



**RESEARCH REVIEW No. 2**

**HAGBERG FALLING NUMBER  
AND BREADMAKING QUALITY**

**AUGUST 1988**

**Price £10.00**



## AUTHORS

### HAGBERG FALLING NUMBER AND BREADMAKING QUALITY

1-3 **ABSTRACT AND INTRODUCTION**

*D. B. STEVENS and the late J. H. BALDWIN, Agricultural Development and Advisory Service, Brooklands Avenue, Cambridge CB2 2DR.*

4. **MILLING AND BAKING**

*D. J. STEVENS, B. A. STEWART AND S. C. W. HOOK, Flour Milling and Baking Research Association, Chorleywood, Herts WD3 5SH.*

5. **VARIETIES**

*A. G. MORGAN, National Institute of Agricultural Botany, Huntingdon Road, Cambridge CB3 0LE.*

6. **HUSBANDRY**

*D. B. STEVENS, R. J. COOK, L. V. VAIDYANATHAN, G. L. JONES and K. A. McLEAN, Agricultural Development and Advisory Service, Brooklands Avenue, Cambridge CB2 2DR.*

7. **GENETICS AND BREEDING**

*M. D. GALE and J. E. FLINTHAM, Institute of Plant Science Research, Maris Lane, Cambridge CB2 2JB.*

8. **BIOCHEMISTRY**

*CAROL M. DUFFUS, Department of Agricultural Biochemistry, School of Agriculture, University of Edinburgh, West Mains Road, Edinburgh EH9 3JG.*

## CONTENTS

<u>Page</u>		
1	1.	<b><u>ABSTRACT</u></b>
		1.1 Introduction
		1.2 Milling and Baking
		1.3 Varieties
		1.4 The influence of husbandry
		1.5 Genetics and breeding
		1.6 Biochemistry
		1.7 Future research and development requirements
6	2.	<b><u>GLOSSARY OF TERMS AND ABBREVIATIONS</u></b>
		2.1 Terms
		2.2 Abbreviations
10	3.	<b><u>INTRODUCTION</u></b>
11	4.	<b><u>MILLING AND BAKING</u></b>
		4.1 Introduction
		4.2 The determination of Hagberg Falling Number (HFN)
		4.3 HFN and milling
		4.4 HFN and baking
		4.5 References
21	5.	<b><u>VARIETIES</u></b>
		5.1 Introduction
		5.2 HFN in the statutory UK National List testing system for wheat
		5.3 HFN in the Advisory NIAB Recommended List Testing system for wheat
		5.4 Breeding progress
		5.5 Spring versus winter varieties
		5.6 Effect of site and season
		5.7 Effect of storage
		5.8 Summary
		5.9 Future research and development requirements
		5.10 References
		5.11 Appendices
43	6.	<b><u>THE INFLUENCE OF HUSBANDRY</u></b>
		6.1 Introduction
		6.2 Cultivations
		6.3 Date of drilling
		6.4 Growth Regulators

		6.5	Spring fertiliser nitrogen
		6.6	Fungicides
		6.7	Time of harvesting
		6.8	Pre-harvest testing
		6.9	Post-harvest manipulation
		6.10	Future research and development requirements
		6.11	References
		6.12	Appendices
63	7.		<b><u>GENETICS AND BREEDING</u></b>
		7.1	Introduction
		7.2	The genetics of grain characters in wheat
		7.3	Pre-maturity alpha-amylase
		7.4	Dormancy
		7.5	Pre-maturity germination
		7.6	Rate of alpha-amylase development during sprouting
		7.7	Conclusions
		7.8	Future research and development requirements
		7.9	References
80	8.		<b><u>BIOCHEMISTRY</u></b>
		8.1	Introduction
		8.2	Review of present information
		8.3	Future research and development requirements
		8.4	References
90	9.		<b><u>ACKNOWLEDGEMENTS</u></b>

## HAGBERG FALLING NUMBER AND BREADMAKING QUALITY

### 1. ABSTRACT

This review includes major contributions from the Flour Milling and Baking Research Association (FMBRA), the National Institute of Agricultural Botany (NIAB), the Institute of Plant Science Research (IPSR) (Cambridge Laboratory) and the Edinburgh School of Agriculture, in addition to a section contributed by colleagues in the Agricultural Development and Advisory Service (ADAS).

#### 1.1 INTRODUCTION

The EEC policy of encouraging home production of quality wheat has resulted in an increase in the price differential between home-produced and imported wheat so that millers are seeking to use the maximum proportion of home-produced grain in their grists. Some deficiencies in the home-produced grain have been overcome by supplements of gluten or modification of the baking process. However a major restraint to any further increase in the use of home-produced grain remains the high level of alpha-amylase enzyme present in many samples, particularly in cold and damp summers. The Hagberg Falling Number (HFN) test is used as an indicator of the level of alpha-amylase enzyme in the sample.

#### 1.2 MILLING AND BAKING

The enzyme alpha-amylase has long been known to be produced in the early stages of germination. It is the most important of a group of enzymes that break down complex food reserves in the grain into simple soluble sugars needed by the developing embryo. At the onset of germination the level of alpha-amylase enzyme can undergo a thousand-fold increase and the presence of only a few such grains can affect the HFN of a large bulk of grain. High levels of alpha-amylase enzyme result in loaves with a sticky interior which causes problems for commercial bakers' slicing machinery.

The HFN test has been developed to provide a reasonably rapid and cheap indication of the level of alpha-amylase enzyme in the grain. However, to provide reproducible values it involves very precise milling of the grain, correction of sample weight for moisture content and strict compliance with procedures for the use of test equipment. Since only 7g of flour are used in the test it is also imperative that the sample accurately represents the bulk from which it is drawn. The test is accurate to  $\pm 5\%$  if conducted correctly, although greater errors are frequently observed because of the difficulties in obtaining representative samples and insufficiently rigid control of the test. It is also important to recognise that HFN numbers are not directly additive. The result of mixing two samples can be estimated but the mixture will have a much lower HFN than a direct mathematical mean.

FMBRA has investigated many methods of minimising the deleterious effects of low HFN but the benefits are generally marginal except in the case of simultaneous combination of microwave and thermal baking. Unfortunately, this method has not as yet been commercially acceptable mainly because of cost.

### 1.3 VARIETIES

HFN is an important factor in wheat breeding programmes and is one of the quality tests that is applied to all varieties within the UK National List and Recommended List testing schemes. After the initial screening year, varieties are grown with and without fungicides. In the years 1981-83 HFN tests were conducted on samples from both treatments but, since then, only the fungicide-treated wheats have been tested. It has long been recognised that pre-harvest sprouting has profoundly deleterious effects on HFN so, since the early 1950s, varieties have been assessed for predisposition to pre-harvest sprouting.

It is apparent that among current varieties, spring cultivars tend to have better HFN values but this is thought to be due to the higher standards that have been required for the minority spring crop to find acceptance. Variation from season to season and site to site has been extremely large in both winter and spring varieties.

While variation from site to site or season to season often had little effect on the ranking order of varieties, it was observed that some varieties were more variable than others. In some cases a low resistance to sprouting is thought to contribute to variable HFN values but in other cases a clear relationship is not established suggesting that in some conditions alpha-amylase is produced independent of visible sprouting.

### 1.4 THE INFLUENCE OF HUSBANDRY

During the last few seasons the HFN test has been included with other routine tests of samples from a wide range of ADAS husbandry trials. In many instances a relationship would appear to exist between treatment and HFN but further detailed examination points to only an indirect effect. For example, at first sight it would appear that growth regulators have improved HFN values but this is actually an indirect result of controlling lodging. Similarly, cultivations can have an effect by influencing the survival of volunteers from a previous crop. Time of sowing trials need to be harvested promptly to avoid the early sowing being at risk to adverse weather. When such details were examined, there was no reliable direct relationship with cultivation, date of drilling or growth regulators.

Fungicides have an effect which seems to be greatest if they are used repeatedly and achieve a marked increase in duration of green leaf area. Moreover, there is evidence that some groups of fungicides have more effect on HFN than others, suggesting a direct growth regulatory effect on enzyme production.

Analysis of agronomy trials has shown that if spring nitrogen application is below the optimum for yield, the HFN will also suffer. Often the response of HFN to nitrogen appears to be dependent on varieties.

Other work has concentrated on predicting HFN values at harvest by extrapolation from samples taken 10-14 days earlier when the moisture content is still 25-30%. Such a prediction service, when perfected would allow the crops from fields with low HFN to be stored separately. The better fields could be given priority and their quality preserved.

A detailed study has been made of the effect of one commercial gravity separator and it was shown to be capable of separating a mixed sample very effectively so that the HFN of a proportion (often 60-70%) of a sample starting with a value of less than 100 can be raised to over 200 while the remainder still sells at a feed wheat price. However, it has also been noted that the breadmaking value of this separated wheat may not be improved to the extent expected from the improved HFN values.

### 1.5 GENETICS AND BREEDING

In recent years, the seasons of 1977, 1985 and 1987 were noted for the high levels of alpha-amylase (low HFN) recorded at harvest. It has been shown that enzyme production has been the result of different causes in each of these three seasons. In 1977 there was conventional sprouting of mature grains post-dormancy but, in 1985, the enzyme production was much earlier in the development of the grain. In 1987 the problem was germination following the onset of ripening but prior to the onset of dormancy. Furthermore, there is evidence that the three different physiological mechanisms are each under independent genetic control.

The mechanism of inheritance in grain tissues, together with the difficulties of manipulating genes to alter levels of alpha-amylase in sprouted grain is discussed. It is likely that the several key UK varieties which have inherited a susceptibility to the expression of pre-maturity alpha-amylase enzyme obtained the genes responsible from the Belgian variety Professeur Marchal. It is suggested that the genes responsible for high pre-maturity alpha-amylase can be removed by judicious selection.

The gibberellin-insensitive dwarfing genes including Rht1, Rht2 and Rht3 all have some action in reducing pre-maturity alpha-amylase levels although only the more extreme Rht3 dwarfing gene is likely to have an economically significant effect in agricultural practice.

The difficulties of measuring dormancy in field conditions are discussed and it is concluded that red grain colour is associated with increased dormancy because either the red phlobaphene pigment itself, or precursors of it, cause a temporary inhibition of germination. Selection for red colour as opposed to white is obviously simple. However, it is likely that 3-gene reds would have greater dormancy than single gene reds and the distinction between 1, 2 and 3-gene red wheats is more difficult. Further sources of dormancy are discussed but it is not yet clear if these can all provide cumulative benefit.

The relationship between pre- and post-maturity sprouting is not understood, thus it is not known whether the same genetic strategies to achieve resistance should be employed.

### 1.6 BIOCHEMISTRY

As previously indicated, there are at least three periods when alpha-amylase enzyme is produced in response to the appropriate stimulus but the appropriate combinations do not occur regularly and therefore the mechanisms have not been well researched. However, it is suggested that variation between varieties may be the result of numerous mechanisms. These include physical differences in the grain surface which affect

water and oxygen absorption, and differences in the rate of embryo development. Many complex biochemical processes are occurring in the developing grain and inevitably these will affect the enzyme production systems. Probably the most investigated but still not completely understood group of effects are those associated with endogenous plant growth hormones, including gibberellins, auxin, cytokinins and ABA. The current understanding of these growth hormones and the ways in which their action is modified by environmental conditions is reviewed.

#### 1.7 FUTURE RESEARCH AND DEVELOPMENT REQUIREMENTS

Detailed recommendations are made under each section but include the following:-

1. While HFN has been used as a convenient rapid indicator of enzyme levels and suitability for breadmaking, there are indications that other factors can be influential. For example, HFN can in some situations be improved by gravity separation while breadmaking may not be improved. This needs further investigation.
2. It is essential that a well co-ordinated national system of variety testing is maintained with samples from a wide range of environments being tested to evaluate the potential level of HFN and stability over a wide range of conditions.
3. Having identified differences in susceptibility to pre-germination enzyme production, the biochemistry of this system should be thoroughly researched and the benefits transferred to plant breeding as soon as possible.
4. Since the HFN test does not always reflect precisely the value of wheat for breadmaking, alternatives should be investigated, preferably identifying a test that could be used by breeders and variety testing authorities as well as grain purchasers.
5. HFN results from stored grain have been rather inconsistent and a more thorough investigation is necessary to determine the effect of storage conditions and moisture content.
6. The effect of fungicide applications in lowering HFN values needs further investigation to determine optimum strategies which could vary for different varieties, diseases and environments. The use of a desiccant should be included as a treatment in appropriate fungicide, nitrogen and time of harvest trials so that its value in aiding the final drying of the crop is more thoroughly investigated.
7. If a reliable pre-harvest sampling service is to be available further work is necessary to confirm details of the appropriate sampling technique.
8. Further research is required to determine precisely the environmental factors that trigger pre-maturity alpha-amylase production so that unequivocal screening methods can be made available for use in research and plant breeding. The major genes involved in the enzyme system should be characterised and the need for still further genetic protection evaluated.



9. Further work is necessary to determine the extent of the dormancy regulating potential of the R genes that provide red seed coat colour. A better understanding of their mechanism will provide guidance for their most effective use.
10. Pre-maturity sprouting in wheat is a rare phenomenon which caused problems in 1987. Further work is required to identify precisely the environmental trigger involved before real genetic analysis can proceed.

This review, completed in August 1988 and with 90 pages in the full article, was funded by the HOME-GROWN CEREALS AUTHORITY, Hamlyn House, Highgate Hill, London, N19 5PR.

## 2. GLOSSARY OF TERMS AND ABBREVIATIONS

### 2.1 TERMS

#### **Abscisic acid**

A plant growth regulator, many of whose effects appear to be the result of inhibition of GA action.

#### **Aleurone (layer)**

A single layer of secretory cells surrounding the starchy endosperm and derived from the same origin (Fig. 7.4).

#### **Alleles**

Alternative genes carried at a genetic locus.

#### **Alpha-amylase**

Cereal: an enzyme (protein) involved in the initiation of starch degradation in the starchy endosperm of cereal seeds.

Fungal: a similar enzyme, not naturally occurring in wheat, that is used as an additive in breadmaking. It is inactivated at lower temperatures than cereal alpha-amylase.

#### **Amino acids**

The sub-units of protein molecules.

#### **Auxin**

A family of plant growth regulators whose best known effect is to promote the elongation of stems and coleoptiles.

#### **Carbohydrate**

Substances containing carbon, hydrogen and oxygen that either are, or can be, broken down into sugars.

#### **Chromosomes**

The microscopic bodies in every living cell which contain the genetic code.

#### **Common Agricultural Policy**

The collective agricultural policy of the European Economic Community (EEC).

#### **Cuticle**

A layer of waxy material.

#### **Cytokinins**

A family of growth regulators with diverse effects including the ability to interact with indole acetic acid (IAA) and to promote cell division and differentiation.

#### **Dextrins**

Carbohydrates formed by partial breakdown of starch, typically containing up to 20-30 linked glucose units. They are viscous and sticky, and can be responsible for giving bread a crumbly structure.

**Diploid**

Having two sets of chromosomes (one maternal plus one paternal, the normal condition in most higher plants).

**Dominance**

The preferential expression of a 'dominant' gene over its 'recessive' allele.

**Dormancy**

The state when mature undamaged seeds fail to germinate under conditions which usually support germination.

**Embryo**

The part of the germ which develops into a seedling following germination.

**Endosperm**

The main storage organ of the grain, mainly starch plus protein.

**Enzyme**

One of a group of complex proteins produced by living cells that act as catalysts in specific biochemical reactions.

**Fructose**

A soluble sugar or monosaccharide.

**Fungicide**

A chemical substance capable of controlling fungi.

**Gelatinisation**

The process whereby starch granules are disordered and swollen by the action of hot water.

**Genetic linkage**

The close association (and co-inheritance) of different genes on the same chromosomes.

**Genome**

The entire genetic complement derived from a single diploid species.

**Germ**

This contains the elements of a young plant; the plumule which grows into a shoot and radicle which becomes a primary root.

**Gibberellins**

A family of plant growth regulators whose best known effect is their ability to trigger synthesis of alpha-amylase in the aleurone layers of germinating cereal grains.

**Glucose**

A soluble sugar or monosaccharide.

**ADP-glucose**

Adenosine diphosphate glucose. Glucose reacts frequently in combination with ADP.

ADP (UDP) - glucose pyrophosphorylase  
An enzyme catalysing the conversion of ADP (UDP)-  
glucose to glucose-1-phosphate or vice versa.

UDP-glucose  
Uridine diphosphate glucose. Glucose reacts  
frequently in combination with UDP.

**Gluten**

The hydrated, water insoluble fraction of the wheat protein.

**Grist**

Wheat, either a single sample or a blend, used in a flour mill to  
convert into flour.

**Heritability**

The proportion of variability caused by genetic differences.

**Hexaploid**

Having six sets of chromosomes (three diploid sets, the normal  
condition in bread wheat).

**Isozyme**

One of a group of enzymatic proteins of differing electrophoretic  
mobilities.

**Monoclonal antibodies**

These are the single antibodies produced from hybridomas derived in  
turn from mutant myeloma cell lines fused with spleen cells. It is  
the spleen cells that specify the particular antibodies produced.

**Nucellar layer**

An epidermal layer lying beneath the testa.

**Pericarp**

A tissue consisting of several layers surrounding the seed. During  
seed maturation the inner layer contains chloroplasts and accounts  
for the green colour of the immature seed. The outer layer is  
transparent during seed growth.

**Pleiotropy**

The ability of a gene to affect two or more different characters.

**Respiration**

The uptake of oxygen and evolution of carbon dioxide that  
accompanies carbohydrate oxidation.

**Scutellum**

The part of the germ lying between the embryo and the starchy  
endosperm.

**Segregation (genetic)**

The appearance of genetic differences between descendants of a  
hybrid.

**Starch**

A carbohydrate of very high molecular weight composed of linked glucose units.

**Starch synthase**

An enzyme catalysing conversion of ADP-glucose to starch.

**Substrate**

The material on which an enzyme acts.

**Sucrose**

A disaccharide (sugar) composed of one molecule each of glucose and fructose.

**Sucrose synthase**

An enzyme catalysing the conversion of sucrose to UDP-glucose and fructose.

**Testa**

A layer of cells lying on top of the nucellus and beneath the chlorophyll-containing cells of the pericarp. The site of pigment deposition during ripening in red-grained wheats.

**Third country**

Country not belonging to the EEC.

**Triticale**

A man-made crop derived from a wheat x rye cross.

**Water potential**

The difference in chemical potential of water in a system and that of pure water at the same temperature and pressure.

**2.2 ABBREVIATIONS**

ABA	Abscisic acid
ADAS	Agricultural Development and Advisory Service
ADP	Adenosine diphosphate
CAP	Common Agricultural Policy (of the EEC)
DUS	Distinct Uniform and Stable (NIAB test varieties for these characters).
EEC	European Economic Community
EHF	Experimental Husbandry Farm
FMBRA	Flour Milling and Baking Research Association
GA	Gibberellic acid
GLA	Green leaf area
HFN	Hagberg Falling Number
HGCA	Home-Grown Cereals Authority
IPSR	AFRC Institute of Plant Science Research (Cambridge)
LN	Liquefaction Number
NIAB	National Institute of Agricultural Botany
UDP	Uridine diphosphate
VCU	Value for Culture and Use (NIAB test varieties for these standards).

### 3. INTRODUCTION

The policy of the EEC has been to promote self sufficiency in the production of our major food needs within the Community. This has been encouraged by high levies on imported grain. Traditionally the UK imported hard wheat from North America, but under the EEC regime, this became uneconomic for millers of bread wheat. It became necessary for them to turn to UK produced wheat and, as UK production turned from a deficit in wheat to a surplus, it became vital for the economy as a whole to maximise the proportion of bread wheat from home-grown sources.

The type of bread demanded in the UK was traditionally produced from North American hard, high protein, red wheat. The wetter, cooler summers here result in higher yields with diluted protein levels.

Modifications to baking methods have made it easier to produce bread from UK wheat and improved varieties and growing methods have led to some improvements in grain quality.

A very major step in the UK's attempt to become self sufficient has been the establishment of starch-gluten plants. The gluten from these plants can be added to UK-produced flour and, for many bread products, this does much to offset the naturally low protein in the UK wheat.

The overriding quality problem remains the high alpha-amylase level of UK wheats in some seasons. This is brought about by rainfall before and during the harvest period and in seasons such as 1977, 1985 and 1987, results in high proportions of potentially good breadmaking wheat becoming unsuitable for this purpose or usable only at low percentage of the grist.

So far, no treatment of the grain or change in baking technology has made it possible to use wheat with high alpha-amylase activity for breadmaking and there is an urgent need to find ways of avoiding this problem.

An indication of the alpha-amylase activity in grain is provided by the measurement of the Hagberg Falling Number which has been of great value to the trade in defining quality but it is a method not widely understood, mistrusted by farmers, and has certain limitations and problems which need exploration and discussion on a wider front.

The intermittent, but serious problems surrounding the production of high alpha-amylase activity in wheat and its measurement prompted the HGCA to commission this review. Studies on this subject overlap various disciplines within crop science and involve different research, development and advisory organisations. It was considered of most value for NIAB, IPSR, FMBRA, Edinburgh School of Agriculture and ADAS to cover the topic from breeding new varieties and on-field advice through biochemistry to the ultimate baking of bread. The review explains the problems, describes research findings to date and discusses advice based on this work and the need for further research.

HGCA RESEARCH REVIEW NO. 2

HAGBERG FALLING NUMBER AND BREADMAKING QUALITY

4. MILLING AND BAKING

*D. J. STEVENS, B. A. STEWART and S. C. W. HOOK, Flour Milling and Baking Research Association, Chorleywood, Herts WD3 5SH.*

*This review, completed in August 1988, was funded by the HOME-GROWN CEREALS AUTHORITY, Hamlyn House, Highgate Hill, London N19 5PR.*

**CONTENTS FOR 4. MILLING AND BAKING**

<b>Page</b>	
13	4.1 <b>INTRODUCTION</b>
	4.1.1 Wheat as a seed
	4.1.2 Wheat as a human food
14	4.2 <b>THE DETERMINATION OF HAGBERG FALLING NUMBER (HFN)</b>
	4.2.1 The principle
	4.2.2 Details of the test procedure
	4.2.3 Accuracy of determination
	4.2.4 Importance of constant stirring
	4.2.5 Blending wheats to give a desired HFN
	4.2.6 Other methods of measuring alpha-amylase
16	4.3 <b>HFN AND MILLING</b>
	4.3.1 Quality criteria for breadmaking wheats
	4.3.2 The consistency of quality of Canadian Western Red Spring (CWRS) wheat
	4.3.3 Replacement of CWRS by home-grown wheat
	4.3.4 Effect of sprouted grains on HFN
19	4.4 <b>HFN AND BAKING</b>
	4.4.1 Attempts to counteract the deleterious effects of alpha-amylase during breadmaking
	4.4.2 The effect of fungal alpha-amylase on HFN
20	4.5 <b>REFERENCES</b>



## 4. MILLING AND BAKING

### 4.1 INTRODUCTION

The Hagberg Falling Number (HFN) test is one of a set of rapid procedures used by flour millers to assess the quality of wheat.

#### 4.1.1 Wheat as a seed

A ripe grain of wheat is intended by nature to produce a new plant. Under the outer bran coats most of the grain is made up of the white endosperm which provides a food store for the early stages of plant growth. The germ, situated at one end of the grain, under suitable moist conditions produces roots and shoots in the process of germination. The food reserves of the endosperm consist mainly of starch and proteins which have to be converted into smaller, soluble molecules needed by the developing germ.

This conversion is performed by enzymes which are synthesised in the earliest stages of germination. The most important of the starch-degrading enzymes is alpha-amylase. It attacks the long starch molecules to produce smaller molecules called dextrans, which are ultimately broken down into simple sugars. The level of alpha-amylase activity in mature grain, before germination, is low. At the onset of germination the enzyme activity increases dramatically, such that a grain with no visible signs of sprouting may develop a thousand-fold increase. The presence of only a few such grains in a sample will have a very significant effect on the overall alpha-amylase activity.

#### 4.1.2 Wheat as a human food

When mankind uses wheat as a food, it is usually made into flour for baking into bread. White flours are produced by separating the endosperm from the bran and grinding it to the required fineness. Wholemeal flours are made using the whole grain; brown flours represent an intermediate category. In all cases, starch is the major component of the flour. On baking dough to make bread, the starch granules take up water and swell, becoming soft and losing their regular shape. This process of starch gelatinisation makes a vital contribution to the structure and texture of a loaf of bread. Unfortunately alpha-amylase very rapidly degrades gelatinised starch, converting it to gummy dextrans and sugars. This process is halted only when the baking temperature reaches a level high enough to inactivate the enzyme.

If the level of alpha-amylase activity in wheat is high it will give rise to bread, which, although as well risen as a normal loaf, has a sticky interior due to the dextrans. Badly affected loaves are almost impossible to slice and are quite unacceptable to the consumer.

Unfortunately there is no practical cure for this problem at the present time and since visual examination of wheat does not allow accurate prediction of the state of germination or level of alpha-amylase activity, an objective test is needed. The HFN test is currently used by flour millers to screen wheat for alpha-amylase activity.

## 4.2 THE DETERMINATION OF HAGBERG FALLING NUMBER (HFN)

### 4.2.1 The principle

The HFN method determines alpha-amylase activity using the starch in the sample as substrate. The method is based upon the rapid gelatinisation of an aqueous suspension of ground wheat in a boiling water bath and the subsequent measurement of the liquefaction of the starch paste by the alpha-amylase in the sample.

### 4.2.2 Details of the test procedure (ICC, 1968)

A representative sample of grain should first be taken according to the ICC Standard method for sampling (ICC, 1982). Dust and coarse impurities should be removed from the whole grain. Approximately 300 g of the grain should be ground. The HFN values are affected by the particle size of the ground grain, which should comply with the following specification.

Sieve aperture (microns)	% sample passing through sieve
710	100
500	94-98
210	55-70

Meal of the required particle size may be obtained by using a hammer-type laboratory mill, equipped with a 0.8 mm screen. Using the mill supplied by the Falling Number Company it is possible to grind grain of moisture content up to 30%. The mill must be fed carefully with grain to avoid overloading and heating. Grinding should be continued for 30-40 seconds after all the sample has entered the grinding chamber. Small quantities (up to 1% of the grain weight) of bran particles remaining in the grinding chamber may be discarded. The ground grain should be thoroughly mixed, and after cooling, its moisture content determined. The sample weight for each HFN determination should correspond to 7 g at 15% moisture content. If the moisture content of the meal is different from 15% then a correction to the weight has to be made.

The water bath of the HFN apparatus should be filled to within 2-3 cm of the top with distilled water. The water is heated to boiling point and must boil vigorously during the test. The test method is then as follows:

- a) Place 25 ml distilled water at 20°C in a viscometer tube.
- b) Weigh out  $7 \pm 0.05$  g of the ground grain (at 15% moisture content) and transfer it into the viscometer tube.
- c) Fit a rubber stopper into the top of the tube and shake vigorously 20-30 times, or more if necessary to obtain a uniform suspension.
- d) Remove the stopper and, with the viscometer stirrer, scrape down any material adhering to the sides of the tube into the suspension.

- e) The stirrer, complete with collar, should then be placed into the tube and the tube inserted into the boiling water bath through the hole in the lid. The timer or stopwatch is started immediately the tube touches the bottom of the rack which holds the tube in position. The tube is secured with the clip.
- f) Exactly 5 seconds after the immersion of the tube, stirring of the suspension is commenced at the rate of 2 stirs per second (1 stir = 1 up and 1 down movement), ie 4 movements per second. The length of the stirring movement is regulated by the lower stop of the stirrer and the bottom of the tube. The stirrer should lightly touch at both ends of the up and down strokes. It is important to keep this exact speed of stirring.
- g) After a total 59 seconds, the stirrer is lifted to the uppermost position, held until the timer reads 60 seconds and then released and allowed to fall under its own weight.
- h) When the lower edge of the uppermost stop is at the same level as the top of the collar, the timer is stopped. The total time, in seconds, including the 60 seconds stirring time is the Hagberg Falling Number. In a sample with low alpha-amylase activity the stirrer falls slowly giving a high HFN. In samples with high alpha-amylase activity the starch paste is liquefied by the alpha-amylase and the stirrer falls more rapidly giving a low HFN.

#### 4.2.3 Accuracy of determination

Repeated tests on subsamples of the same ground sample should give results within  $\pm 5\%$  of the average HFN value when every stage of the test procedure is performed as prescribed. The importance of correct sampling cannot be over emphasised, since differences between the alpha-amylase activity of subsamples of the ground material may occur if the sample is not well mixed.

#### 4.2.4 Importance of constant stirring

The stirring is the most important phase of the HFN determination. Great care must be taken to stir with the correct rhythm as experience has shown that different rhythms can lead to considerable variation in results. Errors from this source are reduced by using an automatic timer, which by means of sound and light signals indicates the correct rhythm. The method is greatly simplified by mechanical stirring. Both semi-automatic and fully automatic versions of the equipment are available, these overcome the above problems.

#### 4.2.5 Blending wheats to give a desired HFN

HFN values are not additive and therefore are not suitable for simple calculation of the HFN of mixtures of known composition. Liquefaction Numbers (LN) defined by the equation:

$$LN = \frac{6000}{(HFN-50)}$$

are additive, because they are directly proportional to alpha-amylase activity over the range encountered in commercial wheats. In this

equation, 50 represents the approximate time in seconds required for the starch to gelatinise sufficiently for it to be susceptible to enzyme attack. Having calculated the LN of a blend of wheat from the values of its components, the LN is converted back to HFN using the above equation.

#### **4.2.6 Other methods for measuring alpha-amylase**

The alpha-amylase activity of wheat or flour can be measured either by following a chemical reaction using colorimetric techniques or by measuring changes to the physical properties of a cooked ground wheat/water mixture. Chemical estimations, although possibly more precise, have the disadvantage of measuring the activity in solution and make no allowance for bound insoluble enzyme. The chemical method commonly used in the UK is described by Farrand (1964). Very recently a new procedure has been introduced by Biocon Biochemicals Ltd, and preliminary findings at FMBRA show it to be faster and more precise than the Farrand method.

Physical methods, which involve heating, are more closely allied to what happens in the baking process. Both the bound and unbound enzymes are measured. The HFN determination was developed to measure medium to high levels of alpha-amylase activity in wheat and has subsequently also been used for flour.

The Brabender Amylograph was developed to measure very low levels of alpha-amylase in flour (ICC, 1984). The test depends on heating a suspension of flour in water under controlled conditions and measuring the resistance to stirring.

More recently an instrument called the Rapid Grain Analyser has been developed in Australia (Ross et al., 1987). The equipment simulates the action of the Amylograph but uses ground wheat. The results are reported to correlate well with HFN values. Although the test is about as rapid as the HFN test for medium to high activity wheats, there appears to be no major advantage over the HFN equipment. Both tests would be subject to the same sampling errors; a major problem with any alpha-amylase activity measurement.

The Canadian Grain Research Laboratory in Winnipeg has also introduced a similar test based on the resistance to stirring of a heated ground wheat/water slurry (Canadian Grain Commission, 1987).

### **4.3 HFN AND MILLING**

#### **4.3.1 Quality criteria for breadmaking wheats**

Wheat destined for breadmaking has to satisfy a range of quality requirements for it to be accepted by flour millers. On arrival at the mill laboratory the wheat will be examined to check for excessive foreign matter, disease, brightness, plumpness, insects, odour and visibly sprouted grains. If the wheat is acceptable visually it will be tested for moisture content, protein content, Falling Number and possibly an indicator of breadmaking quality and variety. The moisture content of wheat for milling is important for safe storage and subsequent milling.

Protein content and quality are of great importance in wheat for breadmaking. Protein content influences the baking quality of the flour milled from the wheat. As protein content increases so does loaf volume. However, although the quantity of protein is important so is its quality. Only certain wheat varieties possess proteins that are suitable for breadmaking. Tests for proteins suitable for breadmaking, either gluten washing or a sedimentation test, may also be performed at mill intake.

If the wheat is satisfactory for breadmaking in all aspects mentioned so far it must also have a low level of the enzyme alpha-amylase. The standard mill intake test for alpha-amylase is the HFN test. Full details of the test and precautions to observe when performing it are given in section 4.2. The HFN (in seconds) for breadmaking wheat should be high, ideally above 220 (HGCA, 1987) in line with the EC standard for Intervention wheat. Lower values will be unacceptable for breadmaking wheats as they will give rise to flours that produce loaves of poor quality with an open textured and sticky crumb.

It is generally assumed that all intake testing should be completed within 20 minutes by which time a decision whether to accept or reject a load of wheat can be made. The HFN test requires only a few minutes to perform and the ground wheat sample prepared for it can be used for other tests. It is therefore well suited for mill intake testing.

#### 4.3.2 The consistency of quality of Canadian Western Red Spring (CWRS) wheat

When large quantities of CWRS wheat were imported into the UK and made up a high proportion of breadmaking grists the properties of this imported wheat were of the greatest importance to bread flour millers. The quality of the home-grown wheat crop had a relatively minor influence on bread properties. The Canadian grain marketing system ensures strict control of the quality of wheats that attain the top grade, CWRS No 1. As it was principally this grade that was bought by UK millers, quality was ensured. An examination of Canadian Grain Research Laboratory reports on wheat quality shows the consistency of quality characteristics over many years. All wheats permitted into the top two grades of Red Spring wheat must be at least equal to the variety Marquis. Hence, breadmaking wheat quality is carefully controlled and the number of licensed varieties is very limited. In most years three varieties account for more than 75% of the crop. The consistently high HFN of CWRS wheat is shown by the mean values of composite samples from 1983-1986 harvests for CWRS No 1 (13.5% protein content) from both Eastern and Western Prairies (Table 4.1).

**Table 4.1** Mean HFN for CWRS No 1 (protein content 13.5%)

Year	Eastern Prairies	Western Prairies
	HFN (sec)	
1983	425	405
1984	415	395
1985	380	375
1986	405	395

#### 4.3.3 Replacement of CWRS by home-grown wheat

The level of imported Canadian wheat has fallen from nearly 2 million tonnes in 1979 to 0.65 million tonnes in 1986. During this period the total wheat usage for flour milling has remained nearly constant. The reduction of imported Canadian wheat is almost completely mirrored by the increased usage of home-grown wheat, although in poor harvest years, for example 1985, imported European wheat was used in increased quantities. The home-grown wheat that has replaced Canadian wheat is much more variable both in terms of varieties and quality characteristics. As an example of variability, the mean HFN of wheats in the National Crop Survey for 1983-1987 are given in Table 2.

**Table 4.2** Mean Falling Numbers for home-grown wheat

Year	HFN (sec)	% of crop with HFN above 220 sec
1983	309	83.6
1984	271	72.7
1985	158	17.3
1986	222	51.8
1987	156	20.3

The two years 1985 and 1987 which had low HFN wheats were characterised by very wet harvest conditions. In addition to the variability of properties such as HFN and protein content the composition of each annual wheat crop changes, with new varieties replacing old. The life span of most UK-grown wheat varieties is 3-4 years, with very few varieties lasting as long as 10 years. Some 8 to 10 varieties make up the majority of the crop in most years. This combination of ever changing varietal composition and variation in quality characteristics such as HFN and protein content emphasises the need for comprehensive mill intake testing.

#### 4.3.4 Effect of sprouted grains on HFN

The impact of a small number of sprouted or even partially sprouted grains on HFN is very considerable. During germination the alpha-amylase activity increases dramatically. The inclusion of only a few sprouted grains into an otherwise sound sample will reduce its HFN greatly. It has been shown at FMBRA that the addition of just 2% of wheat that had been sprouted for two days reduced the HFN of a sample of wheat with a value of over 300 seconds to below 180 seconds. Doubling the level of added two-day sprouted grain or adding 2% of four-day sprouted grain reduced HFN to around 100 seconds. Therefore, the inclusion of very small quantities of lodged and sprouted grain into an otherwise sound crop will drastically reduce the wheat's HFN and hence its quality.

#### 4.4 HFN AND BAKING

##### 4.4.1 Attempts to counteract the deleterious effects of alpha-amylase during breadmaking

The use of home-grown wheat for breadmaking is limited by wet harvest conditions and the cultivation of high yielding wheat varieties having naturally high alpha-amylase activity. The outcome of using such wheats is, of course, a flour with a high level of alpha-amylase and bread with a number of faults. Amongst the deleterious effects of high alpha-amylase activity on bread properties, the most serious in the commercial plant bakery is poor sliceability. Work at FMBRA has shown that the deleterious effects can be divided into two broad categories:

- a) Effects on the chemical properties of the crumb. These result directly from the degradation of starch by alpha-amylase during breadmaking, causing the accumulation of a wide range of soluble carbohydrates in the crumb.
- b) Effects on the physical properties of the crumb. Starch degradation also results in the release of water, thus weakening the dough structure and changing the physical properties of the final bread crumb. As the HFN decreases, the loaf volume increases, causing a decrease in crumb density and strength. In commercial plant bakeries it was observed that a very important factor in the poor slicing behaviour of bread made from high alpha-amylase flours was this decrease in crumb density. This change is probably the major factor causing loaf deformation and uneven slice thickness. The coincidental increase in crumb stickiness serves to compound the effect by causing the mechanically weakened crumb to stick to the slicer blades and to accelerate the failure of the slicing operation.

The effects of various ameliorative measures on both the physical and chemical properties of bread crumb have been investigated. Reducing the amount of water in the dough and adding sodium acid pyrophosphate were predominantly effective in improving the mechanical strength of the crumb, whereas sodium stearoyl lactylate and acid calcium phosphate had an overall improving effect on all crumb properties. Extra fat in the recipe had very little effect.

The lowering of dough temperature decreased loaf volume, and consequently increased crumb density. Extending the cooling period of bread before slicing was beneficial, particularly where only a marginal slicing problem was experienced.

However, all such improvements were minimal, and did not provide a solution to the problem. At present, the only effective solution involves the simultaneous combination of microwave and thermal baking. This completely successful system was developed at FMBRA, but unfortunately no commercial equipment has become available, chiefly because its cost would be prohibitive.

#### 4.4.2 The effect of fungal alpha-amylase on HFN

For many years the milling and baking industries, both in the UK and USA, have supplemented wheat flour when low in alpha-amylase activity with fungal amylase. Because of the lower heat stability of the fungal alpha-amylase, when compared with that of cereal alpha-amylase, over-supplementation with the fungal enzyme does not lead to the bakery problems associated with excess cereal alpha-amylase. Because of the temperature at which the test is carried out, small additions of a fungal alpha-amylase to a flour do not affect its HFN. In recent years, probably because of the changes in the wheat varieties grown in the UK for breadmaking, it has become customary to supplement flours with much higher levels of the enzyme than previously. Studies at FMBRA have shown that even at these high levels there is only a small reduction in HFN. The effect only becomes pronounced at extremely high levels of addition, above those used commercially.

#### 4.5 REFERENCES

1. Canadian Grain Commission (1987). Grain Research Laboratory report 1986-1987.
2. FARRAND, E. A. (1964). Flour properties in relation to the modern bread processes in the United Kingdom, with special reference to alpha-amylase and starch damage. Cereal Chemistry, **41**, 98-110.
3. HGCA (1987). A marketing guide for milling wheat 1987.
4. ICC (1982). No 101/1 Sampling of grains.
5. ICC (1968). No 107 Determination of the "Falling Number" according to Hagberg-Perten as a measure of the degree of alpha-amylase activity in grain and flour.
6. ICC (1984). No 126 Method for using the Brabender Amylograph.
7. ROSS, A. S., WALKER, C. E., BOOTH, R. I., ORTH, R.A. and WRIGLEY, C. W. (1987). The rapid Visco-analyser: A new technique for the estimation of sprout damage. Cereal Foods World, **32**, 827-829.



HGCA RESEARCH REVIEW NO. 2

HAGBERG FALLING NUMBER AND BREADMAKING QUALITY

5. VARIETIES

A. G. MORGAN, *National Institute of Agricultural Botany, Huntingdon Road, Cambridge CB3 0LE.*

*This review, completed in August 1988, was funded by the HOME-GROWN CEREALS AUTHORITY, Hamlyn House, Highgate Hill, London N19 5PR.*

## CONTENTS FOR 5. VARIETIES

Page		
23	5.1	INTRODUCTION
23	5.2	HFN IN THE STATUTORY UK NATIONAL LIST TESTING SYSTEM FOR WHEAT
24	5.3	HFN IN THE ADVISORY NIAB RECOMMENDED LIST TESTING SYSTEM FOR WHEAT
	5.3.1	The testing system
	5.3.2	Reporting of HFN
	5.3.3	Resistance to pre-harvest sprouting
25	5.4	BREEDING PROGRESS
26	5.5	SPRING VERSUS WINTER VARIETIES
	5.5.1	Hagberg Falling Number
	5.5.2	Pre-harvest sprouting
28	5.6	EFFECT OF SITE AND SEASON
	5.6.1	Effect of site
	5.6.2	Effect of season
33	5.7	EFFECT OF STORAGE
	5.7.1	Experiment 1
	5.7.2	Experiment 2
35	5.8	SUMMARY
36	5.9	FUTURE RESERACH AND DEVELOPMENT REQUIREMENTS
	5.9.1	Information on varieties
	5.9.2	Non-sprouting generation of alpha-amylase
	5.9.3	Effect of storage
	5.9.4	Effect of site
	5.9.5	Effect of fungicide
	5.9.6	Effect of admixture
37	5.10	REFERENCES
39	5.11	APPENDICES
		Appendix I
		Appendix II

## **5. VARIETIES**

### **5.1 INTRODUCTION**

Hagberg Falling Number (HFN) has become an increasingly important characteristic of wheat varieties over the last 10 years or so. It forms part of all trading in breadmaking wheats in the UK and is a character in the European Community (EC) Intervention scheme for both breadmaking and feed wheats. Consequently, HFN has been an important feature of breeding programmes and is one of the quality tests applied within the UK National List testing system required by UK and EC law and within the advisory Recommended List testing system operated by the National Institute of Agricultural Botany (NIAB).

It is well known that HFN is a character which can be markedly affected by environment, although the biochemical basis of this variation is little understood at present. This means that it is essential in any system of variety evaluation to provide results from a number of sites and seasons. The specific schemes used by breeders will not be discussed here but they are similar in concept to those adopted by the NIAB for statutory and non-statutory variety evaluation.

### **5.2 HFN IN THE STATUTORY UK NATIONAL LIST TESTING SYSTEM FOR WHEAT**

Performance trials are grown in Scotland (3 trials for winter wheat only) and in England and Wales (7 trials for winter and 5 trials for spring wheat). The trials in England and Wales and all quality tests are conducted by the NIAB, acting as the commissioned agent of the Ministry of Agriculture, Fisheries and Food (MAFF).

In the first year, when the number of new varieties is high (approx. 26 winter and 2 spring) there are two replicates of each variety at each site, grown without fungicide treatment. In the second year the trials include two replicates without fungicide and two replicates with fungicide treatment. The fungicide treatment regime is designed to enable an assessment of the potential performance of varieties in a disease-free situation. For the purposes of quality tests, only the fungicide-treated plots are normally used and the two replicates within each site are bulked to provide a single sample from which a subsample of 1 kg is sent to the NIAB for analysis. The trials are conducted for 2 years, after which a technical report on all agronomic, disease and quality characters (including HFN) is presented to the UK National List Committee, appointed by the MAFF.

The information from National List trials is used to assess the value of a variety for cultivation and use (VCU) and provided it is judged to be better than the bottom third of varieties already on the List it is eligible. The National List Committee also considers systematic botany data to assess the distinctness, uniformity and stability (DUS) of the variety. If it is judged distinct from all other varieties already on the List, and is adequately uniform across all plants and stable between generations, then it is eligible. Varieties have to satisfy both criteria to be placed on the National List.

In the early years of the National List system, wheat trials were grown without fungicide and during the period immediately after the initial inclusion of fungicide-treated plots (1981-1983) HFN was measured on samples from treated and untreated plots. However, since there was such a distinct effect of fungicide (see section 6.5.1) and since it was common farming practice to apply fungicide, it was decided from 1984 that the testing of samples from untreated plots was unrealistic, and potentially misleading and would cease.

### **5.3 HFN IN THE ADVISORY NIAB RECOMMENDED LIST TESTING SYSTEM FOR WHEAT**

#### **5.3.1 The testing system**

At the end of the National List testing period of 2 years a NIAB technical committee, chaired by a member of NIAB Council, selects a small number (up to approx. four each) of the best winter and spring varieties to proceed into NIAB Recommended List trials. These trials (20 for winter and nine for spring varieties) are grown at regional trials centres throughout England and Wales and have two fungicide treatments (as in National List trials Year 2) with three replications per treatment. Fungicide-treated replicates are bulked and sub-sampled for quality analyses, including HFN.

At the end of Recommended List Year 1 the data are added to a 5-year database and subjected to a statistical fitted constants analysis to enable a comparison with varieties already on the NIAB Recommended List. If the Cereal Trials Advisory Committee (CTAC) decide that the overall performance is as good as, or better than, the best variety already on the Recommended List then it is added in a provisional category and continues in Recommended List trials. After a further 2 years in trials, by which time there is data from a total of 5 years, the varietal information is again presented to the CTAC and if the variety has fulfilled its original promise it is promoted to the fully recommended category.

#### **5.3.2 Reporting of HFN**

Up to 1986, the adjusted means were used to derive a 1-9 grade for HFN with 9 corresponding to the highest HFN values (Draper, 1981). However, it was clear from NIAB data that HFN is a very variable character and that such precision in differentiating between varieties was unjustified.

Consequently, the adjusted mean is now used to categorise the variety as having a tendency to produce 'Very High', 'High', 'Medium' or 'Low' HFN values. The variability of HFN is discussed in more detail in sections 5.6 and 5.7.

#### **5.3.3 Resistance to pre-harvest sprouting**

It has long been known that pre-harvest sprouting has a profoundly deleterious effect on HFN values. It also affects other grain characters in the marketability of the sample and, as a result of these considerations, the NIAB has been assessing varietal predisposition to pre-harvest sprouting since the early 1950s. This has, in the past,

been achieved by an assessment of sprouting in the field trials if and when it occurred, or by laboratory tests. However, these systems have not always been entirely satisfactory since, for example, there are many seasons when sprouting does not occur in the field.

Consequently a research project, funded by the Home-Grown Cereals Authority, was conducted to develop a more reliable testing scheme. Many laboratory tests were investigated in an attempt to simulate the field observations but none of these proved an entirely satisfactory representation of experience with field crops. It is possible that these tests could not include the effects of such factors as the physical attitude of the ear in the standing crop, which can affect the retention of rain water on the ear and hence the effective time of exposure to water.

The system which proved most successful, and is currently used at the NIAB, was one in which a field trial is irrigated from above when the grain is ripe (McVittie & Draper, 1982a). The whole trial has to be netted to prevent bird damage and, more importantly, to minimise wind drift of the applied water spray. It is essential to maintain an even irrigation over the plots in order to obtain a true comparison between varieties. Ears from each of the 3-replicate plots of each variety are subsequently examined by microscope for visible sprouting. The data are treated statistically in the same way as for HFN and a 1-9 grade derived by comparison with varieties already on the Recommended List (9 indicating least tendency to sprout). This system has given much more consistent results for varieties than was obtained previously and now provides useful varietal information to be used in conjunction with HFN data to assess the growers risk. The consistency of sprouting results is indicative of a high genetic component in this character.

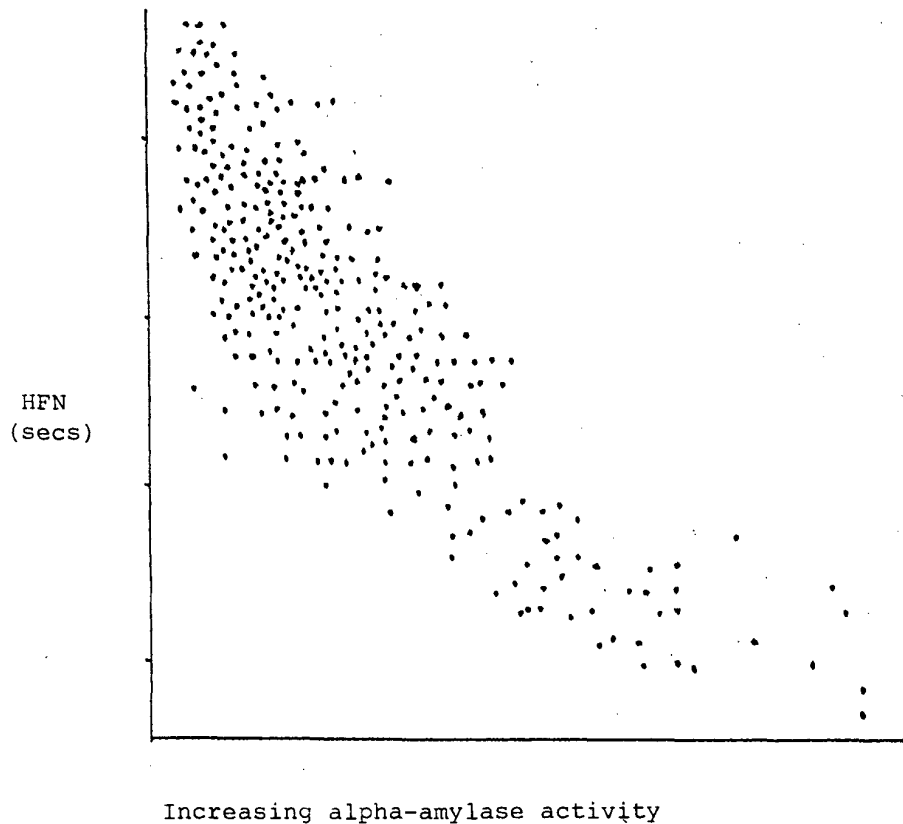
#### 5.4 BREEDING PROGRESS

Very few varieties remain in common use now for more than 7 or 8 years and so a strict comparison under comparable conditions over longer timespans is not possible. However, it is possible to look at the relative performance of varieties as grown in their time. It is clear from National surveys (see section 5.6.2) and from NIAB data that, at least since 1974, there has been no major progress in obtaining higher HFN values or in obtaining varieties which are more resistant to environmental effects.

From the point of view of the baking process, there is probably little reason to improve HFN values above those of the better varieties (approx. 300+) since this could merely necessitate the addition of alpha-amylase by the baker. Furthermore, HFN values of 300+ are associated with very low levels of alpha-amylase and it is probably unrealistic to expect to produce grain with no alpha-amylase at maturity.

In practice, HFN values of 300+ must be treated with caution in relation to alpha-amylase levels. There can be large variation in HFN between samples of the same alpha-amylase level (Finney, 1985; Morgan *et al.*, 1986), particularly when the alpha-amylase activity is low (Figure 5.1).

Figure 5.1 Relationship between HFN and level of alpha-amylase activity



It would seem, therefore, that the criterion most needed is that of consistency of a variety between environments since such a variety would provide the minimum risk for the grower and a more consistent raw material for the processor. Unfortunately there is very little historical data on this aspect of varietal performance but results of NIAB trials from 1982 are dealt with in more detail in section 5.6.

## 5.5 **SPRING VERSUS WINTER VARIETIES**

### 5.5.1 **Hagberg Falling Number**

It is generally considered that spring wheats commonly produce grain of better quality in terms of HFN than winter wheats. However, this generalisation is probably coloured by the range of varieties of each type in British agriculture. Spring wheats have traditionally occupied considerably less acreage than winter wheats and varieties competing for a share of this limited market have generally had to combine good breadmaking quality characteristics with good agronomic features. This

has had the effect that only varieties which generally give good HFN values have been grown, in contrast to the situation with the winter crop where there is a strong demand for high yielding feed wheats. This is illustrated by the information given in the Recommended Lists contained in the NIAB Farmers Leaflet number 8 (NIAB, 1988), and in Table 5.1.

**Table 5.1** HFN and sprouting (from NIAB Recommended List 1988)

Winter Wheats	HFN Rating	Sprouting Grade	Spring Wheats	HFN Rating	Sprouting Grade
Slejpner	High	6	Jerico	V. High	8
Galahad	Medium	5	Minaret	V. High	8
Brock	Medium	7	Axona	V. High	8
Mercia	High	6	Alexandria	V. High	8
Avalon	High	4	Wembley	High	7
*Hornet	Low	(7)	Tonic	V. High	8
*Rendezvous	High	(6)			
*Parade	High	(8)			
*Apollo	V. High	-			

KEY: \* = provisionally recommended  
 () = limited data

In NIAB trials over the period 1982 to 1987, HFN values for the winter breadmaking variety Avalon ranged from 74 at Wye in 1985 to 396 at Sutton Bonington in 1986 (Appendix I). In comparison, the recently recommended spring breadmaking variety Alexandria ranged from 133 at Cambridge in 1987 to 361 at Morley in 1983 (Appendix II). It is evident that both spring and winter varieties are capable of producing grain of high HFN. However, it is also clear that environmental factors can lead to a wide variation in results for the same variety of either type.

#### 5.5.2 Pre-harvest sprouting

A more distinct and consistent difference between winter and spring varieties is the resistance to sprouting in the ear. It has been notable for a number of years that, of the varieties on the NIAB Recommended List and in common use, spring varieties generally have a higher resistance than winter varieties.

This is illustrated in Table 5.1 which gives the 1-9 grades for this character as given in the NIAB Recommended List in 1988. A grade of 9 indicates the best resistance to sprouting. The spring varieties are all classified as grade 7 or 8 whereas the fully recommended winter varieties range from 4 to 7. It is notable that the current most widely grown winter breadmaking variety, Avalon, has a score of only 4, indicating that it is likely to be relatively severely affected by adverse weather conditions. This is reflected in commercial experience and at sites where the weather was cold and damp around harvest, Avalon has shown a tendency to sprout and this has resulted in some very low HFN values. The newer winter varieties, notably Parade, appear to represent an improvement over the established varieties in this respect.

## 5.6 EFFECT OF SITE AND SEASON

It must be stated at the outset that there is little information in the scientific literature specifically on varietal variation in HFN or the effects of site and season. In order to discuss these important aspects, therefore, previously unpublished data from NIAB trials over a number of years has had to be presented in more detail than would be normal in a review document.

Results are presented in detail in Appendix I for winter wheat varieties at the same 5 sites over the period 1982 to 1986. Data from 1987 are included for information but some varieties had ceased to be trialled and hence this year was not included in the tables of means. The mean values in each year and at each site are given in Tables 5.2 to 5.4.

Similar results for spring wheats over the period 1983 to 1987 are given in Appendix II and Tables 5.5 to 5.7.

**Table 5.2** Winter Wheat: Mean HFN results over 5 sites from NIAB trials (treated with fungicide)

Variety	1982	1983	1984	1985	1986	MEAN
Avalon	284	283	313	185	290	271
Aquila	270	261	287	192	243	251
Brigand	278	230	277	170	223	236
Rapier	250	225	281	154	240	230
Galahad	249	240	280	159	207	228
Longbow	115	187	200	90	202	159
Norman	141	169	170	89	174	149
Fenman	86	108	126	65	104	98
MEAN	209	213	242	138	210	

**Table 5.3** Winter Wheat: Mean HFN results over the years 1982-1986 from NIAB trials (treated with fungicide)

VARIETY	SUTTON					MEAN
	CAMBRIDGE	MORLEY	ROSEMAUND	TERRINGTON	BONINGTON	
Avalon	259	301	244	231	321	271
Aquila	226	273	228	244	282	251
Brigand	219	261	205	210	283	236
Rapier	230	257	183	209	270	230
Galahad	234	236	193	200	277	228
Longbow	111	206	161	131	187	159
Norman	105	193	157	104	184	149
Fenman	71	144	106	68	100	98
MEAN	182	241	185	175	238	



**Table 5.4** Winter Wheat: Mean HFN results over 8 varieties from NIAB trials (treated with fungicide)

YEAR	CAMBRIDGE	MORLEY	ROSEMAUND	TERRINGTON	SUTTON	
					BONINGTON	MEAN
1982	166	272	209	183	217	209
1983	198	250	200	163	254	213
1984	192	241	297	204	276	242
1985	128	160	114	129	158	138
1986	225	246	102	194	285	210
MEAN	182	241	185	175	238	

Similar results for spring wheats over the period 1983 to 1987 are given in Appendix II and Tables 5.5 to 5.7.

**Table 5.5** Spring Wheat: Mean HFN results over 4 sites in NIAB trials (treated with fungicide)

VARIETY	1983	1984	1985	1986	1987	MEAN
Alexandria	321	352	271	301	242	297
Jerico	319	293	258	282	265	283
Axona	334	267	269	251	281	280
Tonic	314	297	256	275	242	277
Minaret	296	293	200	293	280	272
Broom	324	252	224	252	243	259
Wembley	294	248	182	214	223	232
MEAN	315	287	237	267	254	

**Table 5.6** Spring Wheat: Mean HFN results over the years 1983-1987 in NIAB trials (treated with fungicide)

VARIETY	SUTTON				MEAN
	CAMBRIDGE	BRIDGETS	MORLEY	BONINGTON	
Alexandria	257	295	307	330	297
Jerico	251	261	323	299	283
Axona	245	270	308	298	280
Tonic	244	267	283	314	277
Minaret	230	269	289	302	272
Broom	229	239	272	296	259
Wembley	217	216	253	242	232
MEAN	239	260	291	297	

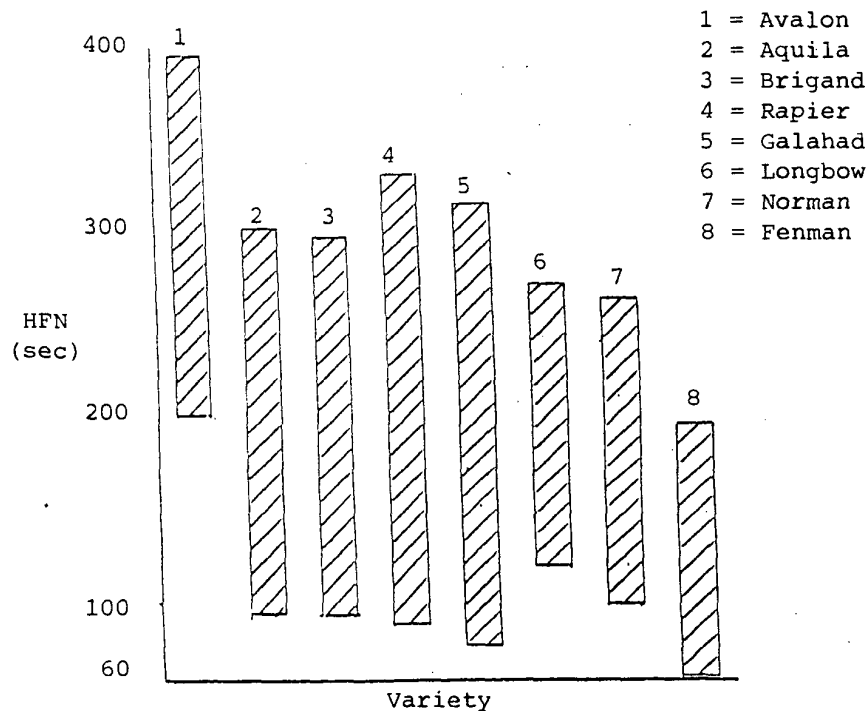
**Table 5.7** Spring Wheat: Mean HFN results over 7 varieties from NIAB trials

YEAR	CAMBRIDGE	BRIDGETS	MORLEY	SUTTON BONINGTON	MEAN
1983	282	281	350	347	315
1984	277	262	315	290	287
1985	212	231	280	189	237
1986	234	224	285	324	267
1987	190	301	224	300	254
MEAN	239	260	291	297	

**5.6.1 Effect of site**

A study of the data clearly shows the variability of HFN values with a given variety between sites (Table 5.6), even within a season (Table 5.7). Rapier in 1986, for example, ranged from 89 at Rosemaund to 333 at Sutton Bonington (Appendix I). This was a reflection of the mean HFN values at those sites which were 102 and 285, respectively (Table 5.4). The range for each variety in 1986 is shown in Figure 5.2 as an illustration of the potential variability between sites within a season. Even in a good season, varieties can show a large range between sites. In 1984, for example, Avalon varied from 246 at Cambridge to 355 at Sutton Bonington and Longbow had a range of about 200, from 89 at Cambridge to 288 at Rosemaund.

**Figure 5.2** Variability of HFN between 5 sites within a season (1986)



It is clear that there is a considerable effect of environment on HFN values which means that results for a particular variety can span a very wide range. The ranking order of winter varieties within environments is fairly consistent (see Appendix I). The situation is not so clear

with spring varieties as a result of their greater similarity in HFN (Appendix II), which means that small variations can change the ranking order markedly. Wembley, however, consistently gave the lowest HFN values. It is also apparent that, even with winter varieties, the ranking order of HFN can vary on some occasions. Thus, although the overall mean for Galahad (228) was considerably less than that of Avalon (271), Galahad gave higher values in 7 out of 25 environments (Table 5.2). An interesting aspect of studies in this area is that the mean level of alpha-amylase in the bulk is often considerably influenced by a few grains in which enzyme activity is relatively high (Gale et al., 1986; Gale & Lenton, 1987; McVittie & Draper, 1982b).

The reasons for the variability in response to environment are not understood but it is possible that weather conditions at specific stages of grain development, which occur at slightly different times for each variety, may be influential. It is certain that visible sprouting is not necessarily connected with this variable ranking order. There is evidence, however, suggesting that alpha-amylase activity can increase in the mature grain of some varieties in adverse weather conditions at harvest without visible sprouting and the extent of this may be a varietal characteristic (Gale et al., 1986; Gale & Lenton, 1987).

Mann (1986) has reported that varieties in NIAB trials have shown differences in sensitivity to site. Results from Finlay-Wilkinson analyses gave a range of regression coefficients from -0.15 for Brimstone to 3.11 for Brock showing that Brock is highly affected by site. These results support those of Medcalf et al. (1968) in the USA whose results indicated a significant site effect and site x year interaction.

#### 5.6.2 Effect of season

It is well known that HFN values vary according to season. This is illustrated by the data in Tables 5.2 and 5.5. The serious effects noted in 1985, particularly with winter wheats, are evident in the year

**Table 5.8** HFN values - national averages 1974-1987

1974	210
1975	289
1976	297
1977	127
1978	203
1979	219
1980	252
1981	263
1982	313
1983	305
1984	276
1985	161
1986	225
1987	(179)

Key: ( ) = provisional

means for each variety in Table 5.2. Results in Table 5.8, based on data from the Agricultural Development and Advisory Service surveys, further demonstrate seasonal changes over the period 1974 to 1987.

The years 1977, 1985 and 1987 were dull, damp summers with poor weather at harvest, resulting in generally low HFN values. In contrast, the years 1975, 1976, 1982 and 1983 were characterised by good weather and HFN values were consequently high.

The ranking order of the winter varieties in NIAB trials, given in Table 5.2, was very similar each year although the magnitude of the seasonal effect was greatest in the better varieties. Avalon, for example, decreased on average by 128 from 1984 to 1985 compared with a drop of only 61 for Fenman. This may be partly a consequence of the non-linear relationship between the HFN test result and the actual alpha-amylase level. As a result, the HFN test is insensitive to changes at high alpha-amylase levels which means that those varieties which have inherently high levels of alpha-amylase activity will show relatively little change in HFN as alpha-amylase increases further (see Figure 5.1). The consistency of ranking order over seasons is supported by Mann (1986) who reported no significant variety x season interaction with varieties in NIAB trials over the period 1981 to 1984.

Although no significant variety x season interaction has been observed it is notable that the seasonal variability of HFN in the spring varieties (Table 5.5) is less than that of the winter varieties. The mean decrease from 1984 to 1985 was only 50 for spring compared with 104 for winter varieties. If only varieties with a mean over years of 200 or more are considered, the mean decrease with winter varieties becomes 117. It would appear that spring varieties are generally less prone to seasonal effects than winter varieties, perhaps partly because of the difference in harvest date and the associated weather conditions. However, spring varieties can show a wide range of results. Alexandria varied from 133 at Cambridge in 1987 to 382 at Morley in 1984 and Wembley was as low as 92 at Sutton Bonington in 1985 compared to 358 at Morley in 1983.

A more likely reason for the difference in magnitude of response to season is the much better resistance to pre-harvest sprouting that is prevalent in spring varieties. This is discussed more fully in section 5.5.2. There may also be differences between spring and winter varieties in the development of non-sprouting alpha-amylase referred to in section 5.6.1. It is interesting to compare the ranges of HFN results for each variety with the grades for resistance to sprouting (Table 5.9).

**Table 5.9** A comparison of HFN with sprouting resistance

Variety	HFN		HFN	HFN	Resistance to Sprouting
	Minimum	Maximum	Range	Mean	
Tonic	194	350	156	277	8
Axona	210	376	166	280	8
Broom	183	350	167	259	9
Jerico	190	358	168	283	8
Fenman	62	239	177	98	7
Minaret	149	341	192	272	8
Norman	64	264	200	149	6
Longbow	62	288	226	159	8
Aquila	95	328	233	251	7
Rapier	89	333	244	230	6
Brigand	94	338	244	236	5
Wembley	92	338	246	232	7
Alexandria	133	382	249	297	8
Galahad	64	332	268	228	5
Avalon	68	396	328	271	4

This can only be a crude comparison, of course, but there is a trend for lower grades of resistance to sprouting to be associated with the larger range in HFN values. On the other hand there is no apparent relationship with mean HFN. It is possible, therefore, that the very low HFN values observed with some of the varieties with a high mean HFN may have been a result of the higher tendency of these varieties (e.g. Avalon) to pre-harvest generation of alpha-amylase under adverse weather conditions.

### 5.7 EFFECT OF STORAGE

A common comment within the agricultural trade is that HFN values can alter after a period of storage. Some trends were observed in a limited number of samples from NIAB trials and consequently this apparent effect was investigated further (Morgan *et al.*, 1986).

#### 5.7.1 Experiment 1

Samples of 14 varieties from harvest 1980 were analysed at harvest and at 7-weekly intervals up to 21 weeks after harvest. Trials, untreated with fungicide, from Myerscough, Rosemaund EHF and Terrington EHF were used in this study and Table 5.10 shows the HFN values at each storage period meaned over the three sites.

Some varieties such as Flanders and Kinsman showed an initial increase followed by a decrease while others such as Aquila and Norman showed an initial decrease followed by an increase. There was no apparent relationship between this behaviour and breadmaking quality. The largest increase was with Avalon which changed from 186 at harvest to 225 after 14 weeks, but this was followed by a decrease to 212. However, most varieties changed very little, with Sentry changing by only 9 from 232 to 241 after 21 weeks. The majority of changes were similar to the normal variability between replicate analyses which is about 10, and caution should be exercised in drawing firm conclusions.

**Table 5.10** Winter Wheat: Variation of HFN with Storage Time

VARIETY	Mean HFN results over samples from 3 sites				MEAN
	AT HARVEST	+7 WEEKS	+14 WEEKS	+21 WEEKS	
Prince	228	245	243	247	241
Armada	234	245	239	239	239
Sentry	232	240	239	241	238
Flanders	202	222	216	208	212
Avalon	186	197	225	212	205
Aquila	194	189	198	210	198
Abele	156	167	188	186	175
Rapier	174	162	170	161	167
M.Huntsman	163	177	178	134	163
Mithras	141	145	158	153	149
Kinsman	125	144	148	115	133
Norman	128	116	125	143	128
Mardler	68	67	77	70	69
Baron	66	69	70	70	68
MEAN	164	170	176	170	

**5.7.2 Experiment 2**

This was conducted in 1985 using 12 varieties from five sites in which samples were analysed at harvest and again in the following March after 28 weeks storage in a well ventilated store at ambient room temperature. The results, given in Table 5.11, showed that only Moulin was consistent in its change in HFN during storage with a decrease in each case, although in most cases the change was very small and within the repeatability of the test.

**Table 5.11** Winter Wheat: Changes in HFN after storage for 28 weeks

VARIETY	HFN after storage minus HFN at harvest					MEAN
	CAMBRIDGE	MORLEY	ROSEMAUND	TERRINGTON	BONINGTON	
Mercia	70	-37	0	-27	128	26
Aquila	64	9	1	10	-7	15
Rapier	17	-6	-22	49	22	12
Galahad	21	29	-8	48	-78	2
Brock	0	-16	-1	3	22	2
Mission	1	-5	9	-8	9	1
Longbow	-24	13	-10	12	10	0
Brimstone	-19	10	-1	21	-18	-1
Slejpner	-50	15	50	1	-25	-2
Moulin	-16	-18	-3	-5	-2	-9
Brigand	11	-5	-57	-17	-44	-22
Avalon	-47	-47	32	15	-75	-24
MEAN	2	-5	-1	8	-5	0

Changes ranged from a decrease of 78 with Galahad at Sutton Bonington to an increase of 128 with Mercia at the same site. Mean changes varied from an increase of 26 with Mercia to a decrease of 24 with Avalon.

The average change at each site and many of the individual changes of variety samples were, however, within the experimental error and in fact the overall mean change in HFN during controlled storage was zero. It is evident, therefore, that varietal changes during storage are not consistent, indicating that other factors are involved. The magnitude of the change is not often very large and it is possible that sample errors or storage conditions could play a part in this apparent effect as observed in commerce. One of the factors which influences the heterogeneity of the bulk is the possibility of contamination with grain of low HFN. Relatively small amounts of such grain included in the sub-sample taken for HFN analysis can reduce the HFN considerably. Table 5.12 shows data from an experiment conducted at the NIAB in 1987.

**Table 5.12** The effect of Mixing Two Varieties

% MINARET	% FENMAN	HFN (sec)
100.0	0.0	275
99.9	0.1	273
99.8	0.2	269
99.5	0.5	266
99.3	0.7	260
99.0	1.0	256
98.5	1.5	244
90.0	10.0	146
80.0	20.0	118
0.0	100.0	64

Similar figures were reported by Finney (1985) in America. Data on this subject is, however, limited.

## 5.8 SUMMARY

A major factor in determining HFN is variety and this should be the first consideration when selecting which wheat would give the minimum risk for a farmer. However, alpha-amylase activity can be considerably affected by weather conditions, particularly near harvest, and this is clearly an important component in the effect of season.

It is possible that the trends observed between sites are a reflection of the prevailing weather patterns at those sites, although other factors may be involved.

It would appear that there are two major possible ways by which alpha-amylase activity can be increased and hence HFN values decreased; a) pre-harvest sprouting and b) non-sprouting generation of alpha-amylase.

Predisposition to pre-harvest sprouting is a varietal characteristic and information such as that provided in the NIAB Recommended Lists for winter and spring wheats should be used by growers in conjunction with the overall varietal assessment of HFN to assess the risk of obtaining low HFN values in the harvested crop. More information is required on varietal susceptibility to the early generation of alpha-amylase in the absence of sprouting but within current varieties there seems to be a relationship with the tendency to pre-harvest sprouting.

## **5.9 FUTURE RESEARCH AND DEVELOPMENT REQUIREMENTS**

### **5.9.1 Information on varieties**

It is essential to British agriculture that independent testing of varieties is continuously maintained to provide information to the grower and processing industry. This information should indicate the average varietal level of HFN, determined from a wide range of sites and seasons, and the degree of stability to environmental influences. It should specifically show the predisposition of varieties to pre-harvest sprouting and, if possible the predisposition to non-sprouting generation of alpha-amylase, both of which are major factors in reducing HFN and affecting the market value of a crop.

### **5.9.2 Non-sprouting generation of alpha-amylase**

The generation of alpha-amylase without evidence of sprouting can be critical in some seasons such as 1985 and 1987. It is essential that the biochemistry of this subject is determined and that the potential for breeding varieties without this tendency is evaluated.

Associated with this is a need for the development of a suitable test for screening purposes in a breeding programme and for use in a National variety testing system such as that operated at the NIAB (see also section 5.9.1).

### **5.9.3 Effect of storage**

It is common commercial practice for wheat to be stored for a number of months before sale. The research into the possible effects of such storage on HFN is limited and generally inconclusive. Further work is necessary to establish the effects of different storage conditions. This study should include the effects of different drying conditions prior to storage and the possible effect of moisture content.

### **5.9.4 Effect of site**

Differences between sites have been observed which are fairly consistent. It was suggested in section 5.6.1 that this may be due to consistent differences in weather pattern and this possibility needs to be investigated together with other potential site-specific factors such as soil type. The possibility of remedial action in sites which do not enable a variety to attain its potential should be explored.

### **5.9.5 Effect of fungicide**

In view of the fact that almost all crops are given a fungicide treatment and that this can have an adverse effect on HFN, this is an area which deserves more attention. Studies should be conducted to determine optimum fungicide regimes and whether this needs to be tailored to particular varieties and/or particular disease situations and market outlets.



### 5.9.6 Effect of admixture

There is an acceptance that relatively small amounts of admixture with a sample of low HFN can markedly reduce the HFN of an otherwise good grain lot. There is a strong need for this to be quantified and for any varietal variation in this effect to be studied. This factor can be very important in consideration of farm-saved seed and in all stages of crop production from contamination in the seed drill or combine to contamination in the store. A greater awareness of this within the farming community backed by good practical evidence would be extremely beneficial to British agriculture.

### 5.10 REFERENCES

1. DRAPER, S. R. (1981). Assessment of the risk of low Hagberg Falling Numbers in samples of unsprouted grain of different wheat varieties. Journal of the National Institute of Agricultural Botany, 15, 385-390.
2. FINNEY, P. L. (1985). Effect of wheat variety on the relationship between Falling Numbers and alpha-amylase activity. Cereal Chemistry, 62, 258-262.
3. GALE, M. D., SALTER, A. M. and LENTON, J. R. (1986). The induction of germination alpha-amylase during wheat grain development in unfavourable weather conditions. Proceedings of a symposium on 'Some aspects of wheat milling quality', NIAB, Cambridge.
4. GALE, M. D. and LENTON, J. R. (1987). Pre-harvest sprouting in wheat - a complex genetic and physiological problem affecting breadmaking quality of UK wheats. Aspects of Applied Biology, 15, 115-124.
5. MANN, G. C. (1986). Recommended Varieties - their mean performance and variation in NIAB trials with respect to Hagberg Falling Number. Proceedings of a symposium on 'Some aspects of wheat milling quality', NIAB, Cambridge.
6. McVITTIE, J. A. and DRAPER, S. R. (1982a). The irrigation of standing plots of winter wheat in order to assess varietal predisposition to pre-harvest sprouting, Journal of the National Institute of Agricultural Botany, 16, 45-49.
7. McVITTIE, J. A. and DRAPER S. R. (1982b). An asymmetric distribution of alpha-amylase activity in bulk samples of grain and sprouted and unsprouted wheat. Journal of the National Institute of the Agricultural Botany, 16, 49-53.
8. MEDCALF, D. G., TOMBETTA, E. E., GILLS, K. A. and FIBBITT, L. D. (1968). A note of varietal and environmental variations in Falling Number values of flour. Cereal Chemistry, 45, 496-499.

9. MORGAN, A. G., MORLEY, J. H. and FLETCHER, P. D. (1986). Observations on the effect of fungicide and grain storage on Hagberg Falling Number. Proceedings of a symposium on 'Some aspects of wheat milling quality', NIAB, Cambridge.
10. NIAB (1988). Farmers leaflet No 8. Recommended varieties of cereals, National Institute of Agricultural Botany, Cambridge.

5.11 APPENDICES

APPENDIX I

WINTER WHEAT

HFN RESULTS FROM NIAB TRIALS IN 1982

(Treated with fungicide)

VARIETY	CAMBRIDGE	MORLEY	ROSEMAUND	TERRINGTON	SUTTON BONINGTON	MEAN
Avalon	272	355	290	184	319	284
Aquila	267	315	280	191	298	270
Brigand	181	329	290	272	318	278
Rapier	181	303	239	232	294	250
Galahad	229	299	251	233	235	249
Longbow	62	199	99	141	75	115
Norman	72	200	161	140	132	141
Fenman	62	174	65	68	63	86
MEAN	166	272	209	183	217	

WINTER WHEAT

HFN RESULTS FROM NIAB TRIALS IN 1983

(Treated with fungicide)

VARIETY	CAMBRIDGE	MORLEY	ROSEMAUND	TERRINGTON	SUTTON BONINGTON	MEAN
Avalon	285	316	273	246	297	283
Aquila	254	294	256	241	258	261
Brigand	264	280	191	148	265	230
Rapier	238	262	147	230	248	225
Galahad	230	281	211	191	288	240
Longbow	120	223	226	97	271	187
Norman	118	200	192	88	248	169
Fenman	74	143	101	64	158	108

WINTER WHEAT

HFN RESULTS FROM NIAB TRIALS IN 1984

(Treated with fungicide)

VARIETY	CAMBRIDGE	MORLEY	ROSEMAUND	TERRINGTON	SUTTON BONINGTON	MEAN
Avalon	246	318	349	297	355	313
Aquila	277	250	308	271	328	287
Brigand	214	279	303	250	338	277
Rapier	275	259	312	244	315	281
Galahad	269	270	316	236	332	285
Longbow	89	230	288	149	245	200
Norman	90	194	264	103	199	170
Fenman	79	129	239	84	97	126
MEAN	192	241	297	204	276	

WINTER WHEAT  
HFN RESULTS FROM NIAB TRIALS IN 1985  
(Treated with fungicide)

VARIETY	CAMBRIDGE	MORLEY	ROSEMAUND	TERRINGTON	SUTTON BONINGTON	MEAN
Avalon	183	214	107	184	237	185
Aquila	97	222	199	221	223	192
Brigand	166	186	146	154	197	170
Rapier	178	180	128	124	162	154
Galahad	172	191	108	109	213	159
Longbow	97	106	73	103	71	90
Norman	72	107	88	75	102	89
Fenman	62	77	62	62	62	65
MEAN	128	160	114	129	158	

WINTER WHEAT  
HFN RESULTS FROM NIAB TRIALS IN 1986  
(Treated with fungicide)

VARIETY	CAMBRIDGE	MORLEY	ROSEMAUND	TERRINGTON	SUTTON BONINGTON	MEAN
Avalon	307	301	202	243	396	290
Aquila	236	283	95	298	303	243
Brigand	268	232	94	224	298	223
Rapier	282	281	89	214	333	240
Galahad	272	141	78	230	316	207
Longbow	185	272	119	164	271	202
Norman	175	264	79	113	241	174
Fenman	77	197	62	63	119	104
MEAN	225	246	102	194	285	

WINTER WHEAT  
HFN RESULTS FROM NIAB TRIALS IN 1987  
(Treated with fungicide)

VARIETY	CAMBRIDGE	MORLEY	ROSEMAUND	TERRINGTON	SUTTON BONINGTON	MEAN
Avalon	69	68	232	115	166	130
Aquila	113	156	296	165	243	146
Galahad	64	80	241	118	232	147
Longbow	108	85	93	92	93	94
Norman	64	73	120	93	89	88
Fenman	76	70	141	65	62	83
MEAN	82	89	187	108	147	

APPENDIX II

SPRING WHEAT

HFN RESULTS FROM NIAB TRIALS IN 1983

(Treated with fungicide)

VARIETY	CAMBRIDGE	BRIDGETS	MORLEY	SUTTON BONINGTON	MEAN
Alexandria	280	289	361	355	321
Jerico	281	284	358	352	319
Axona	298	314	376	349	334
Tonic	283	280	350	345	314
Minaret	253	252	341	339	296
Broom	316	286	345	350	324
Wembley	298	260	316	338	294
MEAN	282	281	350	347	

SPRING WHEAT

HFN RESULTS FROM NIAB TRIALS IN 1984

(Treated with fungicide)

VARIETY	CAMBRIDGE	BRIDGETS	MORLEY	SUTTON BONINGTON	MEAN
Alexandria	340	328	382	356	351
Jerico	302	255	348	265	292
Axona	251	223	319	276	267
Tonic	300	298	304	286	297
Minaret	288	278	324	282	293
Broom	239	229	249	290	252
Wembley	218	222	277	273	247
MEAN	277	262	315	290	

SPRING WHEAT

HFN RESULTS FROM NIAB TRIALS IN 1985

(Treated with fungicide)

VARIETY	CAMBRIDGE	BRIDGETS	MORLEY	SUTTON BONINGTON	MEAN
Alexandria	261	244	323	255	271
Jerico	235	235	331	232	258
Axona	210	274	300	293	269
Tonic	208	281	262	274	256
Minaret	149	208	227	216	200
Broom	196	209	273	219	224
Wembley	227	166	242	92	182
MEAN	212	231	280	189	

SPRING WHEAT  
HFN RESULTS FROM NIAB TRIALS IN 1986  
(Treated with fungicide)

VARIETY	CAMBRIDGE	BRIDGETS	MORLEY	SUTTON BONINGTON	MEAN
Alexandria	270	284	298	350	300
Jerico	248	249	299	332	282
Axona	216	215	280	294	251
Tonic	237	221	296	345	275
Minaret	255	270	310	338	293
Broom	212	183	277	334	251
Wembley	199	145	235	276	214
MEAN	234	224	285	324	

SPRING WHEAT  
HFN RESULTS FROM NIAB TRIALS IN 1987  
(Treated with fungicide)

VARIETY	CAMBRIDGE	BRIDGETS	MORLEY	SUTTON BONINGTON	MEAN
Alexandria	133	331	169	334	242
Jerico	190	280	277	312	265
Axona	252	326	265	279	280
Tonic	194	254	201	318	242
Minaret	203	337	245	334	280
Broom	183	288	214	287	243
Wembley	178	288	194	233	223
MEAN	190	301	224	300	

HGCA RESEARCH REVIEW NO. 2

HAGBERG FALLING NUMBER AND BREADMAKING QUALITY

6. THE INFLUENCE OF HUSBANDRY

*D. B. STEVENS, R. J. COOK, L. V. VAIDYANATHAN, G. L. JONES and  
K. A. McLEAN, Agricultural Development and Advisory Service, Brooklands  
Avenue, Cambridge CB2 2DR.*

*This review, completed in August 1988, was funded by the HOME-GROWN  
CEREALS AUTHORITY, Hamlyn House, Highgate Hill, London N19 5PR.*

**CONTENTS FOR 6. THE INFLUENCE OF HUSBANDRY**

<b>Page</b>		
45	6.1	<b>INTRODUCTION</b>
45	6.2	<b>CULTIVATIONS</b>
45	6.3	<b>DATE OF DRILLING</b>
46	6.4	<b>GROWTH REGULATORS</b>
46	6.5	<b>SPRING FERTILISER NITROGEN</b>
47	6.6	<b>FUNGICIDES</b>
	6.6.1	<b>Cultivar comparisons</b>
	6.6.2	<b>Fungicide programmes</b>
	6.6.3	<b>Discussion</b>
54	6.7	<b>TIME OF HARVESTING</b>
54	6.8	<b>PRE-HARVEST TESTING</b>
55	6.9	<b>POST-HARVEST MANIPULATION</b>
	6.9.1	<b>Summary of results of ADAS tests</b>
	6.9.2	<b>Discussion</b>
57	6.10	<b>FUTURE RESEARCH AND DEVELOPMENT REQUIREMENTS</b>
58	6.11	<b>REFERENCES</b>
60	6.12	<b>APPENDICES</b>
		<b>Appendix I</b>
		<b>Appendix II</b>
		<b>Appendix III</b>
		<b>Appendix IV</b>
		<b>Appendix V</b>



## 6. THE INFLUENCE OF HUSBANDRY

### 6.1 INTRODUCTION

Apart from variety, the weather has a major effect on HFN as indicated by the ADAS/HGCA Survey (HGCA, 1984) which shows annual average values from less than 150 in 1977 to over 300 in 1983. Weather can have a considerable effect even before the crop reaches maturity (sections 7.3 and 8.2).

In addition to variation attributed to variety and weather, measurements of HFN in grain grown in a range of husbandry trials conducted by ADAS have demonstrated further effects on HFN.

### 6.2 CULTIVATIONS

It has not been possible to identify a direct effect of cultivation on HFN although indirect effects have been observed. For example, in a straw disposal trial at Terrington EHF it was observed that straw burning followed by ploughing gave a higher HFN than where chopped straw was incorporated by tined cultivation. Since grain proteins were higher following burning and ploughing than where chopped straw was incorporated by tined cultivation, the difference in soil nitrogen supply following burning and ploughing might account for the higher HFN recorded for this treatment. This relationship would be expected from the evidence on nitrogen given in section 6.5 of this review. It is also likely that cultivations which affected lodging or weeds would result indirectly in changes in HFN since they would affect the rate of drying of the maturing crop. A serious effect is likely to be observed if the previous crop was of a low HFN variety such as Fenman and volunteers survive. In these circumstances, the effectiveness of the cultivation in controlling volunteers will be very important since the depression of HFN would be out of proportion to the percentage contamination (section 5.7.2).

### 6.3 DATE OF DRILLING

In studying individual trials it is very important to separate direct effects from indirect effects. For example, early drilling of wheat can result in more lodging, and as a result HFN values may be reduced. Similarly, in trials, if the early-drilled plots are harvested at the same time as later plots, then the early ones are at risk of sprouting for a longer period.

Comparisons of varieties at different sowing dates have been made at Arthur Rickwood and Terrington EHF's and the results of the latter are shown in Appendix I. These represent two trial series:-

- a) Autumn comparisons of 6-9 varieties sown September/October.
- b) Winter/spring sowings of three or more varieties including Fenman and spring varieties.

In these trials, care was taken to harvest each plot as soon as possible when it was ripe, even to the extent that early maturing varieties were taken before later varieties within the same drilling date. The overall results showed no consistent effect of time of sowing on HFN and it is therefore concluded that where differences have been observed it is the

result of lodging, delay in harvest or chance occurrence of weather at critical times during the ripening period. The apparent improvement with March/April sowings in 1987 (Appendix II) is attributed to the relative improvements in weather at harvest later in September. The improvement following delayed drilling reported by Vaidyanathan (1987) may also be explained by deterioration of the early-drilled plots as there was a common harvest date.

#### 6.4 GROWTH REGULATORS

Many ADAS trials have been conducted using the commercially available chlormequat as New 5C Cycocel, or 2-chloroethylphosphonic acid + mepiquat chloride as Terpal or Cerone. In the absence of lodging, no significant change in HFN has been recorded following use at the commercial rates and timings.

However, where these products have been used to control lodging they have helped to maintain HFN values as shown in an example in Appendix II. In the example given, the HFN was improved from 77 to 140 by reducing lodging from 85% of the plot area to 8%. With better varieties or less difficult weather conditions, these treatments clearly have the potential to contribute towards maintaining acceptable HFN values.

#### 6.5 SPRING FERTILISER NITROGEN

Most winter wheat crops produce economic increases in yield with invariably enhanced grain protein levels in response to additions of spring fertiliser nitrogen. Exceptions are encountered only where soil nitrogen supply is large enough to meet the entire requirement for optimum yield. Although such experiments measuring yield and protein response to added fertiliser nitrogen were not designed to study HFN, several recent ADAS results provide determinations of grain quality features including HFN. Another source of similar, but limited information is from the Harper Adams Agricultural College (Davies et al., 1986; Gooding et al., 1986). Both sets of observations show that HFN increases with increasing spring applied fertiliser nitrogen. These increases usually extend beyond the optimum levels of nitrogen for yield although they are often not statistically significant beyond that level.

Varieties were affected differently. HFN improved with increasing fertiliser nitrogen in Avalon, Moulin, Norman, Wembley, Tonic and Axona. Mission was generally unaffected except in one ADAS experiment where 200 kg/ha N significantly decreased HFN compared with that at 150 kg/ha N. Brimstone was the cultivar in two of the experiments; HFN showed no response to increments of fertiliser nitrogen in one ADAS experiment while in a Harper Adams College experiment HFN showed an increase with increasing spring fertiliser nitrogen applications (Davies et al., 1986).

These experiments were carried out on soils ranging from drought-prone sands to heavier water-retentive clays in different rotational positions. Soil type and previous cropping had no obvious consistent influence in the response pattern of HFN to spring fertiliser nitrogen.

In all these experiments, green leaf area and its duration were increased by the additions of fertiliser nitrogen, as was the HFN of the grain in most of the cultivars. Yet, fungicides used for leaf disease control, while protecting and prolonging green leaf area duration cause

small but significant reductions in HFN. Cook (section 6.6) records a significant negative correlation between green leaf area at mid-grain filling stage and HFN in the harvested grain. There is a conundrum here that may or may not be readily resolved.

Treatments that receive either only small amounts (40 to 100 kg/ha) or no fertiliser nitrogen, usually senesce a few days to a week before the treatments with larger doses of added nitrogen; these latter treatments often retain their enhanced green leaf area even when grain has ripened. Harvesting the treated plots at appropriate times of grain maturity was impractical and the early maturing treatments may therefore have suffered HFN deterioration in situ (McDonald & Vaidyanathan, 1987). However, the response of HFN to nitrogen level was much greater than the effect of a few days difference in maturity and it was observed in seasons when grain was not deteriorating over the harvest period. No critical explanation for the apparent beneficial influence of added fertiliser nitrogen on HFN has yet been made.

Nitrogen applied as foliar urea sprays during the grain growth period for enhancing protein levels did not result in the same apparent benefits in HFN.

## 6.6 FUNGICIDES

Evidence of an interaction between fungicide use and HFN has been cited by NIAB (Morgan et al., 1986). There is also published information indicating that HFN can be adversely affected by fungicide treatment (Gooding et al., 1986; Gooding et al., 1987) without apparent interaction with disease.

ADAS data from fungicide experiments have indicated that most aspects of grain quality are improved by disease control. This is certainly true for the physical aspects, such as thousand grain weight and specific weight (Cook, 1987). HFN is a somewhat arbitrary measure of alpha-amylase activity used to assess milling potential. Measurements of this criterion in fungicide experiments have shown variable results.

This section reviews some of the evidence that fungicides influence HFN and considers some of the factors involved.

### 6.6.1 Cultivar comparisons

ADAS and NIAB variety trials are grown with and without fungicide treatments designed to provide as complete a disease control as possible. Results were obtained from untreated and treated samples during the years 1981 to 1983 (Morgan et al., 1986). The mean HFN values for 11 cultivars are shown in Table 6.1 and show a distinct trend of decrease in HFN with fungicide treatment, the largest effect being a fall of 70 which was recorded in several cultivars. Only the cv. Rapier showed an increase in 1982, which was in fact the result of one exceptional sample.

**Table 6.1** Winter Wheat: Effect of fungicide on HFN in NIAB Recommended List experiments

VARIETY	Average annual HFN							
	1981		1982		1983		Mean	
	UT	T	UT	T	UT	T	UT	T
Avalon	273	202	304	293	298	269	292	255
Aquila	257	219	289	274	272	263	273	252
Brigand	269	220	307	282	264	234	280	245
Armada	265	211	266	240	244	229	258	227
Flanders	270	232	270	245	253	224	264	234
Hustler	259	195	257	238	246	241	254	225
Rapier	228	165	248	254	252	228	243	216
Virtue	245	175	251	226	248	198	248	200
Norman	155	108	214	141	212	174	194	141
Longbow	138	104	197	119	243	185	193	136
Fenman	103	70	134	87	124	116	120	91
Means	224	173	249	218	241	215	238	202
No. of trials	4		6		7		17	

Key: UT = untreated  
 T = treated with fungicide (3-spray programme)  
 (Data from A. Morgan, unpublished)

**Table 6.2** Frequency of a decrease in HFN with fungicide application in NIAB Recommended List Experiments

VARIETY	Number of trials where HFN was decreased by fungicide treatment.			
	1981	1982	1983	Total
Avalon	4	5	5	14
Aquila	4	5	5	14
Brigand	3	4	5	12
Armada	4	6	7	17
Flanders	4	4	6	14
Hustler	4	4	4	12
Rapier	4	3	5	12
Virtue	4	4	6	14
Norman	4	5	7	16
Longbow	4	6	7	17
Fenman	4	5	4	13
Total No. of trials	4	6	7	17

(Data from A. Morgan, unpublished)

The frequency of trials in which the fungicide-treated plots had a lower HFN than the untreated is shown in Table 6.2. These data indicate that in the majority of cases, fungicide application can reduce HFN, although the extent of the change varies between samples.

In the three years 1983-1985, data from cultivar experiments at Doddington, Cambridgeshire suggested that HFN was a highly variable character and was reduced in some years and some cultivars. In these observations, a comprehensive 3-spray programme increased HFN by 8% (untreated 237) in milling cultivars when leaf diseases were not severe in 1984, but reduced HFN by 18% (untreated 156) in the same group of cultivars in 1985, when leaf diseases were severe. There was no consistent effect in a comparable group of feed wheats.

In some early experiments, improvements in HFN were associated with disease control. For example, data from a cultivar experiment on the deep Norfolk peats in the 1979/80 season showed evidence of a significant overall improvement in HFN in relation to effective control of mildew, which is often a problem in crops grown under these conditions. Conversely, however, in the same year the same group of cultivars in an experiment in East Suffolk showed a reduced HFN in response to the same fungicide programme with a lower disease pressure. Crops on the deep peats are often very late to mature and ripen, green leaf surviving long into August or even September.

Other experiments at this time showed a negligible effect of fungicide programme on HFN. It is, however, important to point out that measurements of HFN were made on only a few samples until the mid-1980's.

#### 6.6.2 Fungicide programmes

Salmon & Cook (1987) have summarised results of ADAS fungicide experiments in 1985 and 1986. Table 6.3 extracts some data from this report, suggesting a clear relationship between effective disease control and HFN.

**Table 6.3** Effect of fungicide programmes on yield, Septoria tritici control, and Hagberg Falling Number in hard and soft wheats in 1985 and 1986

	Untreated		3-sprays		2-sprays	
	Hard	Soft	Hard	Soft	Hard	Soft
Yield (t/ha)						
1985	6.66	3.89	9.33*	5.81*	8.67*	5.10*
1986	7.76	8.31	8.65*	9.06*	8.38*	8.91*
<u>Septoria tritici</u> (% leaf 2)						
1985	55.3	41.2	2.4	0.7	8.4	8.0
1986	17.6	3.7	1.7	0.9	1.9	1.2
Hagberg Falling Number						
1985	238.8	181.0	179.3*	121.2	209.4*	170.2
1986	245.8	268.8	225.6	254.1	237.1	269.5

\* Significantly different from untreated,  $p = 0.05$  (disease control figures not tested).

Fungicides:

3-spray - GS31, Sportak Alpha + Corbel; GS39, Bayleton CF; GS59, Delsene M.

2-spray - GS31, Sportak Alpha + Corbel; GS39, Corbel + Bravo + Bavistin

(See Appendix V for active ingredients).

Table 6.3 shows a significant yield benefit and reduction of disease for these spray programmes, although in 1986, there was little financial advantage for the third spray, by comparison with the 2-spray programme. The 3-spray programme, which has given the best disease control, has also given a significant reduction in HFN in 1985. Data from another experiment in the same ADAS series at Doddington in 1985 showed that there was no recordable difference in HFN in the cultivar Avalon. In 1981-1983, experiments at Terrington EHF showed that a series of comprehensive fungicide programmes had no effect on HFN.

The summer of 1985 was wet with significant development of Septoria tritici, and on early-sown crops in Eastern England eyespot (Pseudocercospora herpotrichoides) was pronounced. Data from an experiment in Essex suggested a strong relationship between disease, lodging and HFN. HFN showed a significant correlation with yield, green leaf area and S. tritici ( $r = 0.455, 0.464$  and  $0.573$  respectively). This agreed with the ADAS/FMBRA findings in the same year, suggesting that in high disease situations HFN was highest where disease was most severe. Although eyespot and associated lodging was severe in untreated or carbendazim-treated plots in this experiment, lodging did not have an adverse effect on HFN. Factors affecting final ripening speed were primarily responsible for adverse effects on HFN.

In 1986, an experiment designed to relate a series of fungicide programmes to possible effects on HFN was initiated. At one site where severe *S. tritici* developed (Terrington EHF), the treatment providing the best disease control was associated with a significant reduction in HFN (Cook & Hayward, 1988). Sequential assessments of HFN were made at intervals after harvest by NIAB but there was no significant effect of the time of analysis on HFN in three sequential analyses performed at 3-month intervals.

In 1987, this experiment was repeated at five sites with some revised treatments. At two sites, Boxworth EHF and Burnham-on-Crouch, some treatments effected a significant reduction in HFN. These effects were not consistent between these two sites (Table 6.4), but there is a suggestion that the later fungicides have been associated with the most marked effects.

The table shows that at Burnham, treatments increasing green leaf survival are also those which are adversely affecting HFN with a highly significant inverse correlation ( $r = -0.871$ ). The effect can also be seen at Boxworth, although HFN at this site is extremely low and only those treatments including Sportak (prochloraz) have affected HFN.

**Table 6.4** Effect of fungicide programme on Hagberg Falling Number (HFN) and Green Leaf Area (GLA) at 2 sites, 1987

Treatment	Boxworth (Mergia) HFN	GLA <sup>@</sup>	Burnham (Avalon) HFN	GLA <sup>@</sup>
Untreated/-/-/	81	48.8	267	74.2
Sportak/Sportak	67*	70.0*	243	84.4
Sportak/Bravo + Corbel	76	65.8*	236	82.6
Sportak/Bravo + Triminol	79	63.2*	245	86.4
Sportak/Delsene M	72	67.5*	228*	87.7*
Sportak/Compass	71	56.3	223*	86.6*
Sportak/Bravo + Corbel/ Delsene M	74	66.3*	231*	86.7*
Untreated/Sportak	69*	65.3	237	85.1
Untreated/Bravo + Corbel	82	60.2	243	83.0
Untreated/Bravo + Triminol	75	63.5*	243	84.6
Untreated/Delsene M	70	55.5*	250	83.4
Untreated/Compass	76	52.0	223*	88.3*
Untreated/Bravo + Corbel/ Delsene M	82	68.5*	228*	89.2*
SED	5.0	7.28	18.5	5.09

\* Significantly different from untreated,  $P=0.05$ .

@ leaf 2, GS75

(See Appendix V for active ingredients)

Data from a series of ADAS experiments in 1987 designed to study disease development in relation to crop growth have indicated the nature of the relationship between green leaf area and HFN. The data for two sites is summarised in Table 6.5. These regressions show that early and repeated treatments, which provided the best disease control, have resulted in lowest HFN values.

**Table 6.5** Effect of green leaf survival on Hagberg Falling Number 1987

Relationship accounted for	% variance
Englefield (Avalon)	
y = 277 - 1.73 GLA	73.2 (P=0.0001)
y = 139 + 9.74 wks	80.7 (P=0.0001)
y = 280 - 2.65 GLA-p	82.0 (P=0.001)
y = 302 - 1.88 GLA-c	93.8 (P=0.001)
Terrington EHF (Slejpner)	
y = 198 - 1.22 GLA	49.6 (P=0.0001)
y = 77.7 + 1.68 wks	36.0 (P=0.0001)

y = HFN

GLA = green leaf area, leaf 1, (GS85, Englefield and GS71, Terrington EHF)

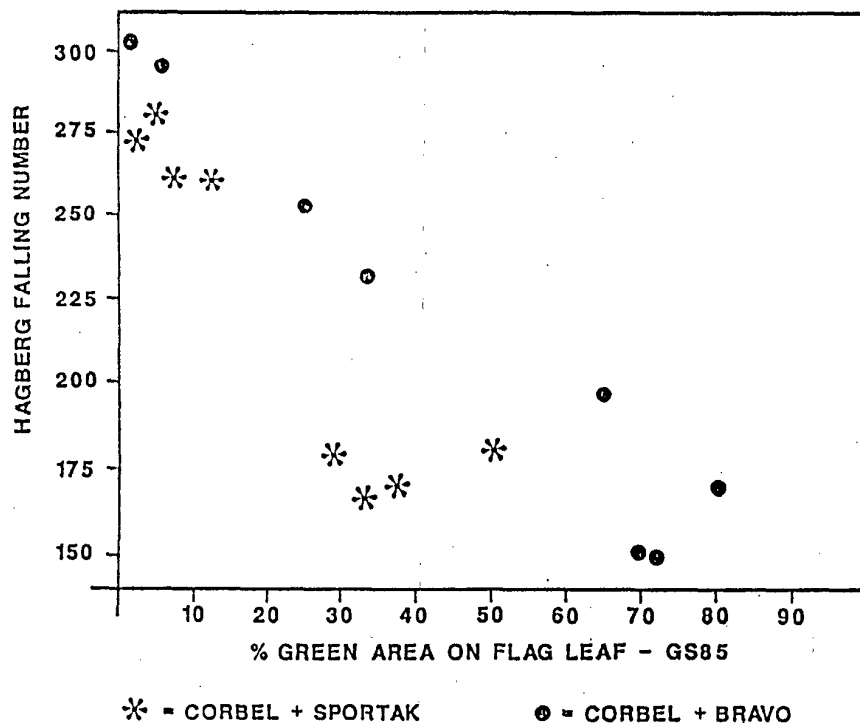
-p = prochloraz + fenpropimorph

-c = chlorothalonil + fenpropimorph

wks = date of start of spray programme in weeks from 1st April

Green leaf survival beyond watery ripe, GS71 (Tottman, 1987) has depressed HFN. At one site (Englefield), analysis has also suggested an effect of fungicide type. Treatment with prochloraz (Sportak) and fenpropimorph (Corbel) had a more severe effect on HFN than treatment with fenpropimorph and chlorothalonil (Bravo) (Figure 6.1).

**Figure 6.1** Effect of green leaf area at GS85 on Hagberg Falling Number





The reduction owing to the prochloraz treatment suggests that the fungicide had a direct effect by giving a lower HFN for the same green leaf area. At both sites, S. tritici was severe and disease severity was well related to yield of grain and green leaf area.

### 6.6.3 Discussion

Although some of the data presented and the results discussed are apparently not in agreement, it is clear that in many situations fungicide application is associated with a reduction in HFN. Late-season fungicides are known to control disease and to increase green leaf survival. Gooding *et al.* (1987) have indicated that these effects on HFN are a result of increased grain moisture in fungicide-treated plots so that the effect seems to be related to speed of maturation of grain, or loss of green leaf area. In this context, high disease incidence will accelerate natural senescence allowing the plant to ripen and mature in the better weather conditions of July or early August, reducing the chance that the natural process will be interrupted by wet or cool weather.

The results from the two ADAS experiments at Englefield and Terrington in 1987 suggest that this effect can be related to the efficiency of the fungicide programme. These experiments have shown (ADAS, unpublished) that leaf disease development and associated yield loss can be prevented if fungicide programmes are started during the middle of May, and that there is a reduction of potential yield benefit from disease control when the start of treatment is delayed from that period. At these sites, the highest HFN was recorded in plots which received no fungicide.

However, at Englefield in 1987 the effect of treatment on HFN was more severe where prochloraz had been used, and this agrees with the results of the 1987 experiment at Boxworth. Prochloraz is an azole fungicide belonging to the same group as other commonly used cereal fungicides such as propiconazole and triadimenol. These products possess some growth regulatory properties and affect gibberellic acid activity (Buchenauer *et al.*, 1984). This last finding thus admits the possibility that effects of fungicides on HFN may also be related to their growth regulatory characteristics. It is, however, clear that the effect of fungicides on disease control and their effect on green leaf survival is the main factor involved in their effect on HFN.

While this group of fungicides embraces the most common and widely used products, evidence for an effect of fungicides on green leaf area in addition to their ability to reduce disease abounds. For example, the carbendazim fungicides are known to have cytokinin activity (Mukhopadhyay & Bandopadhyay, 1977). There is a need to understand the chemistry of fungicide interaction with the host in terms of disease control and possible impact on the grain germination processes.

In the meantime, it is clear that disease control strategies should be designed to provide just enough protection to take the plant to the end of active dry matter accumulation and so preserve yield but not to extend green leaf, and hence retard maturation to such an extent that they interfere with natural amylase decline during the ripening process.

## 6.7 TIME OF HARVESTING

Workers at two EHF's, Drayton and Terrington, have assessed the deterioration resulting from delay in harvest. The Terrington results cover the four years, 1982-85, and are presented in Appendix III. Drayton results are available for two years and follow similar trends.

In view of the known production of alpha-amylase enzyme at the onset of sprouting it is perhaps surprising that HFN values fell only by an average of 16 seconds when harvesting was delayed by 10-12 days. However, this did include the relatively favourable seasons 1983 and 1984 and the 1987 results from Drayton showed a drop of 31 seconds from 186 to 155 in a similar period.

The 1985 Terrington results are interesting in that the variety Galahad which would normally produce a high value had already fallen to only 126 at the first harvest date, thus confirming the evidence of Gale (section 7.3) that, in unfavourable conditions, alpha-amylase production can be stimulated well before harvest is reached. In good conditions such as 1983, there was no detectable deterioration in HFN over a 2-week period at harvest. Lodging was not a feature of the Terrington trials although in 1982 following a storm, the straw was observed to have brackled and a drop of 50 was measured when harvesting was delayed for a further week.

In 1983, the herbicide glyphosate was applied as a desiccant at either 70% d.m. or 74% d.m. of the grain but had no effect on HFN. However, HFN values were generally less variable in that year; thus, it was not a good test of the expectation that a desiccant could alleviate decline in HFN when drying out was protracted.

## 6.8 PRE-HARVEST TESTING

If farmers were provided, pre-harvest, with reliable predictions of the quality of their grain this would enable them to:

- 1) make better decisions on combining priorities.
- 2) know which parcels of grain should be stored separately to avoid mixing grain of differing qualities and thereby risking downgrading.
- 3) have information upon which a marketing plan can be based.
- 4) know which parcels may require further preparation e.g. gravity separation, prior to sale.

Pre-harvest predictions would be of commercial value to farmers.

By the time the moisture content of grain in a standing wheat crop has fallen to 25-30% the deposition of both carbohydrate and protein has ceased. By simulating, but shortening, the natural process of drying, realistic assessments of some quality parameters might be made before combining takes place.

In 1985 a pilot study was undertaken. Hand-grab samples were taken at random from the standing crop when the moisture content was less than 30%. These were carefully dried, threshed and protein levels, Hagberg Falling Numbers and specific weights were determined. These results were compared with those derived from representative samples taken after the sites were combine harvested. The results of this study were sufficiently encouraging to warrant further study. Thus, an experiment on wheat quality pre-harvest testing was carried out in 1986 on 15 sites and in 1987 on 19 sites. The experiment will be repeated in 1988 on 19 sites.

A range of breadmaking varieties was used in the trial including Avalon, Moulin, Mission, Mercia and Axona. All sites were located on ADAS EHF's. In both 1986 and 1987 there was a strong correlation between the Hagberg Falling Numbers of the combine samples and those taken pre-harvest.

The accepted accuracy of the HFN test is  $\pm 5\%$ . In actual terms this means  $\pm 5$  at 100 HFN,  $\pm 10$  at 200,  $\pm 15$  at 300 and  $\pm 20$  at 400. The minimum HFN levels preferred by bread makers range from 225-280 HFN although in recent years they have had to take some wheat with lower levels. In commercial terms, therefore, the level of accuracy of prediction is more important in the range 200-300 HFN and of lesser importance outside this band.

The main commercial value of these predictions is likely to be the identification, pre-harvest, of samples with unacceptably low levels of HFN. These will not improve before harvest and so can be kept apart from more commercially acceptable samples.

The method of sampling, drying and the close observance of the correct analytical procedures are all especially important in deriving commercially valuable predictions, and further work is required to confirm details.

#### 6.9 POST-HARVEST MANIPULATION

Several historical references have been made to the use of gravity separators to improve the HFN of milling wheat and for several years at least one company has been commercially promoting the use of the technique.

In October 1984, FMBRA reported that they had studied the effect of a laboratory gravity separator when used on two samples of mixed wheat (Robinson, 1984). The samples were divided into 5 fractions and it was observed that fractions 1 and 2 contained broken grains while fraction 5 contained plump, vitreous grains. The specific weight rose progressively from fraction 1 to fraction 5 and covered a range of 6.2 and 7.0 kg/hl in the two samples. The increase in specific weight was accompanied by an increase in HFN which was significant for the second sample.

### 6.9.1 Summary of results of ADAS tests (Bailey & Graham, 1987)

**Table 6.6** A summary of grain quality before and after gravity separation

Variety	Initial	Initial	Peak	"Commercial sample" @		
	HFN (sec)	specific weight (kg/hl)	HFN (sec)	HFN (sec)	Specific weight (kg/hl)	% of Initial weight
Avalon (a)	63	71	278	205	73	67
Avalon (b)	79	72	291	276	75	69.5
Wembley	185	73.5	314	247	76	69.5
Avalon (c)	110	73.0	312	175	74.5	99
Brimstone*	164	66	245	242	75	35
Avalon (d)*	74	67	290	273	75	26.5
Avalon (e)	67	69.5	311	187	75.5	78.5

\* Low specific weight prevented a higher percentage extraction.

@ A sample considered suitable for sale in the 1987/8 season.

The fractions across the separation table numbered 1-5 from left to right showed an enormous variation in weight with fraction 3 giving nearly 50% of the overall sample weight. Fractions 1 and 5 yielded a very small proportion of the overall samples at around 5% by weight. The improvement in HFN peaked in fractions 4 and 5. In commercial terms, combining fractions 4 and 5 would generally give about 30% of the weight. However, combining 3, 4 and 5 accounts for a total combined weight of 60% to 80% of the original sample weight with a decreased, but hopefully acceptable HFN reading as shown as "Commercial sample" in Table 6.6. By altering the diverting plate, fraction 3, the largest sample by far, could be further subdivided if it was thought necessary. Alternatively, parts of the original sample could be put through a second time.

Detailed results of the separation of Avalon (c) are shown in Appendix IV to indicate the wide variation in specific weight and HFN that resulted from gravity separation.

In commercial practice a compromise would need to be made. For instance, for sample Avalon (c) taking out the lightest fraction (1% by weight) improved the Hagberg from 110 to 175 and the specific weight from 73 to 74.5. However, taking out fractions 1 and 2 (19% by weight) improved the HFN to 233 and the specific weight to 75.5.

The specific weight of the final ("commercial") sample was also significantly increased over the initial sample. There was a large variation in specific weights from fractions 1 to 5 with generally only minor improvements beyond fraction 3.

Due to the basic principle of operation, the output is intrinsically low varying with these individual samples from 1572 kg to 3704 kg per hour. A major factor in the variation is the "flowability" characteristics of different samples and varieties. The figures in the literature show an eight-fold variation, depending on the type of seed being processed.

### 6.9.2 Discussion

Gravity separators clearly have the potential to remove grain of low specific weight from a mixed sample. In some situations this leads to the simultaneous removal of low HFN grains with a consequent improvement in HFN of the balance. The improvement in HFN has been greatest where there is a wide range of quality between individual grains. This would be expected if patches of the crop had lodged or if there was plant to plant variation in disease incidence.

It should also be noted that a current investigation at FMBRA into the use of gravity separators has shown that there may be problems associated with the process. Although gravity separators clearly yield fractions of wheat with acceptable HFN levels there may be other problems associated with the baking quality of these wheats. In studying a wide range of bread wheats processed on a range of gravity separators, it has been found that all samples produced very poor bread. The doughs prepared from flours milled from these wheats were weak, soft and extensible being more like those required for biscuit making than breadmaking (S.C.W. Hook, pers. comm.).

### 6.10 FUTURE RESEARCH AND DEVELOPMENT REQUIREMENTS

1. Further work should be initiated to assess the effect of fungicide type on HFN and interactions with grain chemistry.
2. The role of alpha-amylase in grain maturation and the influence of weather should be investigated.
3. There is an urgent need to continue current experiments to assess the relationship between disease, crop maturation and HFN.
4. The use of a desiccant should be included as a treatment in appropriate fungicide, nitrogen and time of harvest trials to evaluate its potential in improving the drying rate and hence HFN of a maturing crop.
5. Pre-harvest prediction of HFN values would avoid the mixing of the produce from good and bad fields. Some further work is required particularly on the sampling.

## 6.11 REFERENCES

1. BAILEY, J. and GRAHAM, K. (1987). A pilot study of the use of a Kamas gravity separator to improve the Hagberg of a range of wheat samples. ADAS, Chelmsford.
2. BUCHENAUER, H., KUTZENER, B. and KOTHS, T. (1984). Effect of various triazole fungicides on growth of cereal seedlings and tomato plants as well as gibberellin contents and lipid metabolism in barley seedlings. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, **91**, 506-524.
3. COOK, R.J. (1987). Effect of late season fungicides on winter wheat quality. Aspects of Applied Biology **15**, 417-424.
4. COOK, R.J. and HAYWARD, C.F. (1988). Effect of fungicide and spray timing on control of Septoria tritici on wheat. Tests of Agrochemicals and Cultivars, **9**, Annals of Applied Biology, **112** (Supplement), 42-43.
5. DAVIES, W.P., GOODING, M.J. and KETTLEWELL, P.S., (1986). Responses of Hagberg Falling Number to nitrogen and late-season fungicides. Proceedings of a symposium on 'Some aspects of wheat milling quality', NIAB, Cambridge.
6. GOODING, M.J., DAVIES, W.P., KETTLEWELL, P.S. and HOCKING T.J., (1986). The influence of late-season fungicides on the Hagberg Falling Number of grain from breadmaking varieties of winter wheat. Cereal Research Communications, **14**, 245-249.
7. GOODING, M.J., DAVIES, W.P., KETTLEWELL, P.S. and HOCKING, T.J. (1987). Do fungicides reduce Hagberg Falling Number of wheat by maintaining grain moisture content during grain development? Aspects of Applied Biology, **15**, 413-416.
8. GOODING, M.J., KETTLEWELL, P.S. and DAVIES, W.P. (1986). Effects of spring nitrogen fertiliser on the Hagberg Falling Number of grain from breadmaking varieties of winter wheat. Journal of Agricultural Science, Cambridge, **107**, 475-477.
9. HGCA (1984). Home-Grown Cereals Authority, Marketing Note Vol. 19, No. 21 (11) 31 December 1984.
10. MCDONALD, H.G. and VAIDYANATHAN, L.V., (1987). Effects of nitrogen fertiliser on the Hagberg Falling Number of winter wheat. Aspects of Applied Biology, **15**, 359-364.
11. MORGAN, A.G., MORLEY, J.H. and FLETCHER, P.D. (1986). Observations on the effect of fungicide and grain storage of Hagberg Falling Number. Proceedings of a symposium on 'Some aspects of wheat milling quality', NIAB, Cambridge.
12. MUKHOPADHYAY, A.N. and BANDOPADYAY, R., (1977). Cytokinin-like activity of carbendazim. Pesticides, **11**, 24-28.

13. ROBINSON, I.M. (1984). The Westrup Laboratory Gravity Separator. FMBRA Bulletin No. 5, 193-196.
14. SALMON, S.E. and COOK, R.J. (1987). Effects of fungicides on the milling and baking quality of wheat. Aspects of Applied Biology, 15, 373-384.
15. TOTTMAN, D.R. (1987). The decimal code for the growth stages of cereals, with illustrations. Annals of Applied Biology, 110, 441-454.
16. VAIDYANATHAN, L.V. (1987). Precision and reliability of measuring Hagberg Falling Number of wheat including variability associated with crop husbandry and grain handling. Aspects of Applied Biology, 15, 495-513.

6.12 APPENDICES

APPENDIX I

TIME OF SOWING - TERRINGTON EHF

a) Sept-Oct

1984/5	Sowing	10/9	28/9	12/10
	HFN	97	114	110
1985/6	Sowing	18/9	2/10	15/10
	HFN	247	246	234
1986/7	Sowing	18/9	2/10	15/10
	HFN	104	102	103

b) Nov-April

1984/5	Sowing	11/12	4/2	19/2	2/4
	HFN	147	127	157	149
1985/6	Sowing	5/11	15/1	14/3	11/4
	HFN	128	121	173	148
1986/7	Sowing	8/1	16/2	9/3	22/4
	HFN	202	180	224	260

APPENDIX II

LODGING CONTROL 1985 - VARIETY BRIMSTONE

<u>Treatment</u>	<u>% Lodging</u>	<u>HFN</u>	<u>Yield t/ha</u> <u>@ 85% DM</u>
Nil	85	77	5.86
Early Cycocel*	60	75	6.55
Late Cycocel	25	85	6.46
Split Cycocel	10	105	7.14
Terpal*	15	110	7.17
Cycocel + Terpal	8	140	7.57

\* Active ingredients shown in Appendix V.



**APPENDIX III**

**TIME OF HARVESTING - TERRINGTON EHF**

**HAGBERG FALLING NUMBERS**

Delay beyond approx 80% DM (days)	1982 Avalon	1983 Avalon	1984 Mission	1985 Galahad
0	382	293	202	126
2/3	-	276	197	103
4/5	-	295	221	-
6/7	251	284	211	101
8	294	-	204	-
10/12	321	305	202	114
13/15	288	289	-	-
16	-	-	-	105
18/20	279	-	-	96
22	239	-	-	-
Starting date -	3 Aug	12 Aug	21 Aug	25 Aug

**APPENDIX IV**

**THE EFFECT OF USING A GRAVITY SEPARATOR ON SPECIFIC WEIGHT AND HFN**

Sample Avalon (c)  
 Initial HFN 110  
 Initial Specific Weight 73 kg/hl

Fraction	Specific Weight (kg/hl)	HFN	Weight (kg)	Weight as % of total
1	47	61	0.9	1
2	70	68	17.1	18
3	75	220	47.6	50
4	78	312	26.8	28
5	79	294	2.2	2
Total	-	-	94.6	100
4 + 5	78	279	29	30.5
3 + 4 + 5	75.5	233	76.6	81
2 + 3 + 4 + 5	74.5	175	93.7	99
1 + 2	68.5	-	18	19

Throughput: 2,270 kg per hour.

APPENDIX V

ACTIVE INGREDIENTS OF PRODUCTS REFERRED TO IN SECTION 6.

<u>Commercial product</u>	<u>Active ingredient</u>
Fungicides: Bavistin	carbendazim
Bayleton CF	triadimefon + captafol
Bravo	chlorothalonil
Compass	iprodione + thiophanate-methyl
Corbel	fenpropimorph
Delsene M	carbendazim + maneb
Sportak	prochloraz
Sportak Alpha	prochloraz + carbendazim
Triminol	nuarimol
Growth Regulators:	
Cycocel	chlormequat chloride + choline chloride
Terpal	2-chloroethylphosphonic acid + mepiquat chloride

HGCA RESEARCH REVIEW NO. 2

HAGBERG FALLING NUMBER AND BREADMAKING QUALITY

7. GENETICS AND BREEDING

*M. D. GALE and J. E. FLINTHAM, Institute of Plant Science Research,  
Maris Lane, Trumpington, Cambridge CB2 2JB.*

*This review, completed in August 1988, was funded by the HOME-GROWN  
CEREALS AUTHORITY, Hamlyn House, Highgate Hill, London N19 5PR.*

<b>CONTENTS FOR 7. GENETICS AND BREEDING</b>		
<b>Page</b>		
65	7.1	<b>INTRODUCTION</b>
68	7.2	<b>THE GENETICS OF GRAIN CHARACTERS IN WHEAT</b>
	7.2.1	Hexaploid wheat genetics
	7.2.2	Inheritance in grain tissues
	7.2.3	Alpha-amylase genes
71	7.3	<b>PRE-MATURITY ALPHA-AMYLASE</b>
	7.3.1	Genes causing susceptibility to pre-maturity alpha-amylase production
	7.3.2	Other genes affecting pre-maturity alpha- amylase levels
73	7.4	<b>DORMANCY</b>
	7.4.1	Red grain colour genes
	7.4.2	Germination inhibitor response genes
	7.4.3	Varietal differences in ABA levels
	7.4.4	Other sources of dormancy
75	7.5	<b>PRE-MATURITY GERMINATION</b>
75	7.6	<b>RATE OF ALPHA-AMYLASE DEVELOPMENT DURING SPROUTING</b>
75	7.7	<b>CONCLUSIONS</b>
76	7.8	<b>RESEARCH NEEDS</b>
	7.8.1	Pre-maturity alpha-amylase
	7.8.2	Dormancy duration
	7.8.3	Pre-maturity sprouting
76	7.9	<b>REFERENCES</b>

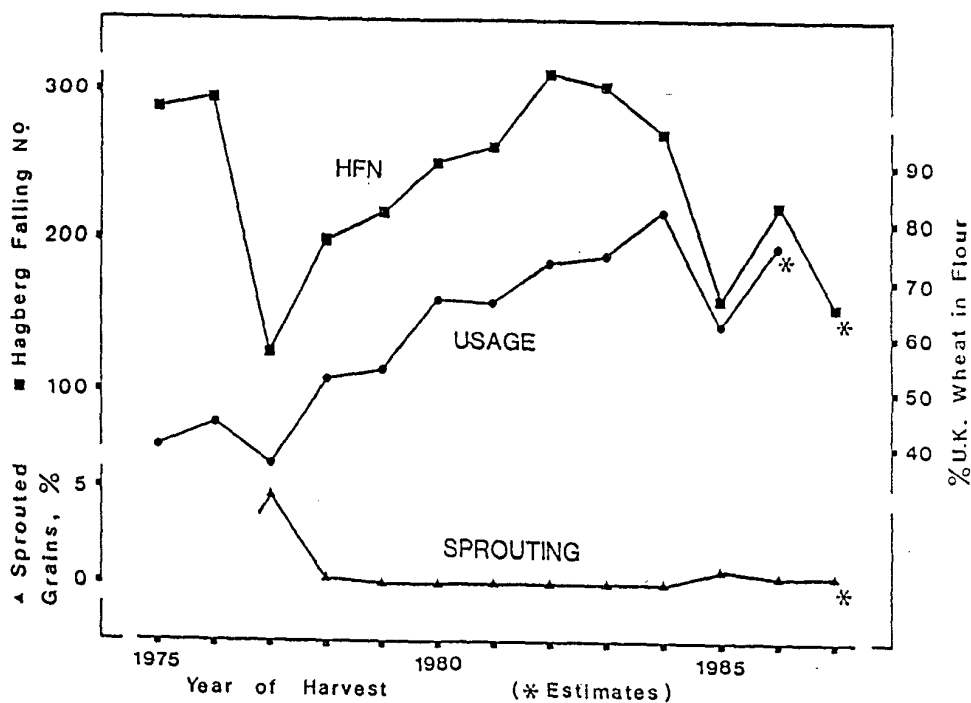
## 7. GENETICS AND BREEDING

### 7.1

#### INTRODUCTION

The mean HFN values in UK harvests from 1975 to date show clearly that in some years our varieties are particularly prone to pre-harvest deterioration in quality. The associated reductions in the proportion of home-grown wheat in millers' grists, which has otherwise tended to rise over this period, indicates the severe economic consequences of the 1977, 1985 and 1987 harvests (Fig 7.1). Analysis of the source of the alpha-amylase in these harvests shows that the causes were different in each case.

**Figure 7.1** Hagberg Falling Number, visible sprouting and millers' usage of home-grown wheat, 1975 to 1987 (HGCA, 1987)

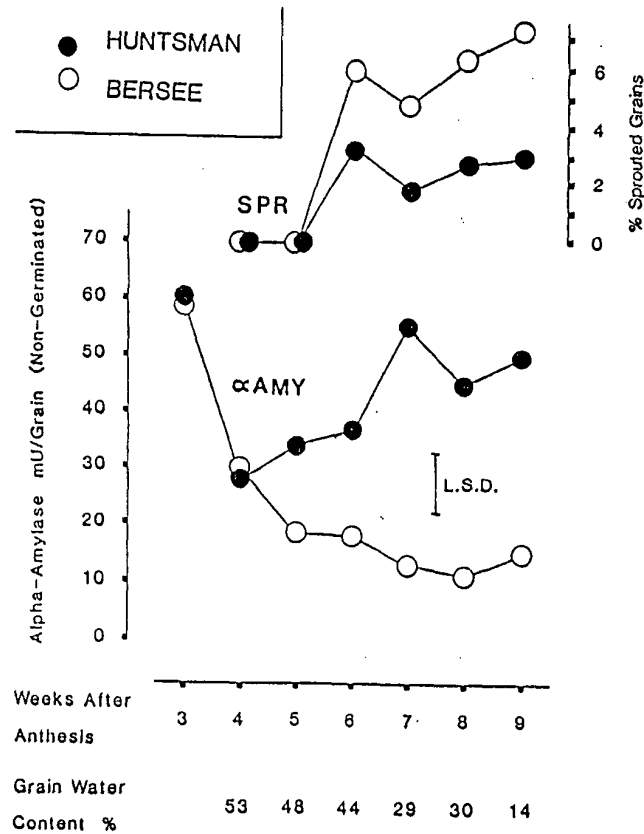


**Note:** Data for wheat usage are taken from August of harvest year to the following July. 1987 data are estimates.

Low HFN in 1977 was due in large part to alpha-amylase produced during premature germination (sprouting) in ripe, post-dormant grain (see % sprouted grains, Fig 7.1). In 1985, alpha-amylase production occurred in the developing grain, prior to ripening and in the absence of embryo germination, so that the harvest had low HFN in the absence of visible sprouting. Serial samples from trials in 1987 showed a third potential risk, with grains germinating in the ear following the onset of ripening but prior to the onset of conventional dormancy. The tendency to prematurity germination appears to be independent of a tendency to

produce pre-maturity alpha-amylase. For example, in Fig 7.2 it may be seen that the variety Bersee showed higher levels of germination than Maris Huntsman during the fifth week of grain development, although the latter showed high levels of pre-maturity alpha-amylase production.

**Figure 7.2** Alpha-amylase and premature sprouting levels during grain development in two varieties in 1987.

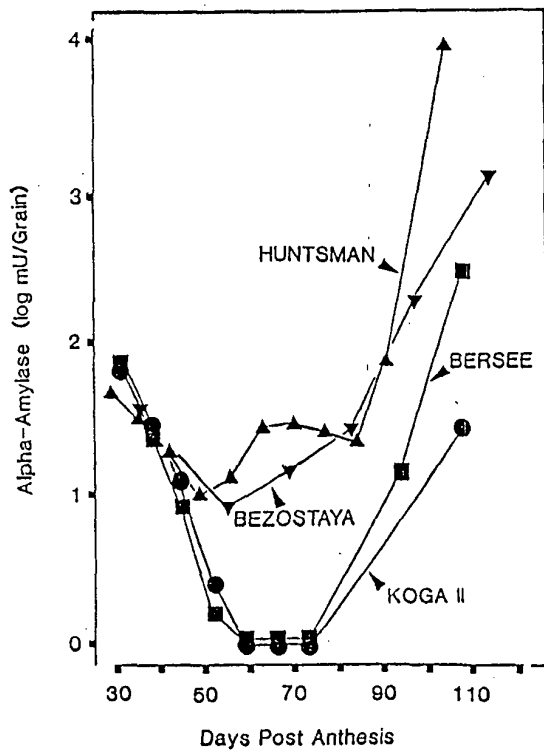


**Note:** Grain samples from plots were first scored for visible sprouting, and then alpha-amylase activity was measured in extracts of unsprouted grains (activity in sprouted grains > 100 mU).

One enzyme unit (U) hydrolyses 1 umol glucosidic linkage per minute at 37°C (Phadebas amylase test).

Figure 7.3 shows alpha-amylase levels during grain development, ripening and sprouting. The varieties Maris Huntsman and Bezostaya I develop high levels of alpha-amylase through the late ripening period while in the varieties Bersee and Koga II, alpha-amylase drops to very low levels. Harvesting was delayed until after the loss of dormancy, when natural rainfall allowed germination with the expected increases in activity. Note that the rate of germination and of alpha-amylase increase was greater in the varieties which exhibited the pre-maturity alpha-amylase syndrome, even though the duration of dormancy was apparently greater in these varieties.

**Figure 7.3** Alpha-amylase levels in grains of different varieties during grain filling, ripening and sprouting in field plots in 1982 (Gale et al., 1983)



**Note:** The plots were allowed to stand after harvest ripeness and sprouting occurred after rainfall. Visible sprouting scores in final samples. Maris Huntsman = 16%, Bezostaya I = 12%, Bersee = 4%, Koga II = 1%.

Thus it appears that high levels of alpha-amylase in flour can arise in three different ways:

- a) Pre-maturity alpha-amylase can be synthesised in pre-ripe, ungerminated grains. Genetic susceptibility to this trait is exposed by conditions which delay ripening. Affected grains synthesise germination-type enzymes without apparent associated embryo germination (Figs 7.2, 7.3).
- b) Sprouting of after-ripened grain can occur when wet weather delays the harvest beyond the decay of grain dormancy. The germinating embryo secretes gibberellin which triggers alpha-amylase synthesis in the conventional manner (Fig 7.3).
- c) Premature sprouting can also occur before the onset of dormancy. This results in high alpha-amylase levels associated with visible germination in pre-ripe grains (Fig 7.2).

To date almost all genetic research in this area has been concerned with alpha-amylase levels rather than HFN. Although it is clear that other factors besides alpha-amylase can affect HFN, particularly at high values, no genetic work concerning these factors has been reported. The remainder of this section deals with alpha-amylase activity, the most important cause of variation in HFN at levels where the character becomes significant as a measure of baking quality. The available evidence, outlined below, indicates that each of the three physiological mechanisms described above are under independent genetic control.

## 7.2 THE GENETICS OF GRAIN CHARACTERS IN WHEAT

In order to develop strategies for breeding resistant cultivars there is a need to understand the genetic and physiological controls underlying alpha-amylase levels at harvest. Desirable genes should be characterised with regard to the magnitude of any beneficial effects, the stability of these effects in different environments and their compatibility with other breeders' targets. A next step must be to develop effective means of recognising beneficial alleles in breeders' populations. Such 'breeders screens' would ideally be efficient in all genetic backgrounds and harvest environments, and must be cost effective.

### 7.2.1 Hexaploid wheat genetics

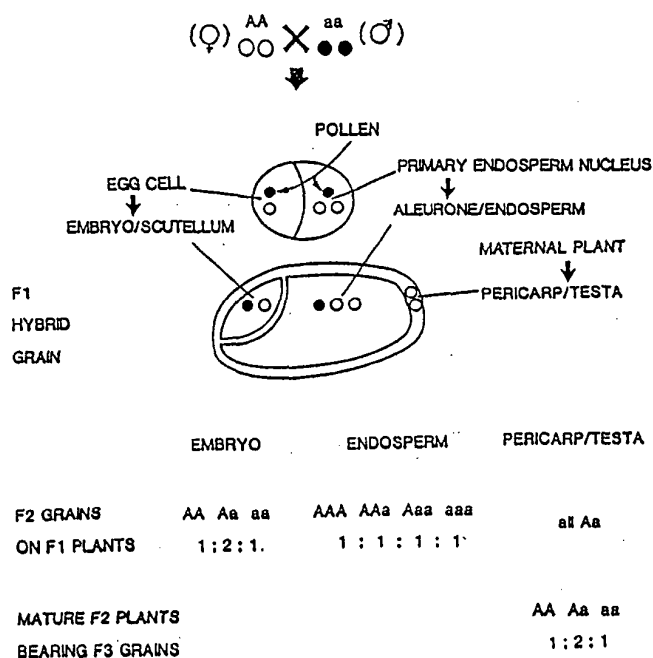
Genetically, bread wheat (Triticum aestivum,  $2n = 6x = 42$ ) is complex, being a hexaploid species with three diploid sets of seven pairs of chromosomes, each set having been derived from a different ancestor. These three sets, the 'A', 'B' and 'D' genomes, were originally derived from a single primateval genome and carry many, probably most, gene sites (loci) in common. Thus many simply inherited characters such as variation in alpha-amylase proteins or red vs white grain colour, which in a diploid species such as rye or barley would be controlled by a single gene, are controlled by three genes in wheat. Besides making genetic analysis more difficult, this means that the presence of null alleles, e.g. deficient alpha-amylase genes, can be masked by effective alleles in the other two genomes and, conversely, that the effects of beneficial genes, e.g. alleles determining red grain colour, may be diluted by disadvantageous or neutral alleles at the equivalent loci in the other genomes.



## 7.2.2 Inheritance in grain tissues

Furthermore the wheat grain, as in all cereals, is itself genetically rather complex. The embryo cells have the normal complement of 42 chromosomes (21 derived from the egg cell and 21 from the pollen), but the endosperm cells however have 63 chromosomes (two copies from the maternal parent plus one from the male). The pericarp and testa are different again, having 42 chromosomes but being derived directly from maternal tissue (Fig 7.4).

**Figure 7.4** Distribution of parental alleles in different tissues of grains derived from intervarietal hybrids.



This poses problems when analysing grain characters related to sprouting damage. For example a single gene which segregates in the ratio  $1 \underline{AA} : 2 \underline{Aa} : 1 \underline{aa}$  in the  $F_2$  embryos will at the same time segregate  $1 \underline{AAA} : 1 \underline{AAa} : 1 \underline{Aaa} : 1 \underline{aaa}$  in  $F_2$  endosperms. Seed-coat tissues will also segregate  $1 \underline{AA} : 2 \underline{Aa} : 1 \underline{aa}$  but not until the following generation (between mature  $F_2$  plants bearing  $F_3$  grain).

Unfortunately it is often difficult to isolate effects from these different tissues. For example, during sprouting the seed coat imposes dormancy which then decays to allow germination of the embryo, which in turn stimulates alpha-amylase production by both the scutellum (part of the embryo) and by the aleurone layer (part of the endosperm).

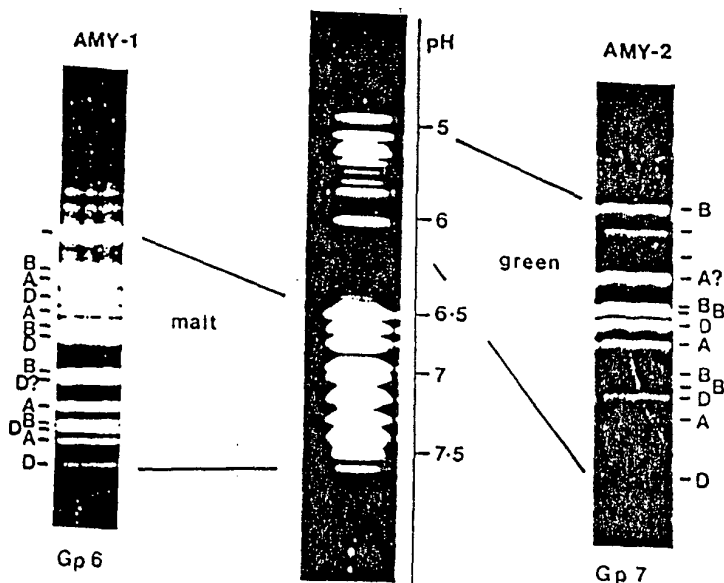
### 7.2.3 Alpha-amylase genes

The structural genes that produce alpha-amylase are fairly well characterised but, at this time, offer little hope for manipulating alpha-amylase levels in sprouted grain.

Two sets of loci,  $\alpha$ -Amy-1 and  $\alpha$ -Amy-2 (Gale & Ainsworth, 1984; Ainsworth et al., 1985), are known to control production of 'malt' and 'green' isozymes respectively. The former enzymes are usually found only in germinating grain, the latter in the pericarp during early grain development and again in the scutellum and aleurone during the later stages of germination. A third set of genes,  $\alpha$ -Amy-3, has recently been recognised which produce a yet further species of alpha-amylase, but only during early grain development in the pericarp (Baulcombe et al., 1987; Daussant & Renard, 1987).

It is probable that the malt isozymes, controlled by the  $\alpha$ -Amy-1 genes on the long arms of chromosomes 6A, 6B, and 6D, are the most significant as regards HFN. However these loci actually comprise small multigene families, each of 3-8 gene copies. Minor differences among the individual gene members are probably responsible for the many different isoforms of malt isozymes seen following isoelectric focusing (Fig 7.5). The green enzyme is similarly produced by  $\alpha$ -Amy-2 multigene families on the long arms of chromosome 7A, 7B + 7D (Lazarus et al., 1985).

**Figure 7.5** Wheat alpha-amylase isozymes separated by isoelectric focusing (Lenton et al., 1987)



**Note:** Extracted isozymes migrate along a pH gradient in an electric field and focus at different isoelectric points. Staining with starch followed by iodine reveals clear bands of amylase activity. Chromosomal control of individual bands is indicated by letters ( $\alpha$ -Amy-1 genes on chromosomes 6A, 6B and 6D;  $\alpha$ -Amy-2 on 7A, 7B and 7D).

Because of the complexity of these structural genes, the possibility of reducing alpha-amylase levels by disabling individual Amy-1 genes, or by selecting heat-sensitive proteins which are inactive at the temperature of starch gelatinisation, is unrealistic at present.

### 7.3 PRE-MATURITY ALPHA-AMYLASE

The early reports by Bingham & Whitmore (1966) describing high alpha-amylase activity in sound, ungerminated grains were confirmed by experience in 1985. Susceptibility to expression of this trait is characteristic of several UK varieties, notably Maris Huntsman and Fenman, and is said to have been inherited from the Belgian parent Professeur Marchal. Similar symptoms have since been described in germplasm from Russia, Australia and South Africa (Gale et al., 1983; Marais & Kruis, 1983; Mares, 1987).

Susceptibility is highly dependent on the environment, and it is probable that any factor which delays grain ripening will enhance both the proportion of individual grains triggered to produce alpha-amylase and the levels of enzyme attained (Gale et al., 1987). Cold temperatures, rainfall, high humidity in a lodged crop and even fungicide applications that delay senescence, all may contribute.

#### 7.3.1 Genes causing susceptibility to pre-maturity alpha-amylase production

The limited available evidence suggests that genetic control is simple, possibly by a single recessive gene. Elimination of this gene from breeding programmes should be straightforward, providing it is appreciated: a) that the recessive nature of the deleterious allele will prevent its detection for at least one generation; b) that expression is dependent on ripening conditions, and; c) that expression of the gene as measured by alpha-amylase levels in harvested grain can be confounded by the presence of grains in which germination has been initiated. Indeed, it is clear that grain which has high pre-maturity alpha-amylase will produce high levels of the enzyme more rapidly during conventional germination (Gale et al., 1983).

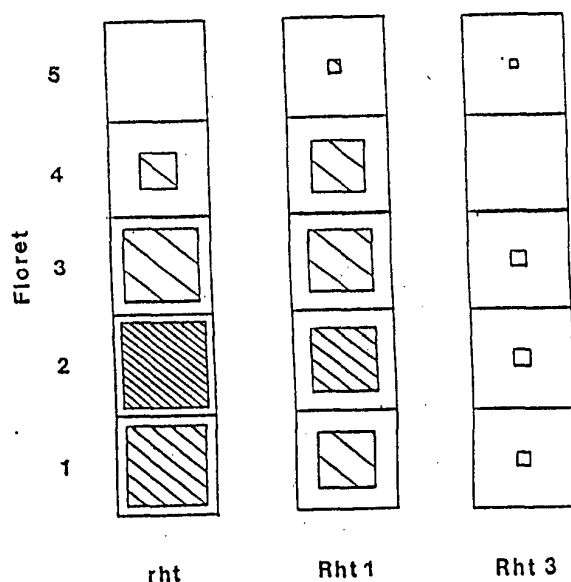
It is not known whether all varieties susceptible to pre-maturity alpha-amylase production carry the same allele, or even alleles at the same locus. The fact that wheats from geographically diverse sources are susceptible, as are almost all triticales, indicates that other alleles probably exist.

#### 7.3.2 Other genes affecting pre-maturity alpha-amylase levels

The gibberellin-insensitive dwarfing genes (Gale & Youssefian, 1985), including Rht1 and Rht2 in widespread use in semi-dwarf varieties, and Rht3, a stronger allele at the same locus, all reduce pre-maturity alpha-amylase levels (Gale et al., 1987) (Fig 7.6).

Figure 7.6

Reduced pre-maturity alpha-amylase in lines carrying Rht1 and Rht3 (Gale et al., 1987)



**Note:**

rht = Maris Huntsman.

Rht1 and Rht3 lines are near-isogenic in the genetic background of Maris Huntsman. Hatched areas represent the proportion of grains with pre-maturity alpha-amylase, the density of hatching indicates the mean enzyme level: low 5 mU/grain, medium 5-10 mU, high 10mU.

Data shown for different florets within spikelets in the middle region of the ear.

For practical purposes only the extreme dwarfing genes are likely to give a significant level of genetic protection. This effect also extends to alpha-amylase produced in germinating grains during conventional sprouting. However, true-breeding wheats of Rht3 genotypes tend to be too severely dwarfed for present day use in the UK.

Rht3 may however be of value for  $F_1$  hybrid (Rht3/rht3) wheats (Gale et al., 1988). In this case alpha-amylase arising prematurely or from sprouting will be accumulated in the  $F_2$  grain on the  $F_1$  crop. Experiments to study the gene dosage effects on this endosperm character (Gale & Marshall, 1975) indicate that protection can be expected in half the grains (endosperms with Rht3/Rht3/rht3 or Rht3/Rht3/Rht3 genotypes). This should reduce alpha-amylase levels by about 40% (Flintham & Gale, 1982).

## 7.4 DORMANCY

Germination before harvest is usually suppressed by post-maturity dormancy and/or the lack of adequate water in the ripe grain. But when harvesting is delayed by wet weather the levels of dormancy in UK wheats can be inadequate and the grain may begin to germinate in the ear, inducing high levels of alpha-amylase. This was the main cause of the low HFN values observed in 1977.

Selection for increased dormancy is usually carried out by scoring sprouting and/or alpha-amylase levels in ripe ears subjected to real or simulated rainfall or simply weathered in the field. This character is very sensitive to the environment, in particular the intensity and duration of dormancy is greatly affected by weather conditions, especially temperature, during ripening (Hagemann & Ciha, 1987). Levels of dormancy in ripe ears from uncontrolled field environments are thus likely to vary from year to year irrespective of genetic protection and, plainly, results from any single year are unlikely to give a true impression of genetic potential.

Many genetic differences are likely to affect the results of such pragmatic tests on field-grown material, further confounding interpretation. For example, variation in heading time will mean that ears of different genotypes may experience different weather conditions during the critical periods of grain development. Such sprouting scores thus reflect the summed effects of a large number of genetic and environmental variables, and thus have low heritability and are prone to genotype/environment interactions.

Present breeding methods are plainly relatively inefficient and labour-intensive. The use of, and direct selection for, major genes with characterised dormancy effects would simplify the selection of resistant genotypes and offer more direct advancement.

### 7.4.1 Red grain colour genes

Red grain colour is controlled by dominant alleles at any of the triplicate set of  $R/r$  loci on the long arms of the group 3 chromosomes (Metzger & Silbaugh, 1970). This character has long been known to be associated with dormancy (Nilsson-Ehle, 1914).

Argument as to whether this association is due to pleiotropy or to close genetic linkage with other dormancy genes appears finally to have been decided in favour of pleiotropy, ie. either the red phlobaphene pigment itself or precursors in the biochemical pathway cause a temporary inhibition of germination.

The effects of  $R$  alleles on dormancy are inherited as a maternal character, ie. segregation is observed between  $F_2$  plants rather than between the  $F_2$  grains on  $F_1$  plants (Fig 7.4). Dominance is, as for grain colour, towards extended dormancy.

Fortunately all present day UK wheats are red-grained, although the number of R alleles present in most varieties is not known. It is probable that the effects are additive and thus selection for 3-gene red varieties will be advantageous.

Unfortunately although selection for red vs white wheats is relatively simple, pyramiding R alleles requires a selection system which can distinguish between 1, 2 and 3-gene reds. No reliable system exists at present, apart from crossing with tester lines of known genotype and carrying out extensive progeny trials.

#### 7.4.2 Germination inhibitor response genes

There is evidence that the response to inhibitors such as catechin-tannins is under simple genetic control (Stoy & Olsen, 1980). Taken together with evidence that some 3-gene red wheats are relatively non-dormant (Noll *et al.*, 1982), it is possible that the red grain colour effect is part of a dual physiological system, with the two components under independent genetic control. This would mean that for maximum protection genes for sensitivity to the inhibitory effect caused by R alleles should be selected along with the R alleles. Little is known of the genetic variation in this character in UK wheats since all the evidence derives from foreign varieties. The embryo response system appears to be determined by one or at most two genes, involving an effect in the endosperm (Stoy & Olsen, 1980; Stoy & Sundin, 1976).

#### 7.4.3 Varietal differences in ABA levels

Abscisic acid (ABA), an effective exogenous inhibitor of germination, reaches high endogenous concentrations in grains prior to ripening. Grain ABA levels drop rapidly with the onset of maturity and there is some evidence that varieties with high levels of ABA show more dormancy than those with low levels (Walker-Simmons, 1987). Although this finding implies genetic control of ABA levels, no information concerning the genes involved, or the association of this character with other factors such as red grain colour, is available.

#### 7.4.4 Other sources of dormancy

There are several reports of improved dormancy in white-grained wheats which may represent genetic resources for resistance to sprouting in UK red wheats. The varieties Kenya 321 and Ford have been reported to carry two recessive genes for extended dormancy in the absence of red pigment (Bhatt & Derera, 1980; Derera *et al.*, 1977). Limited evidence suggests that their effects are smaller than those of individual R genes and it is not known whether they are effective in the presence of red grain pigment.

Other extensive searches among white-grained varieties have however identified a number of genotypes with very high resistance to pre-harvest sprouting (Mares, 1987). No genetic information concerning these varieties is available.

## 7.5 PRE-MATURITY GERMINATION

No information on genetic control is available for this trait apart from the fact that varieties differ.

## 7.6 RATE OF ALPHA-AMYLASE DEVELOPMENT DURING SPROUTING

When dormancy is lost the grain will germinate shortly after taking up water. Various morphological features, such as compact ear type and presence of awns (King, 1984; King & Richards, 1984), increase the rate of imbibition of rain water and thus enhance germination rate. All UK wheats have the common ear type and most are awnless and thus, no obvious means of morphological improvement is available.

Even after sprouting starts HFN can be protected to some extent by reducing the rate of germination. Germinating embryos of certain wheats grow more slowly than others (Mares, 1987); however, no information is available concerning the genetic control of this trait.

In contrast, a class of major genes, the GA-insensitive Rht alleles, which reduce the rate of alpha-amylase production during germination, have been studied in detail. The most effective is Rht3 (see section 7.3.2) which, when tested in 1977, reduced amylase levels by 90% (Flintham & Gale, 1982).

The semi-dwarfing genes Rht1 and Rht2 also have a reducing effect demonstrable in precisely defined genetic stocks on rates of alpha-amylase synthesis during germination. However, in varietal comparisons, the effects of genetic background tend to be much larger than those of these GA-insensitive alleles. Thus Rht1 and Rht2 are unlikely to be useful for this purpose in UK wheats.

## 7.7 CONCLUSIONS

At present about one year in four can be expected to produce a UK wheat crop with HFN below 200. The problem can stem from any one, or a combination, of three different causes related to weather patterns during grain development and at maturity. As our understanding of the syndromes and our awareness of genetic variation for susceptibility and resistance increase, breeding strategies to reduce alpha-amylase levels in UK grists are being formulated. Previously the problem has been considered to be a sporadic failure of certain varieties and as such has received little attention from breeders. Genetic susceptibility is now considered by many breeders as the main constraint to quality in the UK crop.

The genes responsible for high pre-maturity alpha-amylase in UK breeding programmes can probably be removed by judicious selection. Searches should, however, be made for significant residual varietal variation which would offer the possibility of improvements in the future.

The most promising immediate route to longer duration of dormancy is through manipulation of the red colour alleles, probably in the first instance by selection for 3-gene red lines. Other genetic sources of dormancy of value in the red-grained UK wheats probably exist, although their utility should be investigated before clear recommendations can be made.

The relationship between pre- and post-maturity sprouting is not understood; thus it is not known whether the same genetic strategies to achieve resistance should be employed. More work on this problem is urgently required.

Ear types that reduce drying rates after rainfall at maturity should, where possible, be avoided. Of the types currently used by breeders, only awned ears fall within this category.

## 7.8 RESEARCH NEEDS

Some aspects of the pre-harvest sprouting problem, particularly precocious germination during development, still require more precise physiological description. More precise genetic characterisation of variation for the several causes of low HFN is needed. Further surveys of varieties for genes conferring resistance, and possibly of related species as potential donors of genes to bread wheats, are also necessary.

### 7.8.1 Pre-maturity alpha-amylase

More information concerning the precise environmental trigger is necessary to establish unequivocal screening methods for use in research and breeding. The major genes involved in varieties such as Huntsman and Fenman should be characterised. The need for still further protection should be evaluated.

### 7.8.2 Dormancy duration

It must be established whether the R genes constitute an independent means of regulating dormancy, or whether a further genetic system controlling responsiveness to germination inhibition by the R genes operates in UK wheats. Further work on the R genes themselves should quantify the effects of R alleles at each locus individually and additively, and ascertain whether any pleiotropic effects on other agronomic characters can be expected in 3-gene red wheats.

### 7.8.3 Pre-maturity sprouting

Since the phenomenon has rarely been observed in wheat, little genetic analysis can precede a precise definition of the environmental trigger involved.

## 7.9 REFERENCES

1. AINSWORTH, C.C., DOHERTY, P., EDWARDS, K.G.K., MARTIENSSSEN, R.A. and GALE, M.D. (1985). Allelic variation at alpha-amylase loci in hexaploid wheat. Theoretical and Applied Genetics, **70**, 400-406.
2. BAULCOMBE, D.C., HUTTLEY, A.K., MARTIENSSSEN, R.A., BARKER, R.F. and JARVIS, M.G. (1987). A novel alpha-amylase gene (Amy-3). Molecular and General Genetics, **209**, 33-40.



3. BHATT, G.M. and DERERA, N.F. (1980). Potential use of Kenya 321-type dormancy in a wheat breeding programme aimed at evolving varieties tolerant to pre-harvest sprouting. Cereal Research Communications, 8, 291-295.
4. BINGHAM, J. and WHITMORE, E.T (1966). Varietal differences in wheat in resistance to germination in the ear and alpha-amylase content of the grain. Journal of Agricultural Science, Cambridge, 66, 197-201.
5. DAUSSANT, J. and RENARD, H.A. (1987). Development of different alpha-amylase isozymes, having high and low isoelectric points, during early stages of kernel development in wheat. Journal of Cereal Science, 5, 13-21.
6. DERERA, N.F., BHATT, G.M. and McMASTER, G.J. (1977). On the problem of pre-harvest sprouting of wheat. Euphytica, 26, 299-308.
7. FLINTHAM, J.E. and GALE M.D. (1982). The Tom Thumb dwarfing gene, Rht3, in wheat. I. Reduced pre-harvest sprouting damage to breadmaking quality. Theoretical and Applied Genetics, 62, 121-126.
8. GALE, M.D. and AINSWORTH, C.C (1984). The relationship between alpha-amylase species found in developing and germinating wheat. Biochemical Genetics, 22, 1031-1036.
9. GALE M.D., FLINTHAM, J.E. and ARTHUR, E.D. (1983). Alpha-amylase production in the late stages of grain development - an early sprouting risk syndrome? In : Third International Symposium on Pre-harvest Sprouting in Cereals; Eds. Kruger & LaBerge, Westview, Boulder, USA. pp. 29-36.
10. GALE, M.D. and MARSHALL, G.A. (1975). The nature and control of gibberellin insensitivity in dwarf wheat grain. Heredity, 35, 55-65.
11. GALE, M.D., SALTER, A.M., CURTIS, F.C. and ANGUS, W.J. (1988). The exploitation of the Tom Thumb dwarfing gene, Rht3, in F1 hybrid wheats. In : Semi-dwarf cereal mutants and their use in cross-breeding. II. Proceedings of the final IAO/IAEA Research Coordination Meeting, Dec 1985, Rome. IAEA TECDOC,455, 57-68.
12. GALE, M.D., SALTER, A.M. and LENTON, J.R. (1987). The induction of germination alpha-amylase during wheat grain development in unfavourable weather conditions. In : Fourth International Symposium on Pre-harvest Sprouting in Cereals, Ed. Mares, Westview, Boulder, USA. pp. 273-281.

13. GALE, M.D. and YOUSSEFIAN, S. (1985). Dwarfing genes in wheat. In: Progress in Plant Breeding (ed. G.E. Russell), Butterworth, London. pp 1-35.
14. HAGEMANN, M.G. and CIHA, A.J. (1987). Environmental x genotype effects on seed dormancy and after-ripening in wheat. Agronomy Journal, **79**, 192-196.
15. HGCA (1987). Home-Grown Cereals Authority. The quality of wheat and barley from the 1987 harvest. (Also previous reports 1974 onwards).
16. KING, R.W. (1984). Water uptake and pre-harvest sprouting damage in wheat : grain characteristics. Australian Journal of Agricultural Research, **35**, 337-345.
17. KING, R.W. and RICHARDS, R.A. (1984). Water uptake in relation to pre-harvest sprouting damage in wheat : ear characteristics. Australian Journal of Agricultural Research, **35**, 327-336.
18. LAZARUS, C.M., BAULCOMBE, D.C. and MARTIENSSEN, R.A. (1985). Alpha-amylase genes of wheat are two multigene families which are differentially expressed. Plant Molecular Biology, **5**, 13-24.
19. LENTON, J.R., HEDDON, P. and GALE, M.D. (1987). Gibberellin insensitivity and depletion in wheat - consequences for development. In : Hormone Action in Plant Development - A Critical Appraisal, Eds. Hoad G.V., Lenton J.R, Jackson M.B. and Atkin, P.K. Butterworths, London pp. 145-160.
20. MARAIS, G.F. and KRUIS, W.J.G. (1983). Pre-harvest sprouting - the South African situation for seed dormancy in barley. In : Third International Symposium on Pre-harvest Sprouting in Cereals; Eds. Kruger and LaBerge. Westview, Boulder, USA. pp. 267-273.
21. MARES, D.J. (1987). Pre-harvest sprouting tolerance in white-grained wheat. In : Fourth International Symposium of Pre-harvest Sprouting in Cereals; Ed. Mares. Westview, Boulder, USA. pp. 64-74.
22. METZGER, R.J. and SILBAUGH, B.A. (1970). Location of genes for seed colour in hexaploid wheat, Triticum aestivum, L. Crop Science, **10**, 495-496.
23. NILSSON-EHLE H. (1914). Zur Kenntnis der mit der Keimungsphysiologie des Weizens in Zusammenhang Stehenden inneren Faktoren. Zeitschrift für Pflanzenzuchtung, **2**, 153-187.
24. NOLL, J.S., DYCK, P.L. and CZARNECKI, E. (1982). Expression of RL4137 type of dormancy in F1 seeds of reciprocal crosses in common wheat. Canadian Journal of Plant Science, **62**, 345-349.

25. STOY, V. and OLSEN O-A. (1980). Inheritance of a factor affecting the response to germination inhibitors in excised wheat embryos. Cereal Research Communications, **8**, 203-208.
26. STOY, V. and SUNDIN, K. (1976). Effects of growth regulating substances in cereal seed germination. Cereal Research Communications, **4**, 157-163.
27. WALKER-SIMMONS, M. (1987). ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. Plant Physiology, **84**, 61-66.

HGCA RESEARCH REVIEW NO. 2

HAGBERG FALLING NUMBER AND BREADMAKING QUALITY

8. **BIOCHEMISTRY**

*Carol M. DUFFUS, Department of Agricultural Biochemistry, School of Agriculture, University of Edinburgh, West Mains Road, Edinburgh EH9 3JG.*

*This review, completed in August 1988, was funded by the HOME-GROWN CEREALS AUTHORITY, Hamlyn House, Highgate Hill, London N19 5PR.*

**CONTENTS FOR 8. BIOCHEMISTRY**

<b>Page</b>	
82	8.1 INTRODUCTION
82	8.2 REVIEW OF PRESENT INFORMATION
	8.2.1 Structural changes during grain development
	8.2.2 Development biochemistry
	8.2.3 Plant growth regulators
	8.2.4 Coat colour
	8.2.5 Water relations
	8.2.6 Alpha-amylase activity
	8.2.7 Dormancy mechanisms
	8.2.8 Biochemistry of germination
86	8.3 FUTURE RESEARCH AND DEVELOPMENT REQUIREMENTS
	8.3.1 Physiological and biochemical differences between sprouting-susceptible and non-susceptible wheat cultivars during growth and maturation
	8.3.2 Embryo-endosperm relationships and embryo germinability
	8.3.3 Control of alpha-amylase synthesis, secretion and activity during grain development and maturation
	8.3.4 Development of a predictive test for alpha-amylase production and pre-harvest sprouting
	8.3.5 Plant growth regulators
88	8.4 REFERENCES

## 8. BIOCHEMISTRY

### 8.1 INTRODUCTION

Excessive alpha-amylase activity in wheat flours, is now generally taken to be a result of pre-harvest sprouting. In some cases alpha-amylase activity is produced although there are no visible signs of germination. In other cases, characteristically associated with rain at harvest, sprouting is clearly visible, and alpha-amylase activity increases markedly. This is presumably a result of dormancy break while the grain is still on the ear.

The production of alpha-amylase often occurs erratically and unpredictably. This is not unexpected since the onset of sprouting is a consequence of the influence of weather conditions and plant genetic constitution on the biochemical events controlling grain development and maturation. With this uncertainty as a background, it is not surprising that the mechanisms leading to alpha-amylase production during grain development and maturation are poorly understood. One particular problem is that, since the onset of sprouting cannot be predicted, investigation of this phenomenon under field conditions has been infrequent. Indeed much of our knowledge relating to alpha-amylase production during grain maturation comes from work not specifically directed at the problem of pre-harvest sprouting. Current understanding of the biochemistry of excessive alpha-amylase production is based on studies of grain development with or without the use of a rain simulator, as well as on germination studies in vitro using detached grains.

### 8.2 REVIEW OF PRESENT INFORMATION

The sections which follow describe current knowledge relating to the identification of biochemical processes involved in the onset of pre-harvest sprouting and alpha-amylase production in cereal grains.

#### 8.2.1 Structural changes during grain development

Differences in grain structure may be a major cause of susceptibility to pre-harvest sprouting. For example, outer layers of the grain may vary in the extent to which they are capable of taking up water and oxygen in the run-up to harvest. The outer layers which surround the endosperm and embryo during development are of maternal origin. Starting from the outside of the endosperm these are the nucellar layer, testa, pericarp and the glumes and paleae. In wheat, unlike barley, rice or oats, the glumes and paleae come away during threshing. The testa is bound on the inside by a substantial cuticular layer on the outside of the nucellus and by an even thicker cuticular layer on the outside of the outer layer of testa cells. The outside of the pericarp has a thin cuticle and in addition there are small numbers of stomata hidden among the hairs of the crease (Cochrane & Duffus, 1979). It is the lipid-rich, waxy, cuticular layers which are thought to restrict the uptake of water, solutes and oxygen into the endosperm and embryo. However, these cuticles do not offer complete protection to the maturing seed. For example, the pericarp cuticular layer is very thin over the embryo and there are gaps in the testa cuticle at the crease and near the embryo at

the micropyle. It may be that variation in the characteristics of these layers may be related to water and oxygen uptake in susceptible cultivars. Additionally, water may enter the grain through cracks in the pericarp. These appear to be formed during grain maturation (Woodbury & Weibe, 1983) and their presence may be a function of genotype or of environmental conditions.

The cereal endosperm ultimately contains between 75 and 85% of the reserve starch and protein of the mature grain. During the first part of grain growth, cell division takes place throughout the endosperm but eventually becomes confined to the outermost layer which forms the aleurone layer. The susceptibility of a cultivar to pre-harvest sprouting and alpha-amylase production must depend to a great extent on the properties of the immature aleurone cells, since these are the site of alpha-amylase synthesis. During germination, the alpha-amylase moves to the starchy endosperm where it is thought to be responsible for initiating starch degradation. Although aleurone continues to develop over a major part of the maturation period it does seem to be capable of taking part in germination from a very early stage of grain growth. For example, percentage germination in wheat cv. Sappho was found (Black *et al.*, 1987) to be over 60% at 20 days post-anthesis when measured at 5°C.

Differences in embryo development and structure will also influence susceptibility to pre-harvest sprouting and alpha-amylase production. The embryo is located at the opposite side from the groove and at the base of the grain. One particular feature of embryo development is that it grows within the endosperm itself and by maturity is effectively embedded in endosperm. There appear to be no channels for the transfer of nutrients from endosperm to embryo and the sources and identity of the nutrients entering the young embryo are unknown. Embryos develop the capacity to germinate very early in grain development. The threshold of viability in terms of size is between 0.20-0.30 mm in length or around 10 days after anthesis in barley (Cameron-Mills & Duffus, 1977).

Thus the immature grain has the ability to germinate very soon after anthesis. Resistance to pre-harvest sprouting and alpha-amylase production must come from the imposition of other factors presumably related to dormancy.

### 8.2.2 Development biochemistry

Sucrose is generally considered to be the main carbon source for polysaccharide synthesis. Its metabolic fate is not fully understood but it seems likely that most of the incoming sugar is converted to UDP-glucose and fructose in the starchy endosperm in a reaction catalysed by UDP-dependent sucrose synthase. Subsequent reactions may involve the conversion of UDP-glucose and fructose to glucose-1-phosphate and then to starch via ADP-glucose in reactions catalysed by UDP- and ADP-glucose starch synthase. Other carbon-containing compounds which may reach the grain are the amino acids, glutamate, aspartate and serine (Duffus, 1987a). There is presumably much metabolic transformation of these before incorporation into grain protein. Interest in sucrose, starch and amino acid metabolism stems from observations that there may be a relationship between the osmotic potential and composition of the nutrient medium supplying the embryo and embryo germinability (see section 8.2.5).

### 8.2.3 Plant growth regulators

Gibberellins, auxin, cytokinins and ABA have all been identified in immature cereal grains (Duffus, 1985). Unfortunately, however, many, if not most studies describe measurements made on extracts of intact seeds which are then correlated with changes in particular tissues. This is not always valid since the immature seed contains many different types of cell at all stages of growth and differentiation. Of these four groups, the gibberellins and ABA are considered to be most relevant to the problem of pre-harvest sprouting.

The gibberellins have probably been the most investigated of all the plant growth regulators in developing seeds. The greater part of gibberellin activity is distributed fairly equally between the endosperm and pericarp. A high concentration is present in immature embryos but this decreases as the grains mature (Radley, 1976). It remains to be determined if embryo-produced GA<sub>1</sub>, the normal germination gibberellin, is the stimulus for pre-maturity amylase synthesis (Lenton & Gale, 1987). ABA content increases rapidly during the middle and later stages of kernel development in wheat, reaching a maximum value between 25 and 40 days post-anthesis (Duffus, 1985). This is followed by a rapid drop associated with the onset of dehydration. Many suggestions have been put forward for the role of ABA in maturing cereal grains. One is that it may act as an inhibitor of early germination. Evidence in favour of this comes from inverse correlations between seed ABA content and germination. However, such correlations are not always observed and, for example, germinability of wheat may bear little relationship to the ABA content (Duffus, 1985). Attempts have also been made to correlate ABA content with the control of alpha-amylase activity. Premature drying of immature grain leads to inducibility of alpha-amylase in response to added gibberellin, enhances the degradation of applied ABA and is associated with a drop in the ABA content of the grain (King, 1982). Again, such evidence is circumstantial and does not show that ABA is directly responsible for the control of alpha-amylase activity. However, work on the mechanism of action of ABA at the molecular level has shown that it can inhibit alpha-amylase activity (Lenton & Gale, 1987).

### 8.2.4 Coat colour

Traditionally, wheat with a white coat colour is associated with poor sprouting resistance whereas wheat with a red coat colour is associated with good sprouting resistance. It may be that oxidation of the polyphenols present in the seed coat leads to reduced oxygen levels thus inhibiting the biochemical processes involved in germination. If, as seems likely, the red pigments have tanning properties, they may cross-link with seed coat proteins and thus contribute to the observed denser structure of these seedcoats, with reduced ease of access for atmospheric oxygen (Gordon, 1980).

### 8.2.5 Water relations

Pre-harvest sprouting and excessive alpha-amylase production may be a function of endosperm water potential. In turn this depends on the water content of the endosperm and the amount of sucrose and other solutes present. Water may enter the grain from the rest of the plant



via the attachment point or through the pericarp. It may then reach the embryo through the gap in the testa at the micropyle (section 8.2.1). In the later stages, water loss takes place without interrupting the supply of assimilate (Duffus, 1987b). Regulation of the balance between water uptake and efflux is clearly a key factor in the control of endosperm water potential. The factors regulating the levels of sucrose and other solutes in the endosperm are not fully understood but are certainly a function of their rate of entry into the endosperm and their rates of conversion to insoluble material and CO<sub>2</sub>. There is certainly evidence to suggest that assimilate supply and grain water content are inversely related (Duffus, 1987b). Furthermore, studies on germination characteristics of isolated immature cereal embryos indicate that resistance to sprouting may be related to low endosperm water potential (Duffus, 1987b). Whatever the relationship between low grain water and sprouting resistance, it can only be truly effective once the sources of water and oxygen have been sealed off.

#### 8.2.6 Alpha-amylase activity

Much of the work on pre-harvest sprouting in cereals has been concerned with the appearance of increased alpha-amylase activity in immature grains. However the release of amylolytic activity occurs relatively late in germination and it is more likely that the key factors involved in the onset of premature sprouting and alpha-amylase synthesis are associated with earlier events in the process such as water and oxygen supply, embryo and aleurone responses and dormancy mechanisms. It is interesting to note however, that inhibitors of alpha-amylase have been reported in cereal grains and in barley the inhibitor can first be detected at about 12 days post-anthesis. Again however, these inhibitors cannot be involved in the prevention of pre-harvest sprouting since their effect takes place well after alpha-amylase synthesis has begun. In barley, no correlation has been found between pre-germination incidence and level of alpha-amylase inhibitor (Hill et al., 1987).

#### 8.2.7 Dormancy mechanisms

After harvest, mature seeds often fail to germinate when subjected to conditions which normally support germination. This state is known as dormancy. Eventually dormancy is broken and the seeds will become capable of germination. Much work has been carried out on the mechanisms involved in post-harvest dormancy but whether or not the process is the same in maturing grains still attached to the ear is not clear. It is likely that many of the factors described above, and thought to be implicated in the control of embryo germination in immature grains, are part of the dormancy mechanism. Superimposed on these however are environmental effects. For example in wheat (cv. Sappho) low temperatures early in development lead to the production of deeply dormant grains (Black et al., 1987). On the other hand, if there are high temperatures during development (eg 25°/20°C), grains pass through a period of high germinability (approx. 30 days after anthesis) when they would be susceptible to sprouting if wetted. While these observations help us to characterise the phenomenon more precisely they tell us little of the biochemical mechanisms responsible.

### 8.2.8 Biochemistry of germination

The biochemical changes accompanying germination in cereal grains have been subject to much investigation. In general the emphasis has been on barley, in particular naked cultivars such as cv. Himalaya. These studies have led to the conclusion that one of the first events in germination is the synthesis of GA in the embryo. Subsequently, this is transported to the aleurone layer of cells where it triggers the synthesis of alpha-amylase. How far this is true for pre-harvest sprouting and alpha-amylase production in maturing wheat seeds on the ear remains to be discovered. The area of most relevance to the problem of pre-harvest sprouting is that concerned with the mechanisms triggering the synthesis of GA, or alternatively with the mechanisms determining the responsiveness of the target sites in the aleurone to GA. Unfortunately this is probably the area of greatest ignorance.

### 8.3 FUTURE RESEARCH AND DEVELOPMENT REQUIREMENTS

Much of the above information has been derived from work not directly aimed at a resolution of the pre-harvest sprouting/alpha-amylase problem. As a consequence:

- 1) susceptible and non-susceptible cultivars are rarely compared;
- 2) embryo-endosperm water relations during grain growth are largely ignored;
- 3) the nature of the overall control mechanism in wheat grains still attached to the ear, presumably exerted by plant growth regulators and their target tissues, is unknown;
- 4) little work is done under field conditions;
- 5) reliable predictive biochemical tests for pre-harvest sprouting and excessive alpha-amylase synthesis have yet to be developed.

In order to identify the physiological and biochemical factors responsible for sprouting susceptibility and excessive alpha-amylase production in wheat cultivars, further research in the following areas is required (in order of priority):

#### 8.3.1 Physiological and biochemical differences between sprouting-susceptible and non-susceptible wheat cultivars during growth and maturation

Those properties of the grain that may be responsible for susceptibility to pre-harvest sprouting require identification. Key structural and physiological characteristics include husk splitting; pericarp thickness and structure; testa colour, composition and structure; structure of the layers covering the growing embryo; properties of the cuticular layers surrounding the endosperm; embryo damage; aleurone structure and formation.

Biochemical characteristics include determinations of sucrose and amino acid concentrations in the endosperm/embryo; relative rates of sucrose supply and utilisation; water contents of the different tissues of the grain; water potential and composition of the nutrient medium supplying the embryo; differences in respiration.

### 8.3.2 Embryo-endosperm relationships and embryo germinability

The environmental conditions determining embryo germinability need to be defined more clearly for susceptible and non-susceptible cultivars under sprouting and non-sprouting conditions. By using detached ears in liquid culture it is possible to vary both sucrose and water content of immature endosperms. With this technique it should be possible to discover the nature of the relationship between assimilate supply, water potential and germinability. The mechanism whereby, during the later stages of grain filling, water loss can take place from the embryo and endosperm requires investigation. The extent of any modifications to these mechanisms in relation to the retention and further uptake of water in pre-harvest sprouting should be determined. In general, the factors controlling embryo development and maturation require to be identified.

### 8.3.3 Control of alpha-amylase synthesis, secretion and activity during grain development and maturation

The mechanism responsible for inhibiting the gibberellin-dependent synthesis of alpha-amylase during growth and maturation in sprouting resistant cultivars requires further investigation. Questions which require answers include:

- 1) what triggers gibberellin synthesis - if indeed this substance is involved?
- 2) which gibberellin is involved?
- 3) is the mechanism of transport and action of gibberellin similar to that observed in mature detached harvested grain?
- 4) what characteristics of the aleurone cells determine their response to gibberellin?
- 5) what is the role, if any, of ABA in the control of alpha-amylase production?

### 8.3.4 Development of a predictive test for alpha-amylase production and pre-harvest sprouting

The use of immunotests for the detection of specific proteins is developing in the malting and brewing industries particularly for the identification of alpha- and beta-amylases. Such techniques might prove valuable, if cheap, reproducible and effective, for the prediction of the onset of pre-harvest sprouting. These developments require the preparation of monospecific immune serum or monoclonal antibodies. In turn, antigens must be highly purified for testing the clones or for immunisation. Some success has been achieved using immunoaffinity chromatography in the purification of the main alpha-amylase of germinated barley (Kerhardy *et al.*, 1988). Thus we may have the basis of a technique for comparing the content of enzymic groups in different cultivars of wheat. The same techniques may also prove useful in the determination of the alpha-amylase inhibitors known to be present in immature wheat grain. In addition, there is now a readily available monoclonal antibody for the determination of abscisic acid. If indeed ABA proves to have a role in the inhibition of germination then a relatively simple method is now available for its determination.

### 8.3.5 Plant growth regulators

Knowledge of the fundamental mechanism of action of these substances in plants is insufficient to explain the very loose correlations between physiological events and applied or endogenous growth regulators. These problems are compounded by difficulties in analysis of plant growth substances. For example, the gibberellins and cytokinins are groups of substances all with some physiological activity and their determination requires expensive gas chromatography-mass spectrometry techniques for separation and identification. Any significant investigation of the role of gibberellin and cytokinins in the control of pre-harvest sprouting would therefore be costly since it would require extensive analysis of these substances:

- 1) in the different tissues of the grain over developmental time course;
- 2) in sprouting and non-sprouting cultivars;
- 3) under sprouting and non-sprouting conditions.

On the other hand, ABA is a single compound and can be analysed fairly easily (see section 7.3.5). Furthermore, its action at the molecular level is becoming much better understood. It seems likely that a study of its role in pre-harvest sprouting might yield useful results.

It should be noted that any investigations of these substances in grain development and maturation should be carried out under strictly standardised conditions where the structural and physiological changes are fully described. Such conditions might be met most easily by using immature detached ears, grains or embryos in liquid culture.

### 8.4 REFERENCES

1. BLACK, M., BUTLER, J. and HUGHES, M. (1987). Control and development of dormancy in cereals. In: Fourth International Symposium on Pre-Harvest sprouting in Cereals. (Mares, D.J. ed.). Westview Press. Boulder, Colorado. pp. 379-392.
2. CAMERON-MILLS, V. and DUFFUS, C.M. (1977). The in vitro culture of immature barley embryos on different culture media. Annals of Botany, **41**, 1117-1127.
3. COCHRANE, M.P. and DUFFUS, C.M. (1979). Morphology and ultrastructure of immature cereal grains in relation to transport. Annals of Botany, **44**, 67-72.
4. DUFFUS, C.M. (1985). Plant growth regulators and cereal grain development. In: Cereal Tissue and Cell Culture. (Bright, S.W.J. & Jones, M.G.K. eds). Martinus Nijhoff/Dr. W. Junk, Dordrecht, The Netherlands.
5. DUFFUS, C.M. (1987a). Cereal grain structure and development: relationship to the technological properties of the mature grain. Aspects of Applied Biology, **15**, 63-78.

6. DUFFUS, C.M. (1987b). Recent progress in the physiology and biochemistry of immature cereal grains in relation to pre-harvest sprouting. In: Fourth International Symposium on Pre-Harvest Sprouting in Cereals. (Mares, D.J. ed). Westview Press, Boulder, Colorado. pp. 231-244.
7. GORDON, I.L. (1980). Germinability, dormancy and grain development. In: Second International Sprouting Symposium (Gale, M.D. & Stoy, V.). Cereal Research Communications, 8, 115-129.
8. HILL, R.D., MacGREGOR, A.W., WESELAKE, R.J. and DAUSSANT, J. (1987). A review of some properties of an endogenous inhibitor of cereal alpha-amylase. In: Fourth International Symposium on Pre-Harvest Sprouting in Cereals. (Mares, D.J. ed.). Westview Press, Boulder, Colorado. pp. 474-482.
9. KERHARDY, F., BUREAU, D., MEYER, C. and DAUSSANT, J. (1988). Purification of barley malt alpha-amylase by immunoaffinity chromatography. Journal of the Institute of Brewing, 94, 38-41.
10. KING, R.W. (1982). Abscisic acid in seed development. In: The Physiology and Biochemistry of Seed Development, Dormancy and Germination. (Khan, A.A. ed.) Elsevier Biomedical Press, Amsterdam. pp. 157-181.
11. LENTON, J. and GALE, M.D. (1987). Hormonal changes during cereal grain development. In: Fourth International Symposium on Pre-Harvest Sprouting in Cereals. (Mares, D.J. ed.). Westview Press, Boulder, Colorado. pp. 253-264.
12. RADLEY, M. (1976). The development of wheat grain in relation to endogenous growth substances. Journal of Experimental Botany, 27, 1009-1021.
13. WOODBURY, W. and WEIBE, T.J. (1983). A possible role for the pericarp in control of germination and dormancy of wheat. In: Third International Symposium on Pre-Harvest Sprouting in Cereals. (Kruger, J.E., & LaBerge, D.E. eds.). Westview Press, Boulder, Colorado. pp. 51-58.

## 9. ACKNOWLEDGEMENTS

The initial planning of this review was carried out by the late John Baldwin and his sudden death on 4 March 1988 was a sad loss to the team working on this project. Much of the coordination was then taken over by Dr. L. Vaidyanathan until family bereavement necessitated his return to India for about 6 weeks. Other ADAS colleagues have willingly contributed to section 6 of this review.

Authors from the outside institutions have been particularly prompt in providing material at the agreed time and most gracious in accepting the abstract of their work.

The clerical staff of the Agronomy Department at Cambridge have carried out much of the checking of the typing and a final check by Dr. M. J. Griffin and Dr. M. C. Heath has been greatly appreciated.

In order to provide a uniform presentation, the whole review has been typed with much appreciated cooperation by the Block B Typing Pool at ADAS, Cambridge. Their cooperation and efficiency has eased this task considerably.

Finally, of course, without the financial support of the HOME-GROWN CEREALS AUTHORITY this review would never have been carried out.