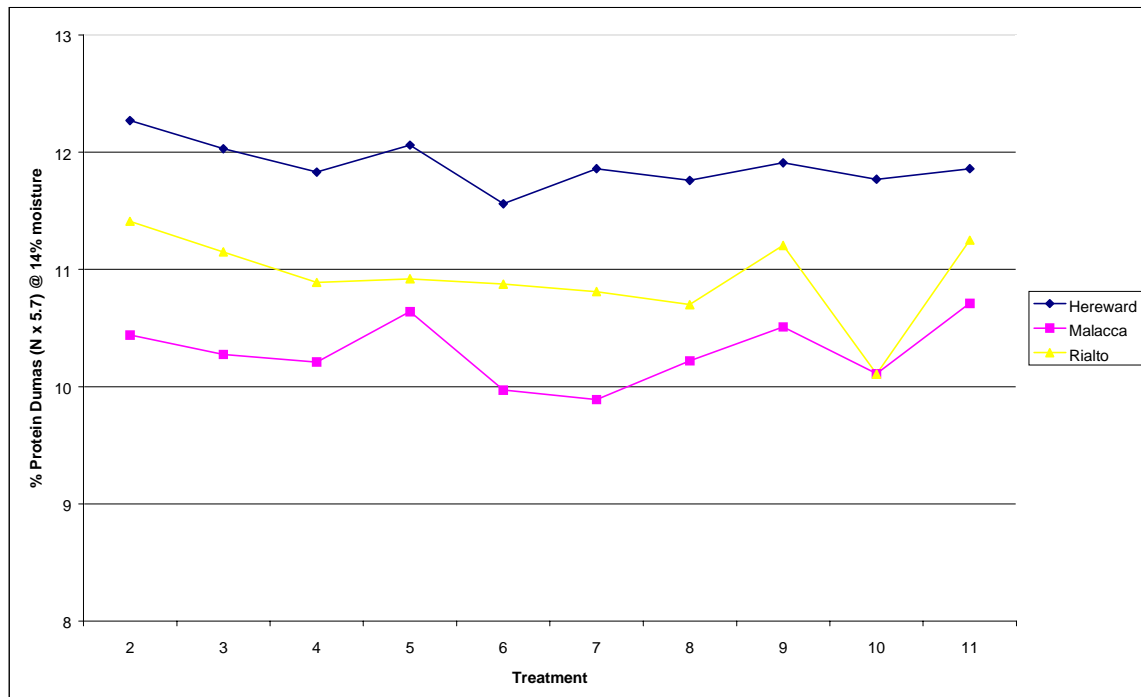


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Flour protein results (Dumas combustion method, Figure 7), confirmed the ranking Hereward>Rialto>Malacca observed in grain intake data. In addition, the same basic trends were observed for individual treatments. Treatment 10 for Rialto resulted in a significant reduction in protein that was also observed in flour tested under NIR methodology (data not shown) and to a lesser extent for wheat protein measurements. This confirms an effect of the Gwent site mentioned earlier. A decrease of nearly 1% occurred under this treatment compared with the average Rialto flour protein value. Treatment 10 did not produce a significant reduction in flour protein content either of the other varieties tested, suggesting a variety specific response for Rialto.

Flour protein levels were consistently above 11.5% for Hereward with little variation between treatments, whilst for Malacca, the average flour protein content was ~ 10.2%, the lowest of the three varieties.

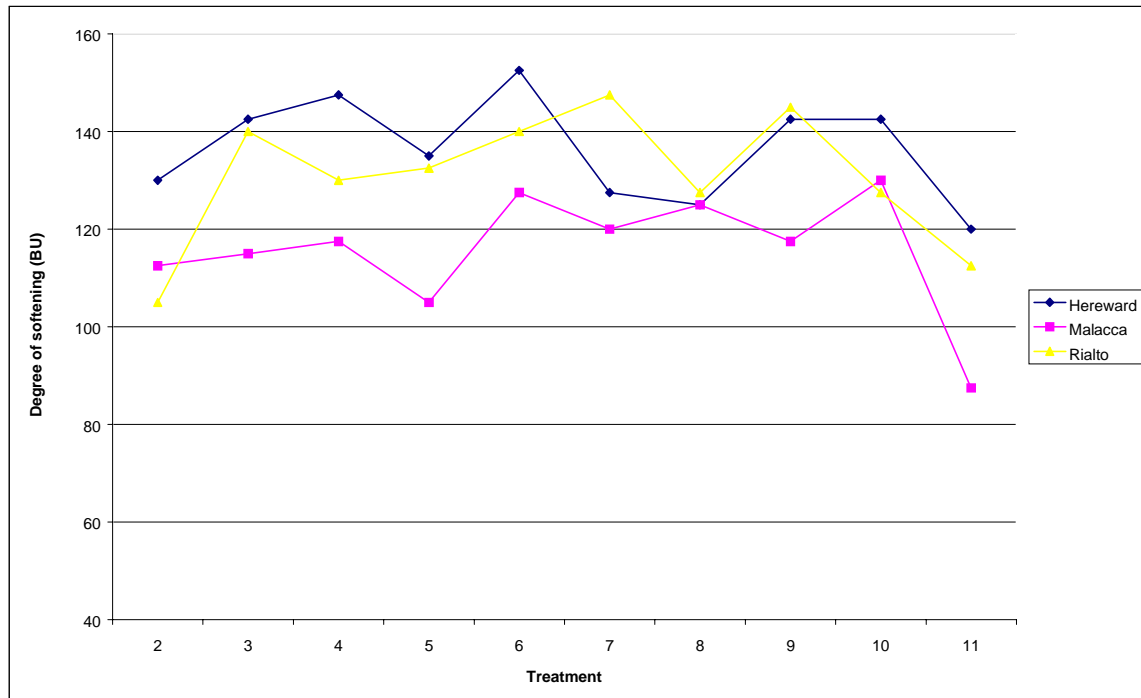
Figure 7. Effect of fungicide treatment on flour protein content



The rheological properties of dough were assessed by Farinograph and Extensograph measurements. Brabender Farinograph evaluation provides four separate parameters: water absorption (WAb), dough development time, stability and degree of softening (DOS). Since stability and DOS basically provide information on the same quality attribute i.e. tolerance to continued mixing only DOS is discussed here.

Farinograph water absorption, presented in Figure 8, is indicative of the amount of water taken up by the flour to create a dough of a fixed consistency. The higher the amount of water absorbed the greater the subsequent yield of bread. High flour water absorption is also preferred for UK style sandwich bread. Water absorption values (600 line) ranged from just over 55 to just over 61% for all three varieties. Hereward and Rialto produced higher water absorption values compared to Malacca, partially reflecting differences in protein content. Whilst differences in water absorption within a variety were often large, there was no pattern to the variation and, hence, fungicide treatments had no significant effect on this parameter. Measured water absorption was generally below that expected for a commercially-milled bread flour, primarily due to differences between the milling processes used.

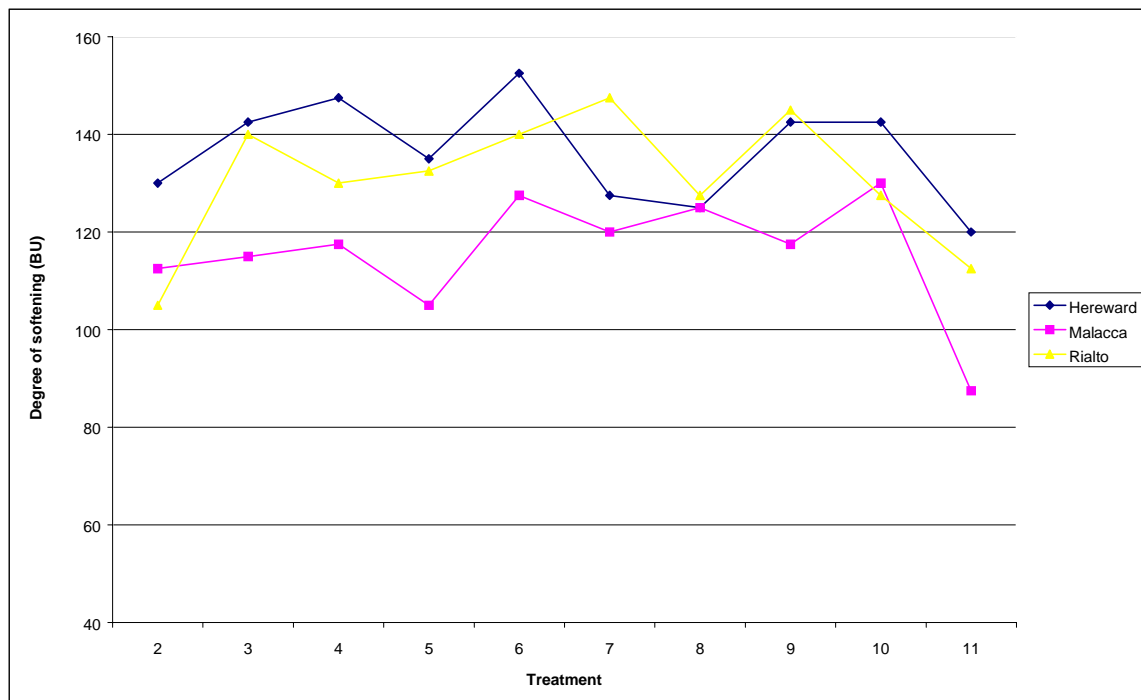
Figure 8. Effect of fungicide treatment on Farinograph water absorption



Farinograph development time indicates the time taken to obtain an optimally mixed dough under the gentle mixing conditions employed in the Brabender Farinograph and thus provides an indication of final mixing requirements during the high-speed mixing of the CBP baking test. For this parameter, varietal differences were very significant i.e. Hereward had consistently higher dough development times than Malacca and Rialto. Variations between fungicide treatments were also greatest for Hereward at between 0.5-1minute. Such differences are within the errors of the test and overall no significant differences between fungicide treatments were observed (see Table 4.)

Degree of softening values (DOS), Figure 9, provide an indication of the strength or tolerance of the dough to continued mixing: high values indicating dough weakness. As for Farinograph stability, which similarly records dough strength, very significant effects were observed between varieties with lesser effects due to individual fungicide treatments (see Table 4). Values were lower for Malacca indicating a stronger dough compared with either Hereward or Rialto. For Hereward and Malacca, the pattern of protein strength, as measured by DOS, was similar for all treatments except 8 and 9 (experimental strobilurin treatments). The picture for Rialto, a variety bred to have unusual dough mixing properties is rather different and more erratic. However, across the three varieties, treatments 2 and 11 (3 and 2 spray triazoles respectively) produced some of the lowest DOS values. These treatments coincide with some of the highest flour protein contents, suggesting that these specific fungicide regimes may produce the best combination of protein quantity and quality. The reduction in DOS is most pronounced under treatment 11 in Malacca that is likely to have had a major impact on the observed significant effect of fungicide treatment on DOS. In general, DOS values were higher than would be expected for a commercial CBP bread flour. The white flours in this study were milled using a laboratory Bühler mill and do not contain any added nutrients or oxidising agents. In particular, the latter would have significant effect on dough strength.

Figure 9. Effect of fungicide treatment on Farinograph degree of softening



Results of Brabender Extensograph measurements are presented in Figure 10 as the ratio of Extensibility to Resistance (100 E/R). This provides an indication of the balance between these rheological properties. Results for the individual parameters are provided in Table 4. Once again, varietal differences were very significant and totally outweighed any differences resulting from any fungicide treatment. Hereward produced consistently higher 100E/R values and the largest difference between individual fungicide treatments. Extensograph 100E/R ratios were very similar for Rialto and Malacca i.e. stronger and less Extensible than Hereward. Whilst there was a general trend towards reducing 100E/R values across treatments 2 – 11 and the largest differences between fungicide treatments were observed for Hereward, no significant differences between fungicide treatments were found.

Gel protein weight, (Figure 11), is a measure of the amount of functional glutenin protein in a wheat sample. Modern breeding techniques, as used to develop Rialto (Payne, 1993), involve combination of the 1B/1R chromosome translocation which produces weak mixing properties with a sequence of HMW-glutenin subunits which impart strong mixing characteristics varieties. As a consequence, this parameter has lost some of its value as a predictor of breadmaking quality. Values obtained confirm previous experience of the three varieties ex 1999 harvest with Rialto producing average gel protein weights of around 7g/5g flour whereas the Malacca and Hereward have average gel protein weights of around 9 and 11 g/ 5g flour respectively. For Hereward and Malacca, there was a tendency for gel protein weight to decrease across the range of applied treatments, such that the amount of gel protein was around 2g/5g flour higher in treatment 2 (a conventional triazole treatment) than in treatment 11 (a 2-spray triazole). This suggests more functional protein for the 3-spray triazole compared to the 2-spray, for these two varieties. However, across the three varieties under test there were no significant differences in gel protein weight.

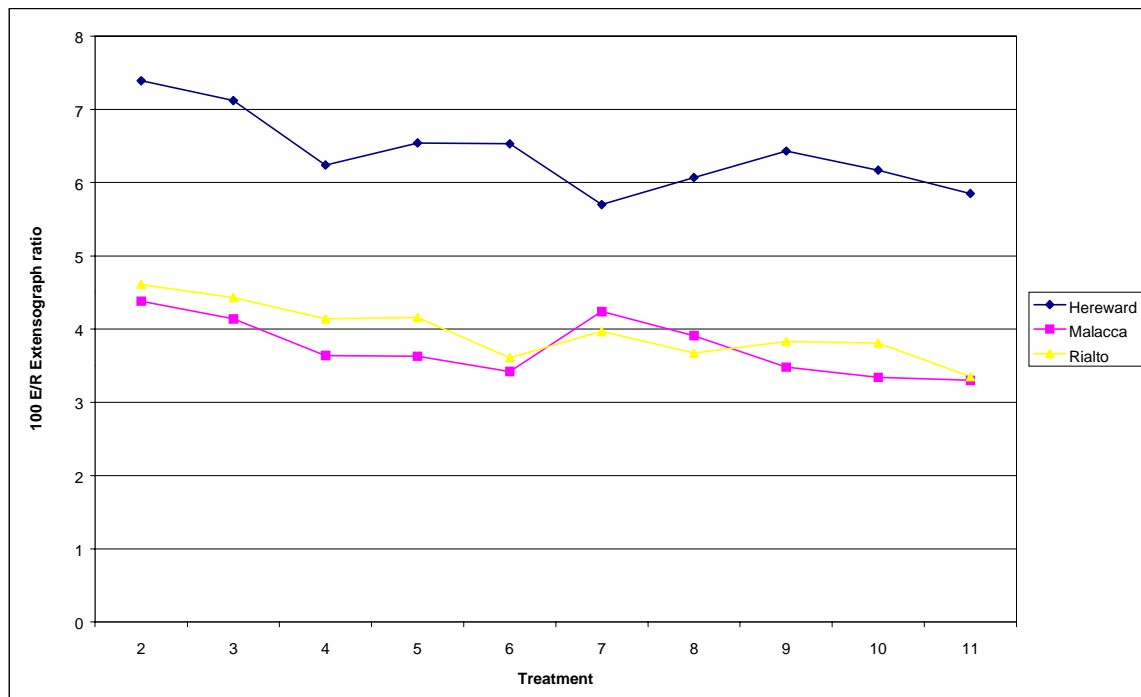
The quality of the gel protein extracted is illustrated in terms of protein strength or elastic modulus (G') in Figure 12. The dominant, strong mixing characteristics of Rialto from the 1999 harvest are evident. Average G' value of 70Pa were recorded for this variety compared with 28 and 24Pa for Hereward and Malacca respectively. The values obtained for Rialto are particularly high for samples

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from the 1999 harvest but are typical of the variability in protein strength associated with this variety. There were minimal differences between fungicide treatments for Hereward and Malacca. Larger differences between fungicide treatments were observed for Rialto; differences of approximately 20% for treatments 8 and 10 (experimental strobilurins, low G') compared with treatments 9 and 11 (experimental strobilurin and 2-spray triazole respectively, both high G'). These four treatments also produced the lowest (8 and 10) and the highest (9 and 11) protein content in the Rialto trial series. These combinations of low protein content plus reduced protein strength and high protein content combined with high protein strength may be expected to have a significant impact on breadmaking quality.

It is interesting to note the inconsistency between the empirical (Farinograph and Extensograph) and more fundamental (gel protein) approaches to measurement of protein strength. Degree of softening (DOS) and 100E/R data suggests that Malacca, with lower DOS and 100E/R, is stronger than Hereward and more similar to Rialto whereas gel protein G' clearly shows Rialto as the strongest variety of the three in this trial and Malacca as the weakest. The predictive ability of the latter appears to be borne out by assessment of breadmaking quality suggesting that the combination of amount and quality of functional protein is more relevant to performance in a CBP process.

Figure 10. Effect of fungicide treatment on Brabender Extensograph 100E/R values



Breadmaking quality assessment

Performance in a standard 800g four-piece CBP baking test was assessed by loaf volume (ml) and visual assessment of crumb score.

As for other quality measures, variety was the overriding factor affecting CBP loaf volumes. In this trial the variety Rialto outclassed Hereward in terms of CBP loaf volume whilst Malacca performed below par with an average loaf volume of around 2700ml approximately 800ml lower than Rialto (see Figure 13). This value is significantly below expectations for this Group 1 variety and reflects the combination of low protein content and protein quality observed in this trial. In this trial, the Group 2 variety Rialto produced the best loaf volume. Whilst there was a trend within Rialto and Malacca for loaf volume to decrease between treatments 2 and 11, there were no significant differences between fungicide treatments for any of the three varieties. Protein content and quality data indicated the

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maximum differences occurred between Rialto grown under Treatments 8 and 10 compared with 9 and 11. Such differences failed to have a significant effect on loaf volume in a CBP process. High speed mixing, as used in CBP, is used to produce the majority of commercial white bread in the UK. Amongst the advantages of the CBP process is the ability to produce more consistent product quality using a slightly lower quality flour i.e. removing the requirement for high protein, strong Canadian wheat in the grist (Cauvain, 1998). Thus, the CBP process for white sliced bread, while representing the major sector of the market is less sensitive to small changes in protein quality.

Figure 11. Effect of fungicide treatment on gel protein weight

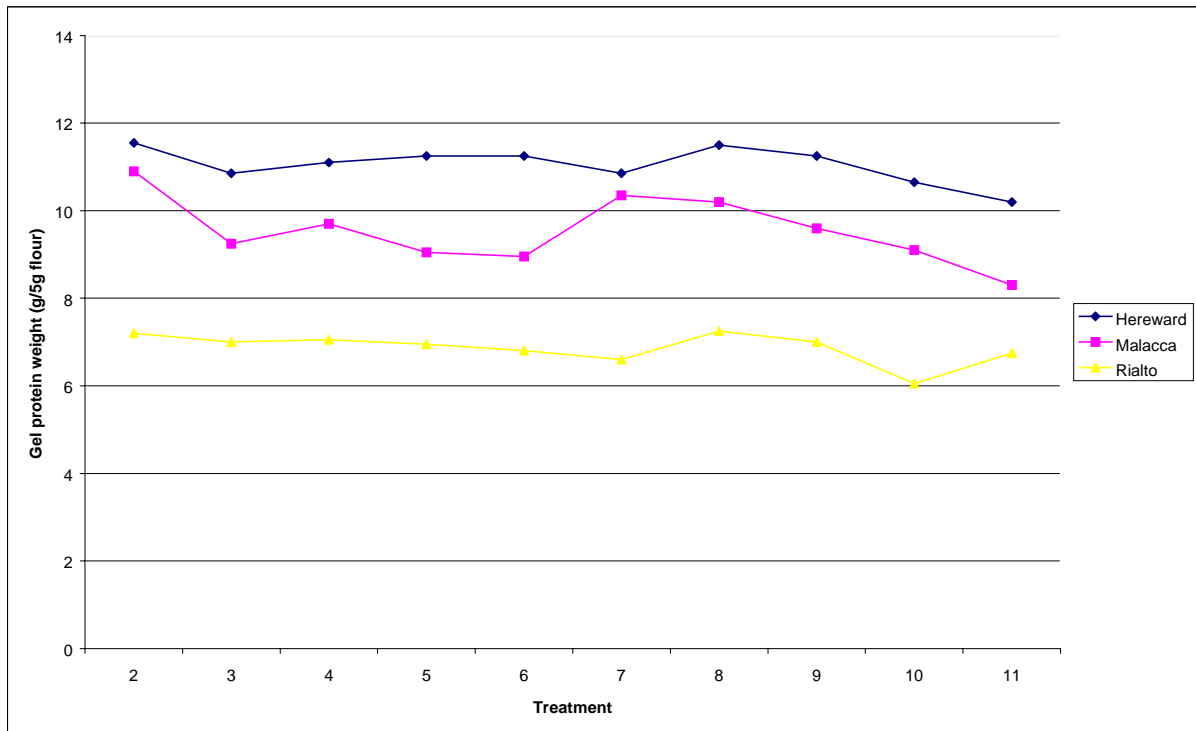
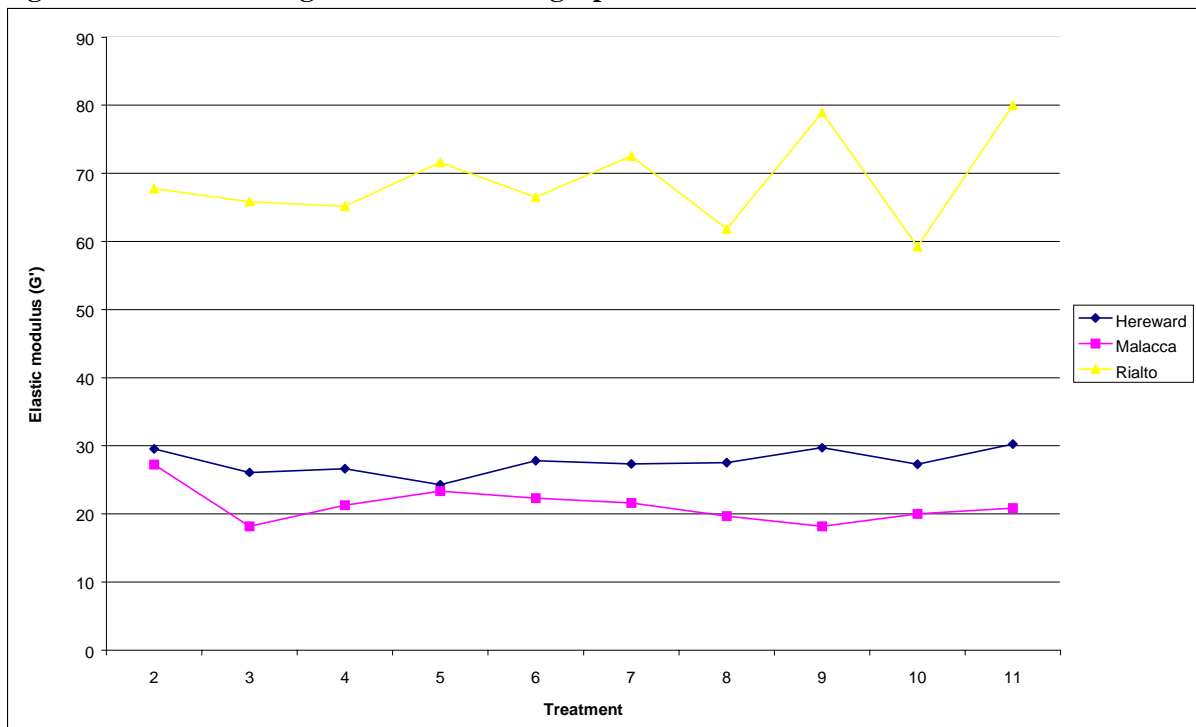
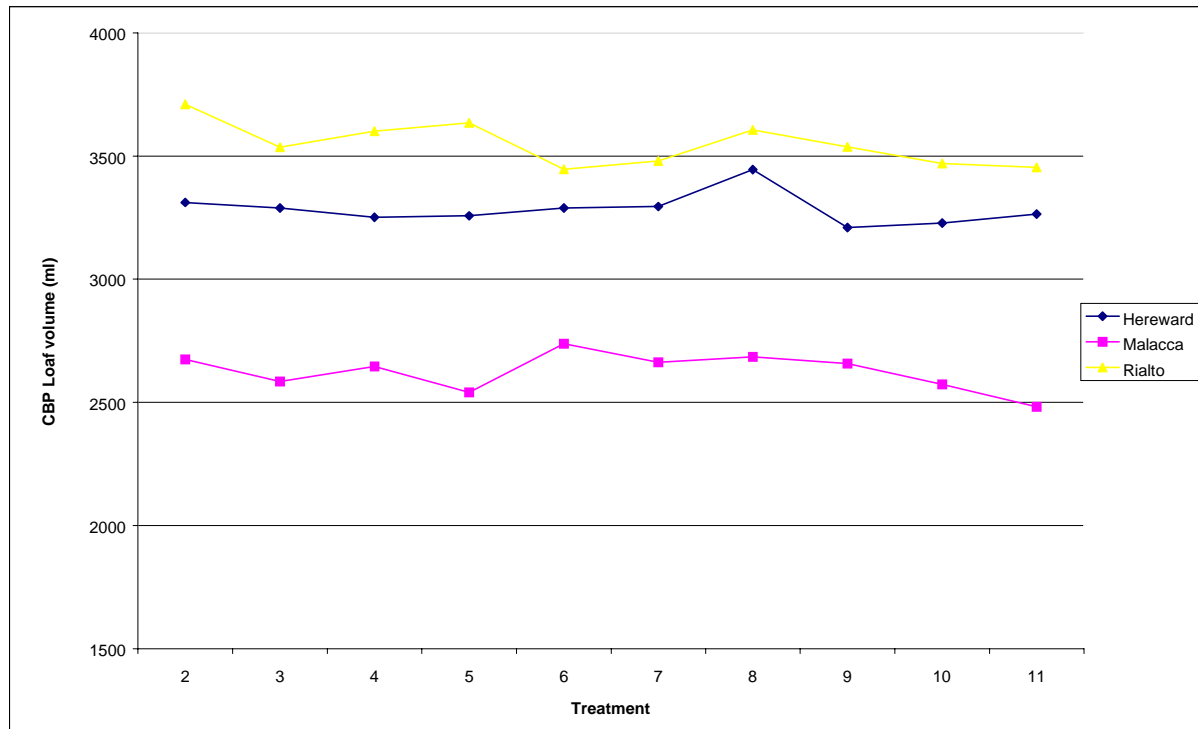


Figure 12. Effect of fungicide treatment on gel protein elastic modulus



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Figure 13. Effect of fungicide treatment on CBP loaf volume



Average crumb score varied between 6 and 7.5 for all varieties and treatments. Therefore crumb structures for all three varieties were classed between acceptable and good. Minor variations within this range made it difficult to establish consistent trends and there was no significant difference between fungicide treatments for any one variety (Table 3).

Contaminant testing

Ochratoxin A was not detected in any of the wheat samples tested (the limit of detection is 1µg per kg or 1ppb).

Total viable counts (TVC) data are presented on a logarithmic scale. Rialto generally produced higher TVC values than Hereward or Malacca (Figure 14). In addition, treatments 4 and 10, a full rate strobilurin and an experimental strobilurin application respectively, yielded lower TVC values than the remaining treatments for Rialto. Data for these treatments were more similar to the other two varieties. Both of these treatments had good control over *Septoria tritici*, the dominant foliar disease at the Gwent site. Taking all varieties into consideration, there was no significant difference in TVC values between fungicide treatments.

Yeast activity in all of the wheat samples from this trial was <5 colonies per gram i.e. below the reporting limit, and as such is not presented.

As for TVC, mould results are presented on a logarithmic scale in Figure 15. Taking all varieties into consideration, there was no significant difference in mould values between fungicide treatments. As for TVC counts, Rialto generally produced higher mould activity than either Malacca or Hereward and treatments 4 and 10 resulted in reduced mould counts in this variety. Within the varieties Hereward and Malacca, mould counts varied considerably between treatments. In particular, treatments 4 and 8 produced above average mould levels. Field data suggests that these treatments could not be separated in terms of their effectiveness against *Septoria tritici*, with both treatments exerting good disease control. These variations may reflect growth conditions in the field, or the predisposition of a particular variety to microbial contamination, or a particular field or storage fungus rather than the effects of fungicide application. Rialto is described as having moderate all-round disease resistance (NIAB, 2000). In particular, resistance to yellow rust, brown rust and *Septoria*

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nodorum is better in Malacca and Hereward than in Rialto. Bayles (1999) suggested that the benefit of strobilurin application was least for low yielding varieties that already possess good resistance to fungal disease. Rialto is an example of the opposite: a high yielding variety that does not possess the best disease resistance profile. Therefore, it is not surprising that particular treatments may have a significant impact on this variety.

Figure 14. Effect of fungicide treatment on Total Viable Counts (TVC)

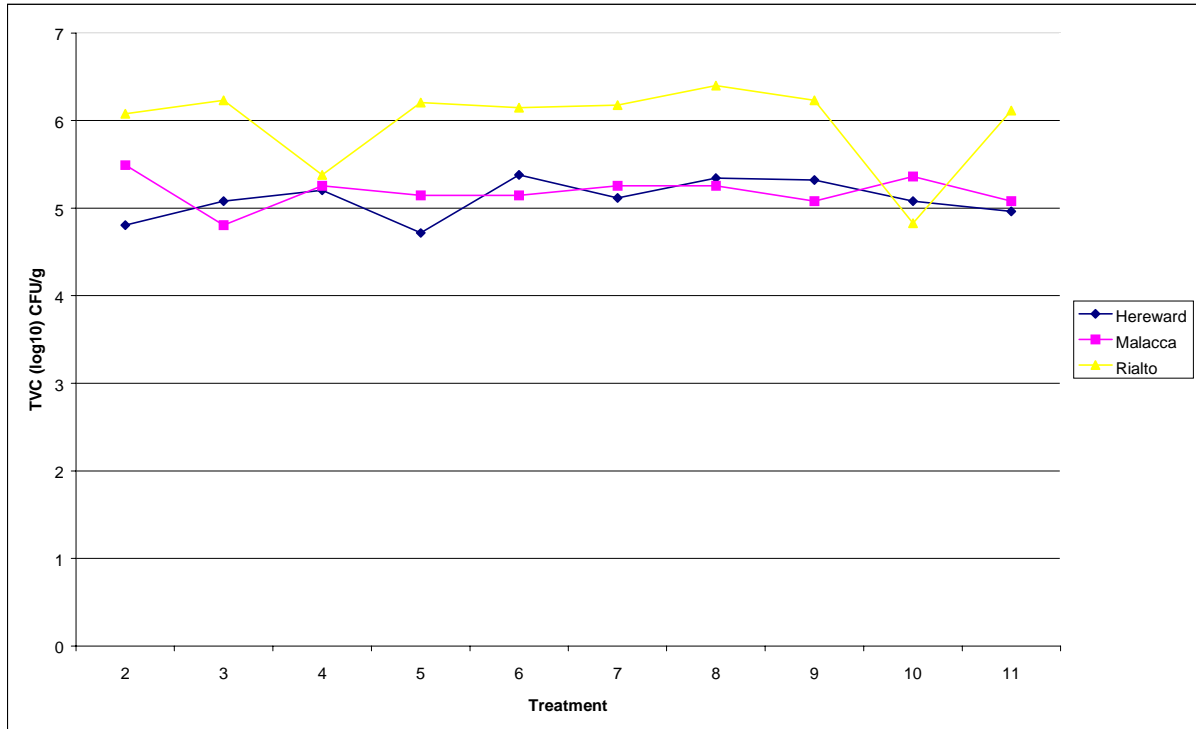
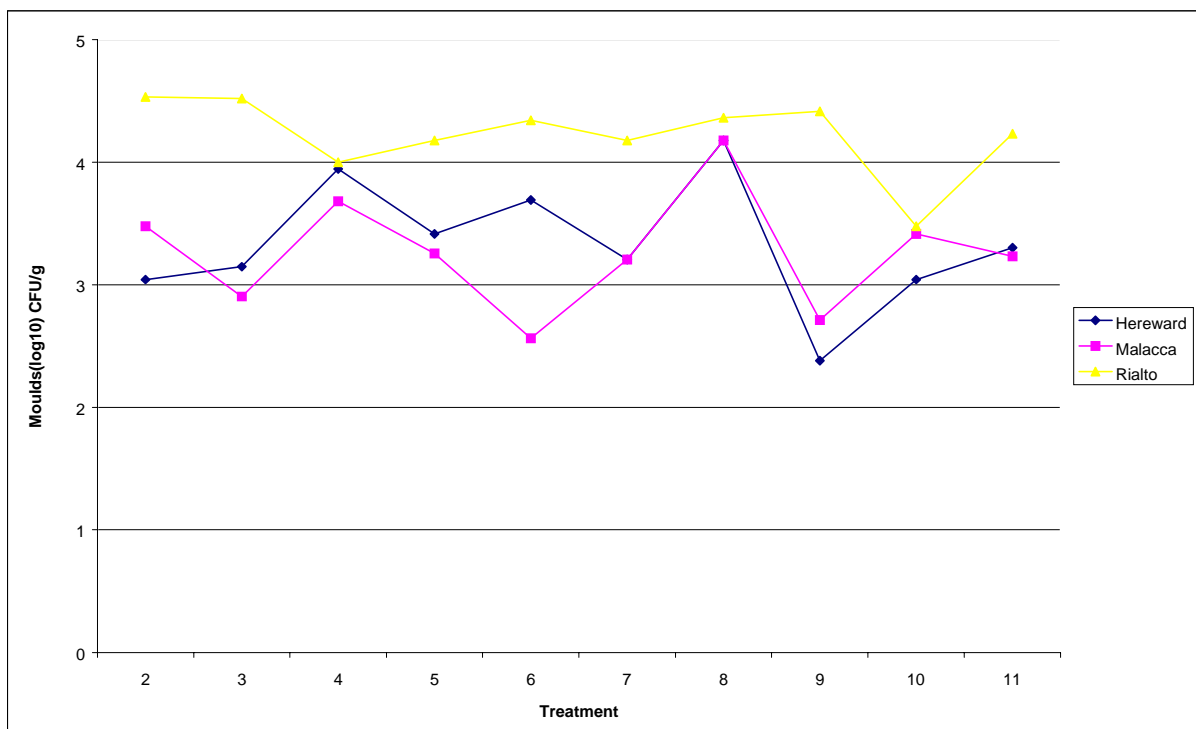


Figure 15. Effect of fungicide treatment on mould counts

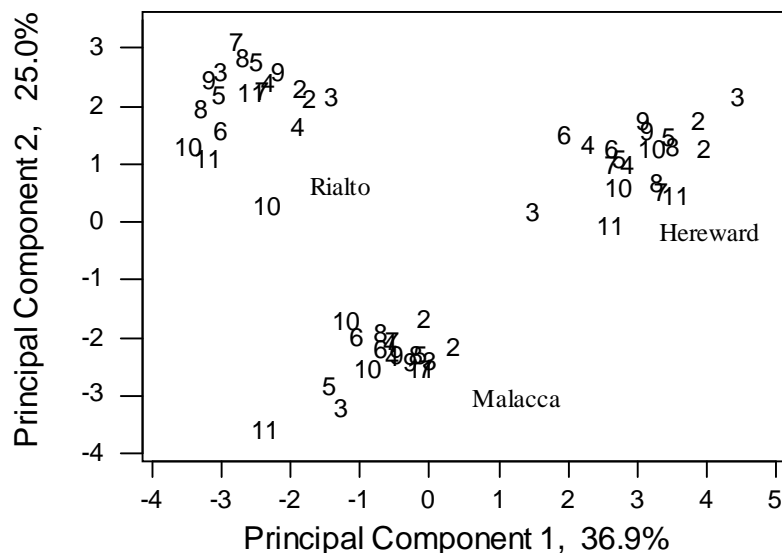


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Principal Component Analysis

Principal component analysis (PCA) was also carried out on all of the data gathered from flour quality assessment, in order to determine the relative responses of each variety to the fungicide treatments applied. A plot of this data, showing both treatment replicates, is presented in Figure 16. PCA suggests that the majority of variation observed across the data sets for all of the responses, can be correlated directly with variety, as opposed to differences caused by fungicidal application. Overall, no consistent picture in relation to fungicide treatment emerged. In some cases, variation between replicates was large e.g. in Hereward treatment 3 (the conventional strobilurin application) and this resulted in little significance between treatments.

Figure 16. Principal Component Analysis Plot of flour quality characteristics



DISCUSSION

Treatment 3 (a conventional strobilurin/triazole application) gave the highest mean yield across the three varieties examined in this study. Treatment 11, a two-spray non-strobilurin treatment, gave the lowest yield and this would suggest a yield advantage for the strobilurin-based control treatment. However, treatment 2 (a 3-spray triazole) yielded 0.5t/ha higher than treatment 10 (experimental strobilurin treatment) at the Gwent site only.

Generally, the majority of the variation in results of quality testing employed in this study, could be attributed to variety rather than effects of fungicide treatments. However, certain measurements and trends within data did reveal differences between treatments.

Intake testing did not illustrate any clear trends between fungicide treatments. Measurement of Hagberg Falling Number (HFN) showed the majority of fungicide treatments for Hereward and Rialto, produced values below 250s. Many of the samples of these vulnerable varieties would therefore be rejected at intake due to potential processing problems i.e. sticky crumb in baked products. The generally poor HFN results are indicative of the poor harvest conditions prevailing in 1999 (premature maturation, wet harvest) as opposed to fungicide treatments *per se*. This is confirmed by the problems experienced in site selection for this study. Treatment 11 yielded the highest HFN values for both Hereward and Rialto and these were significantly different from the majority of other treatments used. It should be noted that treatment 11 was a two spray triazole treatment and did not

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contain a strobilurin element and therefore suggests a beneficial response in terms of *alpha*-amylase levels (i.e. less *alpha*-amylase) which may be particularly important in sprout susceptible varieties particularly when harvesting conditions are difficult.

Trends for protein content (NIR and Dumas methods) suggested a decrease in protein content for Rialto/treatment 10 compared with treatments 2 and 11. This reduction in protein content was not exhibited by either Hereward or Malacca which are lower yielding varieties with better in-bred disease resistance. The fact that strobilurins are known to act by delaying senescence and thus permitting continued photosynthesis which leads to continued production of starch, dilution of grain protein content and increases in grain yield. However, any three-spray treatment with a suitable fungicide (triazole or strobilurin) could produce a later optimal harvest date than a two-spray treatment, due to the enhanced disease control. Reduction of protein content has been a major quality concern for the milling industry associated with strobilurin use. The consistent decrease in protein content for Rialto and fungicide treatment 10, suggests a treatment effect of this type, and it could be that these treatments are responsible for the deleterious effect on protein level. An effect of this type could be ameliorated by the use of suitable nitrogen treatments, e.g. foliar urea, to boost protein levels. However, this appears to be a variety specific response to this fungicide combination in the high yielding fungicide responsive Rialto.

Optimisation of extraction rates is of prime importance to the milling industry. Any fungicide treatment that has an adverse effect on this parameter would therefore be deleterious to mill efficiency. Generally, there were no overall significant differences in extraction rates between fungicide treatments. However, within the variety Rialto a significant decrease was indicated for treatment 8 compared to treatments 4 and 9. The very low extraction rates observed for Rialto grown in this trial would be a cause for significant concern to millers and treatment 8 produced totally unacceptable milling yields. No explanation for this effect of treatment 8, a mixed triazole and experimental strobilurin treatment, on flour extraction rate could be identified.

Flour quality tests of Falling Number and protein content generally reflected previous observations for wheat. For Falling Number, treatments 2-4 were significantly different from treatments 8-11. This was particularly important in the vulnerable varieties Rialto and Hereward. Flour protein content was reduced in Rialto, fungicide treatment 10.

Gel protein data, weight and elastic modulus (G'), showed strong varietal influences. When all three varieties were taken into consideration, there was little evidence of treatment effects. Within the variety Rialto, treatments 8 and 10 produced the lowest gel protein weight and elastic modulus values whilst treatments 9 and 11 produced the highest values of both parameters.

There were clear varietal differences in CBP loaf volume, with highest loaf volumes resulting from Rialto. This example of Rialto would be classified as "extra strong" on the basis of its gel protein G' values and may be expected to under-perform in a standard CBP process. However in this trial series, protein strength in Rialto was counteracted to some extent by above average *alpha*-amylase activity which has a softening effect on dough rheology. As a consequence the rheological properties of Rialto appeared to be optimised for CBP baking and good loaf volume combined with acceptable crumb score resulted. Under the trial conditions at Gwent the variety Malacca produced a combination of low protein content and low protein strength (average $G' = 21\text{Pa}$ i.e. towards the bottom of the protein quality scale for breadmaking). This combination has produced breadmaking performance below that expected for this Group 1 variety. However, fungicide treatments did not have any distinct effect on loaf volume within any one variety. Crumb scores were variable between fungicide treatments for all varieties, but differences were marginal and not significant. Thus in summary, direct comparison between treatments showed no adverse affect on the baking quality characteristics of the varieties utilised in this study. In addition, significant effects observed for both *alpha*-amylase and protein levels during flour quality testing were not evident following subsequent processing.

Ochratoxin A and yeasts were not detected in any of the bulked samples. Moulds and Total Viable Counts were detected but variation between fungicide applications was minimal and not significant

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for all varieties. It is therefore difficult to determine whether fungicide applications were having an effect on the microbial state of the grain, or whether results reflected growth conditions that increased susceptibility to microbial loading in the field. This was particularly evident in Rialto, where microbial levels were higher, but trends were not significant.

Principal component analysis of all flour quality data suggests that there is significant variation between wheat varieties but little difference between fungicide treatments i.e. within a variety. Where differences were shown to occur, they were generally variety specific (i.e. adverse quality effects on Rialto in conjunction with specific fungicide treatments), and these were not shown to exert an effect on subsequent breadmaking quality parameters.

It would seem generally that grain and flour quality were unaffected by the use of strobilurins within this study and any deleterious quality effects shown, were either not significant or could not be separated realistically from traditional triazole use. With the relatively small differences between the strobilurin treatments and the NIAB non-strobilurin standard during unfavourable harvesting conditions, it seems unlikely that strobilurins exert any negative effects on wheat quality or subsequent processing behaviour.

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