HGCA

PROJECT REPORT No. 192

# PRACTICAL TESTS OF A PROTOTYPE SCHEME FOR PRE-HARVEST PREDICTION OF HAGBERG FALLING NUMBER IN WHEAT

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# PRACTICAL TESTS OF A PROTOTYPE SCHEME FOR PRE-HARVEST PREDICTION OF HAGBERG FALLING NUMBER IN WHEAT

by

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## ABSTRACT

If Hagberg falling number (HFN) of wheat grain falls below 250 or 220 s, producers lose bread making or export premia. In the UK, low HFN is caused by four different origins of the enzyme *alpha*-amylase, which digests starch. HGCA project 0056/1/93 investigated the mechanisms of each *alpha*-amylase pathway and designed a prototype scheme for prediction of combine harvest HFN from pre-harvest HFN and germination measurements. The current project was undertaken to fine-tune the prediction scheme and assess the logistics of commercial operation compared to its use at research sites. A range of current commercial cultivars was grown at ADAS Bridgets, Aberdeen University and Harper Adams University College to allow derivation of specific HFN prediction equations and to further test the scheme. Samples from commercial crops were submitted by three crop consultants to NIAB Labtest to assess the logistics of commercial laboratory operation. Crop consultant samples were also used to assess the appropriate sampling method from the field. Methods of determining the stage of 35% grain moisture, required as a marker for earliest possible sampling, were investigated. This included a comparison of microwave and oven drying of ears, grains or milled samples and assessment of accumulated potential evapotranspiration as a marker of grain moisture. The optimum temperature of the pre-harvest germination test for assessment of sprouting risk was studied by analysis of data from the previous HGCA project as well as 1998 germination test data from research sites. Two methods of HFN prediction, either the prediction class system (developed in 0056/1/93 using pre-harvest HFN and germination data) or probability distribution functions (derived from a general HFN prediction equation without germination data) were assessed.

Due to heavy rainfall at Aberdeen, there were too few samples for derivation of significant individual HFN prediction equations for current cultivars, so a general equation derived from the previous project was used. Analysis of sample size showed that the coefficient of variation for pre-harvest HFN was reduced by sampling from five random locations within a crop, but that no further benefit was gained with more samples. Assessment of grain moisture content by microwave drying was problematical due to condensation and charring. However, oven drying of whole ears gave a good estimate of grain moisture content. A strong logistic relationship was found between accumulated potential evapotranspiration (PE) from ear emergence and grain moisture content, indicating that pre-harvest HFN sampling should not occur before 190 mm PE. Analysis of germination test data revealed different responses to temperature for different cultivars, sites and years with the possibility of optima for germination in three days below 10°C or around 20°C. Thus it was concluded that the best compromise temperature for germination testing was 15°C, although the information should be treated with care since temperature optima for sprouting in the field could be very different. Logistically, operation of the scheme proceeded smoothly, although crop consultants were concerned about the large size and time-intensive nature of pre-harvest sampling. Completion of analysis and reporting of data was possible in 4-5 days. Hand threshing and selection of grains for the germination test was the most labour-intensive process, with the three day test setting a minimum time for delivery of results at four days after sampling. Analysis of the HFN predictions made with the scheme showed the prediction class system, using HFN and pre-harvest germination data, to be about 65% accurate. Use of the probability distribution functions, without reference to germination data, gave an accuracy of about 77%, with data from this system available two days after sampling. Consequently, it seems predictions based solely on pre-harvest HFN data rather than with the time-consuming germination test would be most useful, since results could be returned more quickly and rapid re-testing of samples after significant rainfall events would be possible.

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## ABBREVIATIONS

| | |
|---|---|
| AB | ADAS Bridgets |
| ADAS | Agricultural Development and Advisory Service |
| AICC | Association of Independent Crop Consultants |
| CV | Coefficient of variation |
| DW | Dry weight |
| FW | Fresh weight |
| HA | Harper Adams University College |
| HFN | Hagberg falling number |
| HGCA | Home Grown Cereals Authority |
| MORECS | Meteorological Office rainfall-evaporation calculation system |
| NABIM | National Association of British and Irish Millers |
| NIAB | National Institute of Agricultural Botany |
| P | Probability |
| PE | Potential evapotranspiration |
| PMAA | Pre-maturity *alpha*-amylase activity |
| PrMS | Pre-maturity sprouting |
| PoMS | Post-maturity sprouting |
| RPAA | Retained pericarp *alpha*-amylase activity |
| SE | Standard error |
| UA | University of Aberdeen |
| ZGS | Zadoks growth stage |

# 1. INTRODUCTION

## *1.1. Summary of findings of HGCA project number 0056/01/93*

The previous HGCA-funded project number 0056/01/93 '*Development of an Anglo-French scheme for the pre-harvest prediction of Hagberg falling number and sprouting in wheat*' (HGCA Report No. 165, Lunn *et al.*, 1998) investigated the routes of accumulation of *alpha*-amylase activity. This enzyme is responsible for the frequent occurrence of commercially unacceptable Hagberg falling number (HFN) of UK wheat grain, due to its hydrolysis of starch.

Our research identified four different origins, or routes, of *alpha*-amylase enzyme activity, leading to low HFN, relevant to the UK cereal industries (Lunn *et al.* 1999a, 1999b, Major *et al.*, 1999). The *alpha*-amylase isozymes mainly responsible for these routes are shown in **Table 1**. In a series of field and laboratory experiments carried out from 1994 to 1997, we studied the mechanisms of each route and the environmental factors which affect each process. The aim of the research was to identify methods for prediction of *alpha*-amylase accumulation. Such a prediction scheme would allow optimisation of HFN and maximisation of returns to producers, since low HFN causes frequent loss of quality premia in the UK, especially if there is wet weather at harvest (for example, the bread making premium of £15-£30/tonne is not achieved if HFN falls below 250 s). At the start of the research, an *alpha*-amylase/HFN prediction scheme based solely on meteorological data was envisaged. The complex interaction of the four pathways of *alpha*-amylase accumulation and environmental stimuli precluded the design of an accurate model of this type and thus we evolved a two-stage sample-based prediction scheme (Kettlewell *et al.*, 1996, 1999). This system was tested using experimental plots in a research environment during 1996 and 1997, achieving an approximately 75-80% success rate for classification of combine harvest HFN from pre-harvest measurements (Lunn *et al.*, 1998). However, it was evident that further research and modifications were required before the scheme could be offered as a diagnostic test for cereal producers. Thus, this project (No. 2025) was initiated, in collaboration with the Association of Independent Crop Consultants (AICC) and the National Institute of Agricultural Botany (NIAB), to adapt the prototype scheme for commercial use.

**Table 1**: Classification of the different origins of *alpha*-amylase activity and the isozymes responsible for low HFN in UK wheat grain

| Name | Acronym | *Alpha*-amylase isozymes deposited |
|---|---|---|
| Retained pericarp *alpha*-amylase activity | RPAA | α-AMY-2 |
| Pre-maturity *alpha*-amylase activityin the absence of sprouting | PMAA | α-AMY-1 (and rarely α-AMY-2, if severe) |
| Pre-maturity sprouting | PrMS | α-AMY-1 + α-AMY-2 |
| Post-maturity sprouting | PoMS | α-AMY-1 + α-AMY-2 |

### *1.2. Objectives of HGCA project number 2025*

This six month project had five main objectives

1) Development of protocols for commercial operation of the prediction scheme. The basic protocol, developed from modification of the prototype prediction scheme (Lunn *et al.*, 1998) at this start of the project is shown in *Appendix I.* Evaluation of the logistics of sample submission was funded by AICC consultants. Laboratory operation of the tests was funded by NIAB Labtest, who retain intellectual property rights and confidentiality for the development of the scheme in their own laboratories. A recommended procedure that would allow any HGCA levy-payer to initiate their own scheme, incorporating the results of project number 2025, is included as *Appendix II.*

2) Evaluation of optimum sampling size for field scale operation (funded by AICC and NIAB).

3) Assessment of accumulated potential evapotranspiration, domestic microwave drying, or modelling with weather data as possible methods for prediction of the earliest appropriate sample time for pre-harvest HFN and germination measurement.

4) Definition of equations, with confidence limits, for prediction of combine harvest HFN from pre-harvest HFN of current commercial cultivars (Stage 1 of the prototype scheme).

4) Definition of optimum temperature for the germination test (Stage 2 of the prototype scheme).

## 2. MATERIALS AND METHODS

### *2.1. Experimental design, husbandry and sampling*

#### *2.1.1. Experimental sites*

The cultivars Hereward, Soissons, Abbot, Cantata, Malacca, Spark, Charger, Rialto, Riband and Consort, selected to be representative of NABIM Class I and II cultivars currently recommended in the NIAB list, or submitted for recommended list testing (Anon., 1998), were grown at Harper Adams University College, Shropshire (HA) and ADAS Bridgets, Hampshire (AB). At the University of Aberdeen (UA), only the cultivars Hereward, Abbot, Malacca and Rialto were grown. The cultivars were sown as triplicate plots in a randomised block split-plot design, with husbandry similar to that used in the previous HGCA project (Lunn *et al.*, 1998) to limit pests, diseases and lodging to a minimum. The dates of ear emergence (Zadoks growth stage [ZGS 55], Tottman and Broad, 1987) and anthesis (ZGS 65) were subjectively recorded for each plot. ZGS 55 was defined as the date when 50% of ears were 50% emerged from the boot and ZGS 65 as the date when 50% of ears had anthers extruded from 50% of florets. The grain moisture content of the cultivar with the median emergence date was assessed at regular intervals after about four weeks post-anthesis using the standard method described previously (Lunn *et al.* 1998). At 35% grain moisture for the median cultivar, pre-harvest samples were taken and dispatched to Harper Adams University College for processing.

#### *2.1.2. Crop consultant samples*

A range of Class I and Class II cultivars were selected from client farmers' fields from within three crop consultants' areas by the consultant. Agronomy of the fields was not standardised, but was according to the client farmer's normal practice. Sampling date was determined by subjective observation of the crop. Samples were dispatched for analysis by NIAB Labtest.

### *2.2. Operation of the HFN prediction scheme*

#### *2.2.1. Experimental sites*

At 35% moisture content of the median-emerged cultivar, a sample of approximately 400 ears (pooled from 100 ears sampled from each of four randomly chosen positions in the plot) was taken from each triplicate plot for each cultivar and was placed in plastic bags, before dispatch by next-day delivery to Harper Adams University College for analysis. The samples were analysed, and HFN predictions were made according to the experimental protocol shown in Section 2 of *Appendix I.* Combine harvest samples were taken as soon as practicable (moisture content < 15%).

#### *2.2.2. Crop consultant samples*

Crop consultant samples were taken according to the method described in Section 1 of *Appendix I* and despatched to NIAB Labtest for analysis. HFN and germination analysis and predictions were made according to Section 2 of *Appendix I.* Combine harvest samples were taken and dispatched to NIAB Labtest according to the individual farmer's schedule.

### *2.3. Determination of appropriate sample size from the field*

Multiple sampling for analysis of appropriate sample size was only possible in one 21 ha field of the cultivar Hereward (Brian Keen, Bilbury Elms) due to time and field size/cultivar limitations when sampling with other AICC consultants (Steve Cook and Andrew Beeney). Nine pre-harvest HFN samples were taken across the field using tramlines as guides to divide the field. The coefficient of variation (CV%) for the whole data set of nine pre-harvest HFN samples was calculated using GENSTAT 5 statistical analysis software (Lane and Payne, 1996). Data sets of eight, seven, six, five, four, three and two samples were then generated by randomly removing samples. This procedure was repeated eight times, generating eight data sets of eight samples, eight data sets of seven samples, *etc.* The percentage coefficient of variation was recorded for each data set and a mean percentage coefficient of variation calculated for each set of samples

### *2.4. Determination of earliest appropriate sampling time in the field*

#### *2.4.1. Microwave drying of grains, ears and milled samples*

The feasibility of using microwave drying to determine moisture content of grains and ears, and determination of the suitability of using ear moisture content to estimate grain moisture content were both assessed. Samples of 75 ears per plot from three replicate plots of the cultivar Soissons were taken. For each plot, six five-ear sub-samples were removed. Two of these sub-samples were then milled ('milled' samples) using a domestic coffee mill (Kenwood CG 100) and two were ground in a pestle and mortar ('ground' samples). The fresh weights (FW) of all the sub-samples of ears were then measured. The remaining ears were roughly hand-threshed and the resulting undamaged grains, with any adhering glumes removed, were divided into six 5 g sub-samples. As with the ear samples two of these were either milled, ground, or left as whole grains and the FW was measured. One of each sub-sample set of ears/grains was then either placed in metal moisture content tins or trays and dried at 130°C for 2 hours in a forced-air oven, or placed in Pyrex beakers and dried in 650 W domestic microwave oven (Sharp Electronics (UK) Ltd.). The dry weight (DW) of the ears or grains was then measured and the percentage moisture content determined as ((FW-DW)/FW) x 100 after drying.

#### *2.4.2. Accumulated potential evapotranspiration and grain moisture content*

Daily potential evapotranspiration (PE) data was collected from Harper Adams (Shropshire), 1994 and ADAS Gleadthorpe (Nottinghamshire), 1994-1996. PE was calculated by the Penman-Monteith method using MORECS software (Thompson *et al.*, 1981). The Gleadthorpe potential evapotranspiration data was assumed to be similar to that at Sutton Bonington, which is nearby. The PE (mm) accumulated from the date of ear emergence (ZGS 55) was compared to grain moisture content data collected in project 0056/1/93 (Lunn *et al.*, 1998), for the cultivars Pastiche, Riband, Hornet and Haven. Logistic (s-shaped) curves were fitted to the data using GENSTAT 5 curve-fitting software, with calculation of 95% confidence limits.

### *2.5. Optimisation of germination test temperature*

Isolated grain germination tests were completed after sampling at 35% moisture, as described previously (Lunn *et al.*, 1998, 1999b) and in *Appendix 1.* Tests using research site samples were

done at Harper Adams university College at 10, 15, 20 and 25°C were completed exactly as described previously (Lunn *et al.,* 1998). However, data collected from the four research sites in the final year of the preceding project (0056/1/93), using exactly the same test protocol, was also used for this analysis. Germination tests at NIAB Labtest were conducted at 15°C according to the procedures described in *Appendix 1.*

### *2.6. HFN prediction*

#### *2.6.1. HFN prediction equations for current commercial cultivars*

Pre-harvest and combine harvest HFN values from research sites (ADAS Bridgets and Harper Adams University College), sampled and analysed according to the methods outlined in *Appendix 1*, were related by linear regression using GENSTAT 5 software, to derive HFN prediction equations. Aberdeen University samples were excluded from the analysis because heavy rainfalls induced severe post-maturity sprouting and prevented combine harvesting. As some cultivars consisted of too few samples to generate a significant regression, overall equations for NABIM Class I and Class II wheats were also developed, using 1998 data. Finally, all available HFN data was pooled together to allow calculation of generic, Class I and Class II HFN prediction equations. These equations also included the HFN data collected in the previous HGCA project.

#### *2.6.2. HFN prediction classes*

A prediction of the likely class of HFN of combine samples based on their pre-harvest HFN and percentage germination in 3 days at 15°C was made according to the protocol in *Appendix I.* The equations used for prediction of combine harvest HFN from pre-harvest HFN were based on the HFN prediction equations and confidence limits derived for the cultivars Soissons (Class I) and Riband (Class II) in the previous HGCA project (Lunn *et al.*, 1998) using 1995-1996 data..

#### *2.6.3. Probability distribution functions for meeting or falling below HFN criteria*

Probability distribution functions, giving the chance that a crop (with a given pre-harvest HFN value) would exceed or fall below certain given HFN criteria (*e.g.* 300, 280, 250, 220 or 200 s) in the absence of rain after pre-harvest HFN measurement were calculated from the regression equation and confidence limits for the pre-harvest/combine harvest HFN relationship. For the initial analysis, an equation pooling the data from the equations for Class I (Soissons) and Class II (Riband) cultivars used for the prediction class system (*Appendix 1*) was derived (**Table 9**). Subsequently, the effects of using probability distribution functions derived from the individual Class I and Class II equations were assessed. Finally, equations derived from all available data (**Table 9**), both pooled and individually for Class I and Class II equations were analysed to check for any improvements in prediction accuracy.

Only pre-harvest/combine harvest values in crops where no sprouting was identified between pre-harvest and combine harvest sampling were used to generate the prediction equations. Combine harvest HFN (y) was regressed against pre-harvest HFN (x) using the linear regression function in GENSTAT 5 software. This gave a linear regression equation of the form $y = a(x) + b$, with an associated analysis of variance, F-probability *etc.* This equation allowed the plotting of

expected combine harvest HFN from pre-harvest HFN. For this equation, derived from n observations, confidence limits ($\beta$ %, where $\beta = 1-\alpha$ ) can be derived from the formula

$$\text{confidence limits} = y \pm t_{(n-2),\,\alpha/2} * \sqrt{(R(1+1/n + (x-\mu)^2/S))} \qquad ①$$

$t_{(n-2),\,\alpha/2}$ = Student's t distribution (n-2 degrees of freedom, $\alpha/2$ % probability)
R = residual or error mean square (available from GENSTAT ANOVA function)
n = number of pairs of observations
$\mu$ = mean x value
S = corrected sum of squares (available from GENSTAT summary statistics function)

Thus, the lower confidence limit (LCL) is given by the formula:

$$LCL = y - t_{(n-2),\,\alpha/2} * \sqrt{(R(1+1/n + (x-\mu)^2/S))} \qquad ②$$

Rearrangement of the formula gives

$$y\text{-}LCL = t_{(n-2),\,\alpha/2} * \sqrt{(R(1+1/n + (x-\mu)^2/S))} \qquad ③$$

Defining $\sqrt{(R(1+1/n + (x-\mu)^2/S))}$ as SE gives

$$y\text{-}LCL/SE = t_{(n-2),\,\alpha/2} \qquad ④$$

Substituting for y

$$(a(x) + b) - LCL/\,SE = t_{(n-2),\,\alpha/2} \qquad ⑤$$

or

$$(a(x) + b) - LCL/\sqrt{(R(1+1/n + (x-\mu)^2/S))} = t_{(n-2),\,\alpha/2} \qquad ⑥$$

By substitution of a desired value for the lower confidence limit in equation ⑥, with all the other constituents of the equation known from the linear regression analysis of y against x, the $t_{(n-2),\,\alpha/2}$ values associated with that specific LCL were derived (for varying x). The probability of y (*i.e.* combine HFN) falling below the LCL for a given x (pre-harvest HFN value) was thus $\alpha/2$, which was determined from tables of the t distribution (n-2 degrees of freedom). The probability of y exceeding the given confidence limits was 1- $\alpha/2$. This process was repeated for different set values of the LCL (*i.e.* the relevant commercial HFN criteria such as 220 s, 250 s and 280 s), giving probability distribution functions for combine HFN exceeding each criterion given pre-harvest HFN. These functions were plotted graphically.

The probability of combine HFN falling below the criteria of 280, 250 or 220 s was read from the intercept of a vertical line from the relevant test sample's pre-harvest HFN value with the curve of the relevant probability distribution function. A probability of $P>0.95$ for falling below a criterion was reported as 'certain'. For $P<0.05$, the combine HFN was predicted to be certainly above that criterion, with a point awarded subsequently if the prediction of combine HFN was correct in either case. It was possible to predict upper and lower limits of certainty, (*e.g.* > 220 s, < 280 s) allowing two points per prediction. For very high probabilities of exceeding the upper limit of 280 s, two predictions of > 280 s were recorded, awarded two or zero points depending on the combine harvest HFN. For $0.5<P<0.95$ a high probability (h) of falling below the criterion was reported, with one point awarded if HFN did fall, and zero if HFN remained above the criterion. The converse was true for $0.05<P<0.5$, judged to have a low (l) chance of falling below HFN, with one point awarded if combine HFN remained above that criterion. As with the HFN prediction class system, half a point was awarded if HFN remained within 10 s of the predicted criterion. The usefulness of this approach in comparison to the HFN prediction classes used previously (**Section 2.6.2.**) was assessed by comparing the percentage score, since the total number of points available using the probability distribution functions was double that in the prediction class system (predictions of upper and lower expected limits made compared to designation of a single class)

*2.6.4. Assessment of risk of HFN loss from germination data*

In the HFN prediction class system, a high risk of HFN loss due to germination in wet weather was predicted if 2% or more germination was found in the pre-harvest germination test. Germination data was not used when predictions were made with probability distribution functions, although reference to the germinability and rainfall did allow retrospective rationalisation of some of the prediction errors .

*2.6.5. Evaluation of the accuracy of the scheme*

2.6.5.1. HFN prediction classes

The success of the class-based scheme was decided on the basis of combine harvest HFN falling within the expected class limits according to pre-harvest HFN 'potential', the level of germination and whether or not there was rainfall between pre-harvest sampling and combine harvest, with one point awarded for a correct prediction and zero for an incorrect prediction. Half a point was awarded if HFN fell within 10 s of the prediction boundary.

2.6.5.2. Probability distribution functions

Combine harvest HFN values were compared with the probability values for each criterion and points were awarded as described in section 2.6.3. (double the number of points were available compared to the method in Section 2.6.2. as predictions of upper and lower limits were made). As above, a half point was awarded for values within 10 s of the prediction boundary.

## 3. RESULTS

### *3.1. Operation of the HFN prediction scheme*

#### *3.1.1. Logistics of crop consultant pre-harvest sampling and delivery to NIAB.*

Sampling of field crops was carried out by B.J. Major in liaison with AICC crop consultants (Steve Cook - Hampshire, Brian Keen - Cotswolds, Andrew Beeney - East Yorkshire). Problems were encountered in arranging sampling visits with consultants as they tend to take their holiday around the end of July during the optimum sampling period. This lead to some of the samples being taken earlier or later than was optimal, at a greater or lesser moisture content than the desired 35%, respectively.

Definition of the stage of sampling also needed to be clearer for consultants, with the initial visit to Brian Keen on 30th July too early. Consultants expressed some concern at the actual bulk of the sample to be collected and dispatched (15 samples = 20 kg). They were also concerned about gaining a representative sample from a large field (20 ha), where there would be a temptation to take a sample from an unrepresentative area near the gate. Sampling 20 crops from various growers was time consuming, taking all day.

Samples were dispatched in bulk using TNT Express courier depots located *en route* to sampling sites. Samples taken on Monday-Thursday were dispatched the same day for next day delivery to NIAB Labtest. Samples taken on a Friday were dispatched for delivery the following Monday and samples taken on a Saturday were dispatched the following Monday for delivery on Tuesday. Most samples arrived at NIAB within their specified delivery times (**Table 2**).

**Table 2 :** Timing of dispatch and delivery of pre-harvest HFN samples to NIAB from consultants

| Consultant | Date of sampling | No of samples | Date of dispatch | Date of arrival at NIAB |
|---|---|---|---|---|
| Brian Keen | [1] Thur 30 th July | 12 | Thur 30 th July | Fri 31 st July |
| Steve Cook | Tue 4 th Aug | 20 | Tue 4 th Aug | Wed 5 th Aug |
| Brian Keen | [2] Fri 7 th Aug | 20 | Fri 7 th Aug | Mon 10 th Aug |
| Andrew Beeney | Mon 10 th Aug | 15 | Mon 10 th Aug | Tue 11 th Aug |
| Andrew Beeney | Sat 15 th Aug | 5 | Mon 17 th Aug | Tue 8th Aug |

[1]Samples sent, but not analysed, as ears were still green.
[2]TNT can deliver on Saturdays.

#### *3.1.2. Combine harvest HFN samples*

Steve Cook sent combine harvest samples in batches and all arrived safely. Brian Keen delivered combine harvest samples for analysis himself. Samples from Andrew Beeney were sent by

individual farmers. Of these samples, ten arrived safely, five could not be sent due to degradation of the crop caused by post-maturity sprouting in unfavourable weather (extremely late combine harvesting) and five samples were misplaced at an unidentified point in the system. Lost samples therefore accounted for only 4% of the total number of samples submitted by crop consultants, although all pre-harvest samples arrived safely.

*3.1.3. Logistics of laboratory analysis*

During the project, NIAB Labtest assessed the ease of operating the prediction scheme in their laboratories. These results are confidential to NIAB Labtest, due to intellectual property right considerations. However, any HGCA levy-payer is entitled to set up their own prediction system using the information in *Appendices I* and *III,* the protocol on which laboratory analyses were initially based (derived from project 0056/01/93), and the recommended modifications taking account of the results of this project.

***3.2. Determination of optimum sample size from the field***

In large fields (*e.g.* 20 ha) the variations in crops across the field were very marked, with varying degrees of lodging and green tillers apparent in different areas of the field. Consultants preferred randomly selecting ears as they walked through the crop, rather than sampling a larger number of ears at random specified points.

The analysis of the coefficient of variation derived from various combinations of nine HFN samples taken from one 20 ha field shows a sample pooled from sub samples from a minimum of five random locations within the field was optimal, giving the lowest percentage coefficient of variation for pre-harvest HFN (**Figure 1**). There was no evidence that any particular number of samples gave an advantage in minimising variability of the percentage of germinating grains in three-day germination tests (data not shown) and thus no reason to collect a sample from more than five random locations.

***3.3. Determination of earliest appropriate pre-harvest sample time in the field.***

*3.3.1. Microwave drying*

Preliminary tests with microwave drying revealed difficulties in removing moisture from whole ears or grain, with superheating and charring before effective moisture removal. Two methods were investigated to increase the surface area to volume ratio of the sample and to try and ensure more effective drying, namely grinding samples in a pestle and mortar or milling samples using a domestic coffee mill.

There was no significant difference ($P>0.1$) between the moisture contents determined by microwave or oven drying (**Table 3**), or between ear and grain moisture content ($P>0.9$).The method of sample preparation had a significant effect ($P<0.001$) on the grain moisture content recorded. Milled samples had significantly ($P<0.05$) higher moisture content than ground samples, which had significantly higher ($P<0.05$) moisture content than whole ears. This indicates that increasing surface area of samples aided removal of moisture. There was a significant interaction ($P<0.05$) between drying method, sample form and method of sample

preparation.

Microwave drying was problematical compared to oven-drying. A large amount of condensation occurred in the microwave oven as the water vapour removed from the sample accumulated. Constant opening and closing of the microwave door was therefore required to allow its dispersion. Setting at a constant power (high and medium settings) for longer than a seven minute period caused charring of the samples.

Oven drying at 130 °C for two hours appears to be the easiest and most practical method to use, with whole ear moisture content giving a representative indication (though lower value) of actual grain moisture content. This temperature could be easily achieved in a domestic oven or range.

**Table 3**: A comparison of the moisture content of ear and grain samples of the wheat cultivar Soissons determined by microwave and oven drying of milled, ground or whole samples

| Sample | Preparation Method | Moisture content (%) Microwave | Oven | Mean |
|---|---|---|---|---|
| Ears | Milled | 44.4[a] | 43.8[a] | 42.1[b] |
| | Ground | 41.9[a] | 42.7[a] | |
| | Whole | 38.8[a] | 41.3[a] | |
| Grain | Milled | 43.2[a] | 43.7[a] | 42.2[b] |
| | Ground | 42.4[a] | 41.8[a] | |
| | Whole | 40.9[a] | 41.0[a] | |
| Mean | Milled | 43.8[c] | 43.7[c] | |
| | Ground | 42.2[c] | 42.2[c] | |
| | Whole | 39.8[c] | 42.4[c] | |
| Overall Mean | | 42.0[b] | 42.4[b] | |

[a] SED (df = 22) =0.65, LSD = 1.35, [b] SED = 0.27, LSD = 0.55, [c] SED = 0.46, LSD = 0.96, CV = 1.9%

*3.3.2. Relationship between accumulated potential evapotranspiration and moisture content*

A strong logistic (s-shaped) relationship (**Figure 2**), accounting for over 95% of the variance, was found between grain moisture content and accumulated potential evapotranspiration from ear emergence (ZGS 55). An identically-shaped curve accounting for a similar amount of the variance was found for the relationship between moisture content and PE accumulated from anthesis (ZGS 65). Confidence intervals (95%) for accumulated potential evapotranspiration ranged between 191-231 mm at 35% grain moisture (mean 211 mm), indicating that the

probability of 35% grain moisture occurring before 191 mm PE was accumulated from ear emergence was below 2.5%. Regression analysis with groups showed there was no significant ($P$>0.1) cultivar effect, although there were significant effects of site and year with a significant site x year interaction ($P$<0.001). This demonstrated that separate PE-moisture curves could be fitted to the data for each site x year combination to account for most of the variation in the data. However, the improvement in the amount of variance accounted for by this procedure was very small and was not considered beneficial.

The model of Atzema (1993) was not applicable for prediction of 35% grain moisture as it was based on the prediction of changes in grain moisture content due to reabsorption of water from dew or rainfall in ripe grains of 16-18% moisture. At this stage the permeability of the pericarp layers to water is different than earlier in development. Due to the good relationship between grain moisture content and accumulated potential evapotranspiration, the modelling approach was not investigated further.

### *3.4. Optimum temperature for the germination test.*

#### *3.4.1. Analysis of germination results from HGCA 0056/1/93 (1997)*

Analysis of the 1997 isolated grain germination data collected at 10, 15, 20 and 25°C from 1997 revealed an approximately equal division between two distinct responses to temperature between cultivars and sites. In some cases (Type A), a quadratic equation ($y = Ax^2 + Bx + C$, ***Figure 3***) could be fitted to the germination data (number of germinated grains), which could be solved to identify the optimum temperature for occurrence of maximal germination in three days. The average optimum temperature for the samples studied was 17.5°C. In the other case (Type B), germination in three day germination tests was negatively linearly related to temperature, *i.e.* germination decreased with increasing temperature ($y = -Dx + E$, ***Figure 4***). The ideal germination test temperature for three day germination tests, to compromise and ensure recording of as much germination as possible in the event of either relationship to temperature, was therefore 15°C, since the individual separate optima for Type A and Type B responses (~18°C and <10°C respectively) would not allow optimisation of germination by the other type of response. However, as also indicated by the analysis below, these results show that different germination test optima exist for different cultivar x site x season combinations which could limit the utility of germination test data from a single-temperature germination test. A germination response in the field quite different to that expected from pre-harvest germination testing would be possible depending on the germination response-type and prevailing temperature conditions during any crop wetting.

#### *3.4.2. Results from 1998 tests*

Germination tests were carried out on all pre-harvest HFN samples from the sites at Harper Adams (10 cultivars), ADAS Bridgets (10 cultivars) and University of Aberdeen (4 cultivars). The tests were carried at 10, 15, 20 and 25 °C, with the percentage germination assessed after three days. Analysis of variance was performed on the germination percentages of the samples from AB and HA (**Table 4**), after angular transformation of the data to a normal distribution. Overall, temperature did not have a significant effect ($P$>0.5) on germination, but both site and cultivar had significant effects ($P$<0.001). Higher germination occurred in samples from AB

compared to samples from HA. Both of the different responses to germination test temperature described in Section 3.4.1., were observed at each site (*e.g.* Type A: AB Consort, Type B: HA Hereward).

The cultivars Consort and Hereward showed a significantly higher ($P<0.05$) percentage of pre-harvest germination than Charger, which in turn had significantly higher ($P<0.05$) pre-harvest germination than Soissons, Abbot, Cantata and Rialto. The cultivars Spark, Malacca, Riband and Charger did not differ significantly in their pre-harvest germination. A significant site x cultivar x temperature interaction ($P<0.001$) was identified, suggesting that using a single temperature to distinguish between the sprouting susceptibilities of a wide range of cultivars grown at different sites could be problematic. At AB, mean germination in the pre-harvest test was greatest at 20°C, at HA it was greatest at 10°C.

**Table 4:** Percentage germinated grains after three days (mean of three replicates) pre-harvest germination testing at 10, 15, 20 and 25°C for a range of current commercial cultivars grown at ADAS Bridgets and Harper Adams in 1998. Transformed data (used for analysis of variance) shown in parenthesis.

| Site | Cultivar | Germination after three days (%) | | | | |
|---|---|---|---|---|---|---|
| | | 10°C | 15°C | 20°C | 25°C | Mean |
| AB | Abbot | 0 (2.9) | 0 (2.9) | 0 (2.9) | 0.7 (4.8) | 0.2 (3.3) |
| | Cantata | 0.3 (3.8) | 0.3 (3.8) | 4.0 (11.3) | 3.0 (9.9) | 1.9 (7.2) |
| | Charger | 10.7 (19.0) | 7.7 (16.0) | 10.7 (19.0) | 7.7 (16.1) | 9.2 (17.5) |
| | Consort | 12.0 (20.2) | 14.0 (22.0) | 23.0 (28.4) | 20.3 (26.4) | 17.3 (24.3) |
| | Hereward | 9.3 (17.6) | 8.7 (16.5) | 7.0 (14.3) | 6.3 (13.5) | 7.8 (15.5) |
| | Malacca | 5.0 (12.6) | 3.3 (10.1) | 12.0 (20.1) | 5.0 (12.9) | 6.3 (13.9) |
| | Rialto | 1.0 (5.2) | 0 (2.9) | 2.0 (7.5) | 3.7 (11.0) | 1.7 (6.7) |
| | Riband | 6.3 (12.1) | 2.7 (8.0) | 8.3 (16.6) | 8.7 (17.1) | 6.5 (13.5) |
| | Soissons | 1.3 (6.2) | 2.0 (8.1) | 1.7 (7.2) | 1.0 (5.6) | 1.5 (6.8) |
| | Spark | 1.0 (5.7) | 3.0 (9.7) | 4.3 (11.8) | 6.7 (14.9) | 3.8 (10.5) |
| | Mean | 4.7 (10.5) | 4.2 (10.0) | 7.3 (13.9) | 6.3 (13.2) | 5.6 (11.9) |
| HA | Abbot | 0.3 (3.8) | 1.0 (5.6) | 0.3 (3.8) | 0.7 (4.6) | 0.6 (4.5) |
| | Cantata | 0 (2.9) | 0.3 (3.8) | 0.3 (3.8) | 0.3 (3.8) | 0.3 (3.6) |
| | Charger | 1.0 (5.7) | 0.7 (4.6) | 0.7 (4.6) | 0 (2.9) | 0.6 (4.5) |
| | Consort | 8.0 (16.4) | 4.7 (11.8) | 0 (2.9) | 2.3 (8.5) | 3.8 (9.9) |
| | Hereward | 24.0 (29.3) | 17.3 (24.3) | 4.3 (11.7) | 2.0 (11.7) | 11.9 (18.2) |
| | Malacca | 0.3 (3.8) | 0.3 (3.8) | 1.0 (5.7) | 0.3 (5.7) | 0.5 (4.3) |
| | Rialto | 0 (2.9) | 0 (2.9) | 0 (2.9) | 0 (2.9) | 0 (2.9) |
| | Riband | 0.3 (3.8) | 1.0 (5.7) | 1.0 (5.7) | 0 (2.9) | 0.6 (4.5) |
| | Soissons | 0.3 (3.8) | 0.3 (3.8) | 1.7 (7.3) | 2.0 (7.7) | 1.1 (5.5) |
| | Spark | 3.7 (10.9) | 4.3 (11.9) | 0.7 (4.7) | 1.0 (5.7) | 2.4 (8.3) |
| | Mean | 3.8 (8.3) | 3.0 (7.8) | 1.0 (5.3) | 0.9 (5.0) | 2.2 (6.6) |
| | Grand Mean | 4.3 (9.4) | 3.6 (8.9) | 4.2 (9.6) | 3.6 (9.1) | 3.9 (9.3) |

SED (df = 54) = 3.07
(between sites = 0.49, between cultivars = 1.08, between temperatures = 0.69)

### *3.5. HFN prediction*

#### *3.5.1. Prediction equations for current commercial cultivars*

3.5.1.1. Individual regression relationships

The linear regression equations (combine HFN = (*a*)*preharvest HFN + *b*) for each cultivar are illustrated in **Table 5**. Some of the relationships were poor, with only a limited number of samples available for some cultivars, due to the absence of combine harvest HFNs from the University of Aberdeen, where there was heavy pre-harvest rainfall and significant post-maturity sprouting. The only significant ($P < 0.001$) relationships for individual cultivars were for Cantata and Hereward. The general equations developed with all the data, or the subset of research data were also significant ($P < 0.001$).

3.5.1.2 Overall Class I and Class II relationships

The overall pre-harvest/combine harvest HFN regression relationships for Class I and Class II wheats, are also shown in **Table 5.** Only the Class I relationship was significant ($P<0.001$), the Class II relationship was not ($P>0.1$). However, the prediction equation for Class II wheats after removal of the cultivar Rialto was significant ($P< 0.05$).

3.5.1.3. Equations from pooling all available HFN data

Combination of the 1998 data with all data (for pre-harvest/combine harvest HFN pairs in the absence of post-maturity sprouting) from the previous HGCA-funded project produced a second set of general Class I and Class II HFN prediction equations. The pre-harvest/combine harvest HFN equations using all available data, and for all available data divided into NABIM Class I and Class II cultivars, are also shown in **Table 5.** Graphs of the relationships, derived from all available data, with 95% confidence limits, are shown in **Figures 5** (Class I cultivars) and **6** (Class II cultivars).

#### *3.5.2. Probability distribution functions for meeting HFN criteria*

Graphs of the of the probability distribution (P) for combine harvest HFN falling below or meeting (1-P) HFN criteria of 200, 220, 250, 280 or 300 s are shown in **Figure 7**. The relationships shown in **Figure 7**, valid in the absence of sprouting, were based on the pre-harvest/combine harvest HFN regression relationship (**Table 5**) and confidence limits of the pooled data for the cultivars Soissons (Class I) and Riband (Class II) grown in experimental plots from 1995-1996 in the previous HGCA project. This data was used to allow a fair comparison with the HFN prediction class system, with HFN classes assigned using the same data. The relationships allow the probability that combine harvest HFN will meet or fail given criteria to be read off the graph, given an HFN value from a pre-harvest sample. Logistic curves accounting for 99% of the variance in the data could be fitted to the probability distribution functions by GENSTAT 5 curve-fitting software, giving an equation for derivation of probability from pre-harvest HFN. However, these equations allow negative probability values which is not possible, as P must lie between 0 and 1 (an event is either certain to happen, certain not to happen or distributed between the two outcomes). Therefore use of the probability distribution function

graph was preferred for making predictions within the scheme.

**Table 5 :** Relationships between combine harvest HFN and preharvest HFN in the absence of sprouting (combine HFN = (*a*)*preharvest HFN + *b*) for individual cultivars and classes of cultivar in 1998.

| Cultivar | No of samples | *a* | *b* | $R^2$ | *P* |
|---|---|---|---|---|---|
| Abbot | 12 | 0.47 | 161.6 | 0.15 | 0.12 |
| Cantata | 6 | 1.47 | -110.0 | 0.99 | <0.001 |
| Charger | 6 | -0.53 | 414.9 | - | 0.55 |
| Consort | 11 | 0.22 | 210.3 | - | 0.56 |
| Hereward | 28 | 0.91 | 16.2 | 0.39 | <0.001 |
| Malacca | 6 | 0.445 | 242.1 | 0.01 | 0.37 |
| Rialto | 18 | 0.02 | 267.0 | - | 0.67 |
| Riband | 6 | 0.20 | 202.2 | - | 0.72 |
| Soissons | 6 | 0.208 | 278.4 | - | 0.68 |
| Spark | 11 | 1.15 | -20 | 0.20 | 0.09 |
| Class I (research sites) | 36 | 1.12 | 2.93 | 0.76 | < 0.001 |
| Class II (research sites) | 24 | 0.20 | 220.5 | - | 0.33 |
| Class II (research, no Rialto) | 15 | 0.65 | 113 | 0.36 | <0.05 |
| Pooled Class I and Class II (research sites) | 60 | 0.99 | 33.0 | 0.66 | <0.001 |
| Class I (all) | 67 | 1.07 | -8.1 | 0.56 | < 0.001 |
| Class II (all) | 35 | 0.05 | 235 | - | 0.83 |
| Pooled Class I and Class II (all data) | 103 | 0.85 | 50.5 | 0.36 | < 0.001 |

Where no $R^2$ value is reported in **Table 5**, the residual variance exceeded that of the y variate and there was no significant relationship.

*3.5.3. Assessment of risk of HFN loss from germination data*

Risk of loss of HFN was judged to be 'low' if less than 2% of grains germinated in the pre-harvest germination test, and high if more than 2% germination occurred (see *Appendix I*). However, this assessment is very crude and is responsible for most of the erroneous predictions in the scheme. This is due to several reasons indicated earlier:

(a) the 15°C germination temperature is not optimal for each site x cultivar combination and thus does not record maximum possible germination .

(b) the pre-harvest germination test gives no indication of the level of sprouting at other temperatures and rainfall does not necessarily occur in the field at the temperature of the test.

(c) the potential amount of germination may change rapidly and in an unpredictable manner after the pre-harvest test.

(d) the frequency and amount of rainfall which caused sprouting varies and cannot be predicted accurately.

These deficiencies combine to limit the accuracy of the germination test. It is therefore difficult to interpret the accuracy of sprouting predictions since well defined repeatable rules for the quantification of sprouting risk from germination data cannot easily be constructed. Attempts to improve the predictive value of the germination test by use of different levels of germination for assessing sprouting risk, or by consideration of other factors such as orange wheat blossom midge infestation or use of gel test results to identify the pathway of *alpha*-amylase synthesis caused no significant improvements of the prediction scheme.

*3.5.4. Evaluation of the accuracy of the HFN predictions*

3.5.4.1. HFN prediction classes (using *Appendix I* method)

Derivation of the HFN prediction classes from pre-harvest information and interpretation of the sprouting risk used in the project are given in *Appendix I.* A look-up table of the expected combine HFN of the particular prediction classes of wheat used in the scheme in dry and wet conditions is shown in **Table 6.** The pre-harvest HFN values and germination scores, predicted and actual combine HFN results for the 1998 scheme are shown in *Appendix II* (**Tables B.1-B.4**). Samples from Aberdeen University and some from A. Beeney were not combine harvested due to wet weather or were lost, so the accuracy of the predictions for these samples could not be assessed. There was limited weather information from some crop consultant sites, making these predictions difficult to evaluate. Samples from S. Cook in Hampshire were assumed to have experienced similar weather to ADAS Bridgets, also in Hampshire. B. Keen provided some weather data allowing estimation of rainfall where combine harvest date was known. For other samples (including those from A. Beeney) only a rudimentary wet/dry classification was possible and for other samples no weather information was known.

**Table 6**. HFN prediction classes used in the 1998 trial scheme and expected combine HFNs

| Predicted HFN | Sprouting Risk | HFN Prediction Class | Expected HFN Dry | Wet |
|---|---|---|---|---|
| > 250 s | Low | 1 | >250 s | > 250 s |
| > 250 s | High | 2 | >250 s | < 250 s |
| ~ 250 s | Low | 3 | ~250s | < 250 s |
| ~ 250 s | High | 4 | ~250 s | < 250 s |
| < 250 s | High or Low | 5 | < 250 s | < 250 s |

The expected HFN values in **Table 6** were used to assess the scheme predictions. 'Wet' conditions were arbitrarily defined as more than 20 mm rainfall incident in the period between pre-harvest and combine harvest HFN sampling. In the score system, one point was awarded for combine HFN in the expected range and half a point was awarded if combine HFN was within 10 s of the predicted range. If HFN was substantially above or below the expected range, no points were awarded. The different types of errors possible in the predictions are shown in **Table 7**.

**Table 7**: Types of errors possible in the HFN prediction class and probability systems

| Error Type | a (soft) | b (soft) | c (soft) | d (soft) | e (hard) | f (hard) | g (hard) | h (hard) |
|---|---|---|---|---|---|---|---|---|
| Pre-harvest sprouting risk identified (+) | - | - | + | + | - | - | + | + |
| Significant rain (+) between pre- and combine harvest | - | + | - | + | - | + | - | + |
| Combine HFN above (+) or below (-)expected value | + | + | + | + | - | - | - | - |

The points scored and the various errors in the scheme are summarised in **Table 8**. Out of a possible 110 points, the scheme scored 72 (or 65.5%) overall, giving an error frequency of 35.5%. The accuracy of predictions from research sites and crop consultants was roughly equivalent (64.2 and 67.0% respectively). The accuracy rate for Class I wheats (70.7%) was greater than for Class II wheats (56.3%). This level of accuracy is slightly lower than that reported during development of the prototype scheme (Lunn *et al.*, 1998) which is probably the maximal level that can be achieved with the 'HFN prediction class' system.

**Table 8**: Accuracy of HFN prediction classes in the 1998 scheme

| Samples | Points Possible | Score | Accuracy (%) | Total Errors | a | b | c | d | e | f | g | h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Research Class I | 36 | 26.5 | 73.6 | 10 | 0 | 0 | 0 | 6 | 3 | 0 | 1 | 0 |
| Research Class II | 24 | 12 | 50.0 | 12 | 0 | 6 | 0 | 3 | 2 | 1 | 0 | 0 |
| Consultant Class I | 34 | 23 | 67.6 | 13 | 0 | 0 | 0 | 1 | 0 | 0 | 12 | 0 |
| Consultant Class II | 16 | 10.5 | 65.6 | 6 | 0 | 1 | 1 | 0 | 0 | 2 | 2 | 0 |
| Total | 110 | 72 | 65.5 | 41 | 0 | 7 | 1 | 10 | 5 | 3 | 15 | 0 |

Most of the errors in the scheme were due to interpretation of the germination data (see above section), for which there is little scope for improvement. There were 18 'soft' or 'pessimistic' errors (types a - d) where the final combine HFN was acceptable, although a low HFN had been predicted, accounting for 43.9% of the errors. These errors are less serious than the 'hard' errors, as although these crops would have been afforded low priority due to an expected low HFN, the combine HFN remained high. The 23 'hard' or 'optimistic' errors (types e-h) accounted for 56.1% of the errors and were caused when an acceptable HFN was predicted but a low combine HFN was found, either when no risk of sprouting was identified (in the presence or absence of rain) or a high risk was identified but there was little rain. These errors are more serious as commercial value would be expected from these crops which was, in fact, lost. The largest single class of errors was Type g, where HFN fell when there was little rain, although a sprouting risk was identified. These are true errors in the scheme and limit the theoretical maximum success rate to 79.1%. The second largest class of errors (Type d) was where a risk was identified and there was significant rain, but HFN remained unexpectedly high. Some of these mistakes could be due to RPAA in the pre-harvest sanmpleading to underestimation of the combine HFN potential.

3.5.4.2. Predictions using probability distribution functions

The regression equations used to generate the probability distribution functions used to calculate the probability of combine harvest HFN falling below certain HFN criteria are shown in **Table 9.**

**Table 9.** Regression equations used to generate probability distribution functions using 1994-1996 (A-C) and all available data (1994-1998, D-F).

| Equation | Number of samples | *a* | *b* | $R^2$ | P |
|---|---|---|---|---|---|
| A (Soissons and Riband) | 40 | 0.76 | 77.1 | 0.63 | <0.001 |
| B (Class I/ Soissons) | 20 | 0.70 | 135.5 | 0.73 | <0.001 |
| C (Class II/Riband) | 20 | 0.51 | 134.1 | 0.34 | <0.001 |
| D (All available) | 127 | 0.76 | 76.6 | 0.52 | <0.001 |
| E (All Class I) | 71 | 0.58 | 152.7 | 0.38 | <0.001 |
| F (All Class II) | 56 | 0.70 | 75.1 | 0.48 | <0.001 |

The results described below are from assessments of the predictions made with the probability distribution functions (**Figure 7**) derived from Equation A. This equation was derived from pooling the HFN data used to develop the Class I and Class II HFN regression equations specified in *Appendix I.* The use of probability distribution functions to make inferences about combine harvest HFN, without germination data, was at least as successful, if not better, than the above class system which also relied on pre-harvest germination information. Out of a possible 220 points this system of prediction (**Tables B5-B8** of *Appendix II*) scored 166 points or 75.5%, compared to 65.5% overall for the class system. The error frequency (51 errors out of 220 predictions) using the probability distribution functions was thus 23.2%, roughly half that in the prediction class system, even though double the amount of predictions were made, increasing the opportunity for error. Under this system, accuracy of the research sample predictions (77.1%) was comparable to those of the consultant samples (73.5%), although predictions for Class I cultivars were better than for Class II cultivars (81.4% compared to 65.0%) as shown in **Table 10**.

The probability distribution function predictions were subject to the same categories of error as the prediction class system (**Table 7**). Again, the 23 'soft' errors (types a-d), where HFN was eventually better than expected, accounted for a substantial proportion of the inaccuracy (45.1%), with 28 (54.9%) hard errors (an unsuspected fall in HFN, types e-h). The largest proportion of errors (type g, 35.3%) was again caused by a low HFN where sprouting risk identified but there was thought to be little rain. However, some of these errors could be due to inadequate weather data. It would appear from this analysis that the germination data gives little useful additional

data to the predictions derived from the probability functions.

**Table 10**: Accuracy of predictions made with probability distribution functions

| Samples | Points Possible | Score | Accuracy (%) | Total Errors | a | b | c | d | e | f | g | h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Research Class I | 72 | 61 | 84.7 | 12 | 3 | 3 | 2 | 3 | 0 | 0 | 1 | 0 |
| Research Class II | 48 | 31.5 | 65.6 | 15 | 1 | 5 | 0 | 3 | 4 | 0 | 2 | 0 |
| Consultant Class I | 68 | 53 | 77.9 | 15 | 0 | 0 | 1 | 1 | 0 | 0 | 11 | 2 |
| Consultant Class II | 32 | 20.5 | 64.1 | 9 | 0 | 0 | 1 | 0 | 0 | 1 | 4 | 3 |
| Total | 220 | 166 | 75.5 | 51 | 4 | 8 | 4 | 7 | 4 | 1 | 18 | 5 |

Use of separate probability distribution functions for Class I and Class II wheats derived from regression equations B (Soissons) and C (Riband) to make predictions (data not shown) resulted in slightly reduced accuracy (71.8%) compared to the pooled equation. Use of equation D (all available data) did not result in any improvement in accuracy as the equation was very similar to equation A (pooled Soissons and Riband). Use of equations E (All Class I) and F(All Class II) caused a reduction in accuracy compared to the use of the pooled equation D (data not shown).

# 4. DISCUSSION

## *4.1. Logistics of operation of the scheme - sample size, delivery and processing*

An easy method for rapid determination or prediction of appropriate pre-harvest sample time (35% moisture) would be necessary for future operation of the scheme according to the *Appendix I* protocol (see Section 4.2), since consultants were unwilling to regularly visit crops to monitor their readiness for sampling and Zadoks growth stage was too subjective. Too-early sampling lead to inclusion of much green material in some samples. Although these were processed in this experiment, the protocol (*Appendix I*) suggests that high moisture-content/green samples should be rejected due to the presence of pericarp *alpha*-amylase activity which could cause an erroneously low combine harvest HFN prediction (Lunn *et al.*, 1999a). However, as discussed in later sections, use of the probability distribution function system speeds up reporting of the predictions and reduces the pressure for early sampling with 35% grain moisture becoming the *earliest possible* sample point rather than the critical sample point.

The time-consuming nature of the sampling process is a disadvantage, although the sample size experiment showed that ears only need to be collected from five random positions within even a large field to minimise variability of pre-harvest HFN. This is comparable to the value of four sample locations derived from analysis of data from experimental plots used in the protocol (Lunn *et al.*, 1998). The requirement may, however, be very daunting in large (> 20 ha) fields, possibly resulting in sampling from a small area in crop margins close to roads and entrances which might not accurately reflect combine harvest HFN potential of the whole field. This should be discouraged in any commercial application of the scheme. Another point that should be considered is the combine harvest HFN values received by farmers may not be truly representative of a large field, which may mean some discrepancies in predictions around the 200-250 s area.

Use of commercial couriers appeared to be adequate for most next day deliveries and the delivery cost could potentially be included in the price of a commercial scheme. Although a few samples were lost, the rate was very low and could not be conclusively attributed to the courier. Most samples arrived at NIAB Labtest the day after sampling.

The developments made by NIAB in the operation of the scheme in their laboratories are confidential. However, it should be noted that the process-limiting step in the prediction class system, determining the earliest time that results can be returned to the sample sites is the isolation of immature grains and completion of the three day germination test. Therefore, this process should always be initiated before any of the other analyses.

The overall accuracy rate of the scheme in 1998 (see Section 4.4) was slightly lower from the success rate found in the previous tests of the scheme at research sites (Lunn *et al.*, 1998), probably due to errors in sampling time and a lack of detailed weather data for interpretation the results. Therefore, it would seem that the maximum accuracy to be expected is in the range 75-80% and possibly lower. The scheme is relatively simple to operate and could be set up relatively inexpensively by any institution with access to drying ovens, a threshing machine, hammer mill, HFN machine and controlled temperature incubators (*e.g.* grain merchants and co-operatives).

### *4.2. Determination of earliest sample time*

Sampling before 35% grain moisture content has been ruled out by previous research (Lunn *et al.*, 1998), due to the presence of pericarp (α-AMY-2) *alpha*-amylase activity which leads to erroneously low combine HFN predictions. Microwave drying is not a feasible method for rapid determination of grain moisture content due to the problems with condensation and charring. However, oven drying of whole ears at 130°C is a candidate for a rapid moisture test as it did not give significantly different moisture content values to isolated grains. Accumulated potential evapotranspiration is a good candidate marker of grain moisture content. Statistical analysis showed that separate curves for each site x year combination should be fitted to best explain the data, so use of the generic relationship shown in ***Figure 2*** could potentially cause small errors. However, the general relationship accounted for most of the variance, and fitting of site x season specific curves could only be done in retrospect. One way of using this relationship would be to specify an accumulated PE value (191 mm from heading) before which field visits for sampling would be futile due to high grain moisture content and the presence of too much α-AMY-2 *alpha*-amylase activity. Limited testing of grain moisture could occur after 191 mm PE from ZGS 55, thus determining an 'analysis window' for pre-harvest sample time without repeated crop visits. PE data is available from various sources such as the Met. Office (MORECS) and ADAS (Irriguide).

### *4.3. Optimum germination test temperature*

The significant site x cultivar x temperature interaction indicated in the assessment of optimum germination temperature indicates that by using a single germination test temperature, significant variations in the likely amount of pre-harvest germination will be found between sites and cultivars, with optimum germination occurring above or below the test temperature depending on the germination response type. This means that the pre-harvest germination might not accurately reflect the level of sprouting in a particular cultivar at a particular site where the temperature could be nearer the germination optimum in that instance. However, it would not be practicable to specify different test temperatures for specific cultivars and sites, as the optimum temperature would vary from season to season, depending on environmental conditions and it is not presently possible to predict which germination temperature would be the best to use. However, analysis of the pre-harvest germination data set from 1997 illustrated only two types of response of germination to varying temperature. These were either quadratic, with an optimum temperature at about 17.5°C, or linear, with most germination below 10°C. Use of 15°C as a pre-harvest germination temperature should therefore account for occurrence of either of the two possible relationships to temperature to give a reasonable assessment of PoMS risk. The 15°C temperature is also close to mean diurnal temperature that might be expected during showery periods. However, it should be noted that the laboratory germination test will only record a 'potential' germination level. Varying field conditions could lead far greater or far lesser amounts of germination in wet weather than expected from a germination test. Thus, the absence of germination in a laboratory test, or the presence of a very high amount, cannot be interpreted as the complete absence or presence of a risk of HFN reduction in wet weather. Rather, the germination test can only be interpreted by saying that, all things considered, a crop with high pre-harvest germination score is more likely to lose HFN in wet weather than a crop with a low germination score. However, the likely loss of HFN cannot currently be quantified and it is likely that there will be occurrences of HFN loss in crops which showed no pre-harvest germination and

maintenance of HFN in crops with large amounts of pre-harvest germination.

### *4.4. HFN predictions*

Many if the HFN prediction equations for current commercial cultivars were non-significant due to the lack of data from Aberdeen and the consequent small number of sample points. The overall equations for Class I and Class II cultivars developed from 1998 data did not give appreciably better predictions than the general equations from 1995-1996 (Class I - Soissons, Class II - Riband) or from an overall combined equation. Therefore, there would seem to be justification for using a single HFN prediction equation, if the HFN classification system were continued ut also if the probability distribution system were used.

However, the predictions made with the probability distribution functions without pre-harvest germination data were more accurate than the HFN prediction classes, which used pre-harvest germination data. Thus a comparable degree of accuracy was available far more rapidly from a single HFN sample, not requiring the labour-intensive germination test, than with the longer two-stage prediction system. Indeed, consideration of the germination data caused as many false positive errors as false negative errors and appears to introduce a further level of uncertainty in the predictions. Therefore, considering the time consuming nature of the pre-harvest germination test and the problems with interpretation of the data described previously, there appears to be little merit in including the germination data in the quantitative phase of the scheme. However, it may be useful to report samples with high levels of germination to aid explanation of erroneous predictions made with the probability distribution functions, or to report the likely date of dormancy break as a guide to the urgency of combine harvest. With the data available to this project, there appeared to be no benefit in using separate prediction equations for different NABIM wheat classes. However, in future, if more pre-harvest-combine harvest data was archived, the calculations of probability distribution functions for individual cultivars could be beneficial.

### *4.5. Further implementation of an HFN prediction scheme*

The information described in the main body of this report indicates that it would be feasible to initiate operation of an HFN prediction scheme, the only apparatus required being drying ovens, a threshing machine, hammer mill and HFN apparatus. On due consideration of the results, it appears that a prediction scheme based on the reporting of the chance of combine HFN falling below the relevant export and bread making criteria based on pre-harvest HFN measurements and general probability distribution functions would be more efficient than using the two-stage HFN prediction class scheme previously under development. An earliest possible sample time, for growers requiring the timeliest possible intelligence, could be determined by reference to accumulated potential evapotranspiration after ear emergence (data available from the Meteorological Office or ADAS) or by determination of ear moisture content < 35% by oven drying of serial samples. Results could still be returned very rapidly, so a ZGS such as the hard dough stage (ZGS 87-89) could be specified for sample submission. However, samples could be submitted at lower moisture content and throughout the harvesting season to allow prioritisation. Samples of ~ 400 ears, taken from five random positions within a field, could be dispatched by courier for next-day delivery to the analysis laboratory, for overnight drying and subsequent HFN analysis. A general HFN prediction equation or probability distribution function derived from

this research (**Table 5, Figures 5-7** *Appendix I, IV*) could be used to report the probability of a particular crop reaching or failing to reach given HFN criteria, in the absence of significant rainfall on the third day after sampling. A data archive built up by anyone operating the scheme could be used to modify the probability distribution function in the light of the changing pattern of commercially cultivated varieties or local conditions. Repeat sample submission after significant rainfall events wold allow revision of the previous predictions more easily than with the more expensive and time-consuming HFN/germination test system envisaged previously. A procedure for operating such a scheme is summarised in *Appendix III.* NIAB Labtest intend to operate a commercial version of the scheme from the summer of 1999.

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**Figure 1:** The variability in mean pre-harvest Hagberg falling number associated with the number of randomly-selected sub-samples taken from a 20 ha field and pooled for HFN analysis

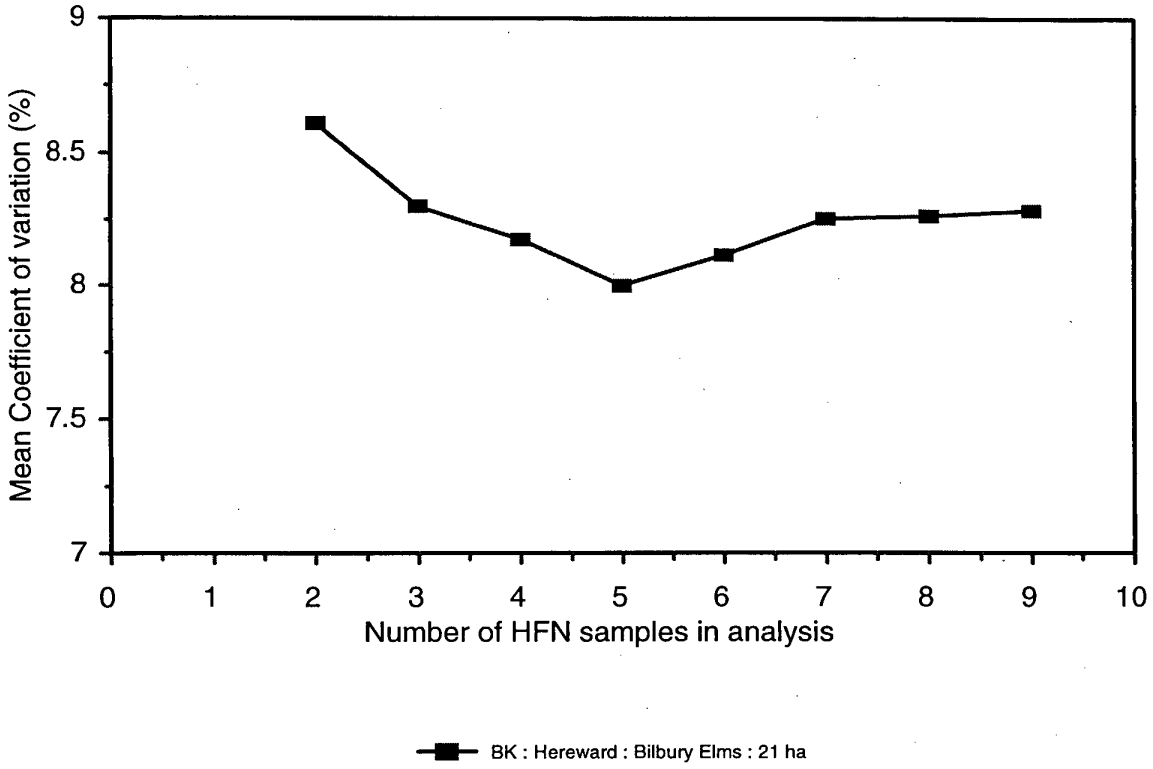


**Figure 2:** General relationship between percentage grain moisture content and accumulated potential evapotranspiration after ear emergence (ZGS 55)

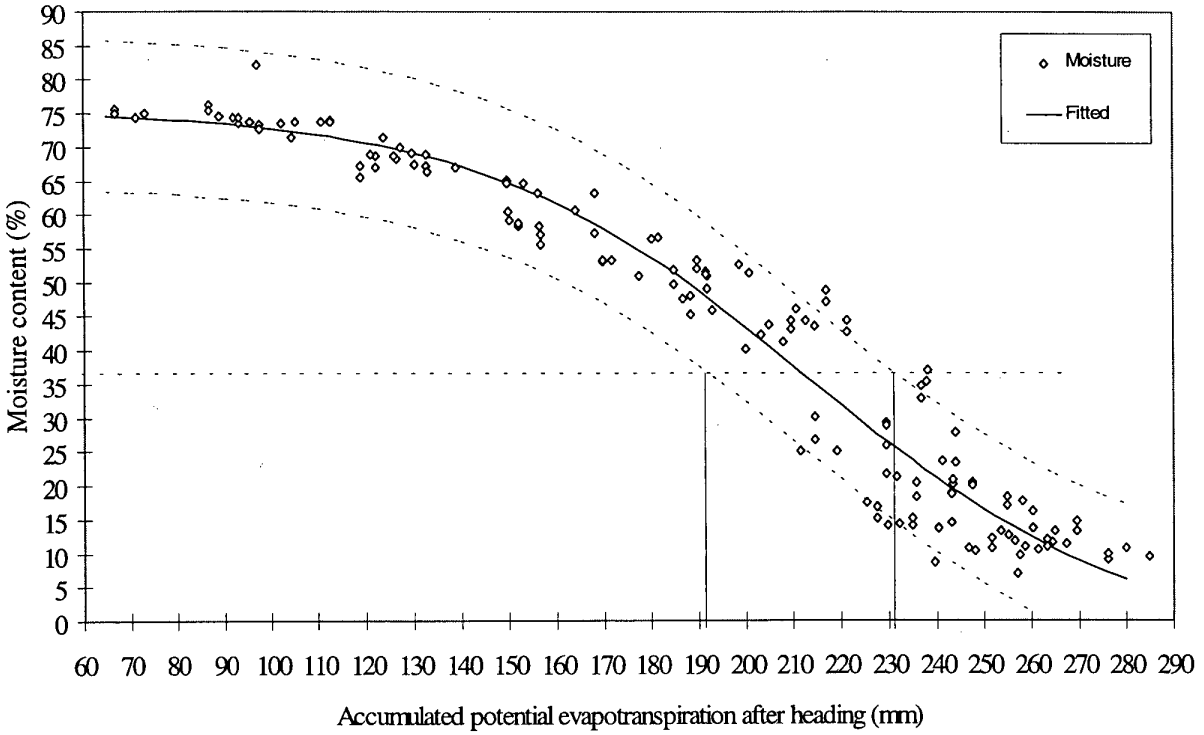


**Figure 3:** Type A quadratic relationship between number of germinated grains in three days and temperature (cv Haven, Sutton Bonington 1997, optimum temperature = 14.6°C).

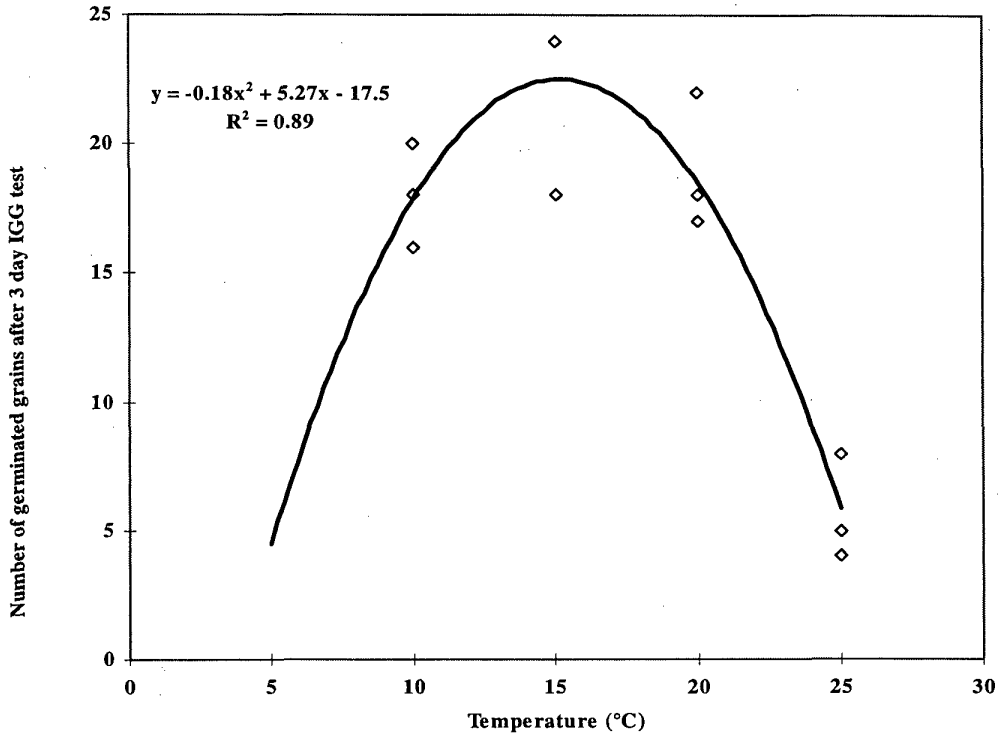


**Figure 4:** Type B linear relationship between number of germinated grains in three days and temperature (cv Riband, Sutton Bonington 1997).

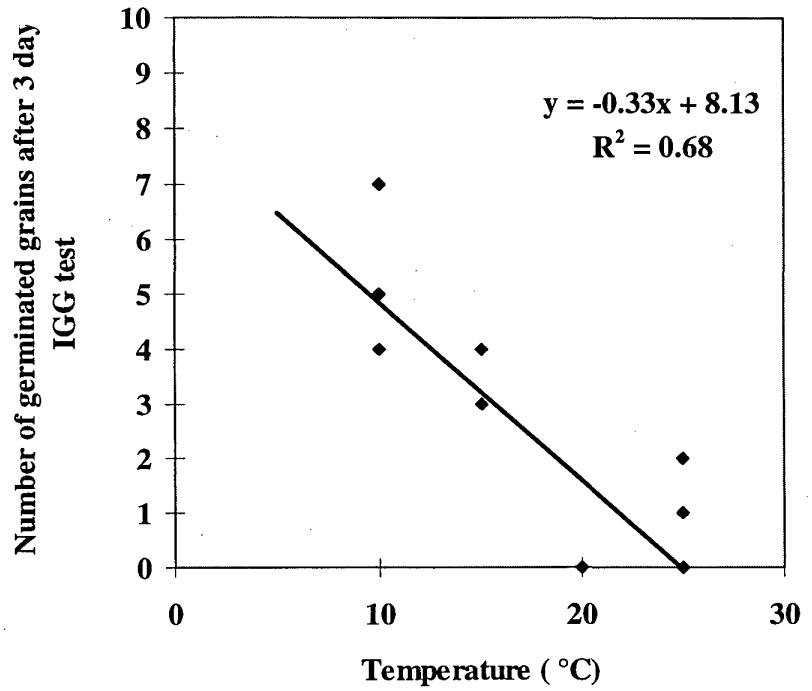


**Figure 5:** Linear regression relationship between pre-harvest and combine harvest Hagberg falling number of Class I wheats (all available data used)

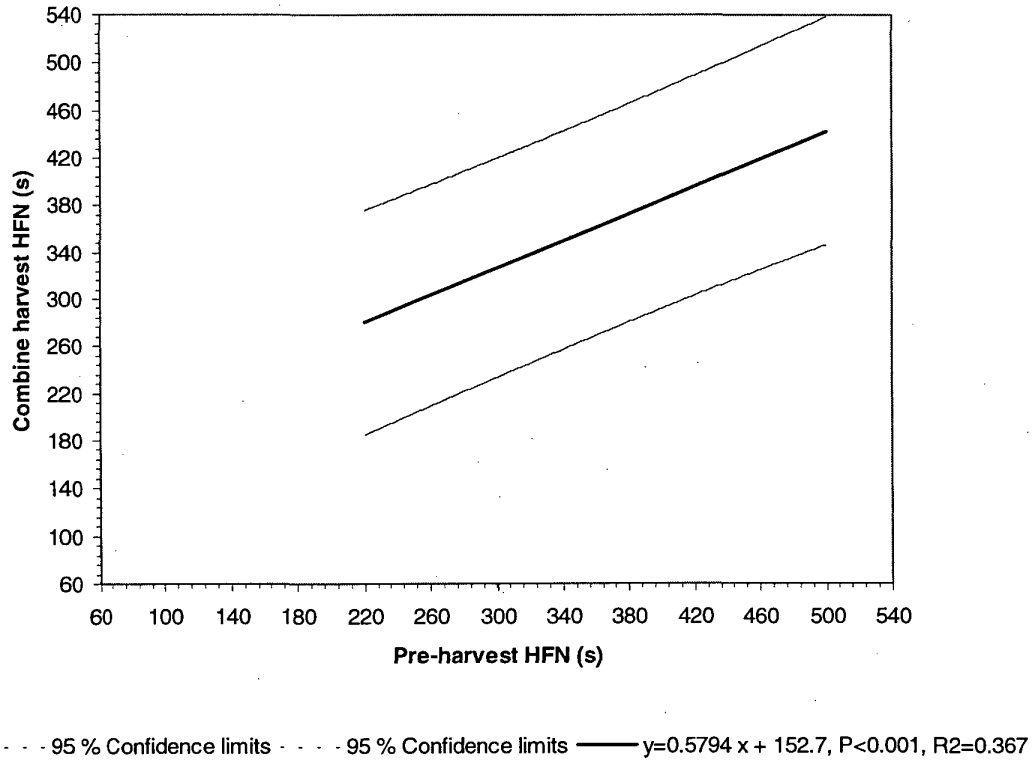


**Figure 6:** Linear regression relationship between pre-harvest and combine harvest Hagberg falling number of Class II wheats from research sites, (all available data used)

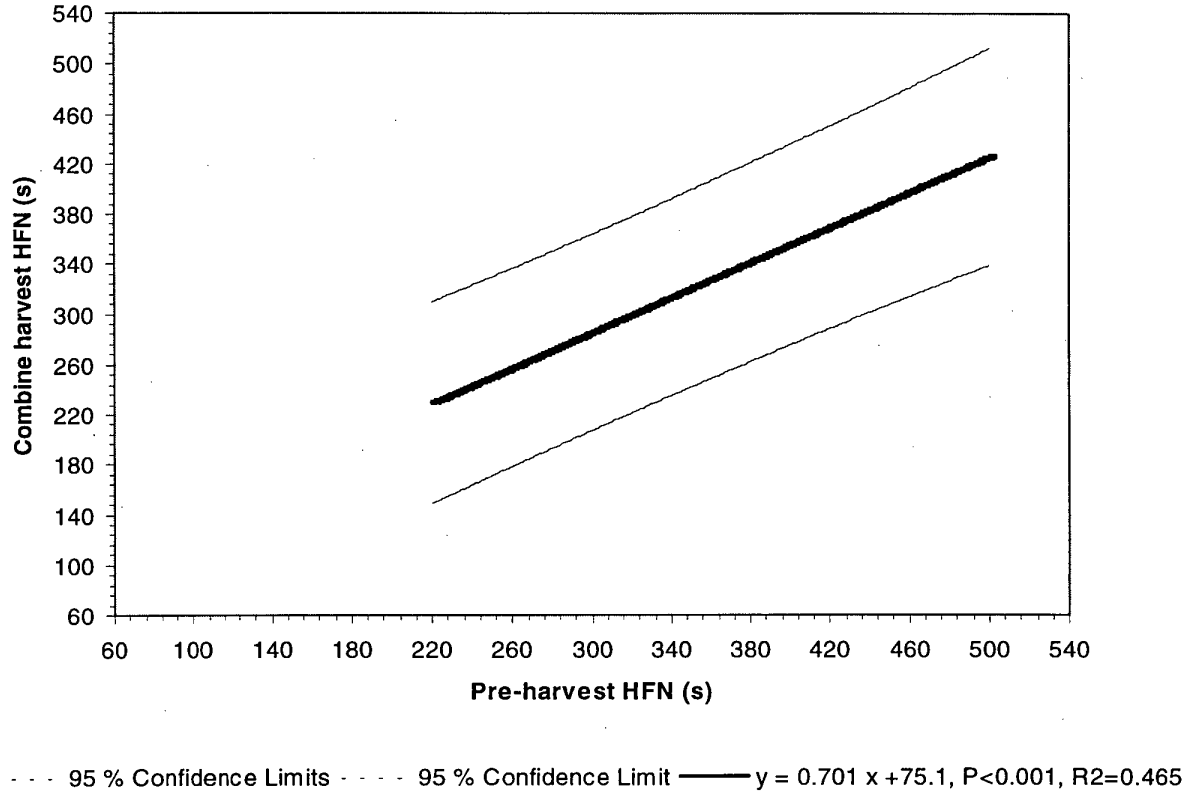
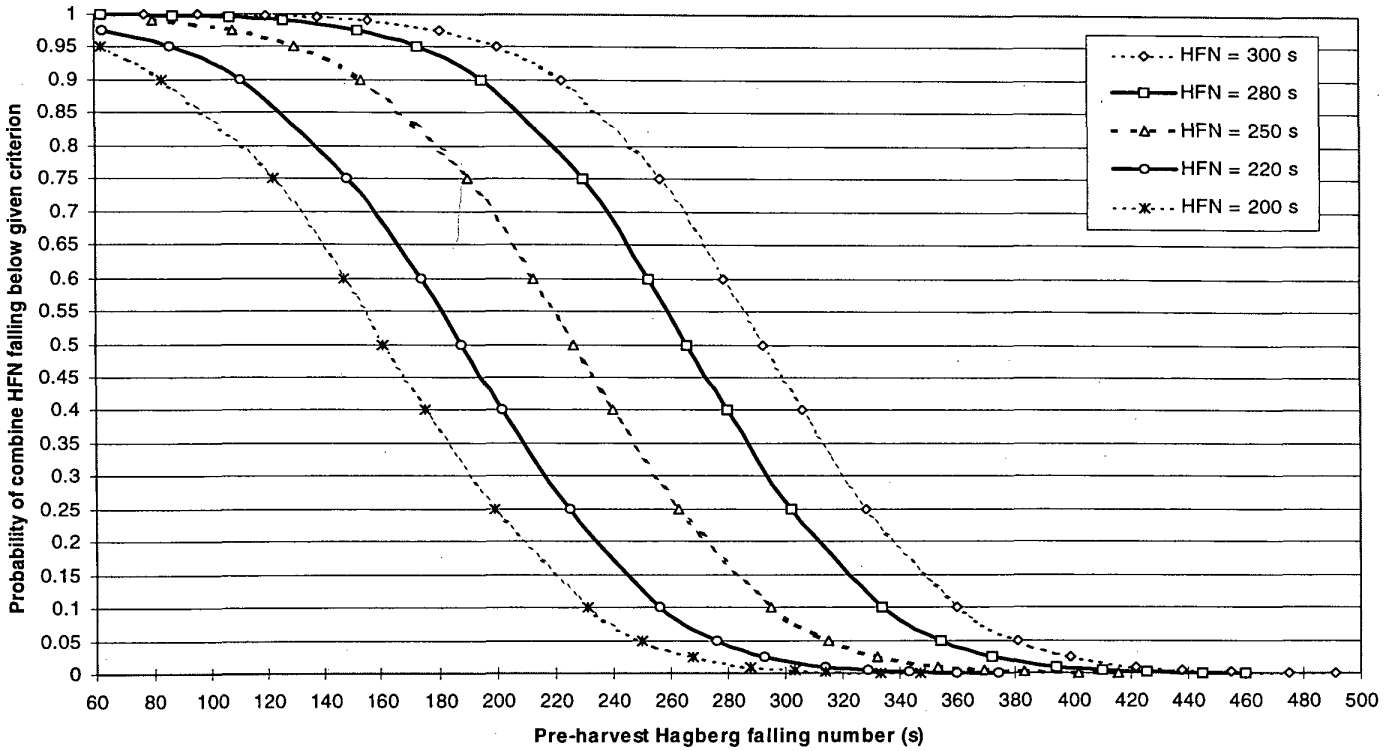


**Figure 7:** Probability distribution functions (P) for combine harvest HFN falling below certain criteria, given pre-harvest HFN. Probability of exceeding the criterion is given by 1-P.



## APPENDIX 1

## PROTOCOL FOR PREDICTION SCHEME TESTS AND APPLICATION OF HFN PREDICTION RULES

### Section 1: Crop Consultant Sampling Protocol for Pre-harvest (A) and combine-harvest (B) HFN Sampling

*A ) Pre-harvest HFN Sampling (2-3 weeks before combine-harvesting).*

Prefered varieties for sampling : Class I : *Hereward, Abbott, Malacca, Spark*
Class II : *Rialto, Charger, Soissons*
Class III : *Riband, Consort,*

Sample ears at ZGS 85-87, from about 20 crops, 2-3 weeks before combine harvesting.

* *Late Soft dough stage (ZGS 85)* - Grain contents are firm and not easily squeezed out, a fingernail impression quickly disappears, wheat embryo clearly visible (about 40% moisture).

* *Early Hard Dough stage (ZGS 87)* - Grain contents cannot be squeezed out, finger nail impression remains, no green colour in grain (about 30 % moisture).

**Select 4 tramlines which roughly divide the field into quarters. Walk up each tramline and at randomly chosen positions collect 100 ears from an area at least 1 m into the crop from the tramline. Collect the ears from all four tramlines in one plastic bag to give a total sample of 400 ears for each field.**

*Cut ears using a large pair of sharp scissors or secateurs to leave as little as possible of the stalk on the ear.*

* *Avoid sampling from* - *lodged and pest grazed areas.*
- *ears with heavy fungal infection.*
- *areas with heavy weed infestation.*

**For 1 field only.**
**Select 10 tramlines which roughly divide field into tenths. Walk up each tramline and at a randomly chosen position collect 400 ears from an area at least 1m into the crop from the tramlines. Collect the ears from each tramline in separate plastic bags to give a total of 10 samples, of 400 ears each.**

*If a small field is chosen, walk up 5 tramlines and randomly select 400 ears from two separate random locations along each tramline, to give a total of 10 samples, of 400 ears each.*

**Dispatch samples to NIAB Labtest by 1 st class post**

Pre-harvest Hagberg prediction and risk of sprouting results should be returned to you within 6 days of the samples receipt at NIAB.

*B) Combine-Harvest Sample (at combine harvesting).*

**Collect a combine-harvest grain sample of 1-2 kg at harvest in a cloth (or paper) bag and fill out combine record sheets.**

Please try and ensure sample is collected at combine harvesting, rather than from in the store afterwards.

**Dispatch combine sample (non-urgent), by Parcel Post /Courier to NIAB for analysis.**

**Section 2: Laboratory Analysis Sampling Protocol**

**2.1. Sample arrival.**

Record the sample arrival date (A). Compare with the sampling date (S). If (A-S) > 5 days, reject the sample and inform the customer/consultant.

Remove the ears from the bag and spread them out for observation on a clean, dry white surface.

Reject the sample and contact the customer/consultant to advise re-testing if any of the following problems are found:

* More than 10% **pure green** ears (not green-tinged)
* A high proportion of weed/volunteer species (*e.g.* barley)
* A severe level fungal growth (musty smell/blackened ears/blackpoint on grain)
* Slimy ears indicating microbial breakdown

Note any minor irregularities on the prediction scheme documentation.

**2.2. Sub-sampling for moisture and germination tests.**

Remove a sub-sample of 50 randomly chosen ears (SS1) for moisture content analysis and isolated grain germination testing. The remaining sample (RS) is required for HFN testing

**2.3. Moisture content analysis (SS2).**

Remove a sub-sample (SS2) of 10 ears from SS1

Roughly hand-thresh the immature grain from the ears (or assess the use of a threshing machine) and remove any adhering chaff. Randomly select 2 x 60 subsamples of grain (GS1 and GS2) and determine the fresh weight (FW) of each sample. Dry the grains in metal moisture cups for 2 hours at 130°C in a forced air oven. Cool the samples in a desiccator and determine the dry weight (DW) of GS1 and GS2. Calculate the moisture content of each

sample as (FW-DW)*100/FW. Calculate the mean moisture content, MMC= [($MC_{GS1}$+$MC_{GS2}$)/2].

If mean moisture content > 40 %, reject the sample and contact the customer to advise re-testing.

In the case of sample rejection, assume a standard rate of moisture content loss of 1.5 % per day.

Calculate a date to advise re-sampling as S +[(MMC-35)/1.5] where S is the date of sampling in the field recorded on the documentation submitted with the sample.

This date may be immediate, *e.g.* if moisture content was 50% sample at S+10 days for 35% moisture; if 40%, S+3 days.

**2.4. Isolated grain germination testing (SS1).**

Isolate 4 x 50 randomly chosen grain samples (GS3, GS4, GS5 and GS6) from florets 1 and 2 of the central spikelets of the 40 remaining ears of SS1. **It is very important that the ears of SS1 are kept at laboratory temperature and not dried or refrigerated before germination testing.**

Two methods of grain isolation are possible:

a) Remove the outer glumes (chaff) of the selected grains before carefully isolating the grains from the ear with a blunt mounted needle, wooden toothpick or similar tool.

b) Roughly hand-thresh the ear after removal of the apical and distal 25%. Remove the adhering palea and lemma (glumes/chaff) from the individual grains

Immerse separated grains in 1 % sodium hypochlorite solution for 2 minutes to surface sterilize the grains. Wash the grains throughly with distilled water after surface sterilization.

Using blunt-ended tweezers place each of the 4 x 50 grain samples (GS3, GS4, GS5 and GS6), on 2 sheets of Whatman No 1. filter paper, dampened with 5 ml of distilled water in a 90 mm Petri dish. Ensure grains are evenly spaced and placed with the embryo uppermost. (NIAB will assess use of 2 x 100 grain tests).

Grains damaged during isolation (split pericarp, burst, squashed or torn at the basal end) should not be used. Previously sprouted, obviously insect-damaged or parasitised grains should also be discarded.

Wrap petri-dishes loosely in clingfilm and incubate in the dark at 15°C for 3 days.

Record number of germinated grains (N) in each test*. Identify germinated grains as those where the pericarp is split above the swollen embryo and those with visible rootlets and shootlets.

Calculate mean percentage germination, MPG = $[(N_{GS3}+N_{GS4}+N_{GS5}+N_{GS6})/200]*100$

NB for rapid operation of the test in the laboratory, counting of all germinated grains is not necessary if % germination is obviously > 2%.

### 2.5. Hagberg falling number (HFN) testing (RS)

Dry remaining 250 ears of the sample (RS) very carefully using two large oven tins for each sample to distribute the ears thinly and aid rapid drying (12-16 hours are required for sample to be dry enough to thresh and mill, < 18% moisture for Falling Number hammer mill)

**THE OVEN TEMPERATURE MUST NOT BE GREATER THAN 50 °C**
(otherwise the *alpha*-amylase enzymes are deactivated)

Mechanically thresh the dried ears and clean samples using an air-aspirated cleaner to remove any chaff.

Observe the clean, dried sample. Reject the sample if there is any blackening or charring (indicating accidental over-heating during drying) and advise re-testing.

If any green grains, orange-wheat blossom midge-damage, or visually sprouted grains are obvious remove random subsamples of 2 x 100 grains (GS7 and GS8) and record the number of green, midge-damaged and sprouted grains on the sample documentation.

* Reject the sample and advise re-testing if there are > 10% green grains.

Mill the grain (250 g) using a Hagberg Mill.

Determine moisture content on two 5g samples of flour using metal moisture content tins and a forced air oven at 130°C for 2 hours.

Determine pre-harvest HFN (PHHFN) on duplicate samples (repeat HFN test if results are not within 5 % of each other) using the British Standard method (Anon., 1987) or ICC methods.

### 2.6. Combine harvest HFN (CHHFN) potential prediction

Use the PHHFN value measured in (4) and HFN prediction graph/equation A (for Class 1 and II wheats, based on *Soissons*) or HFN prediction graph/equation B (for Class II wheat, based on *Riband*) to predict CHHFN with 75% confidence limits. The equations are shown in *Table A1*. A Microsoft Excel v. 5 spreadsheet is available to make the prediction. Graph A is shown in *Figure A1* and Graph B in *Figure A2*.

*Table A1: Equations for prediction of combine HFN (± 75 % confidence limits) from pre-harvest HFN measurements*

x = pre-harvest HFN measurement (PHHFN)

y = predicted combine HFN value (CHHFN), assuming rainfall after pre-harvest measurement is negligible ('potential' CHHFN)

**A (CLASS 1, Soissons):**

$$y = 0.6472[x] + 135.5 \pm 1.18*(\sqrt{(1087.0*(1.0476 + (y - 338.3)^2/13684))}$$

**B (CLASS 2, Riband):**

$$y = 0.5090[x] + 134.1 \pm 1.18*(\sqrt{(1189.0*(1.0476 + (y - 297.9)/17175))}$$

Assign HFN potential with the values produced from the equations, graphs or spreadsheet described above as shown in *Table 2*:

*Table A2: Rules for prediction of CHHFN potential*

| **Predicted HFN ± 75% confidence limits*** | **CHHFN potential** |
|---|---|
| Lower confidence limit < 140 s (equation 1)<br>Lower confidence limit < 163 s (equation 2) | < 250 s probable |
| 140 s < LCL < 230 s (equation 1)<br>163 s < LCL < 237 s (equation 2) | ~ 250 s possible |
| Lower confidence limit > 230 s (equation 1)<br>Lower confidence limit > 237 s (equation 2) | > 250 s probable |

* If up to 10% green grains present, move up to next class.
* If > 10% orange wheat blossom midge damage move down to next lower class

### 2.7. Risk of reduction in CHHFN potential by sprouting

Use the number of germinated grains in the germination test after 3 days to calculate the percentage of germinated grains as described in (3).

Assign low or high risk of sprouting from isolated grain germination test results as described in *Table A3* below.

*Table A3: Risk of reduction in CHHFN potential due to sprouting*

| Percentage germination of grains in 3 days at 15°C | |
|---|---|
| Low risk of sprouting | High risk of sprouting |
| <2% | 2% or > 2% |

### 2.8. Classification of 'Prediction Class'

Collate CHHFN potential (4; *Tables A2, A3*) and sprouting risk (3; *Table A4*) results to make predictions.

Report the prediction class to the customer as shown in *Table A4.*

*Table A4: Rules for evaluation of Combine Harvest HFN prediction classes.*

| CHHFN Potential | Risk of sprouting | Prediction class | Prediction |
|---|---|---|---|
| > 250 s | Low | 1 | Probably > 250 s. |
| > 250 s<br>Maybe in cool, | High | 2 | May achieve > 250 s in warm, dry weather.<br>~ 250 s or < 250 s wet weather. |
| ~ 250 s | Low | 3 | May achieve 250 s (especially if warm and dry) |
| ~ 250 s | High | 4 | May achieve 250 s. Unlikely to achieve 250 s in cool wet weather. |
| < 250 s | Low or high | 5 | Very unlikely to achieve 250 s |

**Notes for modification of prediction class:**

1: If between 5-10% green grains present, increase HFN potential class by 1.

2: If > 10% OWBM damage present, reduce HFN potential class by 1.

Use the sprouting risk determined from germination tests to assign the final prediction class in both cases.

**Reference:**

Anonymous (1982): Determination of the falling number of cereals. British Standard methods of test for cereals and pulses (BS 4317: part 9).

***Figure A1**: HFN Prediction Graph A for Class 1 wheats*

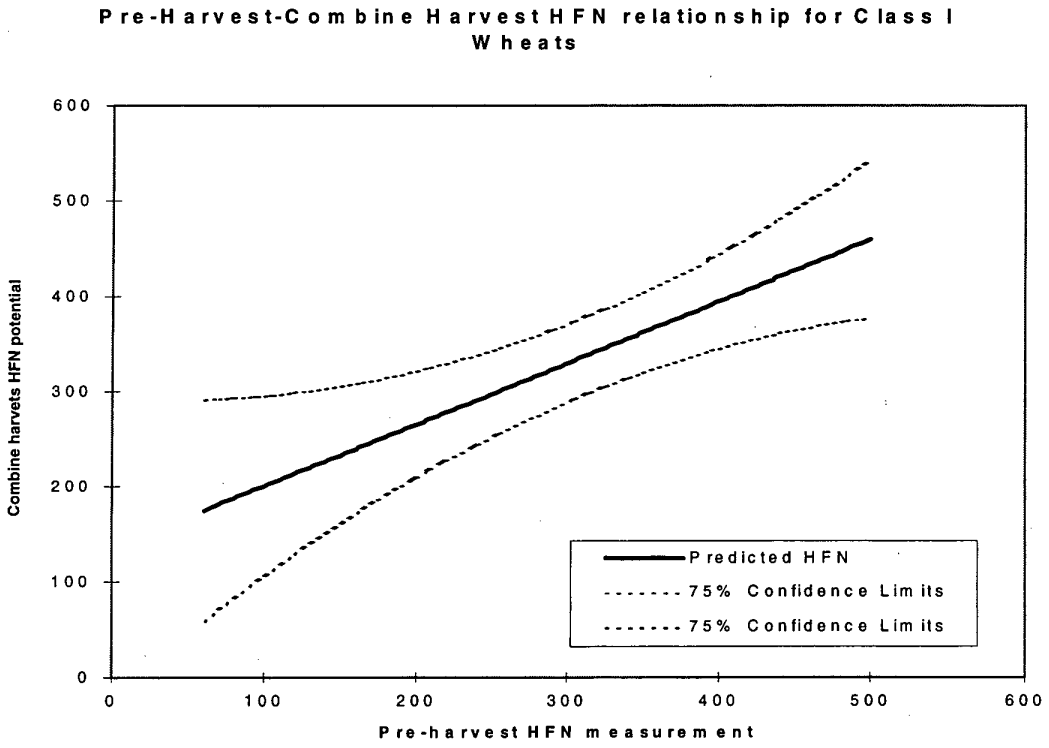
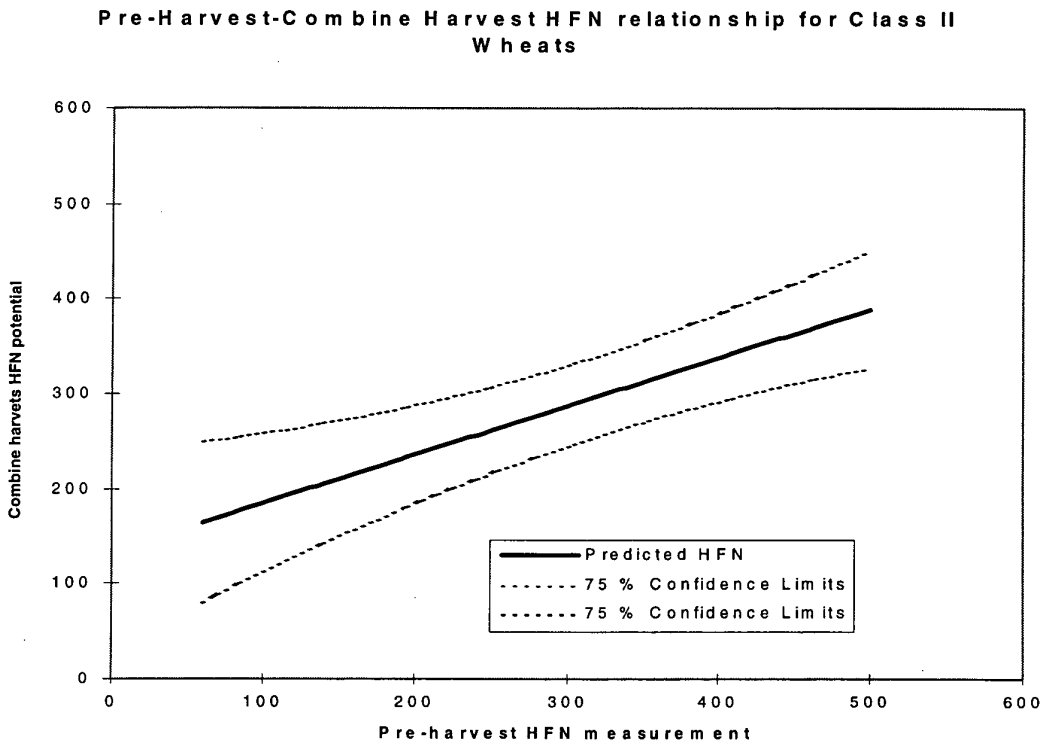


*Figure A2: HFN Prediction Graph B for Class 2 wheats*



## APPENDIX II: HFN PREDICTIONS AND ACCURACY

**Table B.1**: Pre-harvest information, HFN prediction classes and combine harvest HFN (research sites, 1998 - Class I cultivars) for the prediction system using pre-harvest HFN and germination data.

| Site | Cultivar | Pre-harvest data or predictions | | | | | Rainfall[2] (mm) | Combine HFN (s) | Score | Error Type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample HFN[1] (s) | Potential HFN (s) | Pre-harvest Germination (%) | Sprouting Risk | HFN Class | | | | |
| HA | Soissons | 255 | 301 | 1 | Low | 1 | 56.1 | 337 | 1 | |
| | | 249 | 297 | 0 | Low | 1 | 56.1 | 328 | 1 | |
| | | 255 | 301 | 0 | Low | 1 | 56.1 | 313 | 1 | |
| AB | Soissons | 281 | 317 | 2 | High | 2 | 22.6 | 326 | 1 | |
| | | 263 | 305 | 2 | High | 2 | 22.6 | 361 | 1 | |
| | | 229 | 283 | 2 | High | 2 | 22.6 | 324 | 1 | |
| HA | Abbot | 316 | 346 | 0 | Low | 1 | 39.5 | 362 | 1 | |
| | | 314 | 339 | 0 | Low | 1 | 39.5 | 336 | 1 | |
| | | 333 | 351 | 0 | Low | 1 | 39.5 | 336 | 1 | |
| AB | Abbot | 225 | 281 | 1 | Low | 1 | 6.0 | 283 | 1 | |
| | | 243 | 293 | 0 | Low | 1 | 6.0 | 292 | 1 | |
| | | 242 | 292 | 0 | Low | 1 | 6.0 | 301 | 1 | |
| UA | Abbot | 259 | 303 | 2 | High | 2 | - | * | - | |
| | | 256 | 301 | 1 | Low | 1 | - | * | - | |
| | | 248 | 296 | 0 | Low | 1 | - | * | - | |
| HA | Hereward | 377 | 320 | 11 | High | 2 | 39.5 | 416 | 0 | d |
| | | 355 | 313 | 26 | High | 2 | 39.5 | 404 | 0 | d |
| | | 344 | 327 | 15 | High | 2 | 39.5 | 386 | 0 | d |
| AB | Hereward | 266 | 307 | 3 | High | 2 | 6.0 | 289 | 1 | |
| | | 233 | 286 | 11 | High | 2 | 6.0 | 277 | 1 | |
| | | 245 | 294 | 12 | High | 2 | 6.0 | 247 | 0.5 | g |

[1] pre-harvest test [2] between pre-harvest and combine harvest

**Table B.1** (continued)

| Site | Cultivar | Pre-harvest data or predictions | | | | | Rainfall[2] (mm) | Combine HFN (s) | Score | Error Type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample HFN[1] (s) | Potential HFN (s) | Pre-harvest Germination (%) | Sprouting Risk | HFN Class | | | | |
| UA | Hereward | 221 | 279 | 2 | High | 2 | - | * | - | |
| | | 207 | 269 | 2 | High | 2 | - | * | - | |
| | | 214 | 274 | 2 | High | 2 | - | * | - | |
| HA | Cantata | 260 | 303 | 0 | Low | 1 | 39.5 | 275 | 1 | |
| | | 284 | 319 | 0 | Low | 1 | 39.5 | 309 | 1 | |
| | | 264 | 306 | 1 | Low | 1 | 39.5 | 272 | 1 | |
| AB | Cantata | 171 | 246 | 0 | Low | 3 | 6.0 | 149 | 0 | e |
| | | 165 | 242 | 0 | Low | 3 | 6.0 | 128 | 0 | e |
| | | 163 | 241 | 1 | Low | 3 | 6.0 | 126 | 0 | e |
| HA | Malacca | 377 | 279 | 0 | Low | 1 | 39.5 | 416 | 1 | |
| | | 355 | 365 | 1 | Low | 1 | 39.5 | 404 | 1 | |
| | | 344 | 328 | 0 | Low | 1 | 39.5 | 386 | 1 | |
| AB | Malacca | 353 | 364 | 1 | Low | 1 | 6.0 | 405 | 1 | |
| | | 375 | 378 | 4 | High | 2 | 6.0 | 390 | 1 | |
| | | 368 | 374 | 5 | High | 2 | 6.0 | 418 | 1 | |
| UA | Malacca | 317 | 276 | 0 | Low | 1 | - | * | - | |
| | | 294 | 326 | 0 | Low | 1 | - | * | - | |
| | | 332 | 350 | 0 | Low | 1 | - | * | - | |
| HA | Spark | 293 | 325 | 4 | High | 2 | 39.5 | 391 | 0 | d |
| | | 297 | 321 | 6 | High | 2 | 39.5 | 354 | 0 | d |
| | | 268 | 309 | 3 | High | 2 | 39.5 | 327 | 0 | d |
| AB | Spark | 303 | 331 | 2 | High | 2 | 6.0 | 285 | 1 | |
| | | 266 | 308 | 2 | High | 2 | 6.0 | 295 | 1 | |
| | | 319 | 342 | 3 | High | 2 | 6.0 | 307 | 1 | |

Possible points (excluding UA samples, not combined):36
Points scored = 26.5 (73.6%)

**Table B.2**: Pre-harvest information, HFN prediction classes and combine harvest HFN (research sites, 1998 - Class II) for the prediction system using pre-harvest HFN and germination data.

| Site | Cultivar | Pre-harvest data or predictions | | | | | Rainfall[2] (mm) | Combine HFN (s) | Score | Error Type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample HFN[1] (s) | Potential HFN (s) | Pre-harvest Germination (%) | Sprouting Risk | HFN Class | | | | |
| HA | Charger | 264 | 268 | 2 | High | 4 | 39.7 | 300 | 0 | d |
| | | 247 | 260 | 0 | Low | 3 | 39.7 | 291 | 0 | b |
| | | 245 | 259 | 0 | Low | 3 | 39.7 | 297 | 0 | b |
| AB | Charger | 253 | 263 | 9 | High | 4 | 6.0 | 251 | 1 | |
| | | 275 | 274 | 8 | High | 4 | 6.0 | 268 | 1 | |
| | | 266 | 266 | 6 | High | 4 | 6.0 | 269 | 1 | |
| HA | Riband | 242 | 257 | 1 | Low | 3 | 39.5 | 269 | 1 | |
| | | 212 | 242 | 1 | Low | 3 | 39.5 | 237 | 0 | f |
| | | 193 | 232 | 1 | Low | 3 | 39.5 | 254 | 1 | |
| AB | Riband | 220 | 246 | 7 | Low | 4 | 6.0 | 209 | 0 | e |
| | | 187 | 229 | 1 | Low | 3 | 6.0 | 243 | 1 | |
| | | 217 | 245 | 0 | Low | 3 | 6.0 | 259 | 1 | |
| HA | Consort | 262 | 267 | 7 | High | 4 | 39.5 | 304 | 0 | d |
| | | 249 | 261 | 1 | Low | 3 | 39.5 | 289 | 0 | b |
| | | 243 | 258 | 6 | High | 4 | 39.5 | 300 | 0 | d |
| AB | Consort | 244 | 258 | 14 | High | 4 | 6.0 | 274 | 1 | |
| | | 218 | 245 | 13 | High | 4 | 6.0 | 246 | 1 | |
| | | 238 | 255 | 15 | High | 4 | 6.0 | 242 | 1 | |
| HA | Rialto | 283 | 278 | 0 | Low | 3 | 39.5 | 334 | 0 | b |
| | | 183 | 227 | 0 | Low | 3 | 39.5 | 290 | 0 | b |
| | | 226 | 249 | 0 | Low | 3 | 39.5 | 271 | 0 | b |

**Table B.2.** (continued)

| Site | Cultivar | Pre-harvest data or predictions | | | | | Rainfall[2] (mm) | Combine HFN (s) | Score | Error Type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample HFN[1] (s) | Potential HFN (s) | Pre-harvest Germination (%) | Sprouting Risk | HFN Class | | | | |
| AB | Rialto | 225 | 249 | 0 | Low | 3 | 6.0 | 259 | 1 | |
| | | 267 | 270 | 0 | Low | 3 | 6.0 | 251 | 1 | |
| | | 312 | 293 | 0 | Low | 1 | 6.0 | 217 | 0 | e |
| UA | Rialto | 243 | 258 | 1 | Low | 3 | - | * | - | |
| | | 223 | 248 | 2 | High | 4 | - | * | - | |
| | | 236 | 254 | 2 | Low | 3 | - | * | - | |

Total points possible (excluding UA samples not combine harvested) = 24
Points scored = 12 (50.0%)

* = Not combine harvested

**Table B.3**: Pre-harvest information, HFN prediction classes and combine harvest HFN (commercial sites, 1998 - Class I cultivars) for the prediction system using pre-harvest HFN and germination data.

| Site | Cultivar | Pre-harvest data or predictions | | | | | Rainfall[2] (mm) | Combine HFN (s) | Score | Error Type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample HFN[1] (s) | Potential HFN (s) | Pre-harvest Germination (%) | Sprouting Risk | HFN Class | | | | |
| BK | Hereward | 307 | 334 | 14 | High | 2 | 13+ | 271 | 1 | |
| | | 299 | 329 | 14 | High | 2 | 13 | 293 | 1 | |
| | | 207 | 269 | 6 | High | 4 | 13 | 223 | 0 | g |
| | | 295 | 326 | 6 | High | 2 | 13 | 241 | 0.5 | g |
| | | 303 | 332 | 6 | High | 2 | 5 | 207 | 0 | g |
| | | 299 | 329 | 13 | High | 2 | 5 | 280 | 1 | |
| | | 254 | 300 | 5 | High | 2 | 5 | 261 | 1 | |
| | | 303 | 331 | 4 | High | 2 | 5 | 236 | 0 | g |
| | | 299 | 329 | 3 | High | 2 | 0 | 244 | 0.5 | g |
| | | 331 | 349 | 3 | High | 2 | 0 | 349 | 1 | |
| | | 340 | 355 | 7 | High | 2 | 0 | 354 | 1 | |
| | | 293 | 325 | 17 | High | 2 | 0 | 313 | 1 | |
| | | 319 | 342 | 7 | High | 2 | 0 | 315 | 1 | |
| SC | Hereward | 262 | 305 | 12 | High | 2 | 6+ | 271 | 1 | |
| | | 230 | 284 | 22 | High | 2 | 6 | 244 | 0.5 | g |
| | | 180 | 252 | 18 | High | 2 | 6 | 94 | 0 | g[1] |
| | | 299 | 329 | 25 | High | 2 | - | 265 | 1 | |
| | | 165 | 242 | 16 | High | 2 | 6 | 207 | 0 | g[2] |
| | | 243 | 293 | 12 | High | 2 | - | 214 | 0 | g |
| AB | Hereward | - | 340 | 5 | High | 2 | - | * | - | |
| | | - | 321 | 3 | High | 2 | - | * | - | |
| | | - | 294 | 4 | High | 2 | wet | 210 | 1 | |
| | | - | 266 | 3 | High | 2 | wet | 280 | 0 | d |
| | | - | 323 | 5 | High | 2 | wet | 240 | 1 | |

**Table B.3** (continued)

| Site | Cultivar | Pre-harvest data or predictions | | | | | Rainfall[2] (mm) | Combine HFN (s) | Score | Error Type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample HFN[1] (s) | Potential HFN (s) | Pre-harvest Germination (%) | Sprouting Risk | HFN Class | | | | |
| BK | Axona | 306 | 333 | 10 | High | 2 | 13 | 356 | 1 | |
| BK | Abbot | 292 | 324 | 16 | High | 2 | - | 269 | 1 | |
| | | 286 | 321 | 3 | High | 2 | - | 307 | 1 | |
| | | 304 | 333 | 3 | High | 2 | 5 | 262 | 1 | |
| SC | Abbot | 266 | 308 | 7 | High | 2 | 6 | 232 | 0 | g |
| | | 319 | 382 | 6 | High | 2 | - | 290 | 1 | |
| | | 285 | 361 | 6 | High | 2 | 6 | 295 | 1 | |
| SC | Spark | 259 | 303 | 13 | High | 2 | 6 | 242 | 0.5 | g |
| | | 265 | 307 | 15 | High | 2 | 6 | 280 | 1 | |
| | | 286 | 321 | 10 | High | 2 | 6 | 304 | 1 | |
| | | 253 | 299 | 23 | High | 2 | 6 | 206 | 0 | g |
| | | 240 | 291 | 7 | High | 2 | 6 | 271 | 1 | |

Total points available: 34 (excluding two missing AB sample)
Points scored: 23 (67.6)

* = Not combine harvested [1]Lodged crop [2]No weather data so could be right or wrong.

**Table B.4**: Pre-harvest information, HFN prediction classes and combine harvest HFN (commercial sites, 1998 - Class II cultivars) for the prediction system using pre-harvest HFN and germination data.

| Site | Cultivar | Pre-harvest data or predictions | | | | | Rainfall[2] (mm) | Combine HFN (s) | Score | Error Type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample HFN[1] (s) | Potential HFN (s) | Pre-harvest Germination (%) | Sprouting Risk | HFN Class | | | | |
| BK | Consort | 299 | 266 | 68 | High | 2 | 13+ | 251 | 1 | |
| | | 280 | 277 | 53 | High | 4 | 13+ | 271 | 1 | |
| | | 259 | 266 | 46 | High | 4 | 13+ | 262 | 1 | |
| SC | Consort | 234 | 253 | 26 | High | 4 | 6 | 223 | 0 | g |
| AB | Consort | 309 | 291 | 3 | High | 2 | 0 | 302 | 1 | |
| SC | Rialto | 124 | 197 | 14 | High | 5 | - | 198 | 1 | |
| | | 190 | 231 | 5 | High | 4 | - | 261 | 0 | c[2] |
| | | 203 | 237 | 4 | High | 4 | 6 | 245 | 0.5 | g |
| | | 256 | 264 | 3 | High | 4 | - | 257 | 1 | [2] |
| | | 205 | 238 | 18 | High | 4 | - | 148 | 1 | [2] |
| AB | Rialto | 258 | 265 | 3 | High | 2 | - | * | - | |
| | | 304 | 289 | 0 | Low | 1 | wet | 62 | 0 | f |
| | | 262 | 267 | 0 | Low | 3 | not as wet | 278 | 0 | b |
| | | 242 | 257 | 2 | High | 4 | wet | 62 | 1 | |
| | | 241 | 257 | 1 | Low | 3 | - | * | - | |
| | | 248 | 260 | 2 | High | 4 | - | * | - | |
| | | 251 | 262 | 1 | Low | 1 | - | * | - | |
| | | 231 | 252 | 0 | Low | 3 | wet | 161 | 0 | f |
| | | 224 | 248 | 3 | High | 4 | wet | 180 | 1 | |
| | | 180 | 226 | 2 | High | 4 | wet | 192 | 1 | |
| AB | Charger | 314 | 294 | 3 | High | 2 | - | * | - | |
| | | 299 | 286 | 5 | High | 2 | - | * | - | |
| | | 305 | 289 | 3 | High | 2 | - | * | - | |
| | | 306 | 290 | 2 | High | 2 | - | * | - | |

Total possible points: 16 (excluding lost AB samples)
Points scored: 10.5 (65.6%)

**Table B.5**: Probability of combine HFN falling below criteria and scoring of combine harvest HFN (research sites, 1998 - Class I cultivars) for the probability distribution functions derived from Equation A (Soissons and Riband combined)

| Site | Cultivar | Pre-harvest HFN (s) | Probability of combine HFN failing to reach value | | | Combine HFN data | | | Score | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 220 s | 250 s | 280 s | Expected[3] Lower Limit (s) | Expected Upper Limit (s) | Actual HFN | Lower | Upper | |
| HA | Soissons | 255 | 0.1 | 0.3 | 0.57 | >250h | <280h | 337 | 1 | 0 | b |
| | | 249 | 0.15 | 0.35 | 0.64 | >250h | <280h | 328 | 1 | 0 | b |
| | | 255 | 0.1 | 0.3 | 0.57 | >250h | <280h | 313 | 1 | 0 | b |
| AB | Soissons | 281 | <0.05 | 0.15 | 0.40 | >250h | >280h | 326 | 1 | 1 | |
| | | 263 | 0.075 | 0.24 | 0.50 | >250h | <280h | 361 | 1 | 0 | d |
| | | 229 | 0.1 | 0.47 | 0.75 | >250h | <280h | 324 | 1 | 0 | d |
| HA | Abbot | 316 | <0.01 | <0.05 | 0.175 | >250 | >280h | 362 | 1 | 1 | |
| | | 314 | <0.01 | <0.05 | 0.175 | >250 | >280h | 336 | 1 | 1 | |
| | | 333 | <0.01 | <0.025 | 0.1 | >250 | >280h | 336 | 1 | 1 | |
| AB | Abbot | 225 | 0.25 | 0.53 | 0.78 | >220h | <250h | 283 | 1 | 0 | a |
| | | 243 | 0.15 | 0.37 | 0.67 | >220h | <280h | 292 | 1 | 0 | a |
| | | 242 | 0.15 | 0.37 | 0.67 | >220h | <280h | 301 | 1 | 0 | a |
| UA | Abbot | 259 | 0.09 | 0.27 | 0.57 | >250h | <280h | * | - | - | |
| | | 256 | 0.09 | 0.27 | 0.57 | >250h | <280h | * | - | - | |
| | | 248 | 0.13 | 0.35 | 0.67 | >250h | <280h | * | - | - | |
| HA | Hereward | 377 | <0.0005 | <0.0025 | <0.025 | >280 | >280 | 416 | 1 | 1 | |
| | | 355 | 0.001 | <0.01 | <0.05 | >280 | >280 | 404 | 1 | 1 | |
| | | 344 | <0.0025 | <0.025 | 0.07 | >250 | >280h | 386 | 1 | 1 | |
| AB | Hereward | 266 | 0.07 | 0.24 | 0.50 | >250h | <280h | 289 | 1 | 0.5 | c |
| | | 233 | 0.45 | 0.75 | | >250h | <280h | 277 | | 1 | 1 |
| | | 245 | 0.15 | 0.35 | 0.65 | >250h | <280h | 247 | 0.5 | 1 | g |

**Table B.5** (continued)

| Site | Cultivar | Pre-harvest HFN | Probability of combine HFN failing to reach value | | | Combine HFN data | | | Score | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 220 s | 250 s | 280 s | Expected[3] Lower Limit (s) | Expected Upper Limit (s) | Actual HFN | Lower | Upper | |
| UA | Hereward | 221 | 0.3 | 0.55 | 0.82 | >220h | <250h | * | - | - | |
| | | 207 | 0.36 | 0.64 | 0.85 | >220h | <250h | * | - | - | |
| | | 214 | 0.30 | 0.60 | 0.83 | >220h | <250h | * | - | - | |
| HA | Cantata | 260 | 0.09 | 0.26 | 0.55 | >250h | <280h | 275 | 1 | 1 | |
| | | 284 | <0.05 | 0.14 | 0.35 | >220 | >280h | 309 | 1 | 1 | |
| | | 264 | 0.07 | 0.24 | 0.5 | >250h | <280h | 272 | 1 | 1 | |
| AB | Cantata | 171 | 0.64 | 0.85 | >0.95 | <220h | <280 | 149 | 1 | 1 | |
| | | 165 | 0.66 | 0.865 | >0.95 | <220h | <280 | 128 | 1 | 1 | |
| | | 163 | 0.66 | 0.865 | >0.95 | <220h | <280 | 126 | 1 | 1 | |
| HA | Malacca | 377 | <0.0005 | <0.005 | <0.025 | >280 | >280 | 416 | 1 | 1 | |
| | | 355 | 0.0025 | <0.005 | 0.05 | >280 | >280 | 404 | 1 | 1 | |
| | | 344 | <0.001 | <0.025 | 0.08 | >250 | >280h | 386 | 1 | 1 | |
| AB | Malacca | 353 | 0.0025 | <0.005 | 0.05 | >280 | >280 | 405 | 1 | 1 | |
| | | 375 | <0.0005 | <0.001 | <0.025 | >280 | >280 | 390 | 1 | 1 | |
| | | 368 | <0.01 | <0.025 | <0.05 | >280 | >280 | 418 | 1 | 1 | |
| UA | Malacca | 317 | <0.01 | <0.05 | 0.17 | >250 | >280h | * | - | - | |
| | | 294 | <0.025 | 0.1 | 0.3 | >220 | >280h | * | - | - | |
| | | 332 | <0.01 | <0.025 | 0.1 | >250 | >280h | * | - | - | |
| HA | Spark | 293 | 0.025 | 0.11 | 0.33 | >220 | >280h | 391 | 1 | 1 | |
| | | 297 | 0.025 | 0.11 | 0.33 | >220 | >280h | 354 | 1 | 1 | |
| | | 268 | 0.06 | 0.22 | 0.5 | >250h | <280h | 327 | 1 | 0 | d |
| AB | Spark | 303 | <0.025 | 0.07 | 0.25 | >220 | >280h | 285 | 1 | 1 | |
| | | 266 | 0.07 | 0.24 | 0.50 | >250h | <280h | 295 | 1 | 0 | c |
| | | 319 | <0.01 | <0.05 | 0.16 | >250 | >280h | 307 | 1 | 1 | |

Total possible points: 72 (45 samples, 9 missing HFNs, 2 points per prediction)
Points scored: 61(84.7%)

**Table B.6**: Probability of combine HFN falling below criteria and scoring of combine harvest HFN (research sites, 1998 - Class II cultivars) for the probability distribution functions derived from Equation A (Soissons and Riband combined)

| Site | Cultivar | Pre-harvest HFN (s) | Probability of combine HFN failing to reach value | | | Combine HFN data | | | Score | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 220 s | 250 s | 280 s | Expected[3] Lower Limit (s) | Expected Upper Limit (s) | Actual HFN | Lower | Upper | |
| HA | Charger | 264 | 0.07 | 0.24 | 0.50 | >250h | <280h | 300 | 1 | 0 | d |
| | | 247 | 0.14 | 0.35 | 0.65 | >250h | <280h | 291 | 1 | 0 | b |
| | | 245 | 0.14 | 0.35 | 0.65 | >250h | <280h | 297 | 1 | 0 | b |
| AB | Charger | 253 | 0.10 | 0.30 | 0.60 | >250h | <280h | 251 | 1 | 1 | |
| | | 275 | 0.05 | 0.19 | 0.44 | >220 | >280h | 268 | 1 | 0 | g |
| | | 266 | 0.07 | 0.24 | 0.50 | >250h | <280h | 269 | 1 | 1 | |
| HA | Riband | 242 | 0.15 | 0.39 | 0.67 | >250h | <280h | 269 | 1 | 1 | |
| | | 212 | 0.35 | 0.62 | 0.85 | >220h | <250h | 237 | 1 | 1 | |
| | | 193 | 0.50 | 0.75 | 0.93 | <220h | <250h | 254 | 0 | 0.5 | b |
| AB | Riband | 220 | 0.27 | 0.55 | 0.80 | >220h | <250h | 209 | 0 | 1 | e |
| | | 187 | 0.52 | 0.77 | 0.92 | <220h | <250h | 243 | 0 | 1 | e |
| | | 217 | 0.30 | 0.57 | 0.82 | >220h | <250h | 259 | 1 | 0.5 | e |
| HA | Consort | 262 | 0.08 | 0.25 | 0.53 | >250h | <280h | 304 | 1 | 0 | d |
| | | 249 | 0.13 | 0.34 | 0.62 | >250h | <280h | 289 | 1 | 0.5 | b |
| | | 243 | 0.15 | 0.38 | 0.68 | >250h | <280h | 300 | 1 | 0 | d |
| AB | Consort | 244 | 0.15 | 0.35 | 0.65 | >250h | <280h | 274 | 1 | 1 | |
| | | 218 | 0.30 | 0.56 | 0.80 | >220h | <250h | 246 | 1 | 1 | |
| | | 238 | 0.18 | 0.41 | 0.70 | >250h | <280h | 242 | 0.5 | 1 | g |
| HA | Rialto | 283 | 0.025 | 0.14 | 0.35 | >220 | >280h | 334 | 1 | 1 | |
| | | 183 | 0.54 | 0.77 | 0.94 | <220h | <250h | 290 | 0 | 0 | a |
| | | 226 | 0.25 | 0.50 | 0.77 | >220h | <250h | 271 | 1 | 0 | b |

**Table B.6** (continued)

| Site | Cultivar | Pre-harvest HFN (s) | Probability of combine HFN failing to reach value | | | Combine HFN data | | | Score | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 220 s | 250 s | 280 s | Expected[3] Lower Limit (s) | Expected Upper Limit (s) | Actual HFN | Lower | Upper | |
| AB | Rialto | 225 | 0.25 | 0.52 | 0.79 | >220h | <250h | 259 | 1 | 0.5 | |
| | | 267 | 0.07 | 0.24 | 0.50 | >250h | <280h | 251 | 1 | 1 | |
| | | 312 | 0.01 | 0.05 | 0.20 | >250 | >280h | 217 | 0 | 0 | e |
| UA | Rialto | 243 | 0.15 | 0.35 | 0.65 | >220 | <280h | * | - | - | |
| | | 223 | 0.25 | 0.50 | 0.77 | >220h | <250h | * | - | - | |
| | | 236 | 0.19 | 0.44 | 0.72 | >250h | <280h | * | - | - | |

Total points possible = 48 (27 samples, 3 missing HFNs, two points per sample)
Points scored = 31.5 (65.6%)

[1]pre-harvest [2] between pre-harvest testing and combine harvest [3] in the absence of rain
* = Not combine harvested due to rainfall and sprouting

**Table B.7**: Probability of combine HFN falling below criteria and scoring of combine harvest HFN (commercial sites, 1998 - Class I cultivars) for the probability distribution functions derived from Equation A (Soissons and Riband combined)

| Site | Cultivar | Pre-harvest HFN (s) | Probability of combine HFN failing to reach value | | | Combine HFN data | | | Score | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 220 s | 250 s | 280 s | Expected[3] Lower Limit (s) | Expected Upper Limit (s) | Actual HFN | Lower | Upper | |
| BK | Hereward | 307 | <0.025 | 0.06 | 0.23 | >220 | >280h | 271 | 1 | 0.5 | c |
| | | 299 | <0.025 | 0.08 | 0.25 | >220 | >280h | 293 | 1 | 1 | |
| | | 207 | 0.35 | 0.64 | 0.85 | >220h | <250h | 223 | 1 | 1 | |
| | | 295 | <0.025 | 0.10 | 0.30 | >220 | >280h | 241 | 1 | 0 | g |
| | | 303 | <0.025 | 0.08 | 0.25 | >220 | >280h | 207 | 0 | 0 | g |
| | | 299 | <0.025 | 0.10 | 0.30 | >220 | >280h | 280 | 1 | 1 | |
| | | 254 | 0.1 | 0.30 | 0.60 | >250 | <280h | 261 | 1 | 1 | |
| | | 303 | <0.025 | 0.08 | 0.25 | >220 | >280h | 236 | 1 | 0 | g |
| | | 299 | <0.025 | 0.08 | 0.25 | >220 | >280h | 244 | 1 | 0 | g |
| | | 331 | <0.01 | <0.05 | 0.12 | >250 | >280h | 349 | 1 | 1 | |
| | | 340 | <0.005 | <0.025 | 0.07 | >250 | >280h | 354 | 1 | 1 | |
| | | 293 | 0.025 | 0.11 | 0.33 | >220 | >280h | 313 | 1 | 1 | |
| | | 319 | <0.01 | <0.05 | 0.16 | >250 | >280h | 315 | 1 | 1 | |
| SC | Hereward | 262 | 0.07 | 0.25 | 0.5 | >250h | <280h | 271 | 1 | 1 | |
| | | 230 | 0.23 | 0.48 | 0.75 | >250h | <280h | 244 | 0.5 | 1 | g |
| | | 180 | 0.55 | 0.88 | 0.95 | <220h | <280 | 94 | 1 | 1 | |
| | | 299 | <0.025 | 0.08 | 0.25 | >220 | >280h | 265 | 1 | 0 | g |
| | | 165 | 0.67 | 0.86 | 0.97 | <220h | <280 | 207 | 1 | 1 | |
| | | 243 | 0.15 | 0.37 | 0.67 | >250h | <280h | 214 | 0 | 1 | g |

**Table B.7** (continued)

| Site | Cultivar | Pre-harvest HFN (s) | Probability of combine HFN failing to reach value | | | Combine HFN data | | | Score | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 220 s | 250 s | 280 s | Expected[3] Lower Limit (s) | Expected Upper Limit (s) | Actual HFN | Lower | Upper | |
| AB | Hereward | 317 | <0.01 | <0.05 | 0.17 | >250 | >280h | * | - | - | |
| | | 287 | <0.05 | 0.14 | 0.34 | >220 | >280h | * | - | - | |
| | | 245 | 0.09 | 0.35 | 0.65 | >250h | <280h | 210 | 0 | 1 | h |
| | | 233 | 0.22 | 0.45 | 0.75 | >250h | <280h | 280 | 1 | 1 | d |
| | | 289 | <0.05 | 0.11 | 0.33 | >250h | >280h | 240 | 0.5 | 0 | h |
| BK | Axona | 306 | <0.025 | 0.06 | 0.20 | >220 | >280h | 356 | 1 | 1 | |
| BK | Abbot | 292 | <0.05 | 0.11 | 0.33 | >220 | >280h | 269 | 1 | 0 | g |
| | | 286 | <0.05 | 0.14 | 0.35 | >220 | >280h | 307 | 1 | 1 | |
| | | 304 | <0.025 | 0.06 | 0.23 | >220 | >280h | 262 | 1 | 0 | g |
| SC | Abbot | 266 | 0.07 | 0.24 | 0.50 | >250h | <280h | 232 | 0 | 1 | g |
| | | 319 | <0.01 | <0.05 | 0.17 | >250 | >280h | 290 | 1 | 1 | |
| | | 285 | <0.05 | 0.14 | 0.35 | >220 | >280h | 295 | 1 | 1 | |
| SC | Spark | 259 | 0.09 | 0.26 | 0.55 | >250h | <280h | 242 | 0.5 | 1 | g |
| | | 265 | 0.07 | 0.24 | 0.50 | >250h | <280h | 280 | 1 | 1 | |
| | | 286 | <0.05 | 0.14 | 0.35 | >220 | >280h | 304 | 1 | 1 | |
| | | 253 | 0.10 | 0.31 | 0.60 | >250h | <280h | 206 | 0 | 1 | g |
| | | 240 | 0.17 | 0.41 | 0.72 | >250h | <280h | 271 | 1 | 1 | |

Points available: 68 (36 samples, two combine HFN samples lost in the post, 2 points per sample)
Points scored: 53 (77.9%)

[1]pre-harvest [2] between pre-harvest testing and combine harvest [3] in the absence of rain * = combine harvest sample lost

**Table B.8**: Probability of combine HFN falling below criteria and scoring of combine harvest HFN (commercial sites, 1998 - Class II cultivars). for the probability distribution functions derived from Equation A (Soissons and Riband combined)

| Site | Cultivar | Pre-harvest HFN (s) | Probability of combine HFN failing to reach value | | | Combine HFN data | | | Score | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 220 s | 250 s | 280 s | Expected[3] Lower Limit (s) | Expected Upper Limit (s) | Actual HFN | Lower | Upper | |
| BK | Consort | 299 | <0.025 | 0.09 | 0.25 | >220 | >280h | 251 | 1 | 0 | g |
| | | 280 | <0.05 | 0.17 | 0.40 | >220 | >280h | 271 | 1 | 0.5 | g |
| | | 259 | 0.06 | 0.21 | 0.47 | >250h | >280h | 262 | 1 | 0 | g |
| SC | Consort | 234 | 0.19 | 0.42 | 0.70 | >250h | <280h | 223 | 0 | 1 | g |
| AB | Consort | 309 | <0.025 | 0.06 | 0.21 | >220 | >280h | 302 | 1 | 1 | |
| SC | Rialto | 124 | 0.84 | >0.95 | >0.975 | <220h | <250 | 198 | 1 | 1 | |
| | | 190 | 0.50 | 0.75 | 0.93 | <220h | <250 | 261 | 0 | 0 | c |
| | | 203 | 0.39 | 0.66 | 0.87 | >220h | <250h | 245 | 1 | 1 | |
| | | 256 | 0.1 | 0.29 | 0.60 | >250h | <280h | 257 | 1 | 1 | |
| | | 205 | 0.4 | 0.66 | 0.87 | >220h | <250h | 148 | 0 | 1 | g |
| AB | Rialto | 258 | 0.14 | 0.35 | 0.65 | >250h | <280h | * | - | - | |
| | | 304 | <0.025 | 0.06 | 0.23 | >220 | >280h | 62 | 0 | 0 | h |
| | | 262 | 0.08 | 0.25 | 0.54 | >250h | <280h | 278 | 1 | 1 | |
| | | 242 | 0.16 | 0.40 | 0.69 | >250h | <280h | 62 | 0 | 1 | h |
| | | 241 | 0.16 | 0.40 | 0.69 | >250h | <280h | * | - | - | |
| | | 248 | 0.13 | 0.34 | 0.64 | >250h | <280h | * | - | - | |
| | | 251 | 0.13 | 0.34 | 0.64 | >220h | <250h | * | - | - | |
| | | 231 | 0.23 | 0.50 | 0.75 | >220h | <280h | 161 | 0 | 1 | f |
| | | 224 | 0.25 | 0.51 | 0.77 | >220h | <250h | 180 | 0 | 1 | h |
| | | 180 | 0.55 | 0.80 | 0.94 | <220h | <250h | 192 | 1 | 1 | |

**Table B.8** (continued)

| Site | Cultivar | Pre-harvest HFN (s) | Probability of combine HFN failing to reach value | | | Combine HFN data | | | Score | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 220 s | 250 s | 280 s | Expected[3] Lower Limit (s) | Expected Upper Limit (s) | Actual HFN | Lower | Upper | |
| AB | Charger | 314 | <0.025 | <0.05 | 0.17 | >250 | >280h | * | - | - | |
| | | 299 | <0.025 | 0.09 | 0.27 | >220 | >280h | * | - | - | |
| | | 305 | <0.025 | 0.07 | 0.23 | >220 | >280h | * | - | - | |
| | | 306 | <0.025 | 0.07 | 0.23 | >220 | >280h | * | - | - | |

Total possible points: 32 (24 samples, 8 missing combine harvest samples, 2 points per sample)
Points scored: 20.5 (64.6%)

[1]pre-harvest [2] between pre-harvest testing and combine harvest [3] in the absence of rain
* = Not combine harvested due to rainfall and sprouting or sample lost

## APPENDIX IV

**Suggested protocol for implementation of a Hagberg falling number prediction scheme**

1. SAMPLING

EITHER

* Note the date of Zadoks growth stage[1] [ZGS] 55 (50% of ears emerged from 50% of boots) for each crop to be assessed.

* Calculate accumulated potential evapotranspiration (mm) from ZGS 55.

* At 190 mm accumulated potential evapotranspiration from ZGS 55, sample 20 ears randomly from the crop to be tested. Measure the weight of fresh ears (M1), then dry these ears in an oven for two hours at 130°C. Allow the dried ears to cool then measure the weight of dry ears (M2). Calculate the moisture content (%) as 100*(M1-M2)/M1. If moisture content is below 35% the crop is ready for sampling. 35% moisture content is the **earliest possible** sample point, for maximum timeliness of pre-harvest information. Sampling can be done at ant time after the phase of 35% grain moisture has passed.

OR

* Sample after the hard dough (ZGS 87-89) or yellow ripe stage, at moisture content below 30%, or at any subsequent time throughout the harvest period.

* Sample 5 x 80 ears randomly from EACH of FIVE randomly chosen positions across each field to make a pooled sample of 400 ears per field. Sampling may be done semi-systematically, *e.g.* by dividing a field by tramlines. DO NOT SAMPLE FROM ONE LOCATION. DO NOT SAMPLE LODGED OR DISEASED AREAS. DO NOT SAMPLE FROM TRAMLINES OR IF THERE IS A HIGH PROPORTION OF GREEN EARS IN THE CROP.

* Place the ear sample in a plastic bag and dispatch to the analysis laboratory by NEXT DAY DELIVERY for pre-harvest HFN analysis

2. LABORATORY ANALYSIS

* On sample arrival, take a random subsample of 20 ears to determine moisture content as described above. If moisture content is greater than 35% reject the sample and advise re-sampling.

* Reject any samples with unusually large numbers of green, diseased or insect-infested ears.

* Dry the ears for 24 h at 45°C in a fan-assisted oven. DO NOT ALLOW THE TEMPERATURE TO EXCEED 50°C. This would inactivate the *alpha*-amylase activity and give an erroneous HFN prediction.

* Thresh the ears and clean all chaff and debris from the grain after drying

* Reject any samples with large amounts of green grains, pre-maturity sprouting, orange wheat blossom midge damage or other insect damage.

* Mill at least 300 g of grain in a hammer mill.

* Determine the pre-harvest Hagberg falling number according to the British Standard[2] or International Cereals Committee[3] methods.

* Use the HFN probability distribution function (**Figure 7**) criteria to determine the probability that HFN will meet given criteria (*e.g.* export 220 s, breadmaking 250 s) in the absence of rain.

* Re-sampling and re-testing HFN following the procedure above is recommended after any periods of significant rainfall to give more accurate HFN predictions than those based solely on earlier information.

## References


1. Tottman, D.R. and Broad, H. (1987). The decimal code for the growth stages of cereals, with illustrations. *Annals of Applied Biology* **110**, 441-454.

2. Anonymous (1982). British Standard methods of test for cereals and pulses. Determination of falling number of cereals. BS 4317. Part 9.

3. Anonymous (1974). International Organisation for Standardisation - ISO 3093.